FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

TWENTY-SEVENTH MEETING OF THE

BIOLOGICAL RESPONSE MODIFIERS ADVISORY COMMITTEE

8:40 a.m.

Thursday, July 13, 2000

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Gaithersburg, Maryland 20877

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C O N T E N T S

PROCEEDINGS

(8:40 a.m.)

DR. SALOMON: Good morning, everybody. I'd like to welcome you to the latest meeting of the FDA's Biological Response Modifiers Advisory Committee. My name is Dan Salomon. I've got the pleasure of chairing this session today and tomorrow. Again, I'd like to welcome all of you to the Hilton Hotel.

What I always try and do at the beginning of these sessions just so that we get to know each other because a lot of people here don't know each other -- I certainly don't know everybody on the committee. There are some experts here from the field of neural stem cell transplantation and neural science that I'd like to have introduce themselves. So, what we've usually done is just gone around the table so everybody gets a quick idea of who's sitting here. So, I'd like to do that. It's a little more difficult in this setting because of the way we've sort of staggered these chairs. It's usually easier because it's a circle, but if we could start maybe at the last table in the back right and sort of go through there. Can you just tell us briefly who you are and your area of interest?

DR. CHIU: Arlene Chiu and I'm from NINDS, the Neurology Institute.

DR. MULLIGAN: I'm Rich Mulligan from Harvard Medical School and Children's Hospital and involved in stem cell research and gene transfer research.

DR. NOBLE: Mark Noble, University of Rochester. I'm a precursor cell biologist working in both general principles in precursor cell biology and in studying oligodendrocyte biology and repair of demyelinating damage.

DR. GEARHART: John Gearhart, Johns Hopkins Medicine, interested in human embryonic stem cells.

DR. GAGE: Fred Gage from the Salk Institute. I'm a neurobiologist.

DR. GOLDMAN: (Inaudible.)

DR. SALOMON: I should point out to everyone that you press the button. This little red light turns on for all us who forget to turn it back off again after you've spoken.

DR. RAO: Mehandra Rao from the University of Utah. I'm a neurobiologist interested in stem cells in the nervous system.

DR. SNYDER: Evan Snyder, Children's Hospital, Boston. I'm a neurobiologist and also a pediatrician, and I study stem cell biology.

DR. VERFAILLIE: Catherine Verfaillie at the University of Minnesota. I am a hematologist. I'm

interested in stem cells from bone marrow.

DR. O'FALLON: Michael O'Fallon, Mayo Clinic, a member of the committee. I'm a biostatistician. I'm not quite sure what I'm going to make of all this basic science here in the next two days, but I'll be very interested.

(Laughter.)

MS. WOLFSON: Alice Wolfson. I'm an attorney from San Francisco and I'm the consumer representative.

MS. DAPOLITO: Gail Dapolito, Executive Secretary for the committee.

DR. SALOMON: Dan Salomon. I'm from the Scripps Research Institute in La Jolla, California. My interests are in gene therapy, hematopoietic stem cell transplantation, and islet cell transplantation and organ transplantation.

DR. SAUSVILLE: My name is Ed Sausville. I'm a medical oncologist from the National Cancer Institute in the Developmental Therapeutics Program, and my interest is in the development of novel small molecules and biologicals for the treatment of cancer.

DR. AUCHINCLOSS: My name is Hugh Auchincloss, and I'm a transplant surgeon at Harvard Medical School and a very recent member of the Biological Response Modifiers Committee.

DR. CHAMPLIN: Richard Champlin. I'm with the

blood and marrow transplant program at the M.D. Anderson Cancer Center. I'm a hematologist.

DR. DRACHMAN: David Drachman, U Mass Medical Center. I'm a neurologist with an interest in Alzheimer's and other degenerative disorders.

DR. KOLIATSOS: Vassilis Koliatsos from Johns Hopkins. I'm a neurobiologist and clinician interested in neuroplasticity and the mechanisms of regeneration in CNS in the context of neurodegenerative disorders.

DR. KORDOWER: Jeff Kordower from Rush Presbyterian Medical Center in Chicago. I'm interested in gene therapy and cell transplantation.

DR. MACKLIS: Jeffrey Macklis from Children's Hospital and Harvard Medical School. My lab studies the repair of circuitry in the cerebral cortex by neural transplantation or activation of endogenous precursors.

DR. WALKER: Michael Walker, neurosurgeon, Neurology Institute, NIH.

DR. WILCOX: I'm Barbara Wilcox. I'm a neurobiologist with CBER.

MS. SERABIAN: I'm Mercedes Serabian with the Office of Therapeutics Research and Review of the Division of Clinical Trials. I'm a toxicology reviewer.

DR. FINK: I'm Donald Fink with the Division of Cell and Gene Therapy. I'm interested in neurotrophic

factors.

DR. MOOS: Malcolm Moos, also from Cellular and Gene Therapy. My research interests are in pattern formation and cell and tissue fate specification.

DR. NOGUCHI: I'm Phil Noguchi, Director of Cell and Gene Therapy in the Office of Therapeutics.

DR. SIEGEL: Jay Siegel, Director of the Office of Therapeutics.

DR. SALOMON: Thank you all very much. Again, welcome.

I have some administrative things to quickly go over.

First, I'd like to welcome two new members of the Biological Response Modifiers Advisory Committee: Dr. Joanne Kurtzberg and Ms. Alice Wolfson, our new consumer representative.

There are also five panelists today who have been introduced but who are participating as guests, and I'd like to read their names just into the record. It's Dr. Fred Gage, Dr. John Gearhart, Dr. Richard Mulligan, Dr. Mark Noble, Dr. John Trojanowski, and Dr. Arlene Chiu.

I've also been asked to inform you that there are some revised questions in the blue folders. This continues to be an iterative process. Who knows. There may be revised questions tomorrow too.

What we're going to do is have presentations today with some discussion certainly. However, when discussions begin to veer toward things that are very specific questions for tomorrow, we'll kind of get into them a little bit and then decide to put them off or amplify them or return to them tomorrow. We'll just have to see what the chemistry is for that.

Finally, as Chair, I feel like the most important thing that happens in the next two days is that everybody sitting at the table today, as well as those of you in the public, feel like you have had access to make your points clear and add to the conversation. I think that's what we're here to do. Certainly on my part, there's no specific agenda except to address the questions, as best we can, that the FDA has put to us. So, if at any time, as things progress two days, somebody feels like I didn't get my point across or something, I really would rather have you come to me at that point and I will do everything possible to bring the point back around to discussion and have everything included. I say that also for the people in the audience.

So, with that, Gail Dapolito will read us the conflict of interest statement.

MS. DAPOLITO: Yes. Good morning, Dr. Salomon.

I'd like to also take this opportunity to

introduce Ms. Rosanna Harvey, the committee management specialist. She and Ms. Denise Royster will be at the registration table to help out with any questions or assistance you might need for the committee.

I'd like to read the conflict of interest statement. This announcement is made part of the public record at this meeting of the Biological Response Modifiers Advisory Committee on July 13 and 14, 2000.

Pursuant to the authority granted under the committee charter, the Director of FDA's Center for Biologics Evaluation and Research has appointed Dr. Hugh Auchincloss as a temporary voting member.

Based on the agenda made available, it has been determined that the agenda addresses general matters only. For this meeting, general matters waivers have been approved by the agency for all special government employees who are participants. The general nature of the matters to be discussed by the committee will not have a unique and distinct effect on any of the participants' personal or imputed financial interests.

In regards to FDA's invited guests, the agency has determined that the services of these guests are essential. The following interests are being made public to allow meeting participants to objectively evaluate any presentation and/or comments made by the guests.

Dr. Arlene Chiu is employed by the National Institute of Neurological Disorders and Strokes, NIH. Dr. Fred Gage is employed by the Salk Institute. He is a scientific advisor for Cell Genysis, Signal Therapeutics, and Stem Cell, Inc. and has financial interests in several firms that could be affected by the committee discussions.

Dr. John Gearhart is employed by Johns Hopkins University. He receives financial support for his laboratory from Geron.

Dr. Richard Mulligan is employed by Harvard University.

Dr. Mark Noble is employed by the University of Utah. He's also a founding scientist of Acorda Therapeutics. He consults with Acorda and has a financial interest in a firm that could be affected by the committee discussions.

Dr. John Trojanowski is employed by the University of Pennsylvania School of Medicine. He's the co-founder of Layton BioScience, serves as the principal investigator on several federally supported grants, and has a financial interest in a firm that could be affected by the committee discussions.

In the event that the discussions involve other products or firms not already on the agenda for which FDA's participants have a financial interest, the participants

are aware of the need to exclude themselves from such involvement and their exclusion will be noted for the public record.

With respect to all other meeting participants, we ask in the interest of fairness that you state your name, affiliation, and address any current or previous financial involvement with any firm whose product you wish to comment upon.

Copies of the waivers addressed in this announcement are available by written request under the Freedom of Information Act.

At this time we would also like to request that as a courtesy to the participants and to your neighbors in the audience that cellular phones be turned off. Please step outside in the foyer if you wish to use your cell phone. We also ask that pagers be set on the silent mode.

Thank you very much. Dr. Salomon, I'll turn it over to you.

DR. SALOMON: Basically the only other thing I'll say -- you guys got the drill as we went around. One of the things that's very important is that we create a written record. Just to make it easier for the transcriber, try and speak every time into the microphone, and when you're done speaking, turn the thing off, otherwise it picks up the background and she won't be able

to get a clear recording.

Then let's start. We begin with the FDA introduction by Malcolm Moos.

DR. MOOS: Good morning, everyone. I'd like to make a few general remarks aimed largely at the audience because they're not quite as familiar with the process of these advisory committee meetings as some of those of us sitting around the table.

One of the more commonly held models of the nature of the interactions between the Food and Drug Administration and its constituent body as the public/patient advocacy groups and so forth is depicted schematically on the first slide.

Now, although I'd like to point out that if circumstances warrant, we do have the statutory authorities for things to degenerate to this level.

(Laughter.)

DR. MOOS: By and large, that's not how it works.

In fact, if we go back to civics 101, it's important to remember that the public, through the President and cabinet officials and through Congress, delegates to us the job of riding herd on the development of promising new therapies. In fact, one thing that we are empowered specifically to do, in order to help us do our

jobs right, is to go directly to experts in the public in order to gain the expertise that we need to approach difficult, new issues.

Now, certainly the FDA has to be sensitive to emerging technologies. For those of you in the back who can't read the caption, it says, "Look what they're doing," they being the folks who've gotten the clue that flying is faster than walking.

