ought not to do right now, to bank sperm beforehand.

I mean there are a lot of things you can do to encourage, to sort of defeat the likelihood that there is going to be any transmission. So we have got at least two protections there. A third is -- well, think about what if the worst possible scenario happened, we go ahead with the trial, we permit the trial to continue despite being pretty comfortable with the test, despite the promise to do barrier, despite the information given in informed consent, a child is, in fact, conceived and born who is carrying an altered gene here.

We need to think about -- we are not talking about a systemic kind of -- you know, a change in many, many of the births of many children, we are not talking about an intentional modification of a genome, we are talking about, you know, this is an incidental and unintended consequence.

I don't have the answer to that, but that is the thing we would be guarding against, how horrible of a mora affront or of a precedent would that be? I don't have an answer to that one, but I just want to lay it on the table.

One more challenge before us. There is the Avigen issue, which we need to answer. I guess I should hold off for No. 2, but Question No. 2 is going to say, okay, it ain't just men, it's also women, and how are we going to think about being aware with women.

DR. SALOMON: That is Question 2, and we will get to that.

To me, you have raised a couple of really interesting points, so let me go back to this motile sperm thing. The problem I have with any sort of testing strategy in a clinical environment is the more complicated you make it, the more difficult it is, more expensive, more technically challenged, and more often is going to go wrong.

I have heard no data that convinces me that if the semen is positive, that the motile sperm could be negative and that this is such a big advantage. In the absence of that data, I continue to be underwhelmed with the need to be doing this motile sperm test, because what happens then is you say the motile sperm test, because of the fact that there is a whole bunch of manipulation, or you get a patient that doesn't have a large enough volume, although I am not quite sure why they couldn't just

dilute it up to 1 1/2 ml with saline, but anyway -- the bottom line here is if you get into that sort of circumstance, it just tells you why these kind of assay systems are problematic.

We would actually have to tell them -- we would have to have this, I would think rather silly discussion about how to set a quality control for a motile sperm test, because if they let it sit out on the bench for a couple hours and then they do this motile sperm test, and call it negative, and the semen is positive, well, that is bogus.

I am just having trouble with this test, and I don't think it is a minor issue in terms of how they are going to do this trial.

DR. MULLIGAN: Just to directly answer the question, I would say that a clinical hold would not be warranted although I would couple the question in 1A to the first part of 3, and then to answer your question, I think the motile sperm thing is perfectly okay, that if we all think that the likely source of the AAV is probably blood or something, then, the best, you can separate those, I think it helps, and I think there are SOPs, people can come up with something.

But 3, I know how you hate to jump to the

next question, but I think it has to do with at what point, when repetitive tests showed positivity would everyone think that there was something so unanticipated that you would actually want to stop the trial?

My proposal would be no, let the trial go on, but come up with some, maybe it's a year, throw out a year, if after one year there is still positivity, even if that was in the blood mononuclear cells, I think that most people would have grave concerns that something was happening that was not anticipated.

DR. SALOMON: I followed you up until that last little throw-in about peripheral blood and mononuclear cells, I mean because I wouldn't care that much of the patient integrated into a hematopoietic stem cell and was positive.

I think the real issue here is only given, I don't know why a year, because it seems like that is a hell of a vector reservoir to think that you could keep shedding detectable vector for 12 months?

DR. MULLIGAN: That's the point. The point is that everything we have heard here suggests that you won't have persistence of the

vector for that amount of time, simply by having it sitting there in some tissue. Therefore, something is happening that is unanticipated if it is persisting at that point, and that could be an arbitrary point, but I think a year is certainly enough time to think that something is happening that shouldn't happen, at least we don't know what is happening.

My point was just that a critic that would say, well, yeah, that could still be not in the motile sperm, I would say still, we would be worried about the patient because something very unanticipated has now happened, that is, if we are able to get hematopoietic stem cells transduced by AAV vectors, and you have AAV integrated, I think everyone would want to know that and would have great concern.

DR. NOGUCHI: Just to add more confusion to that particular point, the hemophilia trial for factor VIII, and the Chiron proposal, they did report to the same committee that in a situation in which there was a very fractional and very short-lived positivity in the semen, that, in fact, they were able to detect positive peripheral blood samples for I believe well over a year.

That was the unexpected finding in that particular case. Probably an encouraging finding in terms of at least transduction of a somatic cell, I would guess.

