pancreas or bone marrow, for example.

The immunologic issues are different. The host milieu issues are different with advanced diseases. And the mechanisms of functions are completely uncharacterized in many cases or may be a moving target and multifactorial. So, I really do think there are a lot of differences from that point of view.

DR. SALOMON: John, on point, and then Jay.

DR. TROJANOWSKI: After listening to yesterday's discussions and then the questions today, all of which I think have been very valuable to air, I think what we're talking about is a road map for how to proceed in the future.

I would suggest that we may already have a rudimentary road map in the studies that Curt Freed presented, that Tom Freeman and Jeff Kordower presented. I mean, they have gone forward and done studies that are not unmitigated disasters. In fact, I would say the positive effects outweigh the negative. If we could use their guidelines for going into clinical trials as benchmarks for other clinical trials, we would be able to move ahead more quickly with guidelines.

I am aware that the FDA has approved clinical trials for laboratory-grown cells. I don't know all of what they have allowed to go forward, but if you were able

to tell us, without naming names and so forth, where problems arose because you didn't have specific data sets, that too would help I think shape and focus what may be a very, very good rudimentary road map.

The only thing I would finally add is that, remember, we're not talking about treating headaches. We're talking about treating diseases that have no therapies, and individuals who have no life to look forward to. It doesn't mean we should be cavalier, but I think there is a sense of urgency that we all should feel in our bones.

DR. SALOMON: Jay.

DR. SIEGEL: I am pleased to see Don is ready to get on to the specifics. I think that is what will help us the most. I am going to make three very short comments that I think will help set the stage for that.

One is, the agency has shared through guidance documents quite a bit regarding our experience with cell therapies in general, and those are available through our web site.

Secondly, I do agree with Hugh's point about this issue of what you can accomplish in the general and in the specific. So, as we move ahead I hope we'll talk about general principles, but clearly many things will be different for different cell types. To the extent that

time permits and that there is enough known about those differences, it would be nice to bring those out and talk about specific issues regarding specific cell types.

Thirdly and finally, another nonscientific perspective on the difference between this and bone marrow transplantation, or only partially scientific and somewhat more regulatory, to think about is that for many of the products we are talking about here, unlike typically for, say, bone marrow transplant or many of the other cellular products that we've dealt with over the years, including blood products, there is the potential for there to be a single allogeneic donor and tremendous expansion in commercialization, in a sense, of large numbers of like vials of virtually the same product going into the same patients.

And I think Dr. Champlin spoke to the issues in controls in bone marrow transplantation, and if you've obliterated somebody's marrow and you thaw allogeneic cells and they're not quite the quality you want, you think twice before you throw them out, but the issues may be different simply because of the directions of the ability to expand the use of allogeneic cells and the commercialization. That will come to bear in thinking about what are appropriate product and process controls.

In those settings at least, there's more

flexibility to say, this lot isn't quite right. Let's grow up another lot because this one has lower viability than the last three or four. There's more flexibility, for example.

DR. SALOMON: Michael.

DR. WALKER: Yesterday was an extraordinary day, I think, of remarkable science. That science is going to keep investigators going for at least the next decade. I don't think the clinical things can wait. Nor should clinical things necessarily yet be overly influenced by the concerns and the important scientific questions that we pose to the laboratory.

To that end, I think really what we're speaking about is the incredibly complex issue, with the vast unknown that we have to deal with. But we have to deal with it right now, and it really comes down to, what would the prudent investigator want to know at this juncture, with the available data at this juncture in order to proceed forward.

DR. SALOMON: In neurological disease.

DR. WALKER: In neurological diseases. And I think the discussion as to whether the brain is different or not, we really shouldn't be discussing because it is at least perceived by a lot of people as being different.

(Laughter.)

DR. WALKER:

DR. SALOMON: I think we'll warrant that it is different.

Rusty, okay.

DR. GAGE: I'd just like to reiterate the point that we're talking about stem cells, and it's an essential component of the transplantation in bone marrow, that you have a stem cell. We do not know whether or not stem cells are a requirement for the transplant procedures of anything that we've talked about. Stem cells have a property that may be useful in the propagation of the cells, but we have no idea whether or not a stem cell is a requirement for the actual transplantation of the cells and for its viability and its function. I hope that we can keep that in mind in perhaps the separation of the manufacturing and propagation of the cells as perhaps stem cell quality and the actual product that goes into the patient, which may be something required, very different from that which you're actually manufacturing.

DR. SALOMON: I was hoping you would make that point. So, that's a good lead-in right now into where I want to go here. So, just to set some parameters here, we are going to go till 12:30. That is one hour on this. If we can get done before that, which I can't imagine, but if we can, that would be great. But at 12:30 it ends and we

will go on to the second part in the afternoon.

So, the first question is on human stem cell sources. I understand that Dr. Fink is going to put the bullet points up here. I am not that hung up on these bullet points, but they are discussion points.

So, the first question is, I think, obvious to everyone, and that is, what sources are going to be appropriate for these early trials? How far afield are you going to go with, let's say, some of the first studies versus how far afield are you willing to go as the field evolves? I think the latter isn't that important right now because I think without some proof of efficacy it's really sort of a non-issue.

So, I think what we ought to do is just take a minute to consider, in the expertise of the group here, what's practical. I heard somebody say they were going to go up and actually take a piece of brain from somebody and process cells out of it. I mean, I need a reality check on that from my field. That's a hell of a something to do. But this person was very serious about that, and I am not in any way trying to make light of that. It may be something that in neurobiology is okay. So, you guys have got to give us some framework here.

Fetal tissues, embryonic stem cells, et cetera. So, can we start with that, comments along that line? Short.

DR. KOLIATSOS: An obvious thing that comes to mind is that most of those tissues come from sick brains. So, although they come in the neighborhood of illness, they have epilepsy, which propagates beyond the site of origin, and I would be very much concerned about the pathology inherent in these tissues. So, in my mind, I think there's not a way to go about it by going to embryos.

DR. SALOMON: So, you would take the position that in the first phase of trials, which is the only thing that we really can advise the FDA to do because the shape of things change anyway as things go on, you can't see allowing someone to go and take a piece of adult brain in a diseased state.

How about anyone else on that comment?

DR. MULLIGAN: I would take the completely different point of view that it's fruitless to actually begin to pick a particular cell line or primary cell culture. I think the point was made yesterday that everyone has their own favorite source of tissue. There's never going to be any good comparison, which there ought to be, but there will never be such a thing. And so, I think even to kind of move ahead quickly, I would almost say we should defer that issue. I don't think we're ever going to come to any consensus about whether one person's source is more appropriate than another's because it depends

completely on the application. There may be cases where, using epileptic derived stem cells, someone will show that that has a better effect in terms of growth in some tissue.

I would put it, if I can jump just a second to the issue of purity of these cells, that one thing the FDA definitely wants to know is how do we define purity. This is what I think is the most key issue, what is different about these cells. Just to stir up some things, I would say that it's impossible that you'll ever be able to find a pure population of these because biologically, certainly one way it may work is that these cells are undergoing amazingly sophisticated transitions, even in culture. So, people that think, like Evan, that because he has cloned out cells and amplified them that they are more pure than cultures of RSP cells, I think that that's going to be the difficulty.

So, we have to think practically, that when we think of purity, that we can't be thinking in a way that people think about a compound.

DR. SALOMON: Okay, I wanted to clarify one thing, Richard. There is no desire, certainly on my part, to achieve consensus on anything today. If we naturally fall into consensus, that's fine. We're not taking any votes. We can't take any votes, not even spirit of the committee votes.

So, what I want on this topic is merely what you did initially, and that was to say you don't think that you can do this. I mean, just everyone should have a right to pick whatever brain source, come forward with their data and defend it. That's your opinion and that is fine.

So, I just want from the experts these kind of statements, and don't worry about. We're not going to argue about consensus, but I wanted to give the FDA as broad a sense of at least the experts on this committee of where you're willing to go and what you're willing to consider.

DR. RAO: I agree that we cannot say which source of cell you use or what is your favorite choice. But what we should try and emphasize is consistency, and that is that if you are using a source, you should know something about your source and you should be able to type it in some manner or profile it in some manner that you can say, this is --

DR. SALOMON: We'll get to that next, source control. What I need are comments specifically on sources.

DR. SIEGEL: I guess, if you would look at the questions, what we're trying to get here is not, you know, whether one should use live human donor or embryonic, gonadal. But is the issues, you know, well, I think there would probably be consensus here you shouldn't use and HIV-

infected source. What are the other, you know -- whether it's Parkinson's or not. But what are the other issues here? Should there be genetic screening for, say, prion, TSE risk or other -- I'm not a neurologist. I'm not even sure there are others here who could word the question better.

DR. SALOMON: Jay, we'll get to that.

DR. SIEGEL: I thought that's where we were.

DR. SALOMON: I'm taking a little latitude on the part of the chairman just to get a quick sense because yesterday there were sources mentioned from such a wide variety, I was just curious what the response to this question would be. It doesn't have to take too much longer.

DR. SNYDER: Well, whatever the source is, it should probably be well-suited for what the clinical trial is going to be for the particular disease. So, knowing perhaps what you are trying to achieve will then dictate what the logical source would be.

Then the second point, whatever the source is, it should at least be very well understood what its biology is. I am not talking about safety concerns about that. Just understanding what you would expect these cells to do, what your knowledge of the cells is. It should be well understood. Which may mean not just for the first time

pulling out cells, putting them in a dish and saying, this is what we are going to use. It should be well studied before going into a clinical trial.

DR. SALOMON: Are there any other comments on that?

DR. WALKER: I'd agree with Evan, and I'd put one other point in on that, that since we don't know how to fully characterize them. We should also, in some fashion, bank some of that source as future questions come up, that we can then go back and address those issues.

DR. SALOMON: So, what I'm hearing at this point is fine, is that there isn't a sense that there is anything off the table at this early point in stem cells and neural cell transplantation. I think that's fine. That's the question that I was asking. To the extent that there's nothing off the table, it's also a message to scientists and companies in the field, that if they've got a sensible path to defend a specific source, then they will fulfill some of the issues we're going to talk about next.

DR. GOLDMAN: To put something on the table with respect to Dr. Koliatsos' question or remark. I don't know that it's fair at this point to rule out any given source, including the adult ventricular zone, even from an epileptic, given our absence of evidence that there's anything different about that stem cell population or

defined progenitor cell population.

Secondly, I can envisage clinical scenarios where autograft would be reasonable, thinking in terms of nondominant hemispheric biopsy for extraction and implantation into small dominant hemispheric subcortical infarcts, by way of example. So, I don't know that I would rule out clinical scenarios either.

But again, by way of putting something on the table, we discussed yesterday adult sources, we discussed early fetal sources, but mid-gestational or late gestational fetal sources are actually much richer and much more abundant sources of the same types of multipotential progenitors that we've all been talking about in terms of cell therapy. That's something that was not addressed in the course of these two days.

The same types of cells that we see in the adult ventricular zone we can get many, many-fold greater numbers out of late second trimester embryos. That's something that's out of NIH jurisdiction but it's something that may well be an appropriate consideration here.

DR. SALOMON: Again, I think one of the things that we are not going to get into is the ethical issues, just because it's not our purview. I didn't think you were posing an ethical issue. I am just saying --

DR. GOLDMAN: I am trying to divorce it from

the ethics.

DR. SALOMON: So, as long as we, again, in general, are sending the message that if a source is well-characterized and fulfills the rest of the things that we'll get into hopefully now, then a specific source isn't off the table.

DR. KOLIATSOS: Can I respond to that, Dr. Salomon?

DR. SALOMON: Yes.

DR. KOLIATSOS: The way that the chair addressed the question was not ruling in or out. It was a chance of coming up with what we all think of as a homogeneous source. It seems to me that upon the complexity of this cellular sample, you add the complexity of disease. You do know that growth factors are in very different arrangement, including BDNF, FGF perhaps, or the neurotrophins in the epileptic brains, especially in the temporal lobe. So, these tissues have a different trophic profile to begin with.

It seems to me, talking about probabilities of coming up with a homogeneous source of cells, this would be second in my order of priority. It's not ruling in or out. It's just my sense of what would probably give you a more homogeneous and well-controlled, propagated over time cell line.