So, it's natural for us to start looking for new technologies with enthusiasm, but at the same time, it's important to understand that -- the caption reads, "early experiments in transportation" -- there are certain pitfalls that can be anticipated. And perhaps more dangerous than difficulties that are obvious are cases that arise when we are confident in our abilities, we think that we are cruising at altitude and that everything is going to be a smooth flight, when in fact our judgments have blinded us to the obvious potential for great misfortune.

Although I think Mr. Larsen's cartoons make some of these points very elegantly, I don't want anyone to get the idea that we think of this as a big joke. There is great excitement in the issue of stem cell biology, but at the same time, there are various types of hazards, some of which we can foresee, some of which we can't. It is important to let everyone know that we have to be very

sensitive to the fact that the most fundamental tenet of clinical medicine is: "First do no harm."

The task and advisory committee meeting I think is summed well by what a Hewlett Packard executive formulated as the quadrants of confidence. He started with conscious confidence. You know something and you know you know it. I think this quadrant right now is perhaps the smallest of the ones that we have. It will be very useful for us to establish as a group what sorts of things about the biology, the manufacture, the testing of stem cells we are fairly confident in that we think we know.

It will be also useful to address this quadrant, the quadrant of conscious incompetence, things that we know we don't know that we have to find out. In fact, I look at this quadrant as the quadrant of the professional. The professional establishes the things that really need to be taken care of and addressed carefully and one by one systematically pursues this. We want to figure out how we can take precautions about this quadrant, how to be the competent copilot in the previous cartoon, to take things from the arena of where we don't know that we don't know it and place it first into this quadrant and then into this quadrant.

I'll leave this quadrant until later. This deals with issues that we don't worry about until

licensure, and we can talk more about that in a subsequent workshop.

So, the other thing I'd like to establish is certain things that we're not going to talk about. We have a limited amount of time and a lot to get accomplished in that time, and there are certain things that are going to be off the table.

The first thing we're not going to talk about is whether the FDA is going to be regulating these things. There has been a court decision that reinforces the 1993 stem cell policy that we promulgated, and in fact, the entire convening of this workshop is predicated on the fact that we will have jurisdiction over stem cell based products.

Similarly we're not going to talk about xenotransplantation because we've already done that. We are quite sensitive to the fact that many types of manufacture of these products involve things like mouse feeder cells and perhaps other types of technologies that will involve animal tissue, but those issues are not specific to stem cell or neural stem cell biology and so they will not be addressed today.

Similarly, there are some issues that would be important to address in trials involving diseases of the nervous system which are not specific to therapies using

stem cells, and we won't talk about them.

Finally, we are quite sensitive to the fact that certain sources of stem cells are very controversial and certain sources are not. The FDA does not have a position on whether embryonic or fetal tissue is or isn't appropriate. We don't have the expertise to make that call. We don't have the authority. Finally, whether stem cells are coming from fetal or embryonic tissues or from adult tissues or other types of sources or not, the way we look at testing, control of the manufacture, the right types of animal experiments is pretty much the same.

So, to place this in a pithy nutshell, what we want to do in the next day and a half or so is to figure out what we know, if there are areas of consensus. If there's not consensus, are there a few sharply divided viewpoints? What are they? And finally, what must we learn both now -- by that I mean before any human trials can start -- and later, which means before marketing approval. The reason that this last thing is on this slide at this early stage is that there may be some kinds of technical issues that need to be addressed that are going to take quite a while, and we want the whole community to be aware that this may be the case so that they won't be on the critical path to licensure.

With that, I will close and turn the discussion

over to Dr. John Gearhart. I think I'd like to say in closing that we're very excited to have the panel of experts that we have managed to convene and have enjoyed very much working closely with our colleagues from NIH in establishing this. Dr. Gearhart.

DR. GEARHART: Good morning. I'm obviously delighted that there are certain topics that are off the table and that we can talk about some of the biology.

Now, even following an hour conversation with Malcolm the other day, I'm still not sure what I'm supposed to talk about. Now, this may sound strange to a certain extent. There is a lot, as far as the experimental side of things with human embryonic stem cells, going on. So, I think what I'll try to do is to summarize where we are in this field, giving you some examples but, because of restrictions in time, not dealing with a lot of examples.

One of the lessons that I have learned this past year was nicely summed up actually in Sherwin Neuland's admonition to the FDA in Monday's New York Times op-ed piece on a different topic, but dealing with the fact that we should really make a habit of two things when we're dealing with human disease. One is to help, or at least to do no harm.

Our thoughts this past year have really turned to the latter. We are thrilled by what the cells are doing

in culture and in some of our initial experiments with respect to animal studies, but our attention is now more focused on how to demonstrate that these cells will do no more as far as harm is concerned.

In the first part of this talk, I'm going to talk a little bit about stem cells. Now, we have obviously a distinguished group of investigators on stem cells, and anymore I'm almost afraid to define a stem cell. Things have changed so drastically over the past year, but nonetheless, it won't stop me from attempting to do it. So, if I could have the first slide, please.

Well, stem cells have two important properties, the first of which is a capacity for self-renewal, which means that they have the ability to produce more cells like themselves. The second property that is important is that a stem cell is capable of differentiation. It's these two properties and certain degrees of it, which we can talk about, that define stem cells. It's really a definition that's experimental in nature.

Now, in this definition we say nothing about the extent of proliferation. Some stem cells we know can divide indefinitely; others have a very much more short span, or the cell cycling rate of those divisions and also the developmental plasticity of a stem cell. What can it form? As we're learning now, where we thought there was

less plasticity in stem cells, we're now seeing a great deal of plasticity.

In the classic picture of stem cells -- and this really derived from the work of Till, McCollough, Seminovich in the '70s and was borne out by types of experiments, which we need not go into at the moment -- we had a picture that in very early development, we had cells that were capable of forming a large number of lineages. As development progressed, these cells became much more restricted in their lineages, but still had capacities to divide. And finally, we would get into this area we call lineage-restricted stem cells. They would be for neural components or hematopoietic components, et cetera.

Now, obviously, although this type of chart is still illustrative of what we think is going on, maybe the issues of where in this context these cells are are going to vary, and you'll hear some examples of that today.

So, where do we get stem cells? In this drawing, what is depicted is in human terms. We don't know all of this as far as humans are concerned. Most of it is animal work, but we know that we can get stem cells from pre-implantation staged embryos, these structures here, just before implantation into the uterine wall, and we'll talk about that in a few minutes.

We know that the fetus has been a good source

of various kinds of stem cells, and I'll talk about one specific kind this morning. And Evan and others will talk bout other sources here.

Finally, over this past year and a half to 2 years, the realization that the adult is also a very good source of a number of stem cells. The interest of this conference is with respect to the central nervous system, but we know bone marrow, we know muscle and others are now viewed as having stem cell populations with a great deal of developmental plasticity.

Well, let's concentrate on embryonic sources. These structures here represent embryos that are within the first week following fertilization. They are about 100 microns in diameter, just visible to the human eye. These structures here are what you would find in the oviducts on the way to the uterus in human terms.

They consist of a couple cell populations, depicted in this cartoon, in which we have an outside layer of cells here which is responsible for the implantation process, and these are the cells that will give rise to placental tissues. In an embryo of 200 or 300 cells, about 85 percent of the cells are in this layer here.

There is also another group of cells present, referred to as the inner cell mass. These cells are the direct precursors of the embryo proper.

So, we have embryonic structures formed out of these, and extra-embryonic, placental structures out of this outside tissue.

If you remove the outside tissue -- and you do this through immunosurgery -- place this group of cells in culture under specific conditions, you can derive embryonic stem cells. This was initially done in the early '80s in the mouse, and with respect to the human, Jamie Thomson's group in Wisconsin reported this in 1978. Since then, four or five other groups have now obtained human embryonic stem cells using this structure here called the blastocyst, which is derived from in vitro fertilization techniques.

A second procedure that has been found in mammals to work is depicted in this slide with respect to a human embryo. Investigators in the early 1990's were interested in obtaining cultures of primordial germ cells, and their interest here was really to study germ cells. The primordial germ cell represents the lineage that's set aside very early in embryogenesis that gives rise to eggs and sperm. They are diploid. This is before the meiotic events.

It was found in the rodents and subsequently by us in humans that we can identify the stage in which these primordial germ cells can be recovered, placed in culture, and embryonic stem cell lines obtained from them.

I'm going to now talk about our lines in some general terms, to give you a feeling of where they come from and what we've done with them. The end product here, though, is very similar to the type of cell that Jamie Thomson and others have isolated from blastocysts, and I'll tell you about differences as we go along.

This is a 3-week human embryo. It's a drawing from Emil Vecci's classic work illustrating the embryonic axis here with the developing CNS, heart, caudal region, the yolk sac, and this is going to develop into the amniotic cavity. Early, one sees a group of cells, 50 to 100 in number, that's located extra-embryonically. These cells arose embryonically, have been translocated to an extra-embryonic site. And over the next few weeks, these cells divide and then eventually -- this is a sagittal section through a 4-to 5-week human embryo. You see these black dots here that represent the migratory path that these cells have taken from an extra-embryonic site coming through the gut epithelium, the dorsal mesentery, into this large structure here, which will condense down to form either an ovary or a testis.

These cells now number in the thousands. We estimate in humans that there are about 20,000 cells when they come to this ridge or this developing gonad. It's during this period of time that we isolate these cells.

In actual tissue or what one is looking at here is this bundle of tissue that comprises the developing gonad, along with rudiments of the kidney. It's this tissue here that we recover from an embryo, the crown-rump length of about 1 centimeter.

We place these cells in culture. They are individual cells to begin with, these large cells. They are extremely mobile in a dish. This is the mouse equivalence to the human. And over a period of several weeks, under appropriate culture conditions, they sit down and form colonies. And we'll talk about these colonies.

Now, what's important here? Well, there are several issues that are important here.

The culture conditions for these cells we find have a requirement for a specific group of growth factors. We grow them in tested fetal calf serum, and we grow them on feeder layers. All of these, of course, raise issues as relates to how reproducible the results are, and the source, for example, of fetal calf serum and of the feeder layer requirement that Malcolm had alluded to.

These cells have an absolute requirement for the feeder layer, and we use a mouse feeder layer. We can also use human, certain tissues from the human, but this has been a problem. We are trying to grow these cells off of the feeder layer. It has proved to be quite difficult

up to this point, but it is certainly a target of our work.

That calf serum which has been found to be ideal for mouse embryonic stem cells is not good for human embryonic stem cells. So, we now get into this issue of how do you test and what is it you're looking for here without really looking on the human cells. We're trying to find surrogate cells to test this on to begin with, but that's the nature of the beast.