DR. SALOMON: I think that everybody in this field is clear that the amount of time that even the episomes persist, and whatever small integration occurs or whatever, in different populations, I think Jude was very good about pointing out that there is some data, but it is not completely tested. Is that a fair characterization of what you said?

The fact that it would be around, it could be a positive for this as a gene therapy strategy, I am not concerned about it, I think the issue has to focus on the sperm or the semen as a test. My only issues there are technical, but if it is in the sperm at a year, then, it seems to me it is impossible -- well, is it impossible that at that point -- I just can't imagine you are shedding viral reservoir any longer. The implication at that point would be that it has been integrated into the germline.

DR. MULLIGAN: I think the point of setting some long period at which you would stop

and take another look is you want to address it in a way that was not in the clinical protocol.

DR. SALOMON: I am agreeing. I am just bringing up some discussion points on the timing.

DR. DYM: This is an unrelated comment, but it is on the same issue. With the subject No. 2, who has AIDS, I think is what we were told, and spermatogenesis is markedly reduced in patients with AIDS, and this is shown, of course, in the total semen volume, 200 microliter, 150, 150, it is very unlikely that this particular patient will be fertile. I don't know if that is an issue or should be raised.

DR. KAY: Just for a point of clarification on the patient No. 2, the patient is HIV-positive, his CD4 count at the time, that last one that we checked, was around 340. He has had children in the past, and based on his total sperm count, he actually has a normal number of sperm in the ejaculate, but the volume is very low, so from what we can get from that, the spermatogenesis itself is normal, but there is something wrong with the ability to make the fluids. The pH of the fluid has been normal, suggesting that he doesn't have specific obstruction of a prostate versus

seminal vesicle.

DR. DYM: I was interested in this question before that may be relevant. Did you check the size of the testis, does he have a normal testis?

DR. MAY: Well, since I am not the clinical treater on this case, obviously, Dr. Glader, who I think had to leave to catch a plane, is the individual who examined him.

DR. RAO: I actually wanted to retread what Dr. Salomon and Dr. Mulligan said. I don't think that it would be necessary that there should be a clinical hold and that that should be dependent on motile sperm test. There are two problems in my mind with just doing the motile sperm test, is that -- we have already heard about the problems of vector carryover in semen itself, so even if your motile sperm test was negative, you would still worry about that as an issue.

The likelihood from the way the test was presented, is that if your semen is negative, then, your motile sperm fraction is going to be negative. So it doesn't seem that we should be focusing on motile sperm test as a specific test, but rather on total semen.

Do we necessarily need to -- which was part of Question 3, which you put together -- it didn't seem to me that you should include only patients who are incapable of the production, but there should be a time line and that patient consent, the forms should be modified with an emphasis that this is a problem.

DR. JUENGST: I guess my hesitation in originally answering your question was because I was hung up on the qualifier, "or should enrollment be allowed to continue with appropriate modification made to the consent documents."

Well, no, that is not enough to modify the consent documents. It is really a change in the protocol because informed consent is not a reasonable and prudent safeguard. Patients behaving appropriately cautiously is a prudent and reasonable safeguard.

So they need to be more than simply read a few extra lines in the consent form. It needs to be a real concerted program for making this education and sperm banking available, and that sort of thing.

It sounds like what they have been doing.

DR. SALOMON: It sounds like that has been

a part of their protocol even a trial or two earlier, which is good. I think we are getting there.

Does the committee agree that a clinical hold is warranted when motile sperm tests are positive -- let's just call it now "semen" -- you know, if we could vote on this, but I generally don't want to go there, I mean that is a decision I think that the FDA can come back to us. We have had a discussion on this motile sperm versus semen, I am comfortable with the semen.

If there is a clinical hold, is it warranted? The committee to me has said no, that I don't think you need to put a clinical hold on this trial every time the semen is positive. Does anyone want to take a minority opinion here?

DR. NOGUCHI: On that one, we might want to at least have a show of hands to make sure we understand the real kind of sense of the committee.

DR. SALOMON: Then, we will poll. Do you want us to give you a specific on the motile sperm versus semen?

DR. NOGUCHI: I think that is very important. It certainly seems like there is enough discussion that there is some doubt about the extra

value of fractionated versus just whole semen. I think it would be worthwhile for that, too.

DR. SAMULSKI: If we were going to poll, I think it is probably important that I at least make one or two statements about the vector aspect of it.

I am not going to proclaim I know the answers, but I have been working at this for over 20 years, and there are definitely trends that show up that are extremely consistent, that you can begin to feel confident about, and the virus integration is a trend that has been consistent from when this was first studied and now going from tissue culture to animal models, it doesn't integrate very efficiently.