DR. SALOMON: Good point.

Hugh?

DR. AUCHINCLOSS: I was going to suggest we come to the questions.

DR. SALOMON: Thank you for your suggestion.

DR. AUCHINCLOSS: So, I was going to address the first four.

DR. RAO: Just one point, and that's just simply that this deposition of a reference aliquot that Dr. Walker made to of keeping cells is really quite critical, and that we do have an opportunity to do that, which we haven't done with other cells. We should consider that as a generalized requirement.

DR. SALOMON: Now I'm happy, so let's go to the questions.

DR. AUCHINCLOSS: I wanted to address A, B, C, D, with two questions. I started with the question when I read A, B, C, D by saying to myself, why wouldn't you assume that what we do for blood donors is perfectly for what you would do for "stem cell donors." So, that's a question.

Then the second question came about as a result of what you just said, Jay, when you said, well, you know, it is a matter of scale. One blood transfusion from one donor, but this is going to be a pot of a million vials

that could treat, you know, the whole population.

But it seems to me that a special question enters in, so here is the second question. How much of the kind of testing that you mentioned here, say for HIV, or for the genetic defects that might be there from the donor, can you actually do on the sample that you have, rather than needing to go back to the potential donor? Can you solve that problem with the tissue at hand rather than by the source?

DR. SIEGEL: I think for both of your questions, including, why shouldn't it be the same for bone marrow, I would rather hear from the committee than try to answer those myself.

DR. SALOMON: So, why don't we get the committee to start. The first issue is, are existing standards for blood banking and/or organ transplantation appropriate for this new field of neural cell, neural stem cell transplantation?

I'm trying to think about how to frame this. So, there are going to be cell lines. There's going to be fresh tissue, that isn't really a cell line, and then there's going to be some sort of gray area where there will be cell lines that are cultured for maybe even several weeks but not particularly manipulated. And then there will be cell lines that are heavily manipulated by specific

growth factors, along the line that several mentioned yesterday, including Dr. Gage.

So, in those settings, existing standards get a little bit confusing. But the initial thing would be HIV testing of the donor, hepatitis C testing, Epstein-Barr viral testing, CMV. My personal opinion at this point is -- and I think that was what Hugh was saying -- I don't see anything that the blood banking and organ transplantation and stem cell transplantation groups haven't covered. But here is a chance to think about it. Is there anything unique in your area? And there may be, like prions.

DR. NOBLE: It may be that as there are a number of mutations that have been identified that represent relatively later onset neurologic disease, that if one is going to harvest from fetal specimens, one might think of introducing that screen.

DR. KOLIATSOS: That's a wonderful point. I think all we know about genetic predisposition factors for neurologic disease should be out of the pool that we're going to use.

DR. AUCHINCLOSS: Can you test that on the tissue at hand? Do you have to go to the donor?

DR. NOBLE: Most of the mutations, I guess, can be screened. I think they could be screened on the tissue. I don't think you have to go to the donor.

DR. KOLIATSOS: Yes, absolutely.

DR. AUCHINCLOSS: So, now take it to the next part of this question. Supposing you do the screen on the donor tissue, which you have got there and you are developing as your pot of stem cells for therapy, and you find there is a genetic defect there that has hideous consequences. Do you go back to your donor and tell him?

DR. KURTZBERG: I don't think we know what these mutations mean, or what the denominator is that people who have them and what the expression of the disease is, and it gets very difficult. We face the same thing in the cord blood program of not knowing exactly what it means. I think it is easier to say that for mutations definitely associated with neurologic disease, those should be eliminated from the pool and those tissues shouldn't be used. But in terms of what does it mean in the context of fetal tissue, I don't think we know.

DR. SALOMON: That's a really well-put point. So, the question back to the neurology experts here is, is there a short list of genes today -- obviously, we hope they will grow -- that if you see them in a source tissue of any sort, means the disease is going to happen in such a high proportion that you'd all feel comfortable not allowing that to go forward?

DR. TROJANOWSKI: You can screen for 70 PS-1

mutations, 6 PS-2, if I got that right. There are 20 tau mutations. I mean, you can screen for lots of mutations.

DR. SALOMON: If they are positive, John, does that mean that you would not allow transplantation? That's the question because there are things like BRACA II which you could screen for that has some association with breast and ovarian cancer, but not -- what is it? 20 percent?

DR. TROJANOWSKI: There are mutations that we don't know. Fully half the FAD mutations have not yet been identified. But it is important, I think, to consider this in the context of a specific disease and even perhaps an individual's age. A 60-year-old gets a transplant but may have an FAD mutation, presumably it will take another 40 years for that to manifest itself in that graft. So, it's not, I think, an absolute negative. It has to be seen in the context of the disease mutation.

DR. CHAMPLIN: I think the obvious thing is that if you're talking about autologous applications, then you have a much shorter list of things you have to check. Just sterility really if you're giving it back to the same patient. If it's a one-donor/one-patient type transplant, then again it's a different category of risk than if it's one donor now who is going to give a million transplants, with expanded cells. Under those circumstances I would look for prions and everything else I could think of if

you're exposing large fractions of the population. Whereas if it's a single transplant from a relative, then a much more limited list would probably be appropriate.

Also, the sort of timing issues might be appropriate to think about. Say you're going to try to do a related donor transplant for spinal cord injury. You're going to have to do that fast. You're not going to have 6 months to do your infectious disease testing, whereas if you're talking about Alzheimer's disease, where it's not an emergency to do the transplant within 2 weeks, there's a different time frame that could allow more complete testing. So, I think to some extent this has to be common sense for the application and the cell source.

DR. SALOMON: I know there are a couple of comments. Evan, Rusty, Vassilis.

DR. SNYDER: I would just think that if you have the option to screen for genes that even theoretically could be time bombs, at least at the early stages of talking about first approaches for clinical trials, you would take that option to use the best source material possible. Obviously, the universal donor cell approach allows that more than the autologous donor cell approach, but I think one should screen not only for all the genes that we understand now, even those that subsequently may turn out to be problems as our knowledge unfolds. We can

screen for a lot of neurogenetic diseases of childhood, like the lysosomal storage diseases, which are now routinely screened in prenatal testing anyway.

We might even want to screen for deletions of tumor suppressor genes, for instance, or loss of p53, or things that we think may be down the line, if this cell is sitting in the brain of somebody for 10 years, could become a problem later on. So, I would screen as much as possible.

DR. GAGE: I think this could get out of hand in terms of screening for everything, and there would not be any cells available.

One could approach it empirically, and that is, the way it's being done just as an experimental question, and that is, is a gene mutation dependent on the environment for its phenotypic expression, so you can mutate cells, over-express CAGs, for example, and then transplant the CAG repeat cell, transgenic cell, into a normal host animal to see whether or not the expression of that transgene in a normal host results in a deleterious manifestation in that graft itself.

I think we need more empirical evidence supporting the fact that these gene mutations are really damaging to the cell itself autonomously, and whether or not in the context of a normal brain they're going to be

bad. If you eliminate all tau mutations and all amyloid mutations because of their potential, or anything else we are talking about, I think that maybe these are all just issues to bring up, but maybe empirical evidence supporting the fact that that mutation in a cell graft situation has some deleterious effect would be a good reason for excluding it. There are obviously other ones that we have talked about that we already know are bad, and those can be excluded for some reason, but it seems to me that you need to have some empirical evidence that the expression of that gene in that cell is deleterious in a graft context, not just that it is a potential for some genetic abnormality down the road in a normal context.

DR. KOLIATSOS: Actually, Rusty, you know, you take some of those genes that cause familial Alzheimer's, familial ALS, and Huntington's disease and stick them in mice and produce the disease. I wouldn't like those cells in any of the initial transplants. Period. And it's not that many. It is 1 to 2 percent. Those familial forms of neurodegenerative disease are not very common. This would hardly eliminate your basic pool of donors. You certainly have to take out the heterodegenerative diseases that involve childhood neurology and other things. I don't think that would severely limit your pool.

And the other concern I have is, remember, you

put those cells in sick brains. It may very well mean nothing, but at the same time I could argue that it could mean much more than the mutation itself in a normal host. So, I think that we could eliminate those things which we know in transgenic animals can produce the same disease without losing the necessary pool that we need to do our clinical job.

DR. MACKLIS: To reinforce what Rusty Gage just said, putting on my clinical neurologist's hat, in addition to all of the research-based mutations that John Trojanowski told us, and the ones that we may all agree would exclude, there's an increasing list, 50, 70, 100 clinically available tests for polyglutamine repeats, peripheral, central, spinal cord, and I think what Rusty pointed out, that we need to take into account where and how would such a mutation be limiting because I think every month I get a new mailing from Athena Neurosciences with four new tests, and we are going to be chasing our tails if we rule out all of these on every bit of tissue.

DR. SALOMON: That's the kind of input I think the FDA needs to hear.

DR. NOBLE: On a practical note, one of the ways in which one might approach this is with a mutational chip analysis. Maybe, Arelene, with your being here, there'is value in thinking about whether there's value in

assigning a contract for someone to actually develop a kind of chip that everyone would be able to use in these approaches in order to actually turn this into something that's feasible.

DR. SALOMON: Standardization is good.

DR. CHIU: That's a very good idea, Mark. Thank you.

DR. FREEMAN: One other practical issue is, is the hurdle going to be different for phase I trials versus multi-center trials?

DR. SALOMON: They don't need any help from us with that. The hurdles are always different for phase I versus phase II and phase III. I think that is a fair statement?

DR. SIEGEL: Well, they change as a function of phase. The amount of manufacturing controls typically do in terms of the validation. But the level of safety still needs to be there in all phases. When something becomes a safety issue, the amount of validation is what varies more. Of course, knowledge will change by the time a trial is in phase II. This is a field that's rapidly evolving.

DR. SALOMON: John?

DR. TROJANOWSKI: I think we should take disease mutations very seriously. Rusty's comment about empirical data is very important. I think Vassilis knows

that, much to the chagrin of many of us trying to make models of disease, merely putting a transgene in a mouse doesn't guarantee disease. In fact, you have to over-express the protein at a whopping level to see the disease in the 2-year life span of an experimental animal.

From our work with the N-Tera-2 cells, which have an abnormal chromosomal profile including, as I recall, three copies of chromosome 21, and a number of small -- I forget what they're called. We just haven't seen disease in the basic science studies within the 2-year life span of the animals that we've transplanted, and those chromosomes have been stable for a decade. So, I think merely an abnormal chromosomal profile, with other data mitigating the potential side effects of that, shouldn't be discarded. The cells shouldn't be discarded.

DR. SALOMON: I'd like to go on to the next question. I think just summarizing what I've heard, not trying to say any kind of consensus, but the flavor has been that the idea of general use of guidelines already in place for organ, blood banking, sperm, and hematopoietic stem cells is perfectly reasonable to apply to this new field. However, there are some unique features and things like prion, infection, certain specific genetic malformations that could be screened right now.

I thought then things get gray about what would

be the criteria that would allow you to add new genetic malformations to the list. I think that maybe falls into the area we don't know and perhaps further research should be done in that area.

I thought John's last point, in contrast to Dr. Gage's, is just to be careful about, again, the models. I think if you put the disease in and you absolutely got a disease in the animal model, that would probably influence the committee. If you put an abnormal gene in an animal and you didn't get disease in a given time frame, that might not be as easy to interpret.

That's not meant to cover everything, but is that generally okay with everybody? Hugh?

DR. AUCHINCLOSS: I wanted to say a couple of things. There is a danger in a generic conversation that we start acting like academics. I don't know. We were up to about 100 genes that I think we wanted to be looking at. And the notion that we should be using those 100 genes to exclude tissue is, I think, ludicrous. That decision is a matter of individual disease and individual nature of recipient populations, so you can't answer that question.

From a research point of view, the chip I think makes a lot of sense. But that brings you back now to this other question that we haven't addressed on here. When you have a chip and you have all that information, do you go

back to the donor and tell them about it?

