useful to us if we are trying to deal with the questions. The question you asked is whether there is enough data to do clinical research but then you are focusing on the efficacy side. We worded our question somewhat differently, and for a reason, and that has to do with what our regulatory authorities are. I would like to have this discussion within the context of what our regulatory authorities are.

So, your question bears some significant similarity to question number three, which I would like to take just a moment to read and explain the context of why it is worded that way. Are these data, referring to the clinical and preclinical data currently availability, sufficient to determine that ooplasm transfer does not present an unreasonable and significant risk to offspring and mother, and to support further clinical investigations?

The determination we need to make specifically is whether there is an unreasonable and significant risk. That is largely a safety determination, but what risks are reasonably and what risks are not reasonable is clearly linked to the issues of what disease is being treated, what

the prospective outcome is and how strong is the rationale. So, efficacy does figure in but we are not going to decide simply that because we don't think that this is going to work; you shouldn't study it in humans to find that out. So, the question is a little more safety oriented in the context.

DR. SALOMON: Right. We don't always agree on how I get there but I am trying to get there.

[Laughter]

If you will indulge me just a little longer, not too much longer--

DR. SIEGEL: Now that I am on record, you go where you want to go but I hope we will get to where we need to get.

DR. SALOMON: Fair enough. I don't want to delve too deep, I just want to stay on the surface here but I still want to just get a sense of the committee along the lines of where we are starting here. We have been doing a pretty good job of that and we have identified this sort of knife-edge balance between efficacy and safety and, in that case, what Dr. Siegel just said is absolutely true because what we are going to do

then is dive into the safety side. But I would just like to hear a few more minutes of the gut-level feeling at this point of should this discussion go more toward--we need to deal with the safety issues and then step in and say, okay, what is the good clinical design because we are going to go forward with clinical design, or we are going to say, no, this committee does not feel that a clinical design is appropriate now so we had better set a bar in preclinical studies for safety. I am trying to decide where we are going to go as a way of guiding myself. So, Dr. Murray and then Dr. Rao.

DR. MURRAY: I think I want to ask what for me, at least, is a prior question, one that I have to get an answer to before I can answer the one you gave me. There is an expression in my field, bioethics, which is that ethics begin with the facts and I don't know all the facts I need to know at this point. I have heard a lot of raw information. I would really like to hear the considered judgments of a number of the scientists around here about what we actually know about safety and, if not efficacy, about the plausibility of the mechanisms by which this intervention is

presume to have its positive effect.

I certainly have to defer to Jonathan about animal models and what is an adequate animal model, but it seems to me we were getting answers to some of those questions from animal data. They may not be animal models in some very cosmically broad sense but I feel a lot better about the risks for heteroplasmy now having heard the discussion that took place after lunch here. I am much less worried about it than I was when I first read the papers.

So, I think there is a lot of wisdom that has come in front of us today. It would be nice to see that digested, get kind of a best read on it, and then I would be ready to talk about the human trials.

DR. SALOMON: My response to you is you will be one of our bench marks. I will look to you to tell us you have heard enough information. That is important. Dr. Rao?

DR. RAO: As you said, I don't want to dive too deep into this but say that even though we may not have data for efficacy, maybe we have some data from the mouse models for a rationale for why one might want to do ooplasm transfer, and maybe

that may best be addressed by the doctor, I don't know which one; someone right at the end, where they had the mouse model which showed that if you have mitochondrial deficit you actually see degeneration which looks similar, and if you replace those mitochondria you actually see much less degeneration. So, there is a rationale in some sense that, yes, if you transfer something which is present in the cytoplasm you might see some improvement. That certainly doesn't address what happens in human but it does give you a rationale for why you may want to try and address that therapy.

On the safety side too, I think if one defines the problem and says that, well, what you are doing is a procedure which is very similar to what you are already doing in ICSI where you have a lot of expertise, then you have a lot of data, clinical data with humans in the appropriate model on safety. What you don't have in those models is safety in terms of the issues that were raised here in terms of heteroplasmy and in terms of what Dr. Mulligan raised in the sense of what happens with naked DNA transfer or what happens with chromosomal damage.

So, maybe we should compartmentalize it a little bit and say that there is a rationale. We don't have any data on efficacy maybe, and we have some data on safety, except in sort of critical issues.

DR. MULLIGAN: Yes, I think the data issue is very key to think about what would you consider the definition of data versus a rationale. I think that is the mystery we are having here. I think there is no data. I think that every scientist has to figure out where he wants to set the bar. Even if you set the bar really low, there is no data. Yet, there is some rationale, and the rationale, probably my bright ten-year old could come up with listening to me talk about how injecting things into cells can change their function. While we dance around all the embryo work, and whatever, yes, there is a rationale. It is a pretty simple rationale that, of course, you can profoundly affect the way a cell functions by introducing things into it. So, I think there is no data and I would like to have some controversy stirred up about that.

From the safety point of view, I think this is so clearly a gene transfer issue that the

safety issues ought to be focused on essentially what is an unwanted substance in the product that could have a safety effect. I can tell you from a background in gene transfer, and I am an expert in that little narrow part of things, and you can get very, very different efficiencies of gene transfer by doing the method in different ways, things that are typically efficiencies that are one tenth or fifth can be 40 percent if you do it differently. So, I see this no different than the whole regulatory process with gene therapy vectors where having someone say, well, that isn't going to happen, or there isn't enough DNA there, or we do this all the time is and it just can't happen. These guys are laughing. They have heard that before.

So, I would say that my concern, based on the whole process in the gene therapy field, is that this is an analogous case where setting the bar as low as you want for efficacy, there is still no data. But maybe there are some things that can be done. There is clearly some rationale but it ought to be focused on essentially what are you essentially doing? What are you injecting? And, what toxic substances or things that can cause some

risk are in it? I would think that trying to document what kind of tests, checking for whether or not there is chromosomal DNA or naked mitochondrial DNA are things that are supportable. They are not embryo types of things. And, those would be very important, as well as to characterize the consistency, as best you can, of what you are going to use, like count the mitochondria or measure the amount of DNA, just so that in the future you may be able to draw some correlations between some of the most obvious types of things.

DR. SALOMON: I think we will continue. That is a nice beginning to dive into where I promised Jay I would go in a few minutes, the safety issues, because I think that takes us there. Dr. Shoubridge and then Dr. Casper.

DR. SHOUBRIDGE: I don't think the problem with mitochondrial DNA is a real safety issue here. I think the chance of getting naked mitochondrial DNA to do anything real bad, or even getting it, is zero essentially in this kind of a procedure. When you can do subcellular fractionation, and you don't get much more severe methods than this, you just don't get naked mitochondrial DNA unless you isolate DNA. So, certainly the nuclear genomes is

another issue.

For me, the safety issue that revolves around heteroplasmy--it is almost impossible to get that information in humans because if we take our mice as an example and look at the tissue that had the strongest effect for selecting for one genotype, it took basically the mouse's lifetime to do that. It is quite a slow process. So, if we just extrapolate to the human it could take decades to find out whether that is ever going to happen. So, I don't think realistically we are ever going to have that information to go on.

But coming back to something, Dr. Mulligan, that you said earlier on, to me it is crucial to establish, and it would change the whole nature of the enterprise whether mitochondria are important here at all. There, I think Dr. Casper's mouse model, even though it may not be perfect, he has injected mitochondria and shown some effects there. And, I can think of a list of what I think would be pretty decent experiments, some of them genetic and some of them not, that would tell you whether mitochondria or at least the energy metabolism part of mitochondria are at all important in this process. If you could come to

the conclusion that they weren't, then we wouldn't even be having a lot of this discussion because the heteroplasmy issue would be a non-issue. It would be another factor and then maybe we would be interested in the biological effects of putting in pieces of spindles, or having a centriole, or having an RNA population, or something like that.

So, to me, it would be critically important to establish whether or not mitochondria are in fact important in human embryos in a research situation. I don't know if you would call that clinical research because the endpoint here wouldn't be pregnancies. You would have to have some other endpoint, like morphology objectively determined or some biochemical endpoint in an embryo. And you would have to use the mouse models. As imperfect as they are, it is the best we have.

DR. SALOMON: Dr. Casper and then I will take us into dealing with the first question on the safety issue.

DR. CASPER: You asked earlier about gut feeling responses also. I can tell you just from doing clinical IVF for many years and dealing with patients who have repeated fragmented of rested

embryos, it is my impression that it is not a condition that corrects spontaneously. So, I think the fact that there have been pregnancies produced in that group of patients with this procedure suggests to me that there is probably something that is working, although we don't have the numbers to actually support that.

So, I think what we have essentially at this point is the equivalent of a pilot study that demonstrates potential efficacy, and I think it is worthwhile to move on to some more significant research studies.