There are some laboratories that are making progress in using serum-free media with mouse embryonic stem cells, but the rate of growth, the rate of differentiation, and the variety at this point of differentiative products is very minimal, as one may expect, because of the source of growth factors and whatnot in the calf serum.

Not all cells placed in culture grow, although it is a very high rate of growth. Not all grow and some grow to limited degrees as well. Again, we don't know the basis of this at all.

This illustrates the colony morphology of these stem cells. They have a cobblestone appearance and this appears a few weeks after being placed in culture. In the background here, one can see the feeder layers.

Well, how do we define a stem cell? Now, this gets into another area, and that is that we have a series

of biochemical markers, of cell epitope markers, molecular markers, the sum total of which we say you have a stem cell. No one marker is indicative of the stem cell. And this is another issue. Some of these markers are stage-specific embryonic antigens, and we find, for example, that they vary among species, so that what is appropriate for the mouse may not be appropriate for the macaque or the human. So, we look at the consistency of this panel rather than any as an absolute marker.

And this is another issue. We are trying to define these cells from the molecular standpoint, their gene expression profiles. But we're a long way from solving this issue at the moment. So, it's another area that we have to really explore.

Now, having said all this, I would say that we will not use embryonic stem cells as the source of tissues for direct transplantation into anyone. These are pluripotential cells that can form many different structures, and it's really the derivatives of these cells that we are after. So, this is a starting material. This isn't what's going to be a licensed product I believe that's going to go into a patient. The concern here is that if you transplant these cells, without question you're going to get teratocarcinomas. And we've done this extensively in mouse many years ago. So, this is starting

material.

It's important that these cells to me are karyotypically normal and that over many passages they remain so. You can have male or female cells. That's an important issue.

Now, another aspect of this is that we have embryonic stem cells, which is a term used very generally, but it refers specifically to those cells that are derived from the inner cell mass, those cell lines. The type of cell that we've derived from primordial germ cells has another name, embryonic germ cell, and it was given that name to distinguish it from the blastocyst-derived ES cell.

The issue here is how similar or dissimilar are these two cell populations. I would tell you that where both of these in the mouse would go germ line, if you want them, you can get in vitro differentiation, and you can get tumors if you place them in different sites within an animal. They share these features in common. But the cells are not identical. We know of a number of differences between ES and EG-derived cells from mouse studies. No lab has yet had both human ES and EG in them to study together, but they are different. There's no question that they are different. They're derived from different sources, as you can see. I want to emphasize this. So, what maybe come down for ES cells as a uniform

kind of thing will be different from what we would expect from EG. But we do know that they are capable of all of these functions down below here.

Now, some of you may remember this from last fall. In Germany investigators are not permitted to use human ES cells, but they're permitted to use human EG cells. And there are reports that the imprinting within these cells, which is a molecular -- what it is, in alleles that you may inherit from your mom and dad may have a different molecular structure about them that comes on to it subsequently from your inheritance that are then expressed in a specific pattern during development. This phenomenon is allele-specific. It's called imprinting and it's very important. If that imprint is not there, it can lead to birth defects or it could lead to death.

What we have found with the human lines we're working with in looking at several loci, looking polymorphisms of expression at these loci, and the methylation patterns, is that these cells are normally imprinted. But it is an issue.

But I should now back up and tell you that even among mouse ES cells and among mouse ES/EG lines, that there's a variability in a number of parameters. There's no uniformity there either. So, it may not be expected in the human side of things, but it's just something to be

concerned about.

What's in a word? Just to remind you that we refer to these cells as being pluripotent because they cannot form the extra-embryonic structures of the placenta. If they could, we would probably refer to them as totipotent. That means they could form all cell types, but totipotent carries with it the issue of being able to form an embryo. But these cells are pluripotent. Another term that's used is multipotent here.

We've talked about embryonic germ cells, EG, ES. Another one to alert you to are embryonal carcinoma cells. These are all very closely related. The top cell type here is the stem cell of teratocarcinomas, of these special tumors that arise from germ cells.

As many of you are aware, cell lines derived from human EC cells are now used in a clinical trial in stroke at the University of Pittsburgh. Whether there is concern about this with respect to the origin of the cells, the fact that they're hyperdiploid, and whatnot, is something that I am concerned about. We can talk about this later, but they are related in origin.

So, now we have these stem cells in culture, these pluripotent stem cells. How do we get them into these different derivatives that we'd like to use in basic science studies or in therapies? So far to date, the

procedure involves really one of affecting their environment. We know that in mammalian development that the fate of cells in early embryogenesis is really determined by the environment that these cells see. So, by controlling that environment, you can then determine the fate, or at least enhance or affect the fate, of a cell.

How do you do this? Well, different combinations and permutations of growth factors and cytokines. This is what has proved to be most effective.

With embryonic stem cells, the state of the art is that you first work through a structure called an embryoid body. There is an "i" and a "d" on this, just to alert you. It's a pathologic structure. It's not an embryo. What these are are aggregates of these cells that you permit them to aggregate in culture. When you get these little balls of cells, they are depicted like so. You can have hundreds of cells or thousands of cells, and then by treating these embryoid bodies with cytokines or dissociating them and treating them with cytokines, one can then isolate from them different cell types. This is a histologic picture staining for some neuroendocrine cells within an embryoid body. What you then do is go in and isolate and enhance the growth of these cell types.

So, this is done -- for example, you'll hear from John McDonald of taking embryoid bodies, treating them

with retinoic acid and this enhances to some degree the formation of neural structures. About 10 percent of your wells will have some neurons in them under these conditions, and this is an illustration of some of those. That's unimportant. It's been well-documented and published.

Ron McKay came up with a very nice procedure that we use routinely now, of taking embryoid bodies, treating them with a series of cytokines, forming both neurons and glial populations of cells. This procedure takes a few months in culture. And then out of these pots at the bottom, one can identify specific types of neurons or glia. This is the pots that we use for transplantation, and it works very well. But again, the important point here is that this is all being done from the outside of a cell by treating them with different cytokines.

These illustrate then the staining properties that one would like to see in some of these neuronal precursors and finally into some specific types of neuron.

This is not 100 percent. In fact, you're lucky if it's 25 percent. There has been a recent publication from Ron McKay's lab where he's now getting upwards of, I think it is, maybe in the 40 to 50 percent range of dopaminergic neurons by specific growth factors downstream. But again, this is not a process which is 100 percent.

Now, I want to give an illustration here. It's not on your mind, being mostly neural inclined here, but I want to make a point with this. We have been trying to isolate hematopoietic stem cells from our populations of cells. One can do this using techniques that Michael Wiles and Gordon Keller have used. We're again starting with embryoid bodies and treating these with cytokines that we know are important in hematopoiesis. We can get at pots of cells that are enriched for different lineages within hematopoiesis, be they going through the erythropoiesis with macrophages or whatnot.

Now, the important thing here is this. We've been taking these cells that have been isolated and we've been cell sorting at this level here for specific antigens, whatever the latest is for defining a hematopoietic stem cell, and then transplanting these into lethally irradiated animals. So, we've done this with a number of antigens. It's unimportant here.

The important point is this, that we are getting long-term grafts, but along with that, out of over 150 animals that we've transplanted, 2 of these animals have developed teratocarcinomas. And we're concerned about this. What have we done here? Have we carried along a stem cell after literally weeks in culture and then cell-sorting based on antigens that we know embryonic stem cells

don't have? What has happened here? Have we had some kind of reversion or whatnot? Don't know, but it is of concern to us.

Now, let me tell you where our work is currently and where we think that most of the cells that we're going to use in the human story are going to come from. Slightly different than what I just told you about, about taking embryoid bodies, treating them with cytokines, and trying to enhance or direct differentiation into specific lineages.

We know that embryoid bodies can have a variety of cell types within them. So, our current experiments deal with taking an embryoid body, dissociating it, playing it out in conditions that are ideal, or have been reported to be ideal, for specific cell lineages, be they neural, hematopoietic, endothelial, endodermal, whatnot, and then cloning the populations out of this, and using these cells then as the source of material for transplantation studies. So, part of this paradigm is you're growing up these embryoid bodies that come in different varieties. They can be cystic. They can be solid groups of cells. Over time you can enhance within those or select within those populations of cells.

We can use markers. This is an example of different lineages, just PCR-based markers as endpoints to

say that we're into a specific lineage.

This is a very busy slide, but what it shows is some of the markers for different lineages and whether we've used a PCR-based analysis or antibody to detect on the surface of cells or in cells the specific markers.

This illustrates our endodermal markers that we're now using for pancreatic and liver development, some of the markers we use there, an antibody to verify that we have expression.

This is an important slide. What we have done is to take these clonal lines that -- we initially were looking at just different groups of markers to say we have neural, endothelial, whatnot. The cell lines grow robustly, on and on. We were concerned about the following thing, that when we went back and took our neural cells and looked at other markers, hematopoietic markers or anything else, we would find those markers also on many of the cells. So, initially we were just focused on specific subsets of genes, but when we back and tested all of them, we would find they have a lot in common. So, this may say something about using molecular markers for defining a cell population.

But we were concerned that we had a heterogeneous population, but when we did subcloning from these, we found that, indeed, there were characteristics of

all the cells within these populations. I think it's an important lesson here both in the biology maybe of stem cells and in any type of marker system we would like to use to define a given population of cells for product.

Now, we are just in the initial phases of going into culture modeling, into animal models. We're going this with Jeff Rothstein and Tony Ho at Hopkins and a number of other groups. So, we don't have a lot of data other than some preliminary things to say, that when you take some of our human embryoid body derived stem cells, that we can, indeed, form some motor neurons or oligodendrocytes and whatnot under appropriate conditions. We don't have a lot of data on this. We're moving into stroke models shortly with Dick Traytsman, Parkinson's, others. So, this is where our work is at this point in time, defining some of these stem cells and moving into mouse modeling.

These are examples of forming oligos and whatnot and motor neurons.

Now, we're not going to get into these kinds of things. I didn't know what to be prepared for. I want to skip ahead, though, to just a few items.

One is that this field is evolving. There are a large number of investigators working on mouse embryonic stem cells, wanting to work on human, working up

conditions, as I mentioned, of trying to get feeder layer independent cells, of trying to define the growth paradigms to get into different lineages, to use also genetics to try to create transgenic lines, if you will, that would only then give rise to specific lineages but using genes that are early on in pathways. This is sort of where we are from that standpoint.

Now, a couple of things that we're looking at downstream. One is we've already shown that we can get a number of derivatives out of this. Whether or not they're lineage-restricted, we are now giving our hematopoietic cells to the neural people and vice versa to see if they will give rise to appropriate structures.

We're interested down here in the issue of transplantation and what we can do genetically within these cells to try to make, for example, a universal donor. But this involves transfections, gene modifications. That again is an issue to be addressed. It's not unique to the stem cell story, but at least we're using that approach. It has to be considered.