DR. SALOMON: Great. I like that. Let's go on to that question. Maybe, Joanne, given your experience with cord blood donation, do you want to start by making a comment? So, what do we do when the donor sources are fetal? How do you feel about that in terms of confidentiality, keeping records of the person? How far do you go? And then we will turn it back to the group here.

DR. KURTZBERG: There are two levels of answering in cord blood. One is what the specific groups that I am involved with have decided to do, and the other is what the whole community has sort of accepted as a consensus. That still is sort of up in the air.

I think our general approach has been that we've screened for infectious diseases that could pose a risk to the recipient but use the blood donor rules for informing the mother and her physician if an infectious disease was found, like HIV or hepatitis, that had an impact on the health of the mother or baby.

In fact, in our consent form, we require that the mother allows us to tell her and her physician that she has HIV or she has a positive test for syphilis or hepatitis screen so that the baby's interests can be prioritized because some mothers have refused consent on that basis. They let you test as long as they don't have

to know, but they don't want to know.

DR. SALOMON: Have you done any genetic testing?

DR. KURTZBERG: The genetic testing is limited to screening for trisomy 21 and 18 in mothers over 35 who didn't have amnios -- and the baby's cells -- and screening for the leukodystrophies. If the recipient of a unit has a leukodystrophy, then the unit is screened. What the consent form says is that should one of those diseases be diagnosed, and if there is therapy for the disease, the family will be informed.

DR. SALOMON: Do you screen for things like Gaucher's disease, or Niemann-Pick or any of these other things that are hematopoietic stem cell significant?

DR. KURTZBERG: We are screening for globoid leukodystrophy, metachromatic leukodystrophy, adrenal leukodystrophy, but not in all units. Only in a unit that would be selected for transplantation of a patient with that disease. So, if a unit is selected for a patient with leukemia, it's not screened for ALD, but if it's selected for a patient with ALD, then it is screened, both because we don't want to use a carrier, and because we obviously don't want to use an infected unit.

Now, we have really waffled on whether or not to have look forward. Look forward makes sense

intellectually but it is very expensive. It is administratively a nightmare, and it's just not feasible in all cases because it is easy to lose track of these people.

DR. SALOMON: Look forward you define --

DR. KURTZBERG: Look forward means following the baby who was the donor of the tissue for some period of time to see if they have a disease that's not expressed at birth or at the time you harvest the tissue, but that could be important to a recipient of the tissue. The easiest example to give you in our field would be a baby could be born perfectly healthy but at the age of 2 develop ALL, and would that be important to the recipient of that baby's cells. Honestly, we don't know the answer to that, but that's the kind of thing that you wouldn't be able, right now at least, to pick up at birth.

A baby could have an immunodeficiency syndrome and that could be missed at birth. That would certainly be important to the recipient of those cells in the context of marrow transplantation.

So, some of the groups banking cord blood have elected not to have look forward just because of the administrative issues and the confidentiality issues. Others have elected to go forward with it, but it is very expensive, and it's an administrative nightmare to some extent.

It depends what community you are collecting your tissue in. If you have one donor that is going to supply a whole bank of tissues, then it makes a lot of sense. But if you have thousands of donors that supply individual tissues to individual patients and you are in an urban center, it can be impossible, really, to follow all of those babies. And no one knows how long it makes sense to follow those babies.

Here you're talking about diseases there may be no screens for, that may not present until somebody is 60, 70 years of age. Intellectually that might be interesting to do, but I don't see how it is going to be feasible right now.

Our biggest issues have been, I guess, focused on diseases that are either important in real time to the recipient or to the donor of the tissue. We have the added obligation to protect the baby donor, who is a minor and doesn't really have any ability to protect themselves.

DR. SALOMON: I think that is an excellent start from a field that has really definitely done its homework in the last couple of years, so I appreciate that.

How about now taking that area and commenting on it with respect to neural stem cells or neural cell transplants?

DR. WALKER: I'm hearing a principle coming out

of what was articulated, fundamentally that if we have a genetic test, using it as broadly as we want to in definition, that pertains to the specific disease we are trying to treat, maybe that is the very appropriate test to use very specifically in those patients. That is, if we're going to treat a Parkinson's patient, we've got to test for Parkinson's. You surely don't want to be transplanting it in that patient with those cells. That sounds like a good fundamental, straightforward principle.

DR. AUCHINCLOSS: I still haven't heard the principle for what you tell the donor.

DR. SALOMON: Joanne talked about that.

DR. AUCHINCLOSS: Do we tell them about genes that we are not sure whether they -- do we talk about genes that have a 10 percent association with the development of --

DR. NOBLE: What is wrong with saying that the donor has the option, when they are agreeing to be a donor, that they tell you whether they want to know?

DR. KURTZBERG: I agree with that. I think you inform them in advance that if there's a disease you can identify for which there's therapy, you're going to tell them if they have it, and you can list the diseases you're testing for, and then they have the option of deciding whether or not they want that information.

DR. WALKER: I think this is a critical issue that you raise about the donor. On the other hand, that is a generic question that applies to all of these kinds of things which we can't necessarily or shouldn't necessarily solve solely for the central nervous system.

DR. AUCHINCLOSS: I couldn't agree with you more, but it is a question on the FDA's list, what do you tell the donor. But it's a question that is very big, way beyond neural stem cells.

DR. KURTZBERG: There's one more question. There are a lot of diseases we don't have tests for now and we will have tests for later, and how do we handle that. How do you get permission for that testing that you know you're going to want to do but you don't have right now? What we did was put in this generic line that says we plan to do future tests, when they become available, for diseases that are important in this setting. But I don't know if that really covers us or not.

DR. AUCHINCLOSS: Just to comment that when I jump to A, B, C, D in this group here and said blood bank rules apply, at least I would start with that assumption. That includes the assumption that there always is a link between the stem cell product and the original donor, that there are records maintained. And that is the standard in blood banking, is it not? So, is there any reason why that

wouldn't be the case here?

DR. MACKLIS: Two quick comments again from the clinical neurology side. This idea of look forward is very interesting. Many of the degenerative neurologic diseases are anticipated by older members of the family one or two generations ahead, and that comes out in a family history. Would one include that in the look forward kind of procedures?

And the second, if I understand, is only some states have insurance controls that would defend patients from this broad array of genetic tests that we are considering linking to the donors and their families.

DR. KURTZBERG: Well, the linkage can be confidential. So, you can handle that. But instead of calling what you just said look forward, that's really part of the donor screening.

DR. MACKLIS: I was thinking of following them along, the donor and/or members of their family. You could broaden it that much to think we'll look two generations ahead for spinal cerebellar atrophy.

DR. KURTZBERG: Well, you could also use the family history of -- I mean, we define the family history as such and such and such and such in a first degree or second degree relative as an exclusion. So, you would have to think about, for each of these diseases, what makes sense.

DR. SALOMON: I think we have to realize those are great research questions, but we're not going to be like Sweden who has got every patient who has ever been born in Sweden's health care for the last 100 years. Which would be wonderful, and I am not saying wouldn't be great for public health research.

From the back, and then to Tom.

MS. MEYERS: I just wanted to make two comments specifically having to do with fetal tissue. Many of the issues with fetal tissue are similar to things like blood donation or bone marrow transplants. But when you're talking about women who are donating fetal tissue, they may be themselves women having abortions and may have risks that are not pertinent to people who are doing blood donation. So, the whole ethical issue of abortion I think is very relevant to this. There may be real risks to women if it's found out that they did donate their tissues. So, I would urge you to consider those risks.

The other thing is that if you're talking about genetic mutations or any kind of potential markers in using fetal tissue, you need to test the cells or the tissue because you are interested also in the mutations that could be in the father, and not just in the donor who might be available. So, I just would remind you about genetics includes both parents, not just the donor, in this case for

fetal tissue.

DR. SALOMON: Can you please identify yourself?

MS. MEYERS: Carol Meyers, from NeuralSTEM Biopharmaceuticals.

DR. SALOMON: Tom? And then I think we need to go on.

DR. FREEMAN: In terms of blood bank differences, I'd like to amplify that one point, that when the FDA audits records, for example, they do generally go back to the sources. It directly conflicts with confidentiality issues for women that have had abortions. So, that is a special consideration, how to protect that confidentiality, if that is part of the protocol, which from an ethical point of view it should.

Secondly, source sterility is obviously very important for any type of organ donation. Yet, federal guidelines specifically prohibit changing any abortion techniques if fetal tissue as a source is used. So, then you have mutually conflicting regulations, and that has to be taken into consideration.

On the other hand, there are precedents for biologic products where source sterility is not enforced. For example, bovine pericardium, which is used clinically. Obviously, after-the-fact testing of sterility issues is suboptimal, but may be necessary to be considered in this

situation.

DR. SALOMON: So, I think that in general what we've discussed is that the groundwork that has been laid carefully by the groups in doing cord blood banking is fairly applicable to this field, that there are some unique gene markers unique to neural disease, and those could be handled by making it optional in the consent form to the mother. There are some confidentiality issues in maintaining the identity of the mother, the history of the mother, and now we've added the mother's family including, I think quite appropriately from the floor, also the father's family. I think that, however, is relevant, particularly in this sort of cell transplantation, maybe even more than in cord blood stem cell banking. But I don't know. Joanne, you might comment on that last point.

DR. KURTZBERG: I have two comments. One, I would say roughly 10 percent of the time Dad is not Dad. You can't just rely on the history for that.

And two, I think the longevity in some of these diseases is going to make this a little bit different than cord blood.

DR. SALOMON: I agree. I was just saying that in this case there probably should be a provenance, if you will, that the grandfather of the mother had ALS, or had a degenerative disease. That is all I meant. And that would

be unique to this, is what I was trying to articulate.

DR. CHAMPLIN: There is a precedent in bone marrow transplants from unrelated donors in terms of donor confidentiality and issues that result from that. In the early days where that was not foreseen as a problem, there were issues of donors or recipients finding out about each other and undue influence or coercion occurred in one or the other direction.

So, it's actually a federal law that the donor identity needs to be maintained confidentially from the recipient until at least a year after the transplant, and then if there's mutual consent, that they get to know who the donor was. But that has avoided the coercion aspects to a large extent.

DR. KURTZBERG: I just wanted to say one thing. In cord blood we decided not to allow that to happen because the donor is really a minor and can't consent for themselves. Just in the few transplants we've done, we get questions all the time from recipients about can't they know who their donor is, can't they meet their donor. But we've decided no because the donor is a minor, and I think the same should be true of fetal tissue.

DR. KOLIATSOS: I think that the family history is not mutually exclusive. In fact, it's complementary to testing the embryo itself because, for one, you may have a

family pedigree without having the gene and then there's all kinds of unknown genetic causes. We can envision a complementarity here in taking a history, at the same time testing directly the embryo.

DR. SALOMON: That is what I'm saying. I think in this particular case one unique feature of it is that you'll want to know the paternal and maternal histories.

DR. REID: Yes. Lola Reid, UNC.

A suggestion that at least alleviates to some extent what you're describing. It is neonatal tissue. With the help of the neonatal intensive care unit and the surgeons, we've been learning about the postmortem collection of neonatal tissue. That tissue is not used by any transplantation program because the tissue is too fragile for most transplantation procedures. But those tissues are quite replete with progenitor populations, and they are very easy to handle for most of the processing, the studies that most of us do.

Moreover, the parents of those infants who die are usually quite willing to participate in whatever analyses might be necessary for this because in the tragedy, they actually have some degree of comfort in thinking that they may be helping out some other group of people. So, it's just a wholly different situation than fetal tissue where you're dealing with abortuses.

DR. SALOMON: Thank you.

I think we've covered that.

The next question is, are there intrinsic safety concerns for stem cells based on their source of derivation? I think we've kind of talked about that already. I think that it's quite clear that there are.

And if there are differences, describe them. I think then we would deteriorate into a 20-minute discussion of step by step, which didn't go very well at the beginning anyway.

DR. AUCHINCLOSS: Whoa, whoa. This is the fundamental question in the entire two days, it seems to me.

I would have said, listening to you yesterday, that embryonal stem cells are a dangerous product, given the information that we have available right now. Indeed, I was going to ask the committee would it be appropriate to say that embryonal stem cells -- not embryonal tissue transplants, such as we just heard about -- should not be transplanted into humans at this moment, but that there is no intrinsic danger to adult stem cells that I've been able to identify.