I think the most important thing, however, is to find out what it is that actually makes this work. I think it is also important to do away with ooplasm transfer because, first of all, we don't really want to have to subject women to egg donation in order to make this work. If we could figure out what the actual component is we could use that component perhaps without having to get donor eggs. Secondly, the cytoplasm injections also have that small but inherent risk of transferring genomic DNA as well.

So, I think there probably is some efficacy to this procedure. I think it probably

does warrant going ahead with clinical and animal trials, but on a more specific level to try to find out what it is that is actually working in the transfer.

DR. SALOMON: That is good. You touched on something for me. You know, I have been trying to decide for my own self, independent of my job as chair, when I say, well, we should do some clinical research at the same time we are advancing our understanding in the basic models. I am kind of leaning in that direction. Then I think of things like, well, if you really don't know whether it is the mitochondria or some sort of soluble element, maybe you ought to know that before you do the clinical studies and that has all kinds of safety implications, and we will come back to that.

The other thing is if you don't need to use an oocyte donor if you, for example, could do it from a human embryonic stem cell, you know, if you could do that then wouldn't that be an ethical step in the right direction in the sense that now you wouldn't be involving the invisible woman? I thought that was an interesting visual. Or, you could use somatic cells from the mother even.

So, there are some other questions here

that could have really profound implications as to how the procedure was done without saying that this procedure actually would work and, yet, get the benefits for the infertile mothers which I think was well articulated in the public comment period. So, that is a dynamic I guess we will have to deal with for the rest of the next hour or so.

Speaking in terms of risks to the offspring then, the FDA proposes four specific issues that directly affect risks to the offspring, all dancing around the concept of how the procedure might damage or alter the oocyte--mechanical damage, inadvertent transfer of chromosomes and chromosome fragments or cellular constituents, enhanced survival of abnormal embryos and risks with heteroplasmy. We don't have to do an hour discussion of this because we have already touched on a lot of aspects of this, but let's deal with these four specific issues of safety.

Number one, mechanical damage to oocyte architecture. What do you guys think? Dr. Rao?

DR. RAO: I just want to reiterate that there is a lot of data for ICSI and there is no difference in the procedure, except for additional volume injections, in terms of mechanical damage.

So, I would say, from what I have heard, that it seems that the amount of mechanical damage should be the same and there is data from lots of successful births.

DR. SALOMON: So, is that true? I have no clue. I mean, is it true that the amount of physical puncturing of the recipient cells is identical for ICSI as for that? That is a fair point from everything I have heard today. There are issues that you are injecting cytoplasm, whereas before you were injecting the sperm in some sort of natural buffer. Right?

AUDIENCE PARTICIPANT: [Not at microphone; inaudible.]

DR. SALOMON: So, would you say there is an incrementally, albeit incrementally small, difference with the ooplasm injection because of the volume issue? Fair enough.

DR. MURRAY: There are people here more qualified than I am to recite all the data on ICSI's impact on children but, as I recall it, there is some increase in various abnormalities over the natural background rate, although it is not an outrageous increase, and there is I think roughly a doubling of low birth rate among the

children, and low birth weight is a predictor of a lot of later problems. But, again, so far at least those have been deemed to be acceptable I guess by the people who employ them.

DR. SALOMON: So, the point here now is that ICSI is essentially close to, maybe slightly incrementally different but I think we can live with that incremental difference for safety. Now the question is what increase in risk does ICSI cause versus age-matched infertile women?

DR. SABLE: Just to address the ICSI questions, once one factors out the couples who conceive who would never conceive on their own because there is no sperm in the ejaculate, and these are couples where the sperm has to be literally surgically removed from the testicle, once you factor those couples out--and these are not people to be doing cytoplasmic transfer--the risks drop down to the background risk.

Regarding the low birth weight, that is a study that actually included all IVF patients, including the ICSI patients. There did not seem to be an incremental increase in risk of low birth weight versus the background IVF population, just to clarify that.

DR. SALOMON: So, for question number one I assume that there is a fairly high level of comfort here, comfort as defined by mechanical damage to the oocyte cytoarchitecture induced by this procedure is incrementally small over the overall risk of these procedures that are already ongoing.

DR. SAUSVILLE: Right, I would say numbers one and four under the bullet "risks to offspring" are obviously there and are things that are reasonably tolerable or at least known, recognizing the long-term risks associated with heteroplasmy have been extensively discussed that are at one level unknowable but that are intrinsic to the procedure.

I guess I am more concerned with numbers two and three. As Dr. Mulligan articulated, the procedures that are currently in place do seem to be somewhat uncontrolled on whether or not matters of technique or instrumentation can minimize the likelihood of chromosomal fragments being an issue.

Lastly, we heard the figure cited by Dr. Moos about if one just does the crude calculation, there is approximately 20-some odd incidence of major abnormalities in the series that have been

reported so far. So, I am a little concerned that that is a higher level of abnormality than I at least would feel comfortable with.

MS. KNOWLES: I don't want to get off topic if we want to follow this up but since you were taking about number one and four, my feeling about number four, and this may in fact be just a question of my ignorance of the animal models, what I have heard is that we have some limited work in mice that shows that this is not a problem. Yet, I have also heard a discussion that the mouse models are, in fact, not something that we can really use to translate for other questions to the humans. So, I am not a hundred percent convinced that that does away with all of the questions about heteroplasmy. So, I also wonder if there isn't some kind of closer animal model, like a non-human primate, that we could do a study in heteroplasmy that might be quite useful. Perhaps I just don't understand.

DR. SAUSVILLE: I could respond to that, I think we agree that the actual risk or the dimensions in which heteroplasmy would enter being something that could be considered an adverse event are actually unknown. I agree entirely with your

analysis. I guess to me, from the standpoint of writing an informed consent, it becomes at one level something that could be state, look, we don't know anything about this and I could imagine scenarios where, if donors were properly screened for the known mitochondrial issues etc., that one might reasonably take the risk of tolerating that statement, recognizing that it is an unknown.

My issues with respect to number two, that is very much, in my mind, a matter of how the technique would actually be practiced on an individual sense and, therefore, is a potential basis of extraordinary variability.

With respect to number three, I am concerned that the incidence of 20-some odd percent recognizing, if that is true and the issue of how broad the error bars are, ultimately society is going to be asked to, at one level, take care of these children in some way or fashion. So, to countenance a technique that has that level of abnormality generation, if that is truly the number, I think is a matter of concern.

DR. MULLIGAN: On that point, if you drop statistics for the efficacy part of things, that is a gut feeling that maybe there is something to

this, not evoking statistics, then we might as well not evoke statistics for the potential toxic effect too. Since there is not statistically significant info, I think it is important to weigh the data comparably. That is, on one side it looks like there may be difficulty; on the other side there may be some efficacy.

DR. SALOMON: I am happy for this discussion. So, we are still focused now maybe more on questions two and three, the inadvertent transfer of chromosomes or the enhanced survival of abnormal embryos, with the emphasis in the last few minutes on the abnormal embryos. What is the feeling of the panel on that?

DR. RAO: I would just like to second what Dr. Sausville said, that it is really a big issue and what Dr. Mulligan said, that in a system where you don't know, and where you have a spindle and you have DNA, there is a chance of incorporation of extra chromosomal into nucleus is much higher. So, one cannot extrapolate from low amounts and make conclusions, and that we be a really important concern. Likewise, I think the issue of enhanced survival and the society responsibility are really major concerns.

DR. MULLIGAN: Also, I think there are always more or less competent people. You know, for this sort of thing I am sure it makes a big difference and you are going to have people that are going to do this that, I am positive, are going to be much less competent than the experts that we heard. Therefore, you have to have in place some characterization of what damage can occur, what DNA you can get and so forth.

DR. SALOMON: Now speaking for myself, I absolutely agree with that. That is why I said earlier on that no matter how we end up, the field has to accept the mantle toward understanding what it is their product is, what they are injecting. Even if that is not absolutely settled in the first trials, that is fine but that is the direction this has to go for all those reasons. It is not just to do it in three or four really wonderful laboratories, which is where it has been done up to now, but it is doing it in 40 or 50.

DR. CASPER: I think we have to be a bit careful because the numbers are so small in terms of looking at chromosomal abnormalities, and so on. Just as an analogy, there was a paper published concerning sex chromosome abnormalities in ICSI

offspring that showed a 33 percent incidence of sex chromosome abnormalities but it was based on 15 pregnancies, and here we are talking about less than 20 pregnancies. Whether that 20 percent figure is going to hold up or not, I very much doubt it. I think it will be very much lower, probably close to baseline if you got to the position where you had enough pregnancies to actually look at. I understand that we are talking about small numbers but that can just magnify a problem out of proportion.

DR. SCHON: Could you elaborate on why you believe that is a tenable position?

DR. CASPER: Only based on the previous experience with ICSI which really didn't hold up at all. The initial paper that came out, suggesting that there was a 33 percent abnormality rate turned out not to be correct at all when people started to examine hundreds of ICSI pregnancies.