Another issue that many labs are working on is trying to match host to a stem cell, the issue of nuclear cloning or nuclear transfer in which we take a nucleus of an adult, place it into an egg cytoplasm and generate a stem cell out of this, so it would match that patient.

We're also, interestingly, moving on to reprogramming adult cells. Now, this is taking cytoplasm from EG cells, combining it with nuclei of differentiated cells, and beginning to show that you can reprogram these nuclei. Now, what impact will this have if you come up with a population of cells here that are stem cells in nature and you can differentiate them down different pathways? This will happen.

We're concerned here with respect to the origin of tissues, of infectious agents. The interesting thing at Hopkins, we're having some difficulty in being able to gain information in this area from any patients. We are not permitted in the fetal tissue area to take patient records and whatnot. This is completely anonymous in a sense. Although identifiers are kept, we can't go back and get that information. So, we have to do testing on all of our tissue, and even that is controversial. The issue through the feeder layers and the issue through, for example, the serum that we're using.

We're concerned about stable properties with continuous culture. Is the differentiative capacity going to remain unchanged? How do we determine this? Do we have stable gene profiles? And do they remain karyotypically stable over a long period of time? These are things that we're looking at. Can we really regulate or control the

differentiation of these cells?

Non-tumorigenic issues here. We have a number of fail-safe systems that we're now considering that if a cell turns tumorigenic, that we can bump it off internally.

The issue of graft rejection and the fact that we may have to be using genetic manipulation. Of course, that brings with it a number of factors as well.

Now, one last comment. As you know, there are Rhesus ES cells. There has been a push by the NIH to use the Rhesus and Rhesus ES cells as a test before we get into human clinical trials. That would mean that we would take these cells and do the same thing with them that we've done with the human cells or with the mouse cells. That's an issue which I think should be discussed. I'm not in particular favor of it at this point.

There was also the issue of the Rhesus studies themselves of the time frame of taking human cells and putting it into a Rhesus. How long would you want to do this to look for tumors, for example? And on and on. So, there are some other issues that I think we can chat about at this meeting.

Well, I hope in this few minutes I've given you a flavor as to where we are in these studies, where some of the points are that we should be talking about. I still feel that the embryonic source of cells will prove to be a

reliable source and a good source for finally getting into human therapies with transplantation studies.

Thank you very much.

(Applause.)

DR. REID: Lola Reid from the University of North Carolina. I have a couple questions.

One is that you had defined all stem cells as being capable not only of differentiating but also of self-replicating. That's an area where I think there's getting to be increasing controversy over whether that is always true. Certainly for the totipotent stem cells and the embryonic stem cells that is true, that they self-replicate, but for the determined stem cell or, as you're calling them, lineage-restricted stem cells, those are ones in which, at least by assays such as the telomerase assay, they can be restricted in terms of their true self-replicative ability. So, one issue is what is the evidence now that determines stem cells truly self-replicate.

The second issue is that you are going through this elegant procedures from germ cells or from embryonic stem cells into lineage-restricted cells and then getting some evidence that they can be tumorigenic when you inject them back in vivo. But if you were to isolate out determined stem cells from the normal tissue and compare those back with what you think are those lineage-restricted

cells, you should be able to get a better idea of what might be distinctions in them and get better markers for being able to identify the determined or lineage-restricted stem cells from those that are in fact still totipotent.

DR. GEARHART: Let's take your second question first. I would agree we would love to learn from our colleagues here what features we should be looking at at these stem cells downstream that would define a population of, let's say, lineage-restricted cells. We have to wait for that as we're learning. So, that will come I'm sure.

We are not doing that within our own laboratory. We're relying obviously on our colleagues.

The first issue as to whether all stem cells can proliferate where they have a self-renewal capability, I agree with you we're getting into an area now of again trying to define a stem cell. But does it, in a way, really matter? What you can demonstrate or what we would like to demonstrate in the use of a stem cell population is you have to have that capability, if you're going to grow large numbers of cells, to be able to effect any kind of transplantation therapies. A cell has to have the ability to replicate. One isn't going to just remove a few cells and transplant them. You need really thousands, millions. And if you go into any FDA-approved kind of thing, you're going to have to have a bank that's going to be stable and

it's going to be proliferative.

DR. REID: The question of extensive growth potential is separate from self-replication. Obviously, stem cells in general -- I don't know of any stem cells that don't have actually quite extensive growth potential, but the issue of self-replication is that they can form a daughter cell that is absolutely identical to the parent cell. That has certainly been, I think, proven for totipotent stem cells, but I think the evidence is waning on even the most well studied of the determined stem cells, that of the hematopoietic stem cells. So, a "hematopoietic stem cell" isolated from an older animal will have less self-replicative ability than one that is isolated from an embryo.

So, I think that that's an issue that gets muddled, particularly with reviewers or discussions. They keep demanding that that be part of the criteria when, in fact, what we're now seeing is that it's not applicable to lineage-restricted stem cells, or determined stem cells.

DR. SALOMON: Yes. Picking up to try and pick one point that I think we should try and return to tomorrow is that these questions identify an issue for how the FDA is going to look at all of this.

So, one idea that you floated, John, is, well, we'll have a master cell bank, which the FDA would love.

I'm not certain that the biology is going to allow that for many of the types of stem cells that we're going to bring forward into clinical trials in the near future for the reasons that the speaker pointed out, that you may be able to get so many replications of so long a time, and then you're going to have to go back to your source. So, I think that's going to be a very important question to think about because if you regulate from a master cell bank point of view, that's a very, very different prospect than regulating from something in which, let's say, every 2 months, every 10 patients or something, you have to go back to the source. I think we should be careful not to close that off unless the experts in the group say, no, we can really do this master cell bank thing.

DR. NOBLE: Mark Noble, University of Utah at the moment, University of Rochester in real life.

One of the assumptions, John, that's made in a lot of this work is that once you have identified a lineage-restricted precursor cell, you have essentially identified that lineage. Now, we know from current studies in the oligodendrocyte lineage that this just isn't true, that we have thus far a minimum of three -- possibly four, but certainly three -- oligodendrocyte precursor cells with very, very different biological properties, particularly in respect to their self-renewal properties which may have

tremendous implications for the utility in tissue repair. So, as we go on to consider how we're going to develop cell banks, this question of how we're going to prospectively recognize those cells that actually do have the extended capacity for division seems to be part of the discussion we need to have.

DR. SAUSVILLE: Ed Sausville from NCI.

Could you clarify what the HLA restrictions on the use of these types of cells might be and the extent to which immunologic barriers might either require diversity of sources or are not an issue?

DR. GEARHART: Well, it's obviously a major issue here. We would like to think that we in the future can provide cells to patients with a minimum of immunosuppressive therapy out of this technology. We've had discussions with a number of transplantation immunologists as to, if we failed at this, how many different cells we would have to generate from different HLA types and whatnot, that we would have a bank that could service a large population of individuals. And there doesn't seem to be a consensus on this in any fashion of whether we should have 20, 40, 80, 100, or hundreds. So, I can't give you an answer. It has been a topic of debate from that standpoint. That's about as far as we've gone.

We are now looking at gene alterations within

class 1 and class 2 molecules to see how far afield we can go with respect to the mouse work in getting a good degree of transplantation success with a minimum amount of rejection. So, this is where we are in mouse work, not human.

DR. CHAMPLIN: To state the obvious, in bone marrow transplants we use stem cells, even highly enriched stem cells, and HLA restriction there is very important, and even one HLA/allele mismatch leads to a markedly increased risk of rejection and graft versus host disease. So, whether or not that will be true of other stem cell populations is unclear.

One other comment in terms of assaying cells. The phenotype of the cell depends on the culture conditions that they're being prepared and that the growth factors in the milieu will lead to differentiation in one direction or another. So, it's not just the cell itself, but it's the conditions in which they're assayed. So, it is obviously a highly complex question.

DR. GEARHART: I should have pointed out that ES cells and EG cells do not exist in situ. These are artifacts of culture, and that's another issue here. These aren't a naturally occurring stem cell within an embryo or a fetus.

DR. PROCKOP: I'm Darwin Prockop from Tulane

now. I'm here kind of as an advocate of the FDA considering the possibility of using cells from the same patient, that is, stem-like cells maybe lineage-restricted. I think that's a real possibility. It's a possibility that our laboratory and Berber Laboratories are pursuing. So, you'll be hearing the cells that Catherine Verfaillie will be describing quite soon, I believe, in the next talk.

Under conditions we've recently developed, we seem to have no limit on number of cells we can produce. So, from small bone marrow aspirate, we can produce 10 to the 13th cells in 6 to 8 weeks. We have fully characterized those cells, but we don't think we've reached the limit of expansion, and they still stay multipotential. So, I would very much hope the FDA still considers that possibility of cells coming from the patient who's going to be treated.

DR. SALOMON: One thing that you brought up I wanted to get your comment on -- and I'm sure we're not going to settle it here, but I think one of the fascinating things is the observation that if you put these into an adult animal, I'm assuming, that you're getting a teratocarcinoma. As a result, what you're doing is culturing for several months in growth factors prior to transplantation.

One of the things that simplistically has been

said is an advantage of stem cells in many different reviews of the subject has been that the signals for stem cell differentiation exists in a local site. There's nothing simple in stem cell biology. Forgive me for simplicity. But the simplistic idea would be then if you put these in a site, that they shouldn't develop as a teratocarcinoma. In other words, where are these microenvironmental signals? Because I think thinking about these microenvironmental signals is going to be very critical, for example, in neural cell transplantation, right, in order to guide these down the right developmental paths?

Do you have a comment?

DR. GEARHART: My comment on this would be a factor of cell number. We've known for many years working with mouse ES, mouse EC cells that it's an issue -- for example, when you return cells to what's called a blastocyst, if you're making a chimera, you can override that system very easily by placing too many cells within that environment. It may become an issue of how many cells or what the draw-down is with respect to factors within an environment based on the number of cell types that you have there. That's an easy explanation.

The hematopoietic story I gave you is a little bit more complicated I think because we've had these cells

in culture for months, essentially, and then FACS sorted them and then came out at the other end with these two tumors. Now, this was in an initial experiment of which we did 70 animals. We found them there. We've subsequently repeated and haven't found any more tumors.

But is it an issue that we've taken a cell, it's somehow differentiated to a certain degree, and then has dedifferentiated, for a lack of a better term? Or have we carried always along this stem cell -- I mean, this one that's an ES cell rather than a more differentiated type? Don't know.