I think people need to understand that and buy into the fact that if we are going to put a lot of virus into people, the potential of integration is virtually nil to it can happen, but it is not a high risk potential, and then if you move away from that question and look at the question of if we have a PCR-positive signal, which is something like 10 copies in a sample, and we are talking about 0.1 percent of the virus ability to integrate, we are getting down to numbers and the amount of sperm

that one is going to be transmitting, this is like Star Wars in some ways, trying to calculate what is the frequency of the planets lining up again, and stuff like that.

It is so vanishing small, the risk that we are talking about, that from a vector perspective, I think there is no reason at all to put this trial on hold. Where I do have my only concern, and it sounds like it is being addressed, is that Phil brought up, which is if you are mechanically going to have virus tracking along, and you are going to do experiments to see if you can come up with a way of artificially getting this into cells, that is an unknown that needs to be resolved, and I think the onus will fall on the group that is interested to get that data in front of people as soon as possible.

But other than that, I am sitting here saying we are really discussing something that is virtually impossible, and I think that value of what can come out of the studies is a lot more important than us trying to talk this tightrope.

DR. SALOMON: I very much agree with that, too, and I think I am comfortable that the flow of this committee is going in that direction, as well.

You brought up one thing, Jude, that I just wanted to come back to, and that is you suggested, I don't know if you meant it, additional sophistication on this testing strategy, and that would be do quantitative PCR, and if it is lower than a certain number, even that then could enhance one's comfort level. I hadn't thought of that, because up until now, we have been talking about positive versus negative.

Would you be concerned if it came out as a million copies? I mean these guys are detecting it positive at the lowest levels at 14 weeks.

DR. SAMULSKI: So that is where I think it is prudent, and it is not just AAV when you keep dosing biologics, whether they are plasmids of oligos, at some point you are going to have a threshold level where it is going to be unpredictable.

I think that is the kind of information I would like to see keep coming out of this trial, he didn't just persist longer, but here is how he persisted, it was over 1,000 or 10,000 copies per X amount of time, and stuff like that. That is valuable information.

DR. SALOMON: I agree with that, and I

would say that I was not impressed by a statement that it wasn't absolutely quantitative, but the signal was going down. I think today, there are very straightforward ways to do quantitative PCR, so we don't have to really, in these settings, to any longer talk about nonquantitative PCR studies.

Let's do a poll of two questions. One would be the first, and that would be motile sperm versus semen testing, just to get that out of the way.

Starting with you Jude, motile sperm or whole semen?

DR. SAMULSKI: I think whole semen is adequate.

DR. DYM: Yes, same. Not necessary to differentiate between the two.

DR. JUENGST: For what it is worth, my layman's vote will go with these guys. We don't differentiate.

DR. MURRAY: I confess I do not understand the merits of the two tests sufficiently to make an intelligent vote, but it is not because I am torn between two alternatives I understand well.

MS. WOLFSON: I would have to repeat exactly what Dr. Murray said.

DR. RAO: I think whole semen.

DR. SALOMON: I vote whole semen obviously.

I am not certain. Richard gave me what he would say for the second one, but I am not comfortable with that, so we will have to say he is not here.

Let's do the second question, and that was, does the committee agree that a clinical hold is not warranted any longer with a positive semen test? Jude.

DR. SAMULSKI: Not warranted.

DR. DYM: A hold is not warranted, I agree.

DR. JUENGST: A hold is not warranted.

DR. MURRAY: I agree that with the proper additional protections put in place, with Eric's earlier caveat about it is not just adding lines to informed consent, we would not require a hold.

MS. WOLFSON: Again, I agree with Dr. Murray exactly.

DR. RAO: I don't think a hold is necessary.

DR. SALOMON: And I don't agree a hold is necessary either. Richard Mulligan told me that he

also wanted to say that a hold is not --

MS. DAPOLITO: Dr. Mulligan should be here to vote, but Dr. Salomon can put his comments into the record.

DR. SALOMON: Excellent.

The next question here would be discuss the implications of detecting vector sequences due to the presence of contaminating transduced PBMC or vector (either free or on the surface of a sperm) in the motile sperm fraction.

Now, my sense here is we have really pretty much discussed that. If the FDA comfortable with that? I don't see any further discussion as being necessary on that one.

Anyone else on the committee?

[No response.]

DR. SALOMON: Okay.