DR. MURRAY: I am definitely not a statistician but this is the classic case of why take that point of view. I mean, it could be a statistical abnormality in either direction. I don't understand why it is that in this particular case this will turn out to be in the wrong

direction. I just don't get the logic behind why that would be the case. You are saying that in one other case there is a side effect that turned out not to prove to be statistically significant. I mean, how many hundreds of examples of that sort of thing are the case? But there are also cases where the data set shows you a certain percentage and then the next data set shows twice that percentage. I just don't understand it. I don't get it.

DR. CASPER: It just seems to me that that is a very high number. It is out of proportion to the sorts of chromosomal abnormalities that we see with most assisted reproductive technology type procedures. That is all. I am just saying that I think we have to be careful in interpreting the numbers because the numbers are so small at this point.

DR. SALOMON: Dr. Moos?

DR. MOOS: It is worth stirring into the pot the consideration that we don't know the prevalence of chromosomal abnormalities in the population of women presenting these procedures. It may be significantly higher than in the normal, healthy population. So, we don't know the denominator. It is, however, impossible to ignore

this even if, given the sample size, it is a statistically improbably event, not likely to be repeated. Dr. Mulligan's point that the coin could come up heads or tails I think is perfectly well taken.

DR. SAUSVILLE: But to me that is all the more cause for some of the product characteristic issues that we just talked about previously. After some sort of modeling process and after figuring out whether mitochondria are necessary, and whether it is the RNA that is doing it, we come forward with a pristine, let's say, product and there still may be evidence of this occurring, then that would become a more obvious conclusion. As the issue stands now, if this outcome were to occur we would not know whether any of those other things, plus the intrinsic susceptibility of the recipient egg to this sort of thing would be relevant.

DR. MURRAY: I am more focused on the second worry, the worry about chromosomal DNA or the cellular fragments, and I cannot disentangle my thinking about that from exactly the point Dr. Sausville was raising. What is it that is operating here? I mean, we are injecting a soup or, maybe even better, a stew into the egg and it

is full of lots of things, and we sort of roughly know what is in the stew but we have no idea what component or components of the stew are making a difference, if they are making a difference, including the DNA fragments and the other cellular components. Until we have a clear idea, we have a plausible notion of a mechanism and some evidence, and I think it would not be impossible to create some experiments in both animal cells and human embryos that would take us toward answers, it is difficult to justify doing a human trial with the risk of transfer or chromosomal elements until we have a sense of whether they are, in fact, at all necessary in that stew.

DR. SAUSVILLE: To be clear, the issue is not only the transfer of chromosomal elements, but multiple experiments, extending back to some of the classical experiments in bacterial genetics, is that DNA is mutagenic. So, it is not only a question of passively adding something, it is something actively altering something.

DR. SALOMON: I think the other thing that just came out in last weeks is studies on the nature of the algorithms used to call the number of genes in the human genome. Just to explain that

for those of you who didn't catch the last issue of Nature Biotechnology, the call was that there were 30,000 to 40,000 human genes, which upset a lot of humans--

[LAUGHTER]

--because there didn't seem to be enough genes to make us different than mice and everybody was uncomfortable with that concept. It comes down to the fact that when they really began looking at different ways of calling genes that there may be a lot of RNA transcripts in cytoplasm that encode for--

[Laughter]

--see, I told you you would like this stuff! There would be a lot of RNA transcripts that are clearly not called formal genes in the original genome project algorithm. What that also raised was the possibility that a lot of these RNAs wouldn't necessarily have to encode proteins but would encode RNA molecules, like ribosomes for example, that have enzymatic activities that alter different cell functionalities. So, I just bring up to you that one thing that we haven't talked about that is certainly reasonable to put on the table here is that another uncertainty in the

safety issue is RNAs that are not transcriptionally active for proteins but, rather, are important perhaps in other cellular functions. I mean, maybe one of the reasons you are getting these XO chromosome abnormalities is some sort of imprinting phenomenon. That is just a wild speculation, but I think it is more than just mitochondrial DNA that is getting transferred that has a genetic lineage. That is just to make it a little more complicated.

I am told the other mike is now fixed. You will be the experiment on this.

DR. SABLE: I am David Sable, medical director for the Institute for Reproductive Medicine at St. Barnabas. I really want to clarify the very excellent point Dr. Moos made regarding the baseline chromosomal abnormality issue, and I really want to make sure that are assumptions for a control group are appropriate. The pregnancy loss rate in an IVF population at our center, and that is what we are comparing this particular subset to, with a mean age of 37 is 22 percent, and the overwhelming majority of these are chromosomally abnormal, and the single most common chromosomal abnormality in a pregnancy loss is 45 XO. So, these numbers together suggest that we are actually

very close to the middle of the bell curve. The direction of the conversation seems to keep veering to where we have this assumption that there is this huge discrepancy behind the background population and I don't believe the data supports that.

DR. SALOMON: That is an excellent point. Before you sit down, the question then would be if we have a population of infertile women, many of whom are older but not all of whom are older, and we now are capable, with this technique or a technique that we are discussing a few months from now, of rescuing a higher percentage of those oocytes, is it not reasonable then to be concerned about all the implications of rescuing embryos with potential genetic abnormalities?

DR. SABLE: That is an excellent point, however, let's make sure we are not reading too much into a single case. One of the XOs aborted spontaneously.

DR. SALOMON: We will stipulate that your point on the XOs was well taken--

DR. SABLE: No, theoretically I agree completely. I just don't want to imply or allow us to infer that the data supports that that is actually happening. I think in theory, yes, it is

the same point that we would be concerned about ourselves, however, I don't want to take that additional step and say that the data so far, including the losses we have had, really deviates significantly from what the background control should be.

DR. SIEGEL: In that same population though, what is the proportion of 45 XO in the successful live birth pregnancies?

DR. SABLE: I am sorry, repeat the question.

DR. SIEGEL: You said that 27 percent--I don't want to re-quote your numbers but that 45 XO was a common cause in spontaneously aborted pregnancies, many of which were chromosomal abnormalities. What about in successful pregnancies, what has been your incidence of 45 XO?

DR. SABLE: I don't think we have had a report of 45 XO, but we have had pregnancies terminated after second trimester genetic testing. Thank you.

DR. SALOMON: I think that in general here there is consensus on the part of the committee that there are real safety issues potentially that play in this field, and that the amount of data

that we have right now in animal models, which we will talk about a little more a little later but for right now the amount of data in the animal models doesn't really settle the issue adequately, albeit they contribute in some ways positively, and the data in the human system is just really not adequate to make any statements at all about, neither safety or efficacy. That is my attempt to summarize this first part of the discussion. Does anyone disagree? I told you from the beginning you are welcome to disagree. I am just trying to make sure I am giving you a good summary.

5:30 DR. NOGUCHI: Dan, is it true that there are a few safety issues that seem to have been at least allayed to a certain extent? When you are speaking of the human experience I think it is with that caveat that in terms of some of the mechanical parts of ICSI that may be helpful. But you are talking about two and three specifically.

DR. SALOMON: I think two, three and four. I think number one, I think everybody kind of agreed, you are right and thanks for pointing that out, we sort of agreed that that didn't seem to be a big deal in that they have a lot of experience doing ICSI and this is an incrementally small

increase. I think we said that, if everybody agrees with that.

But for two and three there is clearly some real risk there and the clinical data doesn't address it. For four, I don't think we really know. I think it is correct to point out that at least the animals are reproductively active and are overtly healthy, but we are not very good mouse veterinarians when it comes to really know what their kidney, heart, liver and other functions are, and living in little sterilized boxes, being perfect food is not really a measure of health either as judged by SKID animals, fine, but look at SKID children. So, the heteroplasmy thing I think still remains an unclear issue.

DR. MURRAY: Just to follow-up on that point, Lori Knowles observed, and I believe this is correct, that many of the human manifestations of mitochondrial disease are late onset. So, we would have an issue of would we have an ability to follow-up with such children to see if there are early signs of these later onset diseases. That is not, to me, an absolute barrier to doing it; it is a challenge for us.

DR. SALOMON: I think it is an interesting

similarity to all these other fields that we have dealt with in biology, in gene therapy, cell transplantation and stem cells that there is going to be this demand or strong pressure for long-term follow-up of the recipients.

DR. SCHON: I am not that worried about item four, and on the particular case the worry that is being mentioned, let me remind you that this invisible woman is of age 25, 30, 35. She carries the same genotype presumably as whatever is being donated to this child, to this oocyte. The woman donating the cytoplasm is apparently normal. That is why she is donating it. The presumption is that her mitochondria are okay and, therefore, what is being transferred presumably is okay unless there were some random mutation, and these things happen and, in fact, that is what mitochondrial diseases are. So, from that score, I am not all that worried.