DR. GAGE: I think the issue of local environment is really critical in the process of differentiation of the cells. But I also really support John's statement that the ideal cell for transplantation wouldn't be the purified, most primitive stem cell necessarily because that cell may be less able to read that local environment. Particularly when going back into the adult host or the damaged host in some way, while there is a local environment that contains cues that can lead that cell down to perhaps appropriate lineage, it has to be mature enough to be able to read those signals. So, once again, we come back to this idea as to whether or not the most primitive cell is actually mature enough to be read by the local environment that can drive the cell down that

lineage, and understanding how to get from the primitive cell to a cell that can read those environmental cues that remain I think is really a very important challenge for everybody.

DR. SALOMON: Excellent. I think that then should be considered another underpinning of the discussion in the next two days.

DR. MULLIGAN: In the case of the concept of lineage-restricted stem cells from your EG cells, what specific cases do you have where you can actually get from your bulk culture an amplifiable lineage-restricted stem cell population? I'm thinking about cell banking. I think what you were saying was it would be nice if you have something that was focused on a lineage, but I'm not clear whether you've actually shown that that is possible.

DR. GEARHART: Well, our lab has been looking in three areas. One is the neural cells; the second, hematopoietic; the third is endodermal.

What we have been able to demonstrate is we have a number of clonal lines with the neural phenotype that have been working very effectively, long-term culture robustly growing that we've been using in a number of co-culture experiments. They're now into the transplantation experiments. They've been in continuous culture since last August approximately with the same markers and empirically

giving us the same results. So, we do have I think a more general neural line from that standpoint.

The endodermal lines apparently are working the same way.

Is that the question?

DR. MULLIGAN: That's the question.

I guess then the other point is the definition of lineage-restriction, of course, depends on what it's put in and where it sits. So, what's the likelihood that, although in vitro it may look like a lineage-restricted stem cell, again depending on where you put it, it may have great plasticity. So, is it necessarily possible that there is such a thing as a lineage-restricted stem cell?

DR. GEARHART: I worry about this all time, obviously. We are getting examples of this cross-talk of taking a neural cell, giving it to our hematopoietic collaborators, and showing that it will do something different in another environment. So, we are seeing examples of this. But we don't have a lot of numbers to show you, but we are learning that we have some examples of that.

DR. MULLIGAN: On Rusty's point, maybe it's possible that exactly the opposite might be the case, that is, having a more early cell may actually make it more capable of sensing injury and doing the right thing than a

more differentiated. So, I was curious, Rusty, what case in point makes you think that having a more differentiated stem cell would allow it to better sense local environment?

DR. GAGE: Well, a very specific example would be in certain areas of the brain where neurogenesis continues. If you implant into those areas neural cells -- let's say, fetal-derived propagated cells -- that they migrate just to this region, they can differentiate into neurons as evidenced by morphological criteria. If you take a more primitive cell like a hematopoietic cell and put them into that same area, they don't. They get to the area and they don't read, as one example.

So, I'm not saying that there couldn't be other environments where primitive cells could do it, but I submit that in some cases the environment may be quite restricted as to what signals it can demonstrate. It doesn't mean we can't change the environment, but there may be some restriction in those environments.

DR. MULLIGAN: I guess I would caution us that we think that this lineage restriction issue may be very, very complicated and by, for instance, taking the hematopoietic stem cells in vitro and putting them in some other broth or cocktail of factors, they may then behave like a neural-restricted stem cell.

DR. MACKLIS: If I could make a couple of

comments on these last two points. I think another issue we need to raise that comes directly from what Rusty Gage said is how sharply do we define the pie of what we call lineage-restricted. There are estimates of hundreds of types of neurons in the central nervous system, maybe a hundred types in the cerebral cortex itself. Is neural-restricted or neuron-restricted enough?

A second point is, regarding Rich Mulligan's point, there is evidence from our lab and many other labs that later and later stage, partially differentiated neuroblasts or neurons can at least repair certain circuitry with much higher efficiency. I think it would argue that we really need to take those many, many steps from the immature cells up through maturity correctly.

DR. MULLIGAN: But, again, I think the issue that I'm raising is, is that a truly fixed property of those cells? Although they behave in that way, do we know enough about what their state is to make sure that that state is a truly fixed state as opposed to if those cells or another cell type was, again, cultured or manipulated in a different fashion, they would behave in that --

DR. MACKLIS: Right. Well, I assume that's the lower right, knowing incompetence or something. I think we know that we don't know enough yet.

DR. CHAMPLIN: Clearly at some point, cells

become irreversibly differentiated. You can't make a granulocyte into a neural cell, although that may be possible at a more primitive point in hematopoiesis.

At least my view of this is this is a spectrum of differentiation where there's not discrete cells as much as a continuum of cells with different proliferative and differentiative potentials that slowly diminish as the cells mature along a given lineage. So, trying to define each point on this continuum is certainly complex.

DR. MACKLIS: If I could make a quick point on that, there is some new evidence, some of it still controversial, that even what we thought were continuously differentiating glial lineage, away from the neuronal lineage, some of those cells may actually dedifferentiate into precursor cells and then back into neurons, and that's just emerging now. And I think we all have to deal with that.

DR. REID: I wanted to raise the issue on this microenvironment. One of the areas of microenvironment, that at least in the past was not discussed as much, was the matrix chemistry. I listened to some groups from Johnson and Johnson where they were discussing issues of fetal brain transplant, and I was stunned to learn that when they're doing this for Parkinson's patients, they had to use as many as eight fetal brains in order to try to get

some measure of efficacy on some of the patients.

And there have been preliminary studies from Titan in which, when they take particular neuronal cells -- this was not human. I think it was in pig cells -- and bind them in any kind of adhesion state, that they got tremendous increase in their efficacy.

So, I think one of the whole areas of microenvironment that may help in directing toward lineages is that the adherent cell populations, lung, liver, brain, are not going to be ones where we can just simply inject them. We may try that but I think it will be tremendously improved if the injection procedures will involve actually embedding them in some form of matrix before we inject them. It will dramatically improve efficiency and the survivability of the cells.

DR. SNYDER: I think another thing that at least we in the nervous system have to realize is that we don't know exactly what it's going to take to restore function in most diseases, and even though we tend to think of diseases as being one cell type that needs to be replaced, if we simply replaced the neurons, we're home free, the reality is we may need to recreate the whole milieu, which means more than one cell type, which means the neurons, but maybe also the glia that create homeostasis, that nurture, that support, the myelinate. It

may be that we need to do co-grafts. We may need to implant very restricted cells at the exact same time as more plastic cells that play off of these cells, play off of the environment to really reconstruct the milieu. So, it may be more complicated than simply saying let's just replace a dopaminergic neuron or a GABAergic neuron or a motor neuron.

DR. RAO: I just wanted to reiterate what all of this tells us is how little we do know about a lot of the things that we want to regulate. We don't know what's true about the matrix. We don't know what's true about definitions of the cells that we want to use, and we don't know about the environment in which we want to put the cells back in. We should keep all of that in mind.

DR. SALOMON: I think that's probably a good place to stop for the moment. We've got two days to discover what we don't know. I suppose these things are sort of humbling experiences for all of us.

I certainly think that the idea of defining what is the state of knowledge and where the gray areas are is exactly what the FDA wants from us. I don't think anybody here should be concerned about stopping and saying, hey, really I don't have the foggiest idea. I think that's okay.

I'd like to introduce the next speaker. I'm

trying to remember my French. Is it Catherine Verfaillie or Verfaillie?

DR. VERFAILLIE: Verfaillie.

DR. SIEGEL: Could I just add while she's getting hooked up? There may have been some misperceptions from one of the questions from the floor. We're certainly not here to decide which is the right cell source or which is the right matrix or whatever. We're trying to get information about what are the appropriate controls, testing, and whatever to set the ground for safety for human research.

DR. VERFAILLIE: I will also be talking about cells that actually are not embryonal in origin but actually can be derived from some adult tissues which may have potential of differentiating in a number of different lineages, potentially also in neuronal or neuroectodermal cell types.

This is sort of the same that you just saw from Dr. Gearhart. There are totipotent stem cells in an embryo obviously, and then there are embryonic stem cells. Then you have the mature lineage-specific stem cells which are present both in embryos, fetuses, and in adults. There's a lot know, for instance, about the hematopoietic stem cells, and there's a very quickly growing body of evidence or studies that actually address stem cells for other organs

both endodermal, neuroectodermal, and then mesodermal.

My lab has for a long period of time worked on hematopoietic stem cells. A few years ago, we started to also think about a different cell type which is called a mesenchymal stem cell, or a cell that was known to be in the bone marrow that can differentiate to certain forms of mesoderm, cartilage, bone, fat, fibroblasts. So, that's really where the studies I will present to you today and I actually put it in perspective of the field today is starting with cells that we thought initially were mesenchymal cells. But it seemed to have a subgroup of cells that have much broader potential than pure mesenchymal. I will come back to this also in the latter part of my talk.

There is from adult sources of tissues now growing evidence that certain of these cells have plasticity and can maybe become a cell that we never thought they could become. The question ultimately is going to be, is that really a lineage-committed cell or is it a subpopulation of the lineage-committed cells that have more immature features and actually could remain multipotent and actually aren't truly lineage-committed yet? And I don't think there's really any answer in this area at this point in time.

So, when we started this, the area of

mesenchymal cells has been longstanding. Actually the first person to identify the cell is Fridenshtein back in the '70s who isolated from bone marrow a cell he called colony-forming unit-fibroblast which was a cell that, when cultured in the presence of fetal calf serum, adheres to plastic and it makes small colonies. He initially described it in mouse. Lots of people who work in the hematopoietic field have used this because these cells actually support hemopoiesis.

A big group who has done a lot of work on this is the group from Arnie Caplan in Cleveland and some of that technology has actually moved to Osiris where they also used a similar approach to purify mesenchymal cells and then identified a number of antibodies that if used in combination, identifies cells that have the potential to become bone, cartilage, fat, skeletal muscle, and possibly heart muscle, although that's not 100 percent proven at this point in time.

Paul Simmons identified an antibody that he called Stro-1 which was made against what he thought initially a CD34 positive blood cell in the bone marrow, but it ultimately turned out to be something that recognized a stromal precursor. Therefore, Stro-1. And if he uses this antibody together with VCAM, he can isolate, to almost homogeneity, cells that have the ability to make

bone, cartilage, and fat. And he calls these cells osteoprogenitor cells.

Darwin Prockop, who is here, has done a lot of work on these cells too. He also uses the plastic adhesion method. He has been able to show that these cells can differentiate again in bone, cartilage, fibroblasts, fat, and muscle cells and possibly -- and I will get back to that too -- cells with neuroectodermal characteristics, cells that at least express proteins consistent with astrocytes and possibly even other neuronal cell types.