DR. SALOMON: Yes. Hugh, in this case we're not far off. I was thinking in terms of infectious disease and genetic screening was what I thought was in this

section, and I was going to go on to that issue under characterizing of a stem cell, in which case your point is well taken. So, if we agree, then I'd like to go on. I certainly think that that is critical and I agree with you on that.

I think I'd like to go on, just in terms of time, to the next major category, which is manufacturing of stem cells. Well, we've got manufacturing and we have characterization. I'm now just thinking about the way things have gone. I'm going to go to characterization because I think that's the issue that Hugh brought up, and that is if we have these different sources, which I tried to get a sense whether were off the table and the sense was none are off the table, so let's go back and think about the characterization of stem cell preparations.

So, if someone wants to come forward to do a clinical trial, what is it you think should be given in terms of characterization of the cell for transplantation? And then we can talk about manufacturing next.

DR. SAUSVILLE: I think this question is inextricably tied up with model considerations because we're in the position that in contrast to, for example, marrow preparations, which you can define in terms of BFUs and CFUs and things that you can do in a laboratory, we really can't do that here, at least I think.

DR. SALOMON: Well, that's a good question. So, you guys yesterday, though, showing all these beautiful confocal pictures, you know, these are oligodendrocytes and these are dendrocytes and these are astrocytes and these are glial cells -- can you guys comment on that? Do you guys go with what Ed is saying, that you can't distinguish these things, or can you?

DR. MULLIGAN: I think that there are two things. One is the engraftment efficiency. I think there would be great merit to looking at the potency of these cells and their functional purity by looking at, in cases where that's possible, some quantitative measurement of engraftment. Evan and I have been talking about this and I'm not convinced from what I've seen that that is so simple. But when you're talking about purity and the inherent impossibility of looking at real physical purity, functional purity would be the closest you'd come to. So, as you march towards what's functional purity, certainly transplantation, seeing how many cells you get per number of cells that you introduce is a measure of transplantation purity or a specific activity.

But I think the slippery slope is that in all these animal models, the actual issues of what accounts for the therapeutic effect is so questionable and not pinned down that you don't have any important measure of kind of

the potency of the drug in the most important type of context. So, for instance, is it making neuronal connections or is it releasing cytokines? If you knew it was releasing cytokines, then you can measure the ability of these cells, when implanted, to deliver a certain amount of cytokines. But I think that in so many cases we're not going to even have that luxury.

So, I would just come back to if there is a quantitation, a way to really see whether a given number of these cells consistently gives a certain amount of cells being transplanted or persisting, that might be very, very helpful.

DR. SAUSVILLE: Right, although that remains an argument from analogy, unless you actually have the human cells function in the animal environment, which I think is a little dicey.

DR. CHAMPLIN: Well, the fundamental problem with stem cells is that they're defined by what they're not and not usually what they are. At least even in the discussions we've had here today, these are undifferentiated cells that don't have the mature differentiation markers of the end product. So, as much as in hematology we've been searching for the markers of the true stem cell forever and thought we had it in CD34, but now know that that's not the case in terms of identifying

the most primitive stem cells, it's a very difficult matter to quantify the number of stem cells that you have. The readout is in fact engraftment in vivo and reconstituting hematopoiesis or, in this context, neural function. You can obviously look at that preclinically in animals, but how do you know you've got it in a human transplant before you transplant?

DR. SALOMON: Well, we do SCID repopulating cells.

DR. CHAMPLIN: Right, but that's not a quantitative assay and we can't say now this is an adequate bone marrow transplant because it produces so many cells in the SCID mouse.

DR. MULLIGAN: I agree with you, but I'm saying that we do competitive repopulations for bone marrow transplants in mice, and you can definitely get a quantitation of the functional host that you introduce.

DR. CHAMPLIN: I think there are two parts to this question. One is showing that the cells in principle can mediate neuronal function, but the other practical thing is you've got your cells that you're planning to transplant. Is that an adequate graft?

DR. NOBLE: I think that there are two points that are being confused here. One is that in respect to asking what is the repopulation potential of any of the

neural stem cells, we really don't have any limiting dilution assays that have any meaning at this point. You can't do the kinds of things that are being done in the most sophisticated hematological experiments.

What we can ask, however -- particularly Mehandra is being very quiet about this, but I want to give him credit for being really important in this. There are markers that we know distinguish between the totipotent cell and the lineage-restricted cells. And there are not many of these markers. There are things like A2B5 antibody and antibodies against polysialated NCAM that Mehandra and his colleague Dr. Mayer-Proschel have described well as delineating these populations.

It would certainly be nice to know, because we are aware in the community that as these stem cells are grown in culture, a certain proportion of them do become lineage-restricted. It would be nice to know what people are transplanting in that respect. So, similar principles as in mapping the hematopoietic cells, run a few antibodies, so at least we have that information.

DR. SALOMON: Rusty and then John.

DR. GAGE: So, blood reconstitution is one system. It's one objective. And all the neurological diseases we have are uniquely different. So, there's Parkinson's, Alzheimer's. Like you were saying, these are

inextricably linked to the model. So, once you establish a model, then you establish your assay based on that model, and then you can do the reconstitution assay and justify it. Whoever is going to come to the FDA has to justify their model.

Now, 15 years ago, when we were grafting fetal tissue into rats, we tried to establish basically a reconstitution assay, and the question was what is the minimal number of dopaminergic neurons surviving in the striatum that was adequate to drop the rotation behavior in a rat from 7 cycles per minute to below 2. We came up with you have to have a minimum of 300 surviving dopaminergic neurons. Independently of what all the other cells were, there was this threshold, obviously with some variance around that. And you had to get tissue, we found out, between 14 and 16 embryonic day of a certain type of rat, and if you did that, it becomes a high school experiment. You can do that over and over and over again.

DR. SALOMON: So, I think that's excellent. The feedback that you're giving us is that, at least in some animal models, you can be quantitative. That's what we were asking.

DR. GAGE: Now, the question was how does that then translate to the human condition.

DR. KURTZBERG: I think what's important here

is to have a working group of the people who are going to be doing the transplants to come to consensus about what they're going to measure so they can compare their different techniques. I don't think people who don't know the field should be the ones telling them what to measure. I think that's a mistake or something that came late in bone marrow transplantation. Everybody kind of did their own thing and some things matched and some things didn't.

But I think there should be a formal arrangement where there's a group designated who's doing these trials and who decides on the kinds of collective data and even has workshops to make sure you can get the same results in the same assays in your own laboratory, or else have standardized reference laboratories.

DR. TROJANOWSKI: And I think with that in mind, we've come very close to having some guidelines. Again, I would refer to the work of Freed and Freeman and Kordower. There are some numbers that you can go with that are scaled for humans and not rats and that we should benefit from the success I think of their schemes. They're different in each laboratory we've heard, and they inject different sites and so forth. But still I think we can benefit enormously from what's already on the table, published or in applications, to proceed with some of these studies.

DR. KOLIATSOS: It seems to me that we can come up with a consensus in principle here. I refer to Rusty's wonderful non-definition of a stem cell. It's not going to be a pure preparation. It's going to be something enriched in something, and we have to accept impurity as a matter of principle in these procedures.

To add to this, you have impurity in terms of side effects. That's why the hematopoietic precedent is not extremely useful in the nervous system. When I've tried to put the cells in brains, you can see one effect in one side of the cortex and a total different effect in another side of the cortex.

So, I propose that the unit of treatment is not only particular cell preparation but a cell preparation in a particular engraftment paradigm. So, this together should be used as a, so to speak, modular kind of treatment as dose to guide any human experimentation. It's not only the impurity of the population of cells. It's also a site-specific effect you see in the nervous system all over the place. In one side, tumor; in another side, integration into the cortex.

DR. SALOMON: So, I think trying to come to sense of what I'm hearing is that, first of all, we all seem to agree that every disease is going to have to be taken -- now, there are going to be groups that are going

to say I have a stem cell preparation that can treat all diseases. We've already heard that today. Fine. Prove it.

Basically, what I'm hearing from everybody is that you're going to have to start with some sort of a model, with all its limitations. And I think it's important, the idea that these models should be discussed and validated in some sort of working group of experts in your field, not by outside people, including myself.

And there should be some way of quantifying it, albeit the day you step forward for the first clinical trial, we won't be able to answer Dr. Gage's question, so what does that mean in humans. But that's a problem in all translational research. I think all of us have dealt with it in our own areas. So, I think that that works.

What I would like, before we break for lunch in a minute or two, are there any specific markers from the neural experts that are like CD34 and CD45 and CD3, that if you can nail that one, that's really, really useful?

DR. SIEGEL: Can I ask a question about your summary and about the last conversation? In a later set of questions, we're talking about what models are good for safety testing or for proof of concept in efficacy. Here we're talking about cellular characterization to ensure lot-to-lot consistency and perhaps some investigators are

interested in, I think appropriately, also comparing results across centers, a less direct regulatory issue but an important one.

So, in that context, this discussion of models is what I'm hearing, in fact, that in terms of characterizing cells, that the best answers to ensure lot-to-lot consistency, for example, may not just be in surface markers or in vitro functional tests, but actually plugging them into models to look at implantation viability, developing specs --

DR. SALOMON: I think that's exactly what they're saying. My thinking here is that we'll get back to these discussions as we talk about the animal models, Jay, this afternoon. But, again, input if you don't agree with what I'm saying. I'm just trying to capture the threads that came out, and we'll get back to it again in the afternoon. But, yes, that's exactly what I have heard everybody say.

I still wanted some response from you guys. Dick Champlin and I went out to dinner last night. We were saying, oh, it's like a parallel universe. He comes to me and says, we've got these CD34 negatives, Thy 1, and I go, yeah, yeah, yeah, and blah, blah, blah. You guys are talking about a completely different set of markers. So, can you kind of enlighten us? Are there ones like that

that you guys are comfortable with that would be important?

DR. RAO: Like with a lot of stem cells, you have negative markers which are present on differentiated cells but are not present on stem cells. So, you can do a depletion selection and there's a wide variety of markers which people will agree as a consensus.

In terms of a cell surface marker which is specific to stem cells, the data is very weak except for one molecule which was recently described at a meeting, and that is this antibody which is used, recently characterized in stem cells in the hematopoietic system. And that, in our hands and in other people's hands, seems to recognize a subset of stem cells present in the nervous system.

Unfortunately -- and that's the problem I guess in terms of looking at human versus animal models -- antibodies are only human specific. The mouse homologue of the gene has just recently been cloned, and it's not clear that that expression of that antigen in mouse is the same as that in humans. So, it's not perfect.

DR. SALOMON: How about Stro-1? Dr. Verfaillie brought that up as one marker for a mesenchymal stem cell. Have you guys looked at Stro-1, what you'd define more narrowly as a neural stem cell? Darwin, do you have information on that?

DR. PROCKOP: I can speak to that. No, it

hasn't held up. Even Paul Simmons doesn't say it holds up. It was a nice early start.

But as we're studying these cells more and more, the primitive cells -- what Dr. Rao says -- they're pretty negative for almost anything we can look for. A few clues but not many. They're mostly negative. We look in the cultures. We see mature cells which are late progenitors and really don't differentiate well. They do light up with a number of markers, but if we look at the small cells, the real precursors, very few things stain them. Stro-1 is negative. Everyone agrees on that now.

DR. GOLDMAN: I think it's important to remember that the stem cell population, quote/unquote, of the ventricular zone, even in the earliest stages of embryogeny is very heterogeneous itself. So, we're left with requiring many markers in combination to define as accurately as possible progenitor populations. I think in retrospect it hasn't turned out to be very different from the hematopoietic system.

DR. SALOMON: One advantage we have in the hematopoietic system -- Dr. Champlin mentioned that it's incomplete, but CD34. If you purify CD34 cells and transplant them, a population within that heterogenous group reconstitutes the bone marrow and the hematopoietic system. So, at least we have that.

So, if you came to an hematopoietic stem cell group and said, I've got this stem cell, the first thing everyone would want to know is what the CD34 status is, as a general rule. I was just asking if there was something similar to that in neural. I think you've answered it. There isn't.