DR. SIEGEL: Then that is predicated on the assumption that the donor women are screened for mitochondrial disease.

DR. SCHON: No, no, the presumption is that the donor woman looks normal when she walks into the clinic.

DR. SIEGEL: Is that what you would recommend as screening, that she looks normal? Is that what you are saying?

DR. SCHON: I will rephrase it. This is serious. Everybody in this room is different. Everybody in this room had different mitochondrial genotype. We all have a sort of societal consensus presumably--physicians will disagree--that we are fundamentally normal unless proven otherwise. And, for me to, let's say, sequence somebody's genome where there are 16,000 factorial possibilities of genotype, and for me to then say that this genotype is good and this one is not good is just not going to happen. You have to have some kind of rule of thumb. To me, if the physician says she passes my criteria for donation, I have no way of saying at a molecular level, except the most rough molecular level, that she is not a candidate.

DR. SALOMON: That is a key point, particularly as one of the duties we have to this field, to this group of people here is that we don't demand unnecessary testing that is not efficacious or doesn't answer the issue.

DR. SCHON: We certainly could test for the 150 known mutations. Fine.

DR. MURRAY: I am wondering if a pedigree would be useful for the cytoplasm provider.

DR. SHOUBRIDGE: If you look at the pedigree that I showed in five generations, there was one affected individual that happened in the fifth generation. But I think the number that might be important here is the prevalence of these mutations that we know about in the population. No epidemiological studies have been done in North America, but those that have been done in Europe, in Continental Europe and in the United Kingdom, suggest that it is about one in 8,000 or so, one in 8,500. So, the chances of having somebody who looks, to use your words, normal walking into the clinic as a carrier of one of these is pretty slim, and many of these people will manifest some aspect of these disorders which a physician could pick up. So, you have to balance testing the whole genome looking for mutations against the chances that somebody will come in off the street who is a carrier of a pathogenic mutation.

DR. SCHON: This returns to the point that I tried to make before, that I think heteroplasmy is not without risk for the reasons that you cited. I see the risk of an active mitochondrial disease

of being significant is relatively low. What you get into is the unknown of having some sort of interaction between a paternal genome with some maternal mitochondrial genome that would not have gone to fruition otherwise now being in an abnormal context. Again, that is the sort of thing that, in my mind, reflects an unknown procedure and could probably put in some way into an informed consent that could lay that out, not satisfactorily in an absolute sense but in a way that certainly is no different than we attempt to address when we bring an unknown drug to a population for the first time.

DR. SHOUBRIDGE: Just to make it clear, the paternal genome sees a new mitochondrial DNA every generation.

DR. SCHON: But it is a contextual thing. It is mitochondria in the context of a given maternal gene.

DR. MURRAY: I think that your work is so interesting and important to hear because it says that, depending upon the combination of the two, different things can happen. You showed exactly that. Right? So, if you put in something and have a certain maternal copy, it may well behave differently than it had behaved before because

there is some sort of complicated competition or genetic background in the recipient that will maybe accept that.

DR. SCHON: In this case, of course, what we are showing is that there is nuclear genetic control which could just as easily come from mom or dad. You are right. So, I accept the point.

DR. MURRAY: I would just say that on the testing I think you would certainly want to test for whatever it is, the 150 known things even though they are infrequent. That is the least you could do.

DR. SCHON: It is easy to do.

DR. SALOMON: It is easy to do?

DR. SCHON: Yes. You would take a sample from the mother and just sequence her genome.

DR. SALOMON: Sequence her mitochondrial genome which is, what? 7,000 to 8,000 kb?

DR. SCHON: Yes, not kb, 16 kb.

DR. SALOMON: Whatever, right. I don't know how easy that is.

DR. SHOUBRIDGE: No, because you are looking for heteroplasmy and sequencing is the absolute worst way to look for heteroplasmy so it is not a trivial matter.

DR. SALOMON: This is probably a little too technical. This is something the FDA is going to have to deal with but, again, I feel that one of the things you should hear from us is that I don't believe anyone wants to put an unreasonable demand on these people. If it is easy to sequence and find these, then it is easy. Those are the things I hope you will do internally and be fair about it.

DR. HURSH: I just want to get out the point that egg donors in the United States are not tested for mitochondrial disease. There is a lot of egg donation going on. If this was a serious problem I think we would have seen it by now.

DR. SALOMON: That is another good point. I would like to keep going here because time is getting short.

DR. VAN BLERKOM: Just one point, I guess I am not concerned so much about heteroplasmy per se, but I think maybe one issue that needs to be addressed is the extent of heteroplasmy. Is the finding of 50 percent, or 30 percent or 40 percent of donated mitochondria an issue to be concerned with, number one.

I guess the other issue, and maybe Dr. Cohen can answer is, is whether or not in

successful cytoplasmic transfers there have been cases where there are no detectable donated mitochondria, so there is no issue of heteroplasmy at all.

DR. COHEN: I think I said that 10/13 tested are homoplasmic. So, one could argue that the tests are maybe not sensitive enough and that it changes over time and next year it is better again. The samples are stored and we will check them again when the technology becomes available.

DR. VAN BLERKOM: But using the same methodology you were detecting high frequencies, in fact there were ten cases where there was no heteroplasmy.

DR. COHEN: That is right.

DR. SALOMON: The only other issue I would add to that is that you are testing peripheral blood. One of the problems with peripheral blood testing of something as complex as heteroplasmy--

DR. COHEN: Yes, I would like to biopsy all their vital organs twice a year but it is hard.

DR. SALOMON: I wasn't trying to be facetious.

DR. COHEN: What we try to do is go with pediatric care and when they go to the pediatrician

we come along. That is sort of what we do. I hear from bioethicists that we have to follow them for life, well, that is a stigma and we have no intention at all to do that.

DR. SALOMON: That is good to know.

DR. MURRAY: Don't over-interpret what has been said here. I think you are taking that way too far. What I heard Dr. Salomon saying was weighing the pertinence of the data that in peripheral blood you are not finding heteroplasmy, one must take into account that one could find it in other tissues because we know there is differential expression, nor were the ethicists that you have heard from today saying that these children must be hounded for life. That is not the point. The point is we have to think about the issue of late onset and how we are going to deal with it. One way to do it is to say it is just impossible; it would be an unreasonable burden. Another way is to try to at least persuade the parents and eventually they will be young people, not children, that it would be very helpful for the future of this procedure for them to make themselves available voluntarily. There are a lot of approaches.

DR. SALOMON: I would like to go on.

DR. SHOUBRIDGE: One small point, all the data we have on humans, which is very limited, and on mice, which is quite a lot, suggests that if you sample one fetal tissue you have sampled them all. So, if you really wanted to determine whether or not a fetus was heteroplasmic you should be able to do it from embryocytes and then you would know. So, the issue of what to sample after birth to determine heteroplasmy is a thorny one and you won't solve it. You are not going to biopsy perfectly health children; there is no way. But you could determine it from either a CVS sample or amniocytes.

DR. SALOMON: The next big section is the risks to the mother. Might risks to the mother be different from those incurred with established ART procedures? For example, the possibility exists that the ooplasm might enhance the survival of abnormal embryos to incur additional medical risks to the mother, for example late term abortion. Any comments?

DR. RAO: I would say we just don't know. There is just not enough data; the sample size is too small.

DR. SALOMON: In the clinical experience we heard today--I am looking to Dr. Cohen and others for confirmation--it seems like there was one abortion in the group of three that Dr. Lanzendorf presented. Is that correct? There was one in three. One was a miscarriage and one delivered twins. Is that correct?

DR. COHEN: There were a total of 15 pregnancies and two were just confirmation of chemical rise in ACG. That was a biochemical pregnancy. There was one who miscarried before. It was after confirmation of the fetal sac but before fetal heart beat.

DR. SALOMON: That is early, right.

DR. COHEN: That is early, six weeks, five weeks, four weeks. Then there is the one twin that was sustained until amnio.

DR. SALOMON: What I was saying there is not an overwhelming amount of evidence yet, albeit the experience is extremely small, that there is a whole bunch of late abortions due to chromosomal abnormalities.

DR. COHEN: Not yet.

DR. SALOMON: Are the risks to the mother's future fertility or ability to engage in

subsequent ART procedures? Actually, Dr. Cohen, you addressed that specifically, or Dr. Lanzendorf. I remember at least one or two mothers who had failed this and went on to a second procedure and delivered a normal pregnancy, or at least became pregnant. I am not certain they said it was a normal pregnancy. Is that fair?

So, I would say here the only way the risks to the mother are going to get established would be a formal clinical trial. I don't think this is an issue that is going to get settled by any further discussion here, unless someone disagrees.

I would like to go to question number three or four. Three was kind of where I started the afternoon. Are these data sufficient to determine that ooplasm transfer does not present an unreasonable and significant risk to offspring and/or mother, and to support further clinical investigations?