When we started work, we actually thought that we should take bone marrow cells, and since we wanted non-hematopoietic cells, we depleted blood from the bone marrow by depleting 45 and then cultured these cells in relatively defined culture conditions. The initial idea was that we were going to use these cells pretty quickly as stromal support for transplantation of hematopoietic cells or as gene vehicles for certain genetic disorders. So, we wanted to come up with a "defined culture condition" and try to get away from fetal calf serum as much as we could to try to be able to move into the clinic quickly.

So, we used low glucose DMEM, PDGF, beta-beta, EGF, and then a number of nutrients in the culture system. And 7 to 21 days after we started depleting these cells in culture, we see small clusters, some of them spindle-like

and some of them almost star-like cells that grow out of these cultures.

The frequency in an average college student at the University of Minnesota is about 1 in 2,000 to 1 in 5,000 of the 45 negative glyco-4 neo-negative cells. If you take small children, the frequency is actually higher. If you take people that are 50 years old, the frequency is lower. So, it's anywhere between 1 in 1,000 to 1 in probably 8,000 to 10,000.

We now know that what we grow out of these initial cultures is a very much of a mixture of cells of a large population of cells that are truly mesenchymal stem cells, which are cells that can make cartilage, bone, and fibroblasts and adipocytes, and then we believe a very small subpopulation of these cells which has much more multipotent capabilities. So, we actually called this cell a multipotent stem cell, or actually being a hematologist, usually we call it a multipotent progenitor cell because stem cell has a bad connotation to it.

We don't know the phenotype of the cells up front. We've done extensive phenotypic characterization of the cells once we have sufficient cells to actually find out what they are. The multipotent progenitor cell phenotype -- again, it's not a homogeneous population, but if we have these cells in the population, the cells usually

don't express CD44. They don't express HLA-DR type 2. They express little or no HLA-ABC, the beta-2-microglobulin negative. They express a number of characteristics down here. They obviously respond to PDGF, and so they have the PDGF receptor. They have an FGF receptor, EGF receptor. Interestingly, a small subpopulation of the cells expresses the LIF receptor, and there's gp130 present but not any other members of the gp130 family, such as IL-6 or 11 or CNTF receptor. A small subpopulation of the cells is stained with SSEA4, which is an antibody that recognizes ES cells and EG cells.

Once the cells are grown to confluency, they actually only have mesenchymal cell capacity anymore. And at that point in time, the phenotype switches. They lose this SSEA4 expression and they actually become 44 positive. They remain HLA-DR negative but become HLA-ABC positive and beta-2-microglobulin positive. They also lose expression of the LIF receptor.

If we take these cells and culture them, we can generate up to -- we have now made 70, 80 cell doublings, and that's not shown here.

Initially if we grow the cells and we don't subclone cells, we have a cell doubling of the majority of the cells every 40 to 48 hours. If we go beyond here, and actually over time, we actually grow out a cell population

that grows much, much slower, and the cell doubling looks almost like 96 to 100 hours. At this point in time the cells are actually much more homogeneous. Actually the markers that I showed you on the previous slide that would recognize cells with more multipotent potential become expressed significantly more.

What I didn't bring, we actually have molecular markers in cells that are grown out here. They're very low expressed here, but are expressed forms cells that are maintained for very long periods of time in culture. There are markers that would be found in germ cells just like OCT4, SOC-2, and then obviously the LIF receptor. So, you actually can enrich for these markers if we culture the cells for very long periods of time. We actually get a much more homogeneous cell population.

One of the things we've started to look at is whether these cells have extensive self-renewal potential or extensive growth potential. So, the cells express telomerase. If we culture the cells under the right conditions, which means very low density, and split them on a very regular basis, you can see here that we don't use telomere length after 35 cell doublings. We haven't really gone any further than that yet.

This is a 52-year-old donor. We compared telomere lengths at 10 cell doublings and 35 cell doublings

compared to telomere lengths that we found in lymphocytes in the same donor. You can see that the telomere length is much longer in these multipotent-containing cell populations compared to lymphocytes. These is just a cell line with short telomeres and long telomeres as a control.

Now, how do we need to do this to get these multipotent cells?

First of all, we can't grow them at very high cell density. So, they have to remain under 4 to 5 times 10 to the third cells per square centimeter if we grow them.

We have tried to clone them singly, and we have had a very, very difficult time. So, we actually have been able to get down to 10 cells per row and 1 or 2 cells per row, but we can't do this from fresh bone marrow. So, we actually have to grow the initial cell population and then subclone it at that point in time. I think we can now do it at 2 cells per row, and we're trying to get down to 1 cell per row.

If they become confluent they actually lose this multipotentiality and they lose the longevity. They start losing telomere length. They do no longer express telomerase. As I will show you, they actually don't have the ability anymore to differentiate into even endothelium, which is also a mesodermal cell type.

We tried to repeat this in mouse. Actually in mouse it's even more interesting because we cannot even start with CD45 negative cells. There is something in the rest of the bone marrow that supports the initial growth of the cells. But after you start using full bone marrow, you can actually come up with the same cells that actually have the same cell surface markers and obviously have significant cell expansion. We actually haven't been able to show all the differentiations quite yet.

So, it looks like these cells are present in bone marrow from humans anywhere from the age of 2 to 55, as well as in mouse. In mouse, there is suggestive evidence that they may actually be present in other organs as well and that they may be present in organs outside of the bone marrow.

As I mentioned, if we take these cells, 1 and 2 to up to 10,000 cells, depending on the age of the cells, will be mesenchymal cells mainly and a small population of multipotent cells. If we subclone and actually try to come up with the frequency of multipotent cells, we think 1 in the initial 1,000 cells is a multipotent cell. And by doing sequential subcloning, you can actually increase the frequency. So, we're really working hard to come up with populations that are much purer so we can come up with positive selectable markers, whether it's cell surface or

whether is by genetic markers.

The other interesting thing that we found is that we found that these cells express the LIF receptor. If we now sort up front from fresh bone marrow, based on the LIF receptor, we actually enrich significantly for cells with this characteristic. However, LIF doesn't seem to be required in the culture of the human cells even though it is required in the culture of mouse cells.

I'm not going to go through all the different lineages. I just listed them here. The mesoderm we can divide into splanchnic mesoderm and visceral mesoderm. And mesenchymal cells have been defined as cells that can differentiate in most cell types of the splanchnic mesoderm, so fibroblasts, adipocytes, osteoblasts, cartilage, and skeletal muscle.

The cell populations that we have, whether they are containing the multipotent cells or not, can be induced to differentiate along these lineages. We can get homogeneous cultures of bone, and we can get homogeneous cultures of cartilage, which is articular cartilage, but there is also hypertrophic cartilage in there. So, we're actually, aside from looking at some other cell types, trying to understand why we differentiate the cells too far and actually get hypertrophic cartilage rather than regular cartilage.

If you try to induce muscle differentiation, this does not go spontaneous. So, none of these differentiations do happen spontaneously, except that if you let the cultures become confluent, you get fat and fibroblasts. So, in contrast to embryoid bodies where you get spontaneous differentiation with removal of leukemia inhibitory factor of certain cell types, we do not see that. So, to induce skeletal muscle differentiation, we can either actually use 5-azacytidine or induce the cells short term with retinoic acid and maintain them in the same culture, and we get cells that are myoblastic in characteristics. They form myotubes, so they actually have some of the contraction characteristics of skeletal muscle cells.

Cardiomyocytes. We also don't see spontaneous differentiation into this direction. If we treat the cells with retinoic acid and then FGF and BMP-4, we do get cells that express markers of cardiomyocytes. We have spontaneous areas of contraction in the dish, but significantly less so than what you see when you have embryoid bodies that differentiate. And we actually do not have full proof that we get cardiomyocytes at the end.

If we treat the cells -- and I didn't point this out -- the undifferentiated cells express one of the receptors for VEGF, the Flk1 positive, and so if we treat

the cells simply with vascular endothelial growth factor, we can make it differentiate into cells that express von Willebrand factor and a number of other markers consistent with endothelial cells. If we grow them onto collagen gels, they actually make vascular tubes, and so it looks like we can make these cells differentiate into endothelial cells.

For the lineages listed here, we have data, using retroviral marking, that a single cell can give rise to all of the cells with protein characteristics consistent with the different cell types using clonal insertion site analysis of cells that were cultured initially transduced, and then we can find the same insertion location in all the differentiated progeny.

So, compared to mesenchymal cells that have been described, which make usually these types of cells and possibly cardiomyocytes and smooth muscle cells, if we have more undifferentiated cells present, we definitely can make endothelial cells. And I will get back to that too. There is quite a bit of evidence in the literature that there's, indeed, bone marrow-derived cells that can make endothelium, that can make skeletal muscle, and it can make all of these cell types.

Being a hematologist, if we think about mesoderm, the other mesodermal cell type is obviously

blood. So, based on the data from a number of investigators showing the possible existence of hemangioblasts, cells that are initially Flk1 positive but don't express any markers of endothelium or blood, but depending on how you culture them, you can make them become endothelial cells, meaning CD34 positive that then express von Willebrand factor or hematopoietic CD34 positive cells that do not express von Willebrand factor, we thought that we should at least give it a try and see if we could take the same cultures and induce them to differentiate into something that would have hematopoietic characteristics.

To try to do that, we used a couple fetal feeder layers that were from fetal liver and tried a number of different cytokine combinations. Initially we just analyzed the cultures by molecular markers and looked for things that were present in early hematopoietic cells like GATA-1, cKit, and a number of other things. And we were all excited when we saw that these markers came up, but we haven't really made hematopoietic cells out of these cells in any way, shape, or form.

But when we tried to do that, we kept seeing cells that had morphology that didn't look at all like hematopoietic cells to me. I'm not a neuroscientist but even from back in medical school, some of these cells looked like they had at least the morphology of neural type

cells.

So, in this particular culture, we took these mesodermal progenitor cells, which again weren't homogeneous at all -- this was a mixture of cell types -- and cultured them with a number of hematopoietic cytokines, stem cell factor, vascular endothelial growth factor on this fetal liver feeder that supports murine and human hematopoietic stem cells, AFT024. And these cultures also had EGF. So, that's what we kept coming up with.

We had to go to the literature because, as I said, my lab is not at all neuroscience oriented. So, we actually looked in the literature and found out that EGF and basic FGF are two growth factors that are important in neurogenesis. We know that this feeder makes a large amount of basic FGF.