Question 2. There are technical limitations in the ability to monitor women and certain men for evidence of germline alterations. One approach to monitoring subjects for germline alteration would be to restrict early clinical development of certain gene transfer products to subjects who have been shown to be capable of repetitively supplying adequate semen samples for

analysis in order to get good data collection for detecting persistence of vector.

DR. MURRAY: That does leave out a certain number of potential -- about 50 percent of the human population actually, which is not relevant for the Avigen trial, but will be relevant for others.

DR. SALOMON: Correct.

I am going to try and parse it down. So the first issue would be -- I think you guys actually had a slide on this, am I mistaken? You had a slide saying that you were redesigning -- you had to have more than 1 1/2 ml of semen and a certain sperm count, wasn't that right?

They have already incorporated in their protocol, does anyone disagree with that?

DR. SAMULSKI: I think it's admirable that they are doing it, but i also agree with Dr. Murray. I don't think this is something you want to put in as policy because if other things come down that aren't related to hemophilia and the population can't be in an inclusion criteria, but based on something like this, I don't think that would be a direction we would want to go in.

DR. SALOMON: Certainly, men who were

infertile and would have no danger of germline transmission would be excluded from these studies, so I think I could think of one very good reason not to make it policy in the area.

Dr. Rao.

DR. RAO: I was just going to add that one reason for worrying about sample size was because we were doing motility experiments and fractionation. Now that the committee seems to have a consensus that we don't need to do fractionation, I think the tests can be done with a smaller volume, so I don't think that should be an exclusion criteria.

DR. SALOMON: All you need now would be enough sperm to get, let's say, 1 microgram of DNA -- I am sorry -- 3 to 4 micrograms of DNA, so you could do triplicate or quadruplicate at 1 microgram, and from my own experience doing Tacman PCR, which we do a lot of in the lab, I would be comfortable with that, as well.

So, that would be a sperm count of what? Were you guys counting 30,000 sperm for a microgram of DNA?

DR. SAMULSKI: 2 x 106.

DR. KAY: Yes, we were using 2 to 3 x 105

sperm per microgram roughly, and we need 4 or 5 micrograms, and then the issue is recovery.

DR. SALOMON: Would everyone agree there should be a limit of, let's say, no less than 5 million total sperm in an ejaculate as criteria for entering the trials at this point, 10 million? All right.

DR. KAY: Sold.

DR. SALOMON: Any discussion on that point? I think the intention of the committee is clear, and the details we would leave to you.

Depending on the amount of data required, much of the early clinical experience with the vector might be limited to this restricted population. A development program requiring extensive characterization of distribution to germline cells and germline alterations might delay the acquisition of adequate safety and efficacy data in other populations, for example, women.

I guess we can't avoid the very important discussion of where women fit into these trials. So this gets to something that Tom, you introduced to us in your comments, thoughtful comments a few minutes ago, and that is, what is our level of sensitivity here, how big of a deal would it be,

and that has a lot to do with the next trial that comes along that wants to do the study in women, right?

DR. MURRAY: Any disorder that is not x-linked.

DR. SALOMON: Or a male that has no sperm or falls below it, we need to think about them, as well.

The interesting thing here is I mean we are now up against the international consensus that has been supported by many in the lay public, that the line that no one is ready to cross is intentional? Germline transfer certainly. And unintentional germline transfer is probably far enough across that line that it ought to be avoided at all costs, as well.

I am very cognizant of the fact that depending on how this discussion goes, we have to be cautious that we are having an advisory committee advising the FDA that under certain circumstances, it is okay to do germline gene transfer, and I want to put that into context for the committee. You can disagree with it, but that is the question.

DR. MURRAY: There is one piece of advice

I think we could give the FDA. It may not be very useful advice, but that is to --perhaps you have already done it, -- but basically to take stock of the best possible methods for evaluating germline alterations in females, looking at animals, looking at -- we have heard a variety of ways of thinking about looking at such alterations in males, what is the best state-of-the-art in thinking about this with females? Granted that many of those assays are going to be completely unavailable with humans, to think about what would be the most -- morally permissible and without crossing the boundaries of mistreating human subjects, how to involve women, how to monitor potential germline genetic alterations in women.

I don't think that is easy, maybe you have done it, but if you haven't, I would say it is probably an urgent thing. The one thing I could not recommend -- we had a tough choice here -- but one think I clearly could not recommend is that women be excluded from such trials. I suspect national policy would also prohibit us from making such a recommendation.