We began with our gut-level feelings on it, went into the safety as I promised, and we are sort of back here again. Is there more discussion or do we all feel pretty comfortable with the discussion we have already had?

DR. SIEGEL: Well, there has been discussion but of a somewhat different and related question. I would like to know the advice of the committee on this question. I would on that point clarify further that, because I gave a partial clarification but I left an important piece out when I said that we put trials on clinical hold based on unreasonable and significant risks. We also put trials on clinical hold based on inadequate information to determine whether there are unreasonable and significant risks. That is what we will do, for example, if we believe that there are important or critical preclinical studies that could be done that would lead to a better assessment of the risks, a better design of the trial, a better informed consent, and so forth, that need to be done before the trials are done. That is sort of where we are going with this question in asking are there sufficient data to make that determination and, if so, is there a determination that there is not unreasonable--

DR. SALOMON: So, let me make sure that we pose this just right because, as I told you at the beginning, I think this is a very key issue that formed my thinking around the discussion we have

had. If we determined that there is no insufficient data to determine efficacy, regardless of the discussion we have already had about the amount of data sufficient to establish safety, just on the efficacy issue could we advise, or would the FDA agree to put a hold on a set of studies on that basis?

DR. SIEGEL: If you were to determine or advise that the rationale for any benefit is so slim as to not justify the perceived risks, then we could do that. So, we do consider risks in the context of rationale but we are not, in general, terribly aggressive on the rationale piece if the hold is based on the risks, and I think where there is scientific disagreement or where there is scientific consensus, or pretty close to consensus or pretty solid evidence that is one thing, but where there is disagreement we are, I think appropriately, reluctant to assess that our assessment of the rationale is better than somebody else's who is also appropriately assessing.

DR. SALOMON: So, we are back to what I described earlier as a sort of knife's edge here. We have some safety issues. There are some efficacy issues, and we need to think again now in

terms of the discussions we have already had how we are going to balance because that is really an important circle that we have to complete. So, Dr. Murray?

DR. MURRAY: I may jot be formulating in a way that the FDA will find useful but it is the way I am formulating it. I think we have had a good discussion about a number of risks to the offspring and to the woman, to the point where we can say that for most of them, and not all of them and that is a big "but" there is reasonable either combination of evidence or evidence sometimes by analogy that they don't seem to be outrageous risks.

The one piece that remains for me of significant concern is the possible transfer of cellular components, DNA of various forms, etc. I would refer to that as a very poorly characterized risk. We really don't know what we are getting. The problem is the stew problem.

The way I am formulating it that may not be helpful is I feel like we need to know more about what the active ingredient or ingredients are in this stew because at this point we may be exposing offspring to risks that are utterly

unrelated to the therapeutic component of the ooplasm transfer. It is longer than I meant it to be.

DR. SIEGEL: And that is pertinent because risks that are unrelated to a therapeutic are probably less reasonable from the perspective of our regulatory authority than risks that have to be accepted in order to have a chance of achieving the benefit.

DR. MURRAY: And we just don't know.

DR. SIEGEL: No, definitely from contaminants of active ingredients in terms of whether they need to be removed, and if you don't know which is which you are at a disadvantage.

DR. SCHON: I would like to raise something to be sure that we don't lose sight of at least one part of this picture. My lab and a lot of the labs of my colleagues work on mitochondrial diseases because there are women who have children who are destined to die, and some of them die very, very early, and we work on treatment of various kinds. I hope one of these days one of those treatments will be debated in front of you guys. But until that happens the risk to benefit for helping such a woman and using a procedure like OT

is enormous. In the case of a woman who carries a pathogenic mutation we actually know what the beneficial principle is. It happens to be good mitochondria, which is a slightly different way of looking at it but, no matter how the FDA rules or whatever you suggest, I would like you to take into account the enormous benefit that might accrue to those people who really have cytoplasmic transfer, if you will, would really help even knowing that there are these problems of potential chromosomal transfer, and so forth.

DR. MOOS: You are proposing that perhaps pursuing an indication where the rationale is sufficiently strong that we are not on the knife's edge anymore, but the balance is tipped strongly gives us an entree into a human trial that can examine in some kind of a safety series these questions, and then that can be extended to future trials in infertility.

DR. SCHON: As the other Eric pointed out, there are other ways to help these women that do not necessarily require OT but I don't want to eliminate it as a possibility, and some of these other issues might piggyback on that.

DR. SALOMON: Drs. Rao, Mulligan and then

Casper.

DR. RAO: I have one clarification I need about the question. When you say to support further clinical investigations, this is distinct from clinical research. Does clinical investigation mean you are thinking about pregnancies in follow-up and clinical research means you are using human blastocysts and looking at those, or is there no distinction?

DR. SIEGEL: I am not sure we intended a specific distinction, but in this question what we are asking is are there enough data to do clinical research that would involve pregnancies? I am not sure we have consistently made a distinction in the use of those terms but I will tell you that in the context of this, we have IND proposals to do those studies but we have said they can only be done under IND and we are seeking advice as to whether there is more that needs to be done either in terms of human egg research that doesn't lead to pregnancies or in animal models prior to doing that, or whether in fact there are sufficient data to make a judgment that those studies with pregnancies can proceed.

DR. SALOMON: Dr. Mulligan and then Dr.

Casper.

DR. MULLIGAN: I was just going to propose that we will never come to consensus on any animal experiment to find the active ingredient because we are not even at the point really of finding the active ingredient. We are at the point of whether or not there is anything to this. I mean, we are all talking about finding the thing, and I don't think we would ever agree, this group would ever agree on anything that would be compelling, that would definitively document that it is mitochondria that is important or that some other thing is important. So, I would opt just to see if we could get a consensus that that is not an appropriate avenue to pursue--well, it is an appropriate avenue to pursue but it is not something that should limit this going ahead and, rather, focus on what preclinical things do we think really would have to be accomplished before we would want to see the clinical work go back.

DR. SALOMON: So, the question, Richard, that you are getting is, that I want to get to here before it gets too late, is it seems to me, and correct me if my thinking is not straight, that there is this fork in the road and we are not

getting past this fork in the road. Depending where we go on this fork, it seems to me at least, is telling us everything that we have to discuss then.

So, the first fork is there is not sufficient data. The trial designs weren't good; there weren't enough patients, whatever, in the human studies to say anything definitive. I think we have all agreed on that.

Now the question is do we think that we should go ahead and do a study in humans, going all the way to pregnancy, using this field's sense of which are appropriate patients. Or, do we say, no, there are too many unknowns. We are not going down that fork and then we really have to define the bar for preclinical studies. Right? Because they are going to want it and they deserve that. We have to go down one fork or the other, or we ought to agree that we can't agree and we are stuck. That is okay too, I guess.

DR. MULLIGAN: I am saying we could say there is a limited number of things that could be tested that would impact upon the most easily assessable risk.

DR. SALOMON: So, are you saying that we

shouldn't do any human clinical trials until we do that?

DR. MULLIGAN: Yes, but what I am saying that might be is to have people look at the contaminated nuclear DNA content or--

DR. SALOMON: That is what I am saying, if we take that fork, then we can set the bar.

DR. MULLIGAN: I think that we ought to have a consensus on this issue of is there sufficient rationale, and I agree that this probably meets that criteria, that there is some rationale for this and no data.

DR. SALOMON: That is exactly what I trying to get that. Dr. Casper?

DR. CASPER: I hope I can express this properly, but I think one logical thing that follows from Dr. Schon's comments that there could be a huge upside from treating mitochondrial diseases is why not think about mitochondrial transfer, not ooplasm transfer but mitochondrial transfer? That avoids the nuclear DNA issue and you are looking at one specific component. So, if it works, that would help you to determine whether or not that is the right ingredient. If it doesn't work, then you can look at other components of the

cytoplasm but you still might have some information that may help people with mitochondrial problems is because what you are really looking for is a good source of mitochondria for them.

DR. SALOMON: I was thinking about that but it doesn't really address this fork in the road issue, the reason being that a woman with mitochondrial disease may be a candidate for mitochondrial transfer--these guys could go in that direction and maybe they have heard that today and will do that. It might actually be a wonderful thing to be doing, but it won't address this issue because the idea of finding someone with mitochondrial disease is also an infertile couple that would benefit from this.

DR. CASPER: I wasn't suggesting that we go right to healing mitochondrial disease, I was thinking that if you had somebody with fragmented embryos and you do mitochondrial transfer, either it will work or won't work. If it works, then first of all, you have found the active ingredient for ooplasm transfer, and also you have the upside on mitochondrial disease. If it doesn't work, then you have to look in another direction but you may still have some information that will help you in

terms of treating mitochondrial disease.