DR. RAO: Not a marker, but there are mechanical ways of separating cells. I think Rusty alluded to it where you can use density gradients. And then Evan alluded to the fact that you have some kind of transporter which pumps out the bis-benzamide so that you can use absence of bis-benzamide staining -- oxidized staining to select a population of cells, and you can, relatively with reasonable certainty, guarantee that you have a high population of progenitors in those two populations. You can use a combination.

DR. SAUSVILLE: So, the way this would play, if you were going to make some minimalist product description, would be I have a bis-benzamide negative population of cells that, in an immunodeficient animal that's been appropriately lesioned, gives at least 300 dopaminergic neurons per something or other.

DR. MULLIGAN: But this belies the whole concept of the fact that everyone is going to have different favorite cell populations. Just on the

hematopoietic front, what you said is very funny because there are now a lot of people that think that CD34 deficient cells are just as good if not better.

DR. SALOMON: But at least there has been a general experience up until now purifying CD34, injecting them into patients clinically, and using them.

Again, I'm not trying to fight a battle that doesn't need to be fought. If someone came tomorrow and we were all sitting here, a sponsor, and said, I want to do a clinical trial in disease "fill in the blank," and I have a population of stem cells, is there anything that these sponsors should tell you about markers or behavior, Hoechst dye exclusion, that you'd say, yes, I'm on board with you.

DR. TROJANOWSKI: Are you focusing on the right issue? You can differentiate the stem cells in vitro, and we transplant postmitotic neurons and they survive and they engraft. I think a note of optimism here is we may not need to use stem cells. In fact, for many of the diseases that we've heard about in the model systems, we want oligos, we want neurons, we want, in fact, not just generic neurons, we want dopaminergic neurons. I think we have lots of markers for those. And you can very effectively transplant postmitotic neurons into the brains of animals and people and they will survive.

DR. CHAMPLIN: But those aren't stem cells,

which is fine. Maybe that's what you want to do.

DR. TROJANOWSKI: But they can come from stem cells and be differentiated in vitro and then you can transplant them. So, I just would offer this note of optimism that we're not hopelessly lost. We can go for the immature derivatives that go on to become fully mature neurons, oligos, myelinate, what have you.

DR. CHAMPLIN: Getting back to the stem cell issue, one thing is even when you get into these highly enriched populations, when you look by limiting dilution how many of them actually are reconstituting are reconstituting or form colonies, what have you, it's 1 in 1,000 or 1 in 100 if you're in a highly enriched population. So, the word "pure" is not correct here. This is enriched populations and to have a truly pure, uniform population is both technically impossible and it's probably premature to even try to identify such a population.

DR. SNYDER: So, basically when we all sit around in the field and somebody presents cells, we ask, as Steve mentioned, for a battery of proof that these are very immature cells, a Musashi or Hu or nestin or SP or something of that sort, and then as Mehandra mentioned, functionally do they behave like a stem cell should behave?

But ultimately, to reiterate John and Rusty's point, if somebody was going to come to you with a

population and then say it is Musashi positive and all of that, and I want to do it for Parkinson's disease, you would not say, fine, it's Musashi positive, go ahead. You would say, are you now from your population getting at least 300 dopaminergic neurons? And if he says, yes, you would say, okay.

If he then came back and said, now I want to use my exact, same cells to treat Krabbe's disease, look, I have 300 dopaminergic neurons, you would say, well, that's not relevant now, even if it's the same population.

DR. RAO: Can I just add one? I think it's most of the case that since there are so many cells, that somebody is going to come to you and say, I have this cell, and you have to tell him, prove it to me that this is the cell you're saying it is and this is the population that you're going to use. In that sense, we have markers and we can do it. So, the criteria that you use will depend on what the guy says he's coming with and what he wants to do with them.

DR. MULLIGAN: To get back to Jay's question, I think that the surface phenotypic characterization is definitely a reasonable thing, but for like hematopoietic stem cells, there will be maybe 8 or 10 markers, and you'd want to know that every time you use this population, you'll have the same distribution. That's a totally

separate question about whether any of those markers are important to the functional --

DR. SIEGEL: In some sense, so that's the one more critical to us in terms of product control. The science is very important and we'll be reviewing the science. Obviously, people are going to have a rationale to justify experimentation.

But one of the issues that's right on the table -- even if science isn't ready to answer it, we're going to have to answer it in the near future -- is what is your product. One of the critical things in product development is to know that if you make batch after batch and you experiment with a few people and you start modifying things, you know what you had and you know how it changed. That's kind of where at least this set of the questions is focused. How do we know what it is that's going in so we can interpret the results?

DR. KOLIATSOS: It is exactly because we don't have all these wonderful markers that we need more in vitro and in vivo descriptive validation. They used to make mandragora and say it's mandragora because they made it from this and this extract and it puts people to sleep. So, this is why you need to include your animal model together with the preparation.

So, to extend Evan's point, yes, it makes so

many dopaminergic neurons and I stuck it in the nigra of this type of rat and it worked and it functioned and it did this and this. You need more descriptive validation in the absence of specific molecular markers if you are going to start with this as a clinical potential.

DR. SALOMON: I just remind you what Jay is saying to you is part of the frustration from the regulatory side. I don't know if you're quite getting it. What you're saying is really sensible in the sense that I have a population, I want to use it in this disease, and the justification of it is this sort of a validation scheme. I think that really makes a lot of sense to all of us.

What Jay is saying, though, is when we get past the position of saying, yes, you can do your trial, then what the FDA has to do is say -- there are three centers, let's say, in your trial. Is the stuff you're going to give in centers A, B, and C the same? It's not that you didn't 6 months ago in the lead-up data to justifying your trial do all the right stuff, but now is the preparation that centers A, B, and C have the same as the preparation you made. That's what Jay is trying to get at, this idea of product testing.

DR. KOLIATSOS: But you need a model with it. That's what I'm saying. I agree. But it's not enough to

say I give the same cell origin. I also give the same cell origin that has proven to work in a certain model.

DR. SIEGEL: I would just add, by the way, to that explanation. I understand what you're saying and appreciate that. The most critical issue we face in human experimentation in phase I in terms of product consistency is not usually the multi-center issue, but the dose escalation issue. So, once you've established that 10 to the 6th cells is safe, then you're going to say, I want to give 10 to the 6th and a half cells. I'm going to want to know is that really three times as many of the same cells or might they be tenfold more potent because if they're tenfold more potent, that may be unsafe. Or is it a different type of cell, so there's really 30 times more stem cells or dedifferentiated cells there. That's where the critical issue of knowing what you have most commonly comes into play in early clinical studies of cells.

DR. SALOMON: Tom and then Rusty had his hand up.

DR. FREEMAN: Just following up on this dose escalation thing, it's even more problematic with stem cells. First of all, we can't biopsy. Secondly, they migrate. So, if you want to eliminate them, say, by making some lesion where your graft is, you can't do that as opposed to a differentiated cell that doesn't migrate. And

that brings up the need for careful autopsy studies being put into protocols because very often that's missing from a lot of clinical trials at this stage.

It also brings up the point of having ways to eliminate excess cells because with pharmaceutical dose escalation trials, if there's toxicity, you can generally stop your drug. In this case, that won't be possible particularly if cells mature with time, which raises the spectrum of not only suicide genes but also methods of inducing second-set rejection, which is a possibility if you have a purified allogeneic cell line. Can you have a parallel isogeneic cell line of fibroblasts that you bank with each cell line for inducing second-set rejection? There's evidence that an isogeneic skin graft, for example, can make a second-set rejection of a purified cell line. So, these are other considerations.

DR. SALOMON: That would get complicated.

Rusty?

DR. GAGE: I was trying to separate out the manufacturing of the individual cells and the propagation. Then we can talk about stem cells or really any cell that propagates that you can do good blood banking strategies on. Then that doesn't tell you anything about the function of the cell. What you really want is the assay. We've talked about animal models.

But I was just thinking about there is really an intermediate phase, and that is, what are the conditions that you are going to apply to your propagated, well-characterized, GMP quality cell to get them into a state that they're now ready to transplant that's going to give rise to it to assess in your functional assay? Because unlike in the blood system, we're not going to just graft the propagated cell. In many cases, I would argue, if not in most cases, we're going to do something to the cell that's going to take it out of that rapidly propagating state and put it into another state. We're going to do differentiation factors. People use retinoic acid. And we probably need to separate those things out between how you assess the manufacture or the propagation or the cell storage and that next stage where you actually do something to the cell to put into a state that it's now ready to put into the patient. And that's a point of standardization too, it strikes me.

DR. TROJANOWSKI: But I think we're there, though, Rusty. I haven't heard of anyone citing a model that they wanted to use pluripotent -- most of what we want is a specific type of neuron or glial cell or what have you. And I think we have the markers to know after we've proliferated and got a handful of neurons or astrocytes or what have you. We have everything we need, I think, to

characterize, to say those cells are 99 percent neurons or glia at what level of maturity.

DR. GAGE: I couldn't disagree with you more.

(Laughter.)

DR. TROJANOWSKI: Well, I'd like to hear the details.

DR. SALOMON: And that's a good relationship actually.

Evan, I think you were one of these people who actually, I think, would use pluripotential cells, wouldn't you? Or multipotential cells in a --

DR. SNYDER: No. I feel very comfortable using multipotent cells.

In answer to your question to go back to what you were asking, probably what you're asking for is not a stem cell marker. What you want is a surrogate marker that shows efficacy and safety in a particular use. Regardless of how you're going to define the cells that give you that, you're not going to get agreement on a stem cell marker. But that's probably not what you're asking for anyway. You want a marker that they can assay from lot to lot over time that's a surrogate marker that says lot number 1 and lot number 300 both have this marker which is associated with efficacy in this model or this disease and also safety.

DR. SAUSVILLE: But the important point, one

way that this conversation is going is that this field should evolve and the FDA should make that aspect part of what they require people to come to the fore with.

DR. SNYDER: Absolutely, and our job is to come forward with a surrogate marker.

DR. MACKLIS: Well, I think it's very unlikely. I think many of us would agree that there will be a marker. It will, rather, be a big panel, maybe in one or two or three dimensions, of presence and absence of various markers.

DR. SNYDER: Right, but it's going to be markers not defining stem cells. It will be markers defining safety and efficacy for the particular use.

DR. MACKLIS: Agreed.

DR. SNYDER: I agree, yes.

DR. MOOS: I'd like to follow up on that. I think, yes, it's a dream to find a marker or two or three markers. And especially in the interim, it could be a reasonable goal to come up with some kind of -- Dr. Koliatsos used the word "descriptive validation" -- a pattern, perhaps 5,000 randomly arrayed plasmas on a chip that is characteristic -- I'm just throwing that out as a straw man -- okay, 10,000 -- of this differentiated cell type or this type of pluripotential stem cell from this source that is associated reliably in an animal model with

the desired effect and which is not associated with adverse effects.

I would go one step past that. It may be worth considering looking carefully at lots of cells that fail in the validated preclinical model or stress lots of products which can be manipulated in such a way that they do fail and then make careful comparisons using the tools that genomics has given us. We may not quite understand in a reductionist way what that plasmid at position at XY is really doing versus something else, but at least in much the way that anatomic pathologists were doing 150 years ago, we would have some kind of a way to assess consistency of manufacture.

It's perhaps not too fanciful -- maybe a little bit fanciful -- to even envision that one could address not just identity, but purity and impurities profile to the extent that if you have a heterogeneous population, there might be some things on your microarray that are characteristic of certain cell subpopulations and some characteristic of others but could be maintained.

DR. SALOMON: I think we have to be very careful here. Again, this is just discussion. I realize we're not making any sort of regulatory advice to you.

The gene chip thing is great. My lab is working on custom DNA arrays and I love the technology, but

I'm waiting to see what it really gives. I don't think we should get carried away here with gene chip profiling at this point.

DR. MOOS: Please don't misunderstand. It's a paradigm, and I'm just throwing that out as an example that has been touched on. We do not want to foreclose any other very ingenious, multi-dimensional set of -- in fact, we changed all of our wording before because we had carelessly used the term "markers" and replaced it with characteristics.