DR. NOGUCHI: Just in terms of that, FDA, unless there is a compelling reason to exclude one

sex or another or any particular subset of man or woman, we would not use anything other than a very specific reason that is both reasonable and can be defended.

DR. JUENGST: Another thing to think about is where along the research time line we would be willing to take those risks of inadvertent transduction. We do take genetic risks with patients when we give them chemotherapy and radiation, and we justify that by saying, well, we are saving their lives, so in a Phase III trial of gene therapy, we could make a very similar argument.

Now, how about a Phase I safety study, maybe there is a distinction to be drawn there unless we find ourselves in the situation where you can't get to Phase III unless you do the Base I.

DR. SALOMON: That's a good point. Just to highlight that you are pointing out that the quote, "We are doing it because we save human lives" is a clear indication of conviction of efficacy, which would allow you to accept a risk that is very different than in a Phase I or Phase II trial.

Dr. Rao.

DR. RAO: I was just going to say that the general consensus that you want to be able to monitor, it is not an issue of whether it can happen or not happen or the probability, but that you want to have an ongoing monitoring to make sure that there is no evidence of germline transfer, and should we be excluding patients where we can't monitor that, and the answer is that I think we are doing that already when you set up a criteria, whether you set it for males when they can't have a certain sperm count, or whether you can't because you don't have any available tests to do that monitoring.

The question is do we not need to monitor at all. In my opinion, right now, with the available data, it is not clear because there is still enough not known about the virus in the sense what is happening in blood cells, why do we see persistent expression, is there some specific time at which you see better integration, and so on.

So there is a finite, maybe already low probability that there might be germline transfer, but whatever that low probability, at the current situation, with what data is available from animal models, we can't say that we should include

patients where we don't have any monitoring.

DR. SALOMON: If you think about it, there is a couple different ways this could go. None of us, unless some -- I can't imagine are going to give advice that you want to permanently exclude women from certain kinds of trials, right, why even go there, that's impossible, so you can see a couple different ways to try and put this together, and I think the framework is already out on the table, one way is to say in the absence of really good definitive preclinical data that would allow you to say with any sort of confidence it cannot go into the germline, and I think we all agree you cannot say that quite yet, no evidence that it does, but no evidence to say that is can't, and a lot of evidence suggests that it ain't going to be easy and not likely, particularly with this particular class of vectors.

You could say okay, really low possibility, so that takes a lot of the pressure off, but it is not enough. So then you go on and say all right, fine, let's go into Phase I trials and let's restrict the Phase I trials to subject that we can monitor.

I think the company themselves, to their

credit, have taken that view, and the FDA is comfortable with it, and I think we have just refined it a little bit.

Then, the only question left is how much data do we need under our belt before you allow in later phases of the trial, to go into women, pretty much saying, hey, it's not happening, and I don't want to get into demanding someone give us statistical time, like after 100.3 patients we can do it, but I think I am suggesting to you that maybe the best way to think about this is at a certain point, once they get to a Phase III trial, and there is enough confidence that none of these patients with no evidence of germline transfer in these males that can be monitored, that then you could relax the criteria and cautiously open it up to women.

So that would be what I would suggest.

MS. CHRISTIANSON: Janet Rose Christianson. QARA Services, formerly with Target Genetics Corporation.

A brief comment with regard to selecting people for monitoring in Phase I. I think there has got to be another consideration, and I think that has to do with the route of administration.

If there is no dissemination, for example, the present study that Target is doing is an oral aerosolized delivery of an AAV vector in cystic fibrosis patients. I think that the way it is delivered, and any of the preclinical data, indicating if there is dissemination to the peripheral blood distal nodes, or whatever, should also have a bearing as to whether or not monitoring of females or nonfemales or whomever, should be part of that whole process. I think that has got to be a point just to consider. Maybe my glucose was low and it's intuitively obvious, but I did want to make sure that that point was raised.

Thank you.

DR. SALOMON: I think that is an excellent point.

Dr. Rao.

DR. RAO: I actually wanted to add one more piece to the whole monitoring issue, and that was just simply to argue that if the criteria or the worry for which you are excluding patients is because of germline transfer, that perhaps one additional criteria for inclusion is people who would not be, are incapable of germline transfer.

DR. SALOMON: The only problem with that

is, it is kind of a dead end in terms of moving the field forward, because you would never be able to tell whether there was germline transfer, so everyone else would be standing there waiting.

DR. RAO: Maybe you shouldn't exclude them for whatever reason you want to include them in a study. That is all I was trying to say.