DR. SALOMON: I am sorry, I misunderstood you. So, your idea is take the fork in the road that takes you to doing some limited clinical trials now and do it with mitochondria. You went another step, and I don't want to go there yet, about what the clinical trial design should be.

DR. MOOS: With respect to the one issue that I think many agree is significant, the DNA transfer, mention was made of analyzing the donor egg after transfer for cytogenetics and that this was very insensitive. Is there any input that we can get about how we can satisfy ourselves, because Lori Knowles certainly made plain it was important that we are not doing that, using animal model to validate our assay for appropriate sensitivity. You know 10-5 of the human genome is still how many base pairs?

DR. SALOMON: I don't know anymore.

DR. MURRAY: You could do something like Y chromosome, some sort of PCR, to look for whether or not any inoculum that you are going to inject has Y chromosome positively.

DR. SALOMON: You could do genotyping on the transfer and look for genotypes that would be

unique to the donor. You could take the ooplasm and instead of injecting it in an egg just do genotyping on that to see if there is chromosomal DNA that was detectable. You would actually do then just DNA PCR.

DR. SHOUBRIDGE: I just want to make a couple of comments on what Dr. Casper said. One is there is no evidence at all that women who carry mitochondrial DNA mutations have a fertility problem that is different than in the general population.

DR. SALOMON: That is where I was heading before.

DR. SHOUBRIDGE: Yes. The other thing is that I think what you said sort of presupposes that there is a magic bullet here, that all women have the same problem and that by doing one set of experiments you are going to identify it and I would be pretty surprised if that were true.

DR. SALOMON: We have kind of danced up to this fork in the road a couple of different times. A couple of people have walked down it a little bit but it is not like we have rushed down it. Are there some comments from the community? Are you guys satisfied? You have heard our discussion.

You have participated.

DR. WILLADSEN: Well, it is not for us to be satisfied or dissatisfied at this point. We are happy to be here, I guess. But I should say--

DR. SALOMON: No, it is for you to be satisfied.

DR. WILLADSEN: No, the committee is doing its work. One speaker was saying that this type of procedure would not be permitted in Britain, but it is actually interesting that in Britain they left an opening for oocytoplasm transfer in the legislation, I guess on scientific advice. Now, we know those people have been wrong before in the decisions that the government makes there but, nevertheless, they have been thinking about that and this particular procedure has been kept open.

One of the reasons why we have tried to minimize the intervention is that obviously at a certain point if you transfer too much cytoplasm it is no longer a cytoplasm transfer, it becomes a nuclear transfer and nuclear transfer, as we know, has some big problems that are special to itself.

Finally, on the technical side, I think that the chances of getting little bits of DNA, nuclear DNA transfer with this procedure are

virtually non-existent because the chromosomes are aligned in one bundle. You would have to transfer a whole chromosome virtually. I think it would be impossible to tear off a bit of DNA from a chromosome. I am not saying it couldn't happen but I don't think that is a major concern.

Also, what one can do is to check, as we have done, that the donor chromosomes are actually in the remains of the egg. That is not a particularly difficult thing to do. But the concern is not nearly as grave as we may have been led to believe.

I should also say that the possibility that the mitochondrial DNA that is being transferred might somehow interact unfavorably, be it ever so rarely, with the nuclear genome, well the sperm provides disintegrating mitochondria every time you have fertilization in the human. Thank you.

MS. KNOWLES: Can I just clarify the situation in the U.K.? I just want to be clear that they have left open the possibility for mitochondrial disease. The discussion is in the context of mitochondrial disease. In addition, they are not allowing clinical trials. They are

quite expressly not allowing clinical trials until there is more animal and preclinical work.

DR. WILLADSEN: I don't disagree about the purpose of it, but you have to understand that the technique whereby they are going to do it is going to have to be this one or not at all.

DR. SALOMON: Anyone else? Dr. Cohen, at this point you have participated in this discussion--I don't think Dr. Lanzendorf is here--and Dr. Grifo, do you think that you should go forward with a limited clinical trial right now?

DR. COHEN: I think we should consider it. We did a pilot experiment that has been a five-year long pilot experiment. The clinical demand is enormous. There are many patients who have this particular profile have become successful. We didn't do a randomized study but these patients were at the end of their rope and considered egg donation or nothing. And, there are other groups of patients that are similarly interesting. There is, for instance, one group of patients that has recurrent implantation failure but has apparently normal looking embryos and they still don't become pregnant again, again and again. So, this is just one small part of the population but the population

is larger. I think I said in my presentation there is a whole slew of techniques that are waiting at the sideline that has just studied in animal models that has tremendous potential. There are ways of doing egg freezing using cytoplasmic transfer. I won't go into details. It is not just mitochondrial disease treatment that is a potential. There are ways of duplicating sperm genomes so that you can do a genetic test on one duplication and use the other one, once you have tested it, for fertilization. All these technologies, aneuploidy correction, aneuploidy avoidance, all these technologies at this point in time involve, in one way or another, some cytoplasmic transfer.

So, this is a very important decision we are taking, and the biggest concern we have had, and I think you are sharing this, is the safety concern. These are the biggest concerns. The rationale, you can only find out when you do the clinical work, when you do the trials. You can't base it on animal models. And, the safety concerns have been highlighted appropriately today. I get a lot of questions when I give presentations about cytoplasm transfer, but the concern of little

pieces of DNA being slashed off chromosomes that are now being transferred is a concern I haven't heard about in the six, seven years of my presentations. So, I must say I am not well prepared. It is an original concern. The concerns about the incidence of aneuploidy or the issue of heteroplasmy I think were well highlighted today.

DR. SALOMON: As I said at the beginning of the day, our purpose is to make sure that we have adequately presented the whole discussion, and when we get to the end of today, that is what I hope people feel we have done.

How about a few minutes on what would be an appropriate clinical trial? Similarly, what would be the key animal experiments to do to bring the whole group forward to the point where we would all naturally go down the curve in the road that says a clinical trial?

DR. SIEGEL: Before we move on to that, and I know we don't want to be here all night but given that we are going to have to make some difficult decisions, often when there is a consensus of the committee you try to sum up. I haven't heard you do that on this question. Because you started asking the question differently

from the way it is posed, I am not sure I have an appreciation of the consensus. If we move on, I assume the best advice is that we are just supposed to kind of put it all together, but I wonder if it might be helpful--

DR. SALOMON: Well, I put it one way and tried to get at it, and then I put it the other way with your help, and I don't know that we got at it.

DR. SIEGEL: It might be useful to poll the committee members as to whether they think before doing trials in human during pregnancy there is additional animal work to be done. If so, what? That is sort of question number four and I think Dr. Mulligan pointed out correctly that it is hard to ask one question without the other because, in fact, if there is no useful animal work, even if you would like to have more data from animals if there is nothing that is going to be relevant--

DR. SALOMON: Let me just try to get a consensus here, what I have heard from everyone is that this is the fork in the road. That probably based on everything we have heard, most of us would probably be okay with a well-designed, very limited clinical trial going forward, but we haven't talked enough about what a well-designed clinical trial

would be. The rest of us would be much happier if they would put themselves on hold and do the animal work and come back in, you know, six months to a year and reassure us on some of what we have articulated as safety issues. But I think we can certainly poll the committee on that, but that is my thinking. Let's go around. Dr. Casper?

MS. CASPER: I am not sure I am ready to decide yet. I think it would be nice to do some animal work. I am just not sure there is an appropriate model available.

MS. KNOWLES: I think you probably know what I am going to say. I think we should be doing some animal work and some human embryo work before a clinical trial.

DR. NAVIAUX: From what we have heard, there doesn't seem to be a defect in an animal model to try to correct so we would never be able to get an inactive principle in animal studies, which is justification for well-designed basic work in human studies.

DR. SHOUBRIDGE: I think we should be doing all of the above because I don't think there is a right or wrong answer here. As Dr. Mulligan said, no one will agree on an animal model. We

don't know what the principles are, and the only way to move a little inch forward is to do some limited, really good trial in humans I think.

DR. VAN BLERKOM: I would agree also with that. I think the trial should be designed to address the fundamental issue of what defect is being addressed. So, if you are transferring this stew or soup, the point is what are you really addressing? What is the defect? I think if you couple the cytoplasmic transfer with the notion of trying to identify defects, whether it is mitochondrial fragmentation of whatever, I mean, I think that is what is important and I think you could design it in that way so you can get a handle on the problem, if there is one. It is a unique situation because you are not quite sure what is wrong and you are not quite sure if you are fixing it.