DR. SALOMON: Right. I just think, speaking for myself -- and I'm pretty sure I would speak for the group here -- I don't think the FDA should get the idea that anyone here is suggesting to you that today there's any data that suggests that you should use gene chip profiling here. It's a lovely idea. In California, we get to use the word "cool" once a day. It's a very cool idea. That's it for the day for me.

DR. MULLIGAN: I was going to say that I would agree about the gene chip.

But the other twist to the surface phenotype is that there is clearly in hematopoiesis great precedent for a reasonably well defined surface phenotype that, depending upon how you generate the cells, will either not have the activity or have the function activity. This is Sca plus

lineage-deficient, cKit positive. That has been work people have done. If you mobilize cells in a recipient in a different fashion, you get those exact same cells which at one time were the pure cell for transplantation, and they had no long-term potential. So, it's not to say we shouldn't do this, but you really need the surrogate marker. You need to push for trying to associate even a simple marker phenotype with a consistency of function.

DR. CHAMPLIN: We actually use CD34 in that way in that the time to engraftment correlates with CD34 cell numbers, and we all have a sort of minimum CD34 number that we would feel comfortable in a blood stem cell transplant would be adequate then for an individual patient. So, we're not fooling ourselves saying these are all stem cells and we're measuring stem cells, but it's a surrogate marker that correlates with adequacy of the transplant for engraftment.

DR. SALOMON: Yes, and I would add it has been very useful in things like purification strategies and otherwise, including device development.

We need to wrap this up and go to lunch. Is there anybody who has something that just has to be said right now?

(Laughter.)

DR. KURTZBERG: One sentence. I think the FDA

has to realize a lot of this is going to evolve in these phase I clinical trials. I don't think you can demand all of these answers to start the phase I trials. I think you have to come up with some minimum set of requirements but then know that it's going to change and it will be a work in progress.

DR. CHAMPLIN: In the field of hematopoiesis again, a minimal sort of understanding in correlate markers has evolved over 20 years. In the beginning, we were just using cell numbers. We learned from experience in large numbers of cases how to refine that.

DR. SIEGEL: In regard to those last comments, I'd like to second the comment of one of our speakers -- I'm not sure who -- about, where feasible, storing specimens. I think that from the perspective of genomics but also surface antibody or whatever, we may know a lot more 3 years from now, and if we have the specimens to go back, ultimately we're looking for what are the markers, as I think Dr. Snyder pointed out, that predict safety and efficacy. But, of course, until we have clinical data, all we can look for are what are the markers that can ensure consistency and hopefully predict that. We'll measure some, but others, if we have the specimens, we may be glad, as we go back, that they're there.

DR. SALOMON: I think trying to summarize sort

of where we are, so we can go to lunch --

DR. NOGUCHI: Excuse me. I don't think we can let this close. We are very cognizant of the fact that every new technology carries its own price, but tomorrow's technology is yesterday's CD34. Ten years ago, nobody would do that. So, it's incumbent upon people to recognize that, no, we won't require these fancy techniques now, but you've already heard from industry. The claim is they already know how to do everything, which we would find to be very interesting. But it is true that there are going to be large corporate dollars going into this, and if they can characterize something in a way that gives us a better, safer product that's more efficacious, we're likely to be moving toward that in terms of requirements.

So, it's up to the people here, not only to advance the field, but to get down to brass tacks on what can you agree on right now and to evolve that on a continual basis. That way you can control the future of the standards we require rather than have someone who has more money put the standards in place for you.

DR. SALOMON: That's my summary. I like it.

(Laughter.)

DR. SALOMON: I think that's great.

See you guys at, let's say, 1:25.

(Whereupon, at 12:55 p.m., the committee was

recessed, to reconvene at 1:25 p.m., this same day.)

AFTERNOON SESSION

(1:48 p.m.)

DR. SALOMON: So, I'd like to welcome everybody back to the last session of the meeting.

I want to just make one point and that is that nothing from this morning is necessarily off the table this afternoon. It's just that we'll start with the afternoon's questions. My objective is to be done and summarized by 3:00.

Yes?

DR. AUCHINCLOSS: I didn't hear us come back to the source of the cells question from the morning discussion.

DR. SALOMON: But you guys abused the hell out of me over this source of the cells question.

(Laughter.)

DR. AUCHINCLOSS: I'm not going to let that one rest. I really think that's a fundamental question.

DR. SALOMON: Okay, that's fine. I thought it was too. I thought you guys told me it wasn't a fundamental question.

DR. AUCHINCLOSS: I didn't hear that.

Let me explain what's on my mind. This meeting of the FDA is called by the Stem Cell Working Group. It means the FDA has put stem cells in a special box. There's

something particularly important about them. Their regulation might be different. If I were working with stem cells or some variation on stem cells, that would bother me because I'd sit there and I'd say, now I'm going to get special attention. I'm going to get labeled as a stem cell. I'm going to get public headlines to say I'm a stem cell.

So, I've been listening over the course of particularly yesterday about what's different about stem cells compared to other forms of cellular transplantation. What I hear -- what I think I'm hearing -- but it's a question that I wanted to put to the rest of the committee -- is that embryonal stem cells do have a special feature attached to them, an inherent increased risk, that I would put them in a box, separate from other kinds of cellular transplants. But I haven't heard one thing about adult stem cells or variants of adult stem cells or progenitor cells that warrants their being put in a special box, and I think we ought to make clear that that distinction is very, very important.

DR. SALOMON: Actually I like that. Let's try to figure out a strategy then to go at this afternoon. That's a really well-taken point.

I know Mercedes wanted to introduce the topic.

MS. SERABIAN: Yes. I'll be as quick as

possible. Just a couple of minutes, but I just feel a little more comfortable introducing it.

DR. SALOMON: Why don't we do this? Mercedes, why don't you give us your introduction? When she's done, let's start with that point. It's kind of what I was trying to do earlier, but let's see if we can do it better.

MS. SERABIAN: Now, I wrote out some notes, so I won't ramble. I'll stick to what I have.

I just want to introduce myself again very briefly. My name is Mercedes Serabian. I am a toxicologist with the Office of Therapeutics. So, when you submit an IND, there is a large section that, as you have been referring to, is preclinical safety as well as efficacy.

Not to be outdone by Malcolm, I found this when I went home. To me, from listening to everything that went on yesterday, I feel we're not quite ready for prime time yet, getting there, but not quite.

Just in general, to give you a background, because I'm not sure from yesterday, it seemed like a lot from the research aspect. Just exactly when someone is at the point where they feel they need to submit an IND, they can submit an IND, they do, what preclinically from our aspect do we tend to look for?

Well, obviously from this, my main point is

that the preclinical study program is driven by what you want to do clinically. I think that's very important. It's a backward arrow, if you will, from clinically to preclinically.

Obviously, the clinical indication and patient population determines what you're going to do preclinically for your safety studies, and we've been discussing that for a day and a half now. That's quite obvious.

For traditional biotherapeutics, if you will, the duration of therapy has been an issue, whether it's an acute or a chronic therapy. In this case, it's obviously intended to be chronic because of the implantation of cells. So, that brings with it a certain set of issues and concerns.

And then dosing procedures I put down, which is important for such things as route of administration, how many times you're going to be giving an agent in terms of number of injections, et cetera. That may determine at times the species that you use also.

This is kind of a standard slide that I've put up in other talks. Again, it's for traditional therapeutics, just to set the scene. You notice I highlighted the word "safe" because I think that's very important. We've been talking about animal models, proof of concept, but the terminology "safe, safety, toxicology"

we haven't heard too much of.

So, I just made a general list of what we tend to look at in an IND review in the toxicology aspect to get into phase I/phase II trials. Obviously, you want the animal studies to be able to support the recommendation of an initial safe dose and dose escalation scheme in humans, to be able to attempt to determine an acceptable risk/benefit ratio in humans, to identify any potential target organs or tissues of toxicity or activity, as well as to identify parameters to monitor clinically. We've been talking about that as various endpoints, surrogate or otherwise. Identification of inclusion/exclusion "criteria" that should have read. I left a word out, which again is important as to who you're going to enter on the trial. Last but not least, which we've been talking about also, is to be able to discern the mechanism of action. You may have obviously desired pharmacological effects that you want to see, but you may also have some undesired toxicities that you either haven't anticipated, obviously, or crop up either in the clinical trial or earlier.

One last slide. The bottom line then. Toxicology data derived from preclinical models can provide information for the clinical management of potential toxicities. To be able to identify preclinically certain toxicities will, hopefully, be helpful and a requirement

for clinical monitoring as to what kind of a parameters are going to be monitoring and what you're going to be looking for in the clinic.

The predictiveness -- we've been talking about this -- of the toxicology data now, as well as the preclinical efficacy data of the human response from the animal studies to the human response, as well as the potential impact on clinical development. If you see some type of toxicity in these models, what effect will it have on preclinical development? Will it stop it altogether? Will it simply slow it down? Will it affect cohort dosing between patients, patient cohorts, it's a dose escalation, this type of thing?

So, again, the point of preclinical toxicology studies is to be able to address all these issues.

There are three main areas of questions that we have in the package. One is with respect to animal models. This is not just animal models for efficacy but safety. Number two, tumorigenicity, which appears to be cropping up quite a bit. That issue, as well as third which I think in our group we consider probably the most important is the fate of the cells post-implantation as to toxicology studies, what to do, how to do it, how to interpret the data. So, those are the three main.

I guess, depending on how the conversation

goes, it's up to Dr. Salomon in what order he wants to discuss them, but it would be very helpful to get through them all in the hour.

So, I'm just going to leave the first slide, which has some of the bullet points, and then I think Don will help me. We'll go through it as we advance.

Thank you.

DR. SALOMON: What I'd like to do now is go back to what you asked at the beginning and perhaps accomplish what I didn't do a good job of at the beginning. So, let me try and articulate a question out of this, and then you guys help me make sure I've got the right question.

My thinking, from what I've heard in the last two days and what I've read in this area, is that under the rubric of the word "stem cell" for this field, there seem to be several different things. The first kind is either what's been called an embryonic stem cell, which is coming from the inner cell mass of the trophoblast, and a similar, albeit it not identical, cell coming from the gonadal ridge, which they called a gonadal stem cell -- germ line cell. So, there are those, I think for the points of discussion, I personally sort of put together, albeit I accept as a scientist that they're not identical.

Then there are what I would call stem cells

such as those that Catherine Verfaillie explained to us but others not here at the meeting have, for example, found them in muscle cells. So, these sort of noncompartmental stem cells that may have multipotentiality and that could be harvested from adults as well as fetal and your point was even different gestation fetus. So, that would be another one to the mesenchymal stem cell or mature stem cells.

Then there seem to be compartment stem cells. I didn't use the word compartment-specific stem cells because just because they're found in a specific compartment doesn't mean that they didn't derive from bone marrow or from a circulating source in the right arm, but they're in the brain. Those could be induced, as we've heard some people talk about, or they could be harvested, developed in vitro, and put back in.

Then the last group would be products that started with any of these stem cells and then, through in vitro purposeful manipulations, could be driven down a lineage commitment pathway that would be useful for a specific disease.

Does that sort of cover the spectrum now?

DR. GAGE: The latter case is a subcase to all the three prior ones. It's not a fourth category.

DR. SALOMON: Okay, it's not a fourth category.

I agree. It could be thought of as a fourth category in that a clinical trial using that kind of a strategy would be intrinsically different than a clinical trial that would use the other three. So, yes, I think that's fine.

DR. AUCHINCLOSS: That's not what we heard yesterday. What we heard was that even the in vitro derivatives of embryonal stem cells had tumorigenicity. I think it's important to keep the in vitro derivatives of subcategories of each of the other.

DR. SALOMON: That's fine. I wasn't trying to imply anything now. I was just trying to make sure that we all agreed that I wasn't leaving some major group of cells out.

A really interesting question Hugh has asked -- and I agree with it. I was trying to get at it too -- is if clinical trials are going to go forward in this area, is it -- well, maybe back up. That's the way I went at it and it didn't work.