DR. MURRAY: In a way, we should be very grateful to the folks from Avigen for their inadvertent finding, because it really forces us to confront -- I don't mean just this committee, by no means do I mean just this committee -- I mean everybody who thinks about these larger issues of the ethics of research, inadvertent germline transfer, and gender equity in research, and all these things, it warns us about what is probably lurking not too far down the road, and in addition to my off-the-cuff injunction to FDA to sort of think as much as they can, I don't think this committee is the group to decide what the right balance is, but in fact, I mean RAC has had a recent history of doing policy conferences.

This would be a great topic for a RAC policy conference, in my view, about how to balance the concern about monitoring inadvertent germline

modification against an issue of gender equity, is it as we think it is, that it would be much more difficult to monitor in women, are we wrong about that? Are there ways of monitoring this in women? I am not aware of any, but, you know, there are some fact questions there science questions, and then how should one sort of strike the right policy balance.

One emerging suggestion, I think I have heard, is that you do the Phase I -- where this is a possibility -- you do the Phase I on males who have sufficient seminal fluid and sperm that you can test, they make 5 million sperm in an ejaculate.

Now already, that creates some issues of gender inequity, I understand the rationale for that, but I think it would be a mistake to rush forward into that without a chance to really reflect on how to balance.

There are two good things. We are trying to ensure gender equity and participation in research, and we are trying to ensure that we can get a handle on inadvertent germline gene transfer. There are two good things. Somebody has got to figure out what the right balance or plan is, and

it is not a thing we are going to do by 3 o'clock today, and we are not the right party to do that.

DR. NOGUCHI: Steve, shall we work on that as a possibility, what Dr. Murray is talking about?

DR. ROSE: Certainly, it is something that the RAC has been discussing and will continue to discuss, and it is one of the policy conferences we have been thinking about.

DR. NOGUCHI: You are welcome to come, too, Tom, I am sure, and probably most of the rest of the people at the table here.

DR. SALOMON: Jon.

DR. GORDON: Yes, I am commenting. I have recused myself from this discussion because as a committee member, and I am commenting as a member of the public.

I think there are a couple of points. One is that whenever you exclude a certain group of people from a study, regardless of the phase of the study, you at least have to be alert to the introduction of biases in the study, so I think people need to be aware of that.

Is it going to be more safe for the people you study or less safe? I don't think it is necessarily relevant in the present case, but

anytime people are excluded in some sort of overarching parameter, then, that is a risk.

In terms of the addition of females to these trials in hemophilia, not a likely issue to come up, but as people have point out, autosomal disorders it is, I think the committee might consider recommending that good preclinical tests for female germline transmission be encouraged to be developed.

I mean it is not impossible to do that, and there is no reason why, if we have been doing all these things with rabbits and monkeys and all that with the male side, why we couldn't also do things on the female side.

We have a paper where we looked at adeno at the female side, so there is no reason why that couldn't be encouraged by the committee.

DR. SALOMON: I would actually not want to go there. I don't think as a committee, we want to start even getting into whether women as part of participation in a trial ought to undergo laparoscopy and removal of eggs or ultrasound guided biopsies, and things like that, if that is what you were suggesting. I think those are topics for preclinical investigations and not for creating

yet more complex and even potentially risky barriers for participation in a trial.

DR. GORDON: I guess I wasn't clear. I believe, I emphasize preclinical studies in animals that would then give one more confidence that a human could be admitted to a study.

DR. NOGUCHI: Just to say that the issue of women is pertinent to this discussion, albeit it, it is an extraordinarily small population, there are handful of women with hemophilia, and for them, especially they are totally out of any of the normal support mechanisms. They may not even know what hemophilia is because it is not something that they normally know about, but eventually when one of these things works, they are a part of the question. We will have the same question as to whether or not it is an unreasonable risk for that population albeit it might be as many as on this hand here.

DR. SALOMON: I think that at the end, there is no way -- again, I welcome everyone to comment -- from my view, I don't see how one can refine this any further in the sense that it has been put very clearly that, on one hand, the concept of germline transfer as a potential in a

clinical gene therapy trial, there has been a lot of discussion on that, and it is pretty much considered to be a line that the public doesn't want us to cross, and I think we have to respect that.

At the same time, however, we realize that as we gain experience and information, we can begin to feel more and more confident that that is not occurring even though the risk may never be zero, and, of course, we will get into discussion and so I might as well bring it up, that if you show it doesn't happen in the males, does that mean that it won't happen in the females, and, of course, female biology is very different than male biology, we all realize that.