DR. MURRAY: I am actually very close to Jonathan Van Blerkom on this. We have questions five and six, what defects are being addressed, and I agree, we don't know. And number six, do existing clinical data from humans support a rationale? The as is no. So, I would be unwilling to favor any trial in humans that did not have as a

main focus to identify what it is that is actually being addressed by this therapy. In fact, I am in no position to challenge the basic scientists here but it seems to me one could do useful studies, both in animals and in human embryos. Just trot out a few hypotheses, it is the mitochondria. What evidence would we have the mitochondria are working through the mechanism of increased ATP, or calcium ion transport? What sort of surrogate endpoints could we study in either humans or animals to see if, in fact, what in the cytoplasm transfer had these effects? So, I think actually one could have a number of hypotheses, generate a number of interesting research questions. You know, it wouldn't give you the final answer but it would indicate whether the mechanisms we postulated are plausible or not, and I would like to see that happening preferably before we do it in humans, but I wouldn't go to the mat and say that we shouldn't do a human trial to elaborate those questions.

DR. RAO: I looked through the risks with the procedure that is there and I tried to see if there was any real animal model in which one could test this, and it is very clear that if you think there are going to be late pregnancy problems or

childhood defects of chromosomal abnormalities, there is no real clear-cut animal model which would be appropriate. The best animal models are for mitochondrial defects. For those, I think it is worthwhile doing experiments in animal models. But, on the other hand, there seemed to be a consensus that while there might be a finite unknowable risk in terms of heteroplasmy, it is not clear that we should be stopping all experiments because of that data.

So, what one is left with then is to day, yes, you have to do this experiment. We need to get more information, and that information can only come from human testing. So, it seems that the choice was between doing human clinical work and doing human clinical investigations, and it seems that both would be necessary and it is not clear to me that one can do them one after the other or whether one should do them in parallel.

DR. MULLIGAN: I think I concur with that point of view. I would want to see first just better characterization of whatever is being injected, not only the DNA thing but just characterize the consistency, if possible, of DNA content or something like that. Then, I like the

mouse model. I was intrigued by the mouse model and I would encourage people to look at that in more detail. You know, with the history of all the mouse knockouts, if you look hard enough you may well find something. So, that is really worth looking at. But I wouldn't say that you need that information to go ahead.

Scientifically, I think if you could get the people who are going to do the clinical trial to actually perhaps look at--I don't know if this is technically possible--ooplasm without mitochondria, or highly decreased in it by depending on where you poke, or whatever, versus things that are high, it seems to me like that would be interesting too.

DR. SALOMON: I try to be practical about it. So, I see two sides to this coin. On one side, I see some of the most competent clinical investigators out there. This is a field that has moved forward through doing this kind of clinical research up until now. In general, I think everyone respects the fact that it has been done well and done ethically. There really are very few smoking guns in this field. So, I think that the first part of the coin is that I respect that, and

that gives me some sense that a clinical trial could be done, managed properly under FDA guidelines, that would be well designed enough to address the questions, and that would be a step in the direction of the clinical trial.

The other part of me sees the other side of the coin, and that is the reality that I am looking out on a group that are some of the best clinical investigators in the country, and the fact is that I work in mice and I work in non-human primates as well as humans and I think the truth is that when I look at my mouse breeders, at a certain point they start dropping off and I find that very reasonable to document, and I am not at all convinced sitting here that you couldn't find quickly a mouse model of older, less functioning breeder pairs and it wouldn't be that difficult, and you would have your mouse model.

Similarly, I work at UC Davis primate center where they have 3000 rhesus and over 1500 cinos, all of which have got very detailed breeding records and, again, I am not certain that you couldn't find--I don't think this community is really set to look in those directions and that is the other side of the coin.

So with that said, I think that I agree with my colleagues. At this point the people in this field are willing to do these clinical trials and the mothers and fathers that are coming to them are clearly willing, under the right umbrella of consent and well-done trials, to participate in it. So, you know, I think that is an argument for taking that path. But I hope I have put it in some perspective.

I certainly think that we have to do things to insist that animal model work and safety issues--I want to look at messenger RNA transcripts too and how this is affecting the RNA transcriptosome with the oocyte, and I think it is pretty ridiculous how little data there is to support any of this and that worries me because it is kind of a slippery slope that I go through every time, you know, whether it is xenotransplantation and, "oh come on, leave us alone; we are just going to do a little gene therapy", or "you don't know what you are doing; we can just throw some genes in." So, I am just saying I think as an overriding principle if we are eventually going to go down this clinical path, I hope that there is a consensus that there is a real underpinning of

science.

DR. VAN BLERKOM: Just to make a point, I am not aware of mice having menopause or perimenppausal conditions.

DR. SALOMON: In our breeding colony, and we now maintain several different strains which we have maintained for generations, there is no doubt that not only are there better and worse breeding pairs and we cull these out because we are always selecting for good breeding pairs, but also after some certain number of generations the number of pups they have per delivery will decrease, and it is very easy to document. So, I am just suggesting that that might be when you step in and do the ooplasm transfer from a young mother.

MS. WOLFSON: I am not convinced that there are animal studies that need to be done before we go into human pregnancies. I am not a scientist so I can't really go into those, but the paucity of that information frightens me when we look at such a huge outcome.

DR. SALOMON: So, clinical studies or animal studies?

MS. WOLFSON: Animal and human embryo if possible.

MS. SERABIAN: I guess one thing I am concerned with as a toxicologist is what I call worst case scenario. I mean, here we have the best of the best basically that are performing these studies in humans, and when it gets to expanded other sites, again, I am thinking worst case, you know, just going a little too far, etc., that is the kind of thing we would want to look at in animals, assume a worst case scenario maybe not for this initial phase that we are talking about but, for sure, as it expands.

DR. SALOMON: At a minimum also, if they do a clinical trial that they should do it with very specific outcome parameters for the different steps, many of which have been discussed.

MS. SERABIAN: Right. Then, one other comment with respect to the animal studies, it sounds like there is a wealth of data that has been published, maybe a bit of it not published. It would be kind of an interesting idea if there are certain organizations or groups to somehow put this in a document, master files, a certain way to submit to FDA that everyone could refer to in terms of the animal data.

DR. MURRAY: There is one more complexity

that has come up sporadically here but that we need to bear in mind is that I realize that, number one, this isn't the kind of thing people had in mind when they wrote about inheritable genetic modifications but this is plausibly, it will be at least in some children if they have offspring, if they are females if they have offspring, in a stochastic fashion some of the transplanted mitochondrial DNA does in fact end up in eggs that become fertilized and have children later, and I don't know what to do with that but I think it would be a mistake to simply forget that that is on the table.

DR. SALOMON: Dr. Schon, I realize that you were out of the room. What we did was go around and just basically gave some final thoughts about which fork in the road would you be comfortable taking, to clinical trials or no clinical trials, animal or go down both in a parallel way?

DR. SCHON: I have to think about this. Maybe the one comment I would like to make is that it seemed to me that there was--is everybody like me? You don't answer the question, you sort of make up your own question and answer that one?

DR. SALOMON: There have been eight variations of that so far.

DR. SCHON: I have detected sort of a merging of two issues, which are the safety and the efficacy, and I will answer the question. Safety means you have a level of performance which suffers no diminution when you do something. So you are here and you go down. Efficacy is the reverse. You are here and you want to go up. One of the confusions is that when we discuss the analogy to mitochondrial diseases the bar is actually down here because kids are in bad shape, the eggs are in bad shape genetically; they are actually not in such bad shape physiologically. Now, anything you do brings you up. So, to answer the question, for issues of safety clearly I think animal models are the way to go. I mean, the question answers itself. For issues of efficacy what I am hearing, and I am no expert, is that animal models are not the way to go because it is so hard to do. So, some kind of clinical trial for efficacy that followed a preliminary question on safety--you can ask these things about DNA fragments and so forth, although you may not be able to answer questions about aneuploidy, and maybe they can even go on

almost in parallel if you did some of the questions on human embryos, fertilized human embryos without implantation. I don't know of you are allowed to do those kinds of things, but if you were, that is the way I would do it.

DR. SALOMON: I think we have certainly answered almost all the questions. I think the one thing, sitting back here, that we didn't really get to--I mean, we have talked about the preclinical models. I don't know that there would be a lot more. We have discussed the mouse model, talked about the non-human primate models. I don't think that this community has the tools to go into the non-human primate and mouse model, so we would have to interest other investigators around to come into that area, and that is the kind of thing that could be done potentially but those are unknowns.

The only thing that I think we just may have fallen a little short of was exactly what would be the clinical trial. That is not a minor gap. I am sure I will be reminded of this year and years from now about how I failed the FDA on this one. But we have talked a lot about the aspects of what the clinical trial ought to be. I am going to try and get some consensus on that in a minute or

two. One thing I think we are all convinced of, again correct me if I am wrong but I think we are all convinced that there is a population of couples who are not implanting and are not being able to have successful pregnancies. I am not saying that we all agree that there is one problem for all those women, and there may not be, but there is definitely an identifiable population that is the target of this.

I think Dr. Cohen made the very good point that there are a number of other variations that are behind this that are relevant. So, the population is outcome there. I think population choice--I think these guys have that pretty well nailed down. I don't think they have been picking the wrong women to do it in.