The way you put it was? Hugh, can you help me formulate this question so I do a better job than I did this morning?

DR. AUCHINCLOSS: Well, I would ask the committee two things. Can they imagine circumstances in which it would be okay to proceed with a clinical trial of embryonal stem cell therapy for any disease given what we

currently know about the risks associated with tumorigenicity?

On the other side, I would ask them the question, is there any evidence that an adult stem cell therapy, including in vitro derivatives, is any more risky than any other form of cellular therapy?

DR. SALOMON: Comment on it, either way. More risky, less risky.

DR. SAUSVILLE: I have a question pertaining to that actually to members of the panel. Although there is the -- I'll use the word -- theoretical risk from the embryonal stem cells, is it within anybody's experience who has used these cells in any animal system in which it would be either likely or even possible that such a tumor has manifest itself?

DR. SALOMON: Well, they gave us data yesterday that embryonal stem cells produce teratocarcinomas.

DR. SAUSVILLE: But the point is you have to select for that. My question is putting it in the milieu of the animals that you're going to use them in.

DR. SNYDER: John was talking about cells that he had not pre-differentiated, that he had taken as ES cells and put in there.

DR. SALOMON: Let's start with embryonic stem cells.

DR. AUCHINCLOSS: John showed us an experiment in which he induced the differentiation, selected on the basis of markers --

DR. SNYDER: Well, he selected for markers.

DR. AUCHINCLOSS: Selected on markers of differentiation, put those cells in, and said, oh, my God, I've still got three tumors.

DR. SNYDER: But most people working with the ES cells will predifferentiate or precommit them, and the risk of putting in undifferentiated ES cells is not just teratocarcinomas, but for instance, Oliver Brustle has taken ES cells, put them into the brain, and has not gotten a tumor, but has gotten an autonomous neural tube growing within the brain. That doesn't seem to happen if they do these precommitment steps ex vivo.

DR. SALOMON: What I want then is first embryonal stem cells, not embryonal stem cells and this and this and this. Let's start with embryonal stem cells and try and work through these different groups to identify the risk. Like I said, I was trying to do that this morning.

Before Mehandra, Evan, can you just address the embryonic stem cell? If I want to do a clinical trial with an embryonal stem cell, yes or no? I'm not holding you --

DR. SIEGEL: Dan, could I interrupt to broaden out the question you and Hugh ask? We really need a

different answer from that question. The question is framed as do we know enough now to go there. But what will happen to us is that three months from now someone will present a proposal and in that proposal may well be a lot of data that are not before this committee. So, really the type of questions that we're getting into here are not yes or no, do we know enough now, but what do we need to know. Is tumorigenicity an issue that we can define? What tests need to be done to rule it out? So, that's the advice that we could use here.

DR. AUCHINCLOSS: I think that's an excellent way of putting it because I can't imagine that one would ever want to say we'd never want to use ES cells. Presumably we do. But there needs to be more information than I heard today, presumably, before you would go ahead with a trial. I don't know what that information is, but somebody would need to address head on the tumorigenicity problems.

DR. SALOMON: My point only is that there is some information here.

All right. Answer the question the way Jay asked it. What would you want to know about a cell population of any of these stem cell populations that would say no way I'm going to do it?

DR. SNYDER: It's no different than what we

were talking about this morning. You still need to prove that the cell population you're using is efficacious in your model and safe by whatever marker. So, ES cells, yes, can be safe but you need to prepare them ex vivo, probably commit them or predifferentiate them and put them in under the right circumstances. And there are ways of doing that.

Conversely, when you don't do that, for instance, Jonas Frisen has recently demonstrated that you can take adult neural stem cells -- now, we can debate whether we believe the data or not, but what he has published is adult cells, neural cells supposedly committed to the neural lineage can be processed in such a way that he can put them into the embryo where they behave just like ES cells. So, there's at least there the theoretical risk that if they behave like ES cells, can they also give rise to teratocarcinomas and give rise to autonomous organ systems?

DR. SALOMON: So, let's stop there for a second. What everybody is saying is that if they had a cancer-producing potential, it would be bad, but we didn't need to come to Washington to tell the FDA that. So, what we need to tell the FDA is what is evidence, what is a way of determining the cancer-producing potential of any given cell for these kind of transplants.

Mehandra and then Jeff and then John.

DR. RAO: So, I think to answer your question specifically, I think we should make a distinction between ES cells themselves in a naive undifferentiated state and derivatives of ES cells because derivatives of ES cells have the same risk in some ways of getting tumors as any dividing cell population which is maintained in culture for a certain time period. You get spontaneous immortalization, all of those. That risk is probably about the same, at least until there's clear-cut data to the contrary.

In terms of undifferentiated ES cells which have been maintained, I think the data is quite strong because that's a definition that every time you put them in any part of the brain, you're going to get tumors. So, in that sense, if you had to have an absolute answer, I'd say that unless there's data to the contrary, you would not put in ES cells in an undifferentiated way into the brain because there's data clearly saying it.

In terms of differentiated cells which are derived from ES cells or from any other cell type, you use standard criteria to say are you going to get a tumor. Since we're growing them in culture, we have standard sort of ways of looking at things like p53, looking at the levels of telomerase, looking at cell division rates, and alteration of cell division rates, which would be criteria

you would use for looking at frequency, soft agar assays, those sort of things.

DR. MACKLIS: It seems like we already heard part of the answer to your question yesterday. John Gearhart told us that he worked hard, predifferentiated the ES cells for many weeks to make them pure -- they were supposed to be pure oligos? I can't remember, but to purity. Then he did a great experiment. He transplanted them into 150 animals and 2 of them got tumors. It would seem like that's exactly what one would want to insist upon.

DR. SNYDER: He was selecting for hematopoietic lineage.

DR. MACKLIS: That's correct.

This might be the only time that I'll disagree with something Mehandra said over these two days, but it's not clear that we have the data to say that ES cell-derived proliferating cells have more or less or the same risk as other proliferating cells unless somebody does a head-to-head experiment.

DR. SALOMON: Well, again, I'm following sort of the idea of it's not really important to get hung up on whether we think embryonic stem cells, this or that. But I think we need to still give specific things. Now, Mehandra has responded to say, okay, do karyotyping, do p53, do gel

assays. Fine, that's what I want to hear.

DR. MACKLIS: How about transplantation into animals in large numbers.

DR. SALOMON: That was good.

John, Michael, and Vassilis.

DR. TROJANOWSKI: Virginia Lee and I in Philadelphia have eight years' experience with the N-Tera-2 cells in animal models on normal nude mice. These, as I mentioned, have an abnormal karyotype. They are differentiated. We have never seen a tumor with the postmitotic neurons beyond 1 year.

In a parallel life, I've worked for 15 years on medulloblastomas. These are stem cell-like tumors, and I've tried very diligently to create animal models of tumors. I've looked side by side at the N-Tera-2 cells. I know of no precedent for a cell sitting in the brain for over a year transplanted and suddenly becoming a tumor cell. It has not happened. It may be formally possible.

DR. SALOMON: John, you're making a key point, that in my listing of stem cells, I apologize I left those out. That would be some sort of a transformed or accidentally naturally immortalized cell line. That should be on the list.

DR. TROJANOWSKI: Right. I think it applies to stem cells.

So, you do 100 animals, you leave them for a year, if they don't make tumors -- and in fact, I would say we've taken the retinoic acid-naive N-Tera-2 cells, which are considered to be neuronal progenitor cells, not stem cells, and depending upon where you inject them in the brain, they will or will not form tumors. If you confine them to the caudate, we have shown they will differentiate. There's some retinoic acid-like factor there. And those animals too survive for over a year and a half without erupting into malignant tumors.

So, I think we have very good screens for tumorigenicity, and it ain't mysterious and it ain't problematic. I'm just telling you that.

DR. SALOMON: What you're talking about are rodent studies.

DR. TROJANOWSKI: These are human cells -- human cells I would emphasize -- into mouse brains. I would challenge you to show me a model in monkeys, squirrels, zebras, whatever. Everything is formally possible. We have to talk about what is realistically possible, and there just is no precedent in brain. Maybe in marrow, maybe somewhere else, but not in brain.

DR. SALOMON: We're going to keep going. I just want to point out already there's one inconsistency here. We like the idea that we would take cells and look

at karyotyping and p53 expression, and the implication there, Mehandra, was that if we found these changes, those would be arguments against going forward with that as a trial. Rusty yesterday showed us data in which 1 of 2 that had karyotypic changes, 1 of 7 total that he characterized did form tumors. However, what John is saying is that here's a cell line that has a karyotypic change, and he's saying that it's safe. So simply finding a karyotypic change is not necessarily saying that it's not safe.

DR. TROJANOWSKI: The data is king, and I would listen to empirical evidence for or against some of these things. And be mindful of formal possibilities and really seriously consider practical problems. But if there's data to the contrary, I think you're going to pass.

DR. SNYDER: There are functional assays that you guys probably even know better than we do for transformation, which is growth in soft agar and lack of contact inhibition, producing a tumor in the brain or in the muscle of a nude mouse. So, we would subject these cells to exactly what you would do to define transformation.

DR. SALOMON: Michael and then Vassilis.

DR. WALKER: We're talking about what, in essence, is a very low incidence phenomenon. Therefore, we're always going to be approaching it from the null point

of view, namely that we haven't found anything but we might find something in the future. So, from that point of view, any cell that to me has the potential for unlimited and uncontrolled replication is of concern. That doesn't mean we don't do it. It just is of concern.

Second of all, we're used to considering the brain as fully and irreversibly differentiated, and now we're finding out that it clearly is not and that there are cells that you can get out of there that have all sorts of wonderful potentials. If they were pushed back to acting like ES cells, I wonder what might happen.

That being the case, I think we have to start thinking about simply the kinds of things that John was talking about, that when a model is set up, for example, that's going to be showing whether this particular treatment has in that particular model efficacy, I'd like to have an aliquot of those models totally set aside for long-term follow-up simply to find out if there is an incidence that we could find or not. It doesn't prove there isn't any, but I think we need to set some of those kinds of things up. I think to try and break it up into each individual cell, each individual cell type, each individual cell source, and sort of say for each one of those, we're going to have a different parameter, that's too difficult.

DR. SALOMON: Vassilis and then Rusty.

DR. KOLIATSOS: Actually I feel obliged at this point to release some published information from my lab. I really want to make a plea that John Gearhart's deposition is not misinterpreted. These things were not tried in the nervous system.

We tried in my laboratory some of these ES-derived cells, not human but mouse, and the person who did it was trained with Ron McKay and did exactly what Ron McKay does to get neural precursors from ES cells. We had a 90 percent UN positive population before we put the juice in the brain. In some parts of the cortex, we saw some nice integration, and in some other parts of the cortex, we saw something that I don't call it a cancer, but I will send to John the slide, if you want, John. It's clearly unintegrated. It's round. It pushes. And it doesn't look like nervous tissue to me, so clearly not cortex.

This is not to say that this is going to happen all the time. It is to say that even when, Mehandra, we do all these steps to differentiate, even one or two cells can remain that can do a job not necessarily to form tumors, but unintegrated globs of tissue, which are not going to be of any functional relevance and may be deleterious. The potential will be there and the picture is very complicated.

DR. GAGE: In our case, they were cells that had been passaged for long periods of time and karyotyped. In one of the two aneuploidy cells showed tumors sometimes. I should point out that they didn't tumor all the time. Probably 1 out of 30 animals had a tumor, but that was marked in the protocol saying that there was a tumor found in that animal that received that cell type.

The other point about using rats or small animals as an index for safety of tumorigenicity concerns me, that generally our graft sizes are about 200,000 cells per microliter. You might be putting in 3 or 4 microliters, and that's not comparable to the number of cells that you'd actually be putting into the human.

So, if you come to a point where you have a question of aneuploidy, p53, you've done your first-pass screen and there's something that is highlighted and you begin to do your tumor assay to see in 100 animals what percentage of them give tumors where, I think the number of cells that you put in should at least be taken into consideration. It may not be that a mouse or a rat with 100,000 of those cells is adequate to determine whether or not that cell has tumor cells in it. Because it's not that every cell is going to be tumorigenic, but some small fraction.