I think there we need to put more energy, I think as John and others have already said, and to some of the preclinical models anticipating what is around the corner for this field, and that I think a reasonable leadership position from the committee.

I guess the last thing we have to talk about, and if there is anything else, please jump in, but the last thing I feel we have to talk about is okay, so we come back in here a year from now,

and we get presented data from company XYZ now, it is not Avigen any longer, but they did a trial like this and 10 of the first 50 patients are persistently positive in their semen at one year, and so they do an in-situ hybridization on motile sperm on these particular 10 patients, and 8 of them are positive in 10 percent of the sperm. Now what?

DR. DYM: I will answer the question by asking a question of the virologists. Does that clearly mean that it is coming from the earlier germ cells, or can the virus persist?

DR. SALOMON: We might as well get that question on the table. Jude, do you want to comment on that?

DR. SAMULSKI: My feeling would be that it would have to be in a germ cell to persist that long and consistently come up positive, and for it to just persist, it would get diluted with time. All those cells kept dividing. So this would be the same as the trial, they would come down over time, so I think you are now talking about a completely different situation.

DR. SALOMON: And they do a testicular biopsy and it is positive in the spermatogonia.

Now what?

DR. JUENGST: It's at least time to stop and take stock and look at where the gene is being integrated, you know, study what is happening, if it is consistent, those sort of things.

DR. SALOMON: That's fine. Remember what Dr. Samulski pointed out very clearly is that there is no evidence that these vectors will integrate in some specific spot. They will integrate in some specific spot. They will integrate in multiple concatemers in many areas.

DR. RAO: There is two aspects to this. You don't know what you are doing now because the assumptions are wrong in some sense. You assume that there will be a very low probability of integration, there wouldn't be germline transmission, and that if it did occur, there will be a clear-cut barrier and it wouldn't be 10 percent. So that I think is pretty clear.

The question then is what do you do with the participants, right? I mean what happens with the 10 patients that were persistently positive and who presumably have germline transmission, and that I think is a very hard question. I don't know that the FDA has any authority and whether we can do

anything after the fact.

DR. SALOMON: That, we know the answer to that. They can't do anything. But the question would be now, 50 of the 50 patients haven't had a bleeding episode in the last six months.

DR. MURRAY: So, it worlds.

DR. SALOMON: It works.

DR. MURRAY: I think this is not a far-fetched hypothetical life here. There are scientists here who understand different vectors that may, in fact, operate very differently even than AAV, if I understand correctly, and some of then might be much more likely to incorporate to work themselves into spermatogonia, and so this scenario with the different vector system might not be so far fetched at all.

So you have done right by the committee to ask this extremely difficult question. I don't feel at all qualified to answer it right now. I would have a number of other questions. I would want to know, look, we are talking about a potential random, you know, incorporations at some random place in the genome of foreign DNA.

I would like to know how many copies integrated, are we talking about 1, are we talking

about 1,000 in each genome? If it is thousands, it would seem to me that increases the chance that some of these mutations are, in fact, may be pathological. A thousand hits is more than one hit.

Do we have any analogies? Are there other bits of DNA that get incorporated into the genome in a similar random fashion, and how do they -- and spermatogonia, and what we do know about their fate, and what do they know about the impact they might have on the health of any child born. If it is absolutely horrendous, then, that is one thing, if it is, well, it happens all the time, and rarely really leads to any harm, that is another thing.

So, there are still a lot of factual questions we would ask. That will help, I think, help us sort out, but you are right, we should be thinking about them now.

DR. GORDON: As a member of the public, I would like to sort of suggest that the committee, in facing such a circumstance, should consider this the way other risks of drug treatment are considered. Now, if you give cisplatinum or bleomycin to somebody, you can probably damage their DNA, or adriamycin to them, and there are

precautions to be taken.

In the case of germ and a gene transfer, which I think is a little bit exceptional because you provide acquisition of function, not simply alteration in the existing genome, there are precautions to be taken before the procedure is performed, and there are precautions that can be taken if, in the event, such a thing is discovered.

If 10 percent of sperm had a new gene in them, that would mean that there is a 10 percent chance that a conceptus would have it, let's say, presuming those sperm function equally well, well, there are people carrying recessive traits around where there is a 25 percent chance that there is actually going to be genetic disease, and there are approaches to that problem - pre-implantation, genetic diagnosis, conception followed by abortion.