So, we've repeated some of these studies in more purified conditions and actually used basic FGF together with EGF and then looked over time whether we would again be able to induce differentiation to cells with morphology and markers that would be consistent with neuroectodermal cells. If you do this under defined conditions, 80 to 90 percent of the cells probably died during the initial phases of the culture, and then we get cells to differentiate into cells that express markers that are consistent with neuroectodermal cells, like tubulin-

beta-3, neurofilaments, NSE, and MAP-2. So, the vast majority of the cells under these conditions have neuronal markers, although there's a small subpopulation of cells, usually about 10 to 20 percent of the cells that survive, that have markers consistent with astrocytes and oligodendrocytes. We don't have any functional data on the cells at this point in time.

We confirmed this by PCR and Western Blot and showed again that you find myelin basic protein, GFAP, and neurofilament-200 in the cells that are induced to differentiate, again here with EGF and basic FGF. And we can find at the protein level the same markers to be expressed.

In collaboration with a person at the Neurosurgery Department at our institution, we have started to take some of these cells and implant them into the brain of immunosuppressed rats. We've implanted them mainly as undifferentiated cells. We've taken some of the basic FGF induced cells and implanted them, and they do survive in vivo, but we don't really have much more data than that.

We've put them in undifferentiated. We're not sure what we actually see. We see that cells survive, and these were GFAP-marked. Unfortunately, the way they treated the brain, we've had a hard time getting around the problems with autofluorescence, and so we actually haven't

really been able to use the green fluorescent protein and have had to come in with a secondary antibody.

So, you can see here, these are the cells that were implanted 2 weeks before. These undifferentiated multipotent cells are very large and the cells that you see here are very large too. Interestingly, some of these cells express markers consistent with neural differentiation even though at 2 weeks of time in the brain they're very large still, and we're actually not sure whether this is differentiation or whether this is nonspecific staining at this point in time.

This is 2 weeks after transplantation into the rat brain, nestin staining, and this is 6 weeks after. This is the area where we put the graft. You can see cells with a much more elongated morphology than the cells that you saw on the previous slide. They're nestin positive, but we couldn't prove at this point that these are not rat in origin and cells that were recruited from the normal rat brain into the area where we put the cells in.

Interestingly, though, if you looked at an animal in whom we had caused a stroke and actually asked the question whether implantation of these undifferentiated cells in the brain would have a functional effect, this is shown here. So, this is a sham animal, and this is just one test that was done 6 weeks after implantation of the

multipotent cells. This is limb placement in a sham animal. This is an animal in whom we caused a parietal infarct 2 weeks prior to implantation of the cells infused with saline. This is the medium that comes from the cultured cells. So, there's just a medium from the multipotent cell culture. And this is where we implant the multipotent cells in the brain.

Again, this shows that there is improvement functionally, but this doesn't prove in any way, shape, or form that the improvement is due to neural connections or actually recruitment of cells present in the rat brain to the area because of the implantation of multipotent cells here.

Now, do we have proof that any of these cells are stem cells? In the previous discussion, there were a lot of questions about how long self-renewal has to be. Does it have to be unlimited? Does it have to be for a certain period of time? We have evidence that in the most undifferentiated cells telomerase is present. We don't see shortening of telomeres, so we've only looked really at 35 to 40 cell doublings, but we can grow the cells to 70, 80, or more cell doublings if we grow them under the right conditions, low density and with subcloning.

We have evidence that these cells remain cytogenetically stable at least in 3 of the donors that

we've tested currently.

We have shown multi-lineage differentiation, but to prove that it was a stem cell, we would have to really be able to show this at the clonal level. We haven't been able to sort them singly, but using retroviral markers, we've at least shown that the mesodermal differentiation is single cell-derived. And I can't prove to you today that actually we can get some of these neuroectodermal marking cells also from the same cell derived.

We have no data currently on if we implant these systemically in an animal what will happen to this. We know that they survive and you can find them in different tissues, but we do not know currently whether they will differentiate into the right phenotype. Obviously, that's why we're working on the mouse model, to try to be able to do this in a more rigorous fashion than trying to do human into an immune deficient animal.

So, how does this fit with what we all thought? We all have thought about tissue-specific stem cells present in postnatal individuals, whether it's humans or nonhumans. We've always thought that hematopoietic stem cells were hematopoietic stem cells and mesenchymal stem cells were mesenchymal stem cells and neural stem cells were neural stem cells, and that there wasn't really this

ability within this population to take on other fates.

But over the last two or three years, there is a very significant number of papers that have come out suggesting that there is more plasticity in stem cells than we thought to be present beforehand. What I don't know is whether these cells are committed, dedifferentiate, and redifferentiate, or if there is really a subpopulation of cells that will have this more multipotent potential that would be more similar to that of the multipotent ES cell or embryonic germ cell.

There is the paper that came out two or three years ago now by Ferrari, showing that at least in a model where they cause damage in the muscle, bone marrow cells could contribute to muscle regeneration in the animal.

Richard Mulligan's group has shown that a population of cells that is very quiescent in the bone marrow that contains hematopoietic stem cells, which he calls side population cells, if that is infused in animals that have muscular dystrophy, that these very rare side population cells from the bone marrow, which have hematopoietic potential, can contribute to muscle formation in these animals. It can actually improve the muscular dystrophy in these animals.

The Pittsburgh group a couple of years ago has shown that in a mouse transplant model, that cells that

were transplanted with bone marrow into an animal can contribute to liver regeneration. This has been confirmed by a group from Yale and New York both in mouse and possibly in the humans.

Irv Weissman and Marcus Grompe have the same data. If they use a, quote, hematopoietic stem cell population for mouse that is fairly well phenotypically defined, that these cells not only can reconstitute the hematopoietic system, but may also be able to reconstitute the liver.

There's a number of groups that have shown that bone marrow can contribute to endothelium. As I mentioned in the beginning, Dr. Prockop's group, Dr. Kopen and his group have shown that if you implant mesenchymal cells in the brain of a rat, that you have cells with astrocytic characteristics.

And my lab has shown that, again, the cells at least have the phenotype of neuroectodermal cells. Whether they function as neuroectodermal cells is still a question.

The other cell source that has been looked at is Peggy Goodell who has shown that SP cells from muscle can actually differentiate in blood.

Then there is a number of groups who have suggestive evidence that neural stem cells might actually have the ability to differentiate into hematopoietic cells.

So, the question really will be is there plasticity, and can bone marrow become endothelium? And is it actually a cell that switches its genetic makeup and becomes another cell, or is there a small subpopulation of cells that can be identified phenotypically and by genetic markers that is a multipotent cell that is a descendent somehow from ES cells or embryonic germ cells that is present in multiple different organs, and if you are able to select the mouse and put them back into the right environment, can take up the fate of that environment? So, it goes it round and round that it is indeed a subpopulation of cells and most organs that have this pluripotent potential is still a question.

So, since this is a neural stem cell oriented meeting, what is the evidence that marrow cells can become neural cells? There is, I would say, currently soft evidence that that might be possible. As I mentioned, Dr. Prockop's group has shown cells with astrocyte morphology and astrocyte markers and possibly even neural markers that can be derived from mesenchymal cells derived from humans. And our lab has the same evidence possibly in vitro as well as in vivo.

But I don't think there is any data currently to indicate that we go through the regular neural cell development. We have cells that stain positive with

nestin, but we don't know whether there is a neural stem cell-like cell derived from multipotent cells or whether the differentiation is actually correct.

We obviously have no data whatsoever at this point in time that these neuroectodermal-like cells have functioned as such cells.

So, I think in this area the biggest question really is going to be to try to, first of all, come up with a much more better defined cell population. Currently a lot of laboratories are using plastic adhesion, which is a fairly crude method to purify the cells. What we did initially was CD45 depletion, and that is a very crude method too. The frequency of the cell that has this multipotentiality is extremely low in there.

So, a major effort in multiple labs is ongoing to try to come with positive selectable markers, because they're cell surface markers or gene trap methods to try to come up with a more purified cell population such that we can actually evaluate the cells much better and actually define exactly whether a single cell has this differentiation potential.

So, there is really very little known about cell surface markers. There's very little known about the expressed gene profile. Currently we really are depending on functional definitions, which means that you have to

grow the cells for 6 months in the laboratory and be able to show that you get lineage differentiation from a single cell. But actually coming up with these markers will be of extreme importance to try to really nail down what we have.

There is some suggestive evidence that cells might be able to differentiate into neuroectodermal cells, but that will need to be nailed down much more extensively before these cells could be used in a clinical setting.

The studies that I showed you on the functional recovery of some of these animals are consistent with some of the studies that Dr. Prockop has where he also shows functional improvement after implantation of these cells in the brain, but it could essentially be that you implant the cell that produces certain cytokines that recruit local stem cells or progenitor cells in the brain itself that then ultimately make functional improvements.

Once we have nailed down the cell, then the same questions will come up as what came up with the embryonic stem cells and embryonic germ cells: At which point do you then use these cells?

We have given mixtures of cells to now SCID mice and looked at them 6 to 8 weeks later and haven't seen teratomas. The cells we injected, though, knowing what we know, back then had very few of these multipotent cells present. So, I think if we get better at purifying the

multipotent cells, we may see incorrect differentiation as has been shown for embryonic stem cells, although currently we don't really have any data to suggest that. We haven't really seen any teratomas being formed.

But if you think about a cell that has the potential to make bone and cartilage and to use that cell and take it undifferentiated and implant it in the brain or any other organ, it may not be something that you want to do. There may be some problems with even more primitive cells like ES cells and embryonic germ cells to do that.

So, I think these questions will only be able to be answered once we have a better handle on what these multipotent cells that are present in individuals or animals have as characteristics.

I think that's it. I'll stop there.

(Applause.)

DR. SALOMON: Yes, please.

DR. KOLIATSOS: Thank you.

Dr. Verfaillie, your approach raises acutely the issue of who is what and who becomes what in this field. In particular, the first point I would like to raise is that I would be very careful, very cautious before I characterize any type of immunoreactivity in the brain, be it GFAP or nestin or what have you, as originating from the cells you put in the brain, unless if you use dual

labeling procedures or have wonderful cytological and morphological evidence. I don't know if these are the cells you put or the reactions of the brain to the cells you put or, more importantly and perhaps more interestingly, induction of indigenous neurogenetic gradients and other processes by the cells you put in the brain. We have a tendency to focus too much on what our cells do and we don't forget that we may change fundamentally neurobiology by putting these cells, be they hematopoietic or neural stem cells, in the brain.

DR. VERFAILLIE: Yes, I fully agree with that. I think I was trying to be very cautious in trying to say what we have at this point in time. We have cells that have these markers, but we don't have dual labeling. The main reason is we're actually doing everything over with cells that haven't been GFAP-labeled because that seems to be a major problem in us trying to find out. So, we're planning to either use BrdU-labeled cells or specific human antibodies to try to double label and prove that the transplanted cells are indeed the cells that have these markers.