Certainly in cancer studies, even with gliomas,

if you take a mouse glioma or a rat glioma and put it into a rat at 200,000 cells, you're not going to get a tumor. You need to get up to a certain number of cells before you get that kind of growth.

So, at least in the consideration of things and then the assays are being developed to determine whether or not the cells that reach a certain stage that they need to be evaluated -- we've got to make sure we're looking at enough cells and enough animals to feel comfortable that it has passed some sort of criteria.

DR. TROJANOWSKI: So, I think we have to distinguish between tumors and whatever is going on in Curt Freed's poor patient. I don't think that's a tumor. That may be exuberant growth or overproduction or what have you. I'm talking about tumors that kill people and not hamartomas, not benign growths, not aberrant differentiation. You can do bromodeoxyuridine labeling and if you do that, as we have done, it's negative in animals that survive beyond a year, the postmitotic neurons, when they put them in there. I would again challenge anyone in this room to show me a tumor that you can get after you put a million cells of whatever your favorite benign -- and let them survive a year without a death. That I have not heard of.

DR. KOLIATSOS: John, this is a very important

point. I'm afraid that the less BrdU I see, the more I get concerned because it tells me that the cell has gone through several generations in the brain. In fact, I see much better BrdU in well-integrated, graft-derived cells than in the ones that I'm afraid of.

DR. TROJANOWSKI: I'm not saying one or the other. I'm saying do both. Bromodeoxyuridine, pulse them several times.

DR. SALOMON: Phil?

DR. NOGUCHI: In this conversation, I think we must be very careful to distinguish between rodent cell lines and human cell lines and embryonal human cell lines. These are all new things.

But in terms of classical cell biology, there is, as far as I know, no documented case of a human cell line, normal cell line in culture being transformed by any means, shape, or form into a malignant line. Now, you have malignant lines who start that way and you have normal lines who start that way. But people have been trying by many means. Human cell lines are clearly different than mouse cell lines. We lost about 20 years in using any kind of continuous cell lines because of the concern that we had for mouse.

The same goes I think for testing for tumorigenicity. What we can say is if it forms a tumor in

nude mice, you probably don't want to use that as it is, putting it into the brain, but it could be differentiated like the N-Tera-2.

So, there is a fundamental difference of human cell lines, and I think what we really don't know at this time is for an embryonal stem cell or any other kind of human stem cell, other than hematopoietic, does that kind of transformation ever really occur in vitro or not. I would posit that so far we haven't seen it, even when people tried desperately to do that.

DR. SALOMON: So, Phil, just as a point of clarification, because this came up in a conversation, is it then what you just said, that mouse cell lines may have a much higher potential of malignant transformation in culture than human cell lines?

DR. NOGUCHI: I would say that the common factor is a mouse cell line carried long enough will become tumorigenic. That's how all the original lines were derived. It's not the same for humans. You cannot really transform them in vitro.

DR. SALOMON: Darwin?

DR. PROCKOP: I'd just like to make a couple comments.

I think a lot of interesting points of view have been raised here, but I keep coming back to Dr.

Auchincloss' point. How is this different than what we've been doing for 50 or 100 years in developing therapy for patients? There are some differences, but I don't see very many.

So, yes, in the beginning, we'd like to thoroughly characterize what we start with. It would be great to have a single crystal organic compound whose structure we know. We're not going to get that out of these cells. I think it's more like Premarin, pregnant mare's urine, which is still used in place of estrogen. It has compounds in it; we don't know what they are. So, we're going to get an approximation of what the cells are, but it's only going to be an approximation.

Our experience fits with the hematopoietic stem cell line. We can see two cells divide. They look the same but they're already committed down different routes. Our data fit with that kind of paradigm. We're going to wind up with mixtures of cells no matter what we do, so we can give plus or minus what the percentages are, define them. We have to do that for dosage and all the rest. But beyond that, we're going to do standard pharmacology in effect. We're going to do as much as we can in isolated cells, look for karyotypic changes. And, oh, yes, that all helps, but you've got to go from there to the animal and you've got to give doses to the animal. You've got to look

for toxicity. Yes, if you've seen changes of changes in the karyotype ahead of time, you've got to do much more studies for tumors. But it's all the same thing. As John was saying, it's an empirical game which is not so different than we've been doing for nearly 100 years. I really don't see a difference.

DR. SALOMON: The only point, Darwin, is that if this field came forth to successful clinical trials, having done all of that, that would actually not have been done for any of the things that you're talking about. It hasn't been for islet transplantation. It hasn't been done with hematopoietic stem cell transplantation. So, the fact is if this is done, it certainly builds on the shoulders of all the hard work that's been done in those other areas. But it's not been done before, so it is unique.

DR. PROCKOP: The other step is this. The lung transplant field is one lesson. You can do as much as you can in animals. You go to the patient, you see something different. I think you have to go to the patient who's extremely ill, be very attentive to what's happening, and get an idea how you might go back again and improve things.

It's part of the story I was telling about osteogenesis imperfecta. We got ahead in the clinical trials of what the animal trials are about. It's not the way I would have liked to have done it. That's the way it

happened.

Bone marrow transplants went the same way. As I understand the field, before you answered basic fundamental questions in mice, you went on to humans. And you still can't make that transition very cleanly.

So, it's going to be a whole game that way, but I don't think it's that different than we and scientists in the past have faced before developing therapies. I don't see the big differences.

DR. SALOMON: I understand the point. As a clinical investigator myself, I'm always concerned that we regulate things out of business. If that's Darwin's point, I agree with that.

Ed?

DR. SAUSVILLE: I just had one follow-up to the point may be Phil. Recent work by Bob Weinberg has actually shown that humans differ from mouse cells in the telomerase regulation, and if you control for that, you can actually transform human cells. So, I just would point that out.

I guess I'd turn it back to Hugh. You asked two questions. Do you feel like this conversation has clarified things?

DR. AUCHINCLOSS: For question number 1, I think I got an answer. The group thinks that an

undifferentiated ES cell has an intrinsic capacity to form tumors and would be an unlikely candidate for a source of tissue at this point. Maybe somebody can put in a suicide gene or a regulatory gene, et cetera. I can imagine lots of things that could change that, but right now an undifferentiated ES cell has an intrinsic difference.

But what I think I heard is a differentiated ES cell puts you into the world of empiricism where you do lots of tests that you people have talked about, both in vitro and in vivo, and it may well be that you can come up with sufficient numbers of indicators to say I think this is safe based on what we've seen so far. You may turn out to be wrong, but there are assays that you can do.

Then I thought that you made the correct point that the real intrinsic property that we're looking for is the capacity for uncontrolled proliferation and that you might get there in vitro with any cell line, an islet cell or hepatocyte, that is in culture long enough to transform, if in fact that were to happen in humans, and that would put you into the special box category.

So, now back to the second question. So, that's what I had gotten on question number, but now there's question number 2. What I thought I heard yesterday or what I didn't hear yesterday was any evidence that an adult stem cell is any more risky than any other

form of cell transplantation.

DR. SALOMON: Mercedes?

MS. SERABIAN: I just want to make a comment that if you have a tumor in the brain, you can't remove the brain. There is a difference that and the other organs, other tissues.

The gold standard is in vivo testing. We can talk back and forth here about what cell type, what would be required, what not. But if an IND gets plopped on my desk and it's a certain cell type and someone has or has not done tumorigenicity, then we have to make a call as to whether it's appropriate or not, what animal species, what's the study duration? Is it an immunosuppressed animal and we watch it for a year? I've seen some they claim after 6 weeks no tumorigenicity. You think that convinces me?

DR. SIEGEL: Let me follow up actually on that question and on your remark, Hugh, on this area of adult-derived cells. Somebody commented that at least if you put adult-derived cells in a certain environment -- but we've talked a lot about environment, so I'm not sure where in the brain you want to inject these cells for tumorigenicity, but that's another issue. Or do you want to inject them in the brain? But probably you'll get different results based on all we've heard about

microenvironments.

But somebody pointed out you could put them in the embryo and unlike, I would guess, a pancreas cell, you can get dedifferentiation of these adult stem cells to totipotential stem cells, which presumably at least we're concerned do have some risk of tumorigenicity. So, that raises the question is that risk or concern high enough that there ought to be similar types of -- your question. Should the similar types of safety testing be done regarding tumorigenicity and dedifferentiation if the cells are from an adult neurological source?

DR. AUCHINCLOSS: I think your question is just right. That's the first I know of that data and it seemed to me it began to move the adult stem cell back into a more risky category again. How do you interpret that data?

DR. SNYDER: Even the cells that Rusty was talking about were adult neural progenitors passaged many, many times. So, simply being adult or not adult is not the decision.

DR. AUCHINCLOSS: Passaged many, many times puts me back into the category of in vitro potential for transformation. It's taking the adult stem cell out and putting it into the patient that I so far haven't heard any indication that was a risky procedure.

DR. SNYDER: I think the distinction maybe is

not so much whether it's adult or not. There are many steps in between. Perhaps organ-committed, organ-specific, or lineage-committed. And the ES cell clearly has the intrinsic potential to give rise to many lineages by definition. Shortly after that, under normal circumstances, there may be stem cells that are at least organ-committed or organ-specific that under normal circumstances do not seem to give rise to inappropriate cells in the respective organ. Those can be brought out. They need to be expanded, and you would subject them to same kind of safety and efficacy studies.

Now, we're not certain, however -- and it's still an open question -- whether a neural stem cell from a fetus, let's say, which is beyond the embryo but certainly not an adult, is better or worse than an adult stem cell. There's a division now and it's going to be an empiric decision. There's some evidence that maybe an adult stem cell doesn't have quite all of the potential that a fetal neural stem cell does. Others believe that they're completely synonymous and that a stem cell is a stem cell.

DR. SALOMON: We're going to need to move on in a minute because there's too much to do and it's almost 2:30. So, if there are a couple really pithy comments on this, I'd like to close this and move on.

DR. CHAMPLIN: I have a pithy comment.

DR. TROJANOWSKI: I would just like to say I think we have the data that Dr. Auchincloss is looking at. We've had 40 mesencephalic transplants. I was just looking at a fetal mesencephalon last week, a section thereof, in my laboratory where I'm study alpha-synuclein expression in the fetal mesencephalon. And I saw dopaminergic TH positive cells in the fetal nigra, but I also saw them moving -- or at least what could be interpreted -- away from the central canal. I don't know if you guys, Jeff, have done marker studies, but I can't imagine that there aren't fetal neural progenitor cells in those mesencephalons that we've already done the experiment to show that they don't form tumors by virtue of doing fetal mesencephalic transplants in 40, 50 patients, however many.

DR. SALOMON: Well, just maybe to jump forward here, I think that what I'm hearing -- I just can't imagine you guys would disagree with it -- is that if you have a stem cell, wherever you get it, as defined by its ability to differentiate in a very plastic way in many different directions, i.e., multipotent, whether it's for adults or whatever, even if you manage to keep it in that primitive state in culture rather than differentiating down a specific lineage, that cell will have to be tested by the same standards of tumorigenicity as any other cell you want to use that's a stem cell. Right? I think everyone agrees

with that.

DR. RAO: Yes, but it shouldn't be held to the same standard that you're trying to hold the embryonal stem cell to. That's all. Despite the data around the --

DR. SALOMON: I think you're going to find it very difficult to get the FDA to set too many different standards. I think that Mercedes is going to have a standard for tumorigenicity. Jay Siegel and Phil Noguchi are going to have a standard for tumorigenicity. Meet that standard, you can use the cells. Don't meet that standard, get back to work. I think that's how it's going to be. You can't say, well, let's see this, and then you'll argue, but it really fits into this standard. I don't think that kind of thing works in a regulatory environment.

DR. RAO: I thought in the big thing of this conversation we agreed that embryonal stem cells formed a special class. Right? I just don't want every cell to be held to that standard. It's okay to hold embryonal stem cells to whatever standard you're holding all the other cells to, but --

DR. SALOMON: I think this is something that the FDA should respond to. My comment is that they're going to hold you to the same standard. But, Mercedes, tell me I'm wrong.

MS. SERABIAN: I don't know what the standard