So there are ways of addressing it if it occurs, but I think the committee is well advised to consider that a hold should be placed while those considerations are formalized.

DR. SALOMON: I think that is what Dr. Rao said. I mean I realize this is -- I think the major point that I was getting at was just to introduce the question, and I think there has to be

a limit, and I think that limit for me is that. I mean if the hypothesis is wrong for any vector now, I am not talking about the Avigen trial, then, probably we should put it on hold and there should be discussions at the highest level, whether it be at the RAC, or be here, or in every place.

That includes the appropriate science experts, as well as policy people and ethicists, because I think there, we really have crossed a line that has been set for us in gene delivery.

I think what is critical to the FDA, though, unless someone wants to disagree with me, is the advice that you better make sure that any trial that you allow to go forward is adequately designed to make sure that you don't miss this from happening. I mean if you are going to recognize it, the trial had better be designed and monitored properly enough to make sure we recognize it, because then, you have got to deal with these issues as a reality instead of as a theoretical risk.

Any other comments from the committee, from the public? Does the FDA feel like we have answered their questions? Are there any additions or refinements, et cetera, that we should deal

with?

DR. NOGUCHI: Once again, on behalf of CBER, I do want to extend our heartfelt gratitude for helping us over our current and future difficult issues that we seem to face on an increasingly more frequent level.

I think the discussion yesterday and today is going to enable us to move forward in a much more cohesive and responsive and responsible way, and for that I only can say we are again very thankful.

Based on your last comments and the questions you raise, however, Dan, I don't think you are going to necessarily be able to get away from this committee that easily, so I am sure we will see you again.

DR. SALOMON: There are laws that will govern eventually.

Dr. Couto, did you have a comment?

DR. COUTO: Well, it is actually a question that I wanted to just ask the committee, because it was raised earlier, and that has to do with the most optimal PCR assay for detecting vector sequences in semen, because it was raised that maybe a better assay would be a quantitative

PCR assay.

One of the reasons why we are not doing that now is because the FDA asked us to develop a spiked plasmid that has a deletion in the coating region, and so that we could differentiate between our vector sequence and our spiked sequence.

Now, we wouldn't be able to do that with a quantitative PCR assay, but as you have seen, most of our other assays, biodistribution studies are all done with quantitative assays, so I guess I would just like a little bit of clarification as to what people think would be the best assay in the clinical sample.

DR. SALOMON: There is two answers to you, but I mean there is certainly multiplex PCR where you could design your probe. If they wanted the spiked sample deal going, I mean you could easily do that these days, and I can help you figure out how to do that if you don't know.

Dr. Rao.

DR. RAO: I was just going to add exactly the same thing in some sense is that spiking is a method of quantitation, so it is a quantitative method of estimating what you have against a known standard of DNA, so I don't think there is

confusion. I mean you can even do spiked multiplex PCR on a quantitative fashion using Tacman type of assays if you want to.

DR. SALOMON: If you did a Tacman assay, I mean just to educate me a little, if you did a well-validated Tacman assay or I mean there is now other technologies, I am not doing a commercial blurb for Tacman, just quantitative PCR based, there is fluorescence, there is Cybergreen, there is a bunch of different ways of doing it.

If you did that, what is the spiking thing for?

DR. TAKEFMAN: Well, the spiking, we just say run samples without spike, but one sample at least with the spike just to test for inhibitory effects. So you could run QPCR on some of the samples.

DR. SALOMON: Just that same control, that was the main reason. I mean sometimes you need spiking because there is endogenous transcripts that are confusing your sample, but that is not the issue here when you are looking at vector.

DR. NOGUCHI: No, semen does have a history of sometimes inhibiting viruses. HIV detection in semen actually for many years could

not be done because of inhibition.

DR. SALOMON: That point is well taken. We have been suffering with serum and plasma for stuff in my lab, looking at retrovirus, that is well taken.

Did we miss anything? I mean is there anything else people want to get on the table here at the last minute? No.

Again, I want to thank everyone at the committee table, and Avigen particularly. I hope we haven't beaten you up too bad, but I think you are going home with pretty much you were hoping for, and I hope for the community that your studies go safely first and then demonstrate efficacy next, as I think it is clear that your stakeholders need a viable therapy. If it's not Avigen, then, let's pray it is going to be for somebody else doing gene delivery doing it.

Anyway, good luck. Good luck to everyone else out there. Travel safe and be healthy.

[Whereupon, at 2:03 p.m., the proceedings were adjourned.]

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