We want to know efficacy. We have talked about what the safety issues are. So, whatever that clinical trial design is that you do, it has to give us safety and it has to give us some insight into the nature of the product, what is in that ooplasm--DNA fragments, RNA transcripts? How many mitochondria are in there? Does mitochondria have anything to do with this? What kind of measures would give you mitochondrial function? We

heard ATP and then we heard, come on, there are 50 other things that mitochondria can do; get a grip. We heard about apoptosis testing, all of which is commercially available, etc. So, I think that is the kind of thing that would come relatively easy is you sat down and said what are the aspects of a clinical trial.

Actually, I have just talked myself into the fact that we did answer all of the questions and I don't want any grief later.

[Laughter]

DR. SIEGEL; Well, I could come back years later or now, I guess--

[Laughter]

I don't want to keep the committee forever and, obviously, there are a lot of unanswered questions and we are not going to answer all of them. One or two that stand out in my mind is that we did hear a comment, I think from Dr. Cohen, that there is no intent for long-term follow-up of these children. I guess it would be useful to know from the committee whether they think that is an acceptable way to move forward, and if we allow trials to be done without long-term follow-up, then in the long term we still won't know what the

long-term effects are.

DR. SALOMON: We fought and died over this one in gene therapy in xenotransplantation so I can't believe I am back again discussing this problem. Fro Dr. Cohen's sake, xenotransplantation now is follow-up forever, and we are really not interested in whether the investigators want to do that or not. That is what has been said. In gene transfer studies it is a movable target depending on some of the issues of an integrating vector, non-integrating vector etc., but it is as long as 15 years in some vector classes. But the good news is that in these trials, just to give you the background here so you guys don't faint, a lot of the long-term follow-up came down to sending a postcard once a year kind of thing: "are you alive?" That sort of thing. So, you guys might ask "are you alive? Do you have mitochondrial defect."

DR. SABLE: Just to give an idea how seriously we do take it, we had one of our investigators in the delivery room, breach delivery, and the investigator has gone to the pediatrician's appointments. So, we don't mean to imply that we are not serious about follow-up, I

think it is just a matter of degree.

DR. SALOMON: With that background, I also wanted to educate those of you who are not privy to these other long discussions at multiple BRMAC meetings of long-term follow-up. What do you guys think? Again, we can just get some quick opinions. Why don't we just go around? Dr. Casper, long-term follow-up?

DR. CASPER: Yes, I think it is reasonable.

MS. KNOWLES: Yes, I think obviously there should be a very rich informed consent procedure about what long-term follow-up would look like up, particularly when we are talking about inheritable genetic modifications, how long that might have to be.

DR. NAVIAUX: Yes, I think long-term follow-up is going to be required, and there should be a default pathway. After doing the routine monitoring, if anything abnormal comes out in development, if there is abnormal growth of the child or abnormal cognitive development, then there should be an intensified examination to look for why.

DR. SHOUBRIDGE: I think so too. If you

could demonstrate that you haven't actually transferred DNA, then that would, of course, change how long might want to follow-up.

DR. SALOMON: I just want to add that that is one of the concepts that came out very clearly in the gene transfer experiments as well.

DR. SCHON: I don't think I am competent to answer the question. It seems to me that whoever designs the clinical trial, it is incumbent on them to figure out what the nature of the follow-up is. I can't do it.

DR. VAN BLERKOM: It would be nice to have long-term trials, but I just would put in a caveat that in this field, in IVF in particular, compliance is an issue because, believe it or not, patients disappear, regardless of what they signed in their informed consent, they leave their embryos in storage behind. So, it is a complicated issue to get the type of follow-up. Yes, you can put it there in writing but whether you actually get that on the other end is a different story.

DR. SALOMON: I don't know that this group is any less likely or more likely to disappear than our gene transfer patients or the patients who eventually will be candidates for

xenotransplantation. But there certainly is, on the other hand, a precedent for really extraordinarily successful long-term trials and, as a principle, it is quite possible to do, and I don't think we should approach it by saying, you know, all these patients disappear; there is no way to do it.

DR. VAN BLERKOM: It is not what I meant, but it may be a different category because it may not be perceived on the part of the couples that this is a pressing issue.

DR. SALOMON: They won't be able to put it on the income tax return either.

DR. MURRAY: No, but we can use the internet. Years later it is eerily possible to find you or anybody else if you know how to look and you are determined. So, I would say, yes, there should be long-term follow-up. It should not be onerous on either the investigators or the families, but reasonable thought needs to be given to what would be an effective program of long-term follow-up and I think that is all one can reasonably ask of either party.

DR. RAO: I can only second that. I just wanted to add one more thing. There were some

issues raised by Dr. Lanzendorf about selection criteria and controls, and I think those are going to be important issues. Given that we don't think there is a great amount of data on actual benefit or efficacy, that means you have to select your patient criteria for any kind of trial and you have to really define it very carefully, along with appropriate controls. That is going to be something that needs to be factored in.

DR. MULLIGAN: Yes, and with your point, I think the consent form--I don't know if we are going to get to that but I think it really ought to deal with this issue of the data that does exist. I am interested in whether or not patients and families would actually find anything interesting about the issue that I think you raised about the evolutionary uncertainty. I think there ought to be something about the evolutionary things that could occur.

DR. SALOMON: I certainly agree with long-term follow-up. As I said, I have been chased around and around on that already and I just accept it as being a part of the responsibility I think we have. I don't mean to be facetious about it. I think that in the end the arguments for long-term

follow-up, when done in a way that is not onerous on the patients, don't provide stigma, that carry then anywhere from school to insurance etc., if it is done right I think long-term follow-up is important to the community at large for these sort of cutting edge gene transfer experiments.

In terms of a clinical trial, the only other thing that I would add to the picture is if we go ahead with a clinical trial in this area, I really hope that when you say, for example, that here is a patient with repeated failures to implantation and then we did the oocyte transfer and we got such and such a result, that those patients are really much better controlled than the data we have seen so far. I want to make sure that it is all done at your center under optimal conditions and then at your center you do it.

I was also very concerned that 9 of your 28 patients in your study, Dr. Cohen, were patients who supposedly had male infertility problems. I wouldn't understand why you were doing oocyte transfer. Now, I may have misunderstood that slide, but that is an example of something I hope you will design out of a clinical trial.

DR. COHEN: Thank you for mentioning that.

It is a very good point. This was discovered after the fact.
eggs were treated with ooplasmic donation and the remaining eggs from the donor oocytes were injected with the husband's sperm. So, it is like a control with the purpose of freezing those embryos for years clinically later. But what we found is that in nine cases the embryos of those controls developed as badly as the embryos of the patient, and I think that is what I was trying to say. So, it is sort of after the fact. Looking at it closer, some of these were borderline male factors and we could have probably figured it out before but that is a very grey area.

DR. SALOMON: Again, that would be something that you presumably could exclude on the way to deciding this is a repeat implantation failure and won't benefit from ICSI.

DR. COHEN: Yes, you can do that but then you have to do a really big experiment, which is get an egg donor and test the sperm, yes.

MS. WOLFSON: I think there should be long-term follow-up in whatever way is possible, and insofar as there could, in fact, be a DNA transfer that is involved, I think the follow-up

should go into the second generation.

DR. SALOMON: Anyone else?

DR. NOGUCHI: What I do want to say is that I think this has been an extraordinarily open and frank meeting, and is exactly the kind of discussion and interplay back and forth with the community, the practitioners and our colleagues to really obtain advice that we need, because these are the questions that my colleagues face daily and actually are going to have to do the reviews, and this has been just an invaluable experience. So, I personally want to thank all of you, all the participants from the public as well. This was great. Thank you very much.

DR. MOOS: One quick extension on a comment Mercedes made a bit ago, it seems as though there are a couple of issues that could be addressed in preclinical models, like validation of DNA and so forth, that could be done once definitively in a sort of platform mode and people in the field could, in fact, work together to present us with some useful approaches to validating this. The quicker that some of these safety issues, which can be addressed in animal models, can be laid to rest, and it sounds like it

might be fairly easy to do the DNA one for example, the better for all of us. Then we can begin with a kind of staged approach in clinical models that we have all talked about, and we have heard a lot of discussion that it can only be evaluated there. So, think about it and come talk to us.

DR. SALOMON: Are there any last comments from anyone that have to be made before we adjourn? If not, I would like to thank everyone who came, both the expert panel, my committee, the FDA staff, particularly staffers like Gail and her group who put all this together, and everybody else. Thank you very much for a successful meeting. That group of you who will be here tomorrow, we will see you tomorrow. Otherwise, everyone travel safely and good health.

[Whereupon, at 6:45 p.m., the proceedings were recessed, to reconvene on Friday, May 10, 2002.]

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