The cells I showed you were 2 weeks after transplant.

DR. KOLIATSOS: Which is not trivial to do, by the way.

DR. VERFAILLIE: Right, I know. So, that's one of the reasons why we don't have it yet.

(Laughter.)

DR. KOLIATSOS: The second point I'd like to say is that here we tend to be very particular about what constitutes a germinal cell or an embryonic stem cell. Having a neuroscience background, I would like not to be less strict in how I define a neuron. Neurons are cells that make all these wonderful phenotypic markers and at the same time make connections in the afferent and efferent sense and also generate, propagate potentials and transduce electrical into chemical in the synapse. And for our patients in particular, I would like nothing less but our cells, whatever we put into brain, to eventually become, if they are going to be used as neurons, to become neurons with all the features of neurons.

DR. VERFAILLIE: Correct. That's right. I fully agree there too. I think we have data to suggest that proteins get turned on that are consistent with T-cells, but we have no functional data. Even though we saw functional improvement, I would be the last one to say that this is due to a nexus between the new cells and the host brain cells. It might well be cytokines being produced by the undifferentiated cells you place into the brain.

DR. SAUSVILLE: So, to pursue that thought from

our questioner, do you therefore recommend or do you think we should consider actual assays of function for particular uses? Because if you pursue your argument, a substantia nigra neuron is not the same as a caudate neuron is not the same as a spinal cord neuron. So, how would you address the uses, or do you think we should be addressing potential uses with respect to the functions we would assay?

DR. KOLIATSOS: The answer is in some form, yes. It depends on the clinical question. It depends on the context. But you need certainly more information, and certainly you need to have an idea what generates your functional advantage or benefit. Is it the cells you put or what you've done to the host tissue? You have to have some measures to figure those things out because I believe they have clear implications in terms of longevity of the treatment approach, complications, et cetera, et cetera.

The argument here again is that I believe I have the sense that here we're facing a dark area, a new biology which needs to be defined in a very fundamental, basic way, not what the cells are, but what also they do to the entire circuitry and whatever is going on in the brain. It's a totally new area and needs to be treated as such.

DR. MACKLIS: If I could simply reinforce that comment, I would argue not just in some cases yes, but I'd say absolutely yes, that we have to figure out the disease

process we'd like to reverse and whether that can be reversed simply by a neurotransmitter production locally as a mini pump versus rebuilding circuitry, and that we need to look at that anatomically and functionally. Also, individual markers may not at all define a neuron. In fact, a cell may express a neuronal marker and might express a glial marker at the same time.

DR. GAGE: Did you say that your cells make FGF?

DR. VERFAILLIE: No, they do not make FGF. They're responsive to FGF, but they do not make FGF. Actually we looked at it. When they are treated with FGF on the date and start making it, I don't really know that.

DR. GAGE: Do you know what other cytokines are made by --

DR. VERFAILLIE: In the undifferentiated cells, we find KGF, VEGF.

DR. GAGE: Is VEGF secreted? Do you know if any of these are secreted from the cell?

DR. VERFAILLIE: We don't know if it's secreted, but they do make it. They make keratocyte growth factor. They vascular endothelial growth factor, and I'm blanking on the third one that we've actually found to made at the RNA level and protein level. We don't know the amount that is there. We haven't looked whether they make

BDNF or GDNF or any of the other neuronal growth factors and cytokines. So, we haven't really looked at that.

DR. SALOMON: Have you looked at angiopoietin, by the way? That came up in a gene screen in Science a couple months ago in CD34 stem cells.

DR. VERFAILLIE: We've done several arrays and I can't remember whether angiopoietin is on there. I would have to look that up. I don't know. But they definitely do make VEGF.

DR. GAGE: So, in a general sense, ideally in neurobiology we'd like the cells to differentiate into competent cells for the local environment and reconstitute the area. But maybe another goal we should be at least aware of or attuned to is that some of these cells might in and of themselves provide local factors which could have some reparative properties locally. We're sort of casting this off as an artifact of the cell and, in fact, maybe in some cases that's exactly what we'd like the cells to do. If we can learn more about what the cells do and differentiate them down lineages where they persist, perhaps in an undifferentiated state, but secret factors which can induce repair, that should be or could be a target for utilization as much as a completely differentiated cell which may be even a more difficult task to achieve.

DR. MACKLIS: I didn't mean in any way to cast that aside. It was more that I think we should have that as the goal. Oh, in this circumstance, we'd like these cells to make X and Y factor and prove that they do that. Or maybe it will be in the upper left. It will be -- whatever it was -- unknowing competence, that maybe we'll just find it out serendipitously and then we'll figure that out.

DR. SALOMON: Dr. Prockop?

DR. PROCKOP: I'd like to congratulate Catherine on summarizing a huge amount of data very beautifully for us here. This is a new area. There are many very big questions that we really can't speak to.

But on one point, I think the data are quite good that at least some of these cells take on the characteristics of astrocytes in the central nervous system. Don Phinney and Ausim Azizi have been able to do experiments with double labeling with BrdU and markers. Ausim Azizi, who is here, has actually been able to grow out astrocytes and infusing human astrocytes in rat brain and grow out human cells that have the morphology of astrocytes and stained for GFAP.

That doesn't prove functionality, but coming back to Fred's point there, our thinking about these cells is, yes, maybe as wild type cells in the brain, they may do

nice things. They may repair the brain. We don't know that. There are many big questions to be answered. But I would submit that they really last a long time and they migrate and integrate in the brain.

So, one appeal to us is just vectors for the kinds of growth factors that might be helpful in one condition or another. They do last long periods of time. Ausim Azizi has been able to recover them after 6 months, even human cells in rat brain, but we of course prefer to stay with rat cells in rats and so on.

But I think the potential is very broad with a lot of big questions still unanswered.

DR. DRACHMAN: The other side of that is where we put those cells. Why did the, say, hippocampal neurons die to begin with? We are hoping or making the assumption that all we need to do is put in at least pluripotential cells. The circuitry will be regenerated, but we're putting it into a microenvironment that no longer supported the cells that were there to begin with. What is our belief or what is our faith that whatever is needed to redirected even wonderfully defined, really pluripotential cells or cells that have gone down a further pathway that whatever microenvironment it is needed in order to reestablish circuitry exists in the host?

DR. VERFAILLIE: Well, I would like to answer.

I don't know anything about circuitry in the host. I think in other organs, for instance, the data on bone marrow cells repopulating endothelial cells happens in areas where there is damage to the endothelial cells. So, there is obviously the cues locally to make primitive cells from bone marrow become endothelial cells. The data that is out there on bone marrow cells differentiating into skeletal muscle would suggest the same thing.

So, there's something about a damaged microenvironment, at least inflammatory damage, that causes that. Well, muscular dystrophy that might be degenerative -- you can maybe that under the same category. But that wasn't in a transplant setting. So, there was, again, probably inflammatory damage done first for the cells to get there.

DR. DRACHMAN: Yes. That makes perfect sense, but we're talking about things like Alzheimer's disease where something has gone wrong that has enabled cells to die. So, that's a little bit different.

DR. CHAMPLIN: There's precedence for other organ systems in hematopoietic transplants where we transplant stem cells to treat hematopoietic diseases. Aplastic anemia is the classic example of a disease we can treat by just giving more stem cells, at least in some patients. Some patients have stem cell injury from a virus

or something, and if that virus is then gone, you can then reconstitute hematopoiesis with stem cells. On the other hand, if they have an autoimmune disease that's ongoing, just giving stem cells will not work.

So, in the analogous situation, if you have a neural disease where there is an active disease process that will affect the new stem cells, of course they wouldn't work unless you could overcome that underlying process. If it is a degenerative disease or if it is a toxic injury that is no longer present, then perhaps stem cells would be successful in and of themselves.

DR. RAO: I think there are two reasons to be hopeful. One is that there's been a lot of recent data which suggests that there's ongoing neurogenesis. We also know from a lot of data in the past, there's ongoing synaptogenesis. So, we know that there is remodeling of circuits and connections which are being made and that these are being made all stages of development. So, cues exist.

The other, I think, positive note to remember is that a lot of the neurodegenerative diseases are very slow and ongoing processes which take decades to appear. So, if you could provide a reasonable number of cells, even if they responded the same in cells, the likelihood of seeing symptoms would be long enough that there would be

therapeutic value. Does that make --

DR. DRACHMAN: Not really.

(Laughter.)

DR. DRACHMAN: That's a little bit different. That's not quite the point. The point isn't whether we supply viable cells, but whether the microenvironment to redirect them exists at that point. That's different. Those are host factors that are really important where circuitry is key. That may not be so with Parkinson's disease where really you're looking for a dopamine pump, to be crude.

DR. RAO: No. What I was pointing out is that there is ongoing synaptogenesis and ongoing circuitry involvement or control. So, in that normal environment, irrespective of whether you put in cells or not, there are signals which are directing synaptogenesis and growth of cells, and that's been clearly evident.

DR. DRACHMAN: Synapse is really one of the keys of things like Alzheimer's disease.

DR. RAO: And that's a slow, ongoing process which takes over 25 years perhaps to happen. Therefore, even if you transplanted cells and they integrated, it might take 25 years before you get Alzheimer's again.

DR. DRACHMAN: It's worth a try.

DR. KOLIATSOS: With all due respect, I

disagree. I really disagree. I think this point actually is probably irrelevant because at the time you're called to do the treatment, the patient already has reached a threshold. Unless if you have biological markers to predict when somebody starts having Alzheimer's or ALS 10 or 20 years ahead of time, this point is irrelevant. Unfortunately, when we're going to be called upon to put the cells, the patient is already symptomatic. So, there are plaques forming. There is cell death going on. The whole environment is totally different and has been different for decades.

DR. MULLIGAN: I just wanted to get back to the question of mechanism. It was reminiscent of a lot of the gene transfer work in the past where there has been, I think, the continued argument about how much do you need to know about mechanism. If it works, go for it. I think that has led to some difficulties.

Here I think the FDA has asked us to think about the question of how much preclinical work is necessary, and I think we're going to get right back to that very issue of when you implant neural stem cells, is it injury, is it really cytokines, is it forming the right connections? I think that we're going to have to think very, very carefully about how much we do want to know.

A good case in point is, I'm reminded by, the

TK suicide gene therapy that people worked on where there is very clear mechanism thought to be accounting for the preclinical success, which I think in time proved clearly not to be the mechanism. And this led to a very large, costly pursuit that is of questionable ultimate value.

In this case here, I think you really have to ask the question how useful would it be to know that your neural stem cells are releasing several growth factors, and that's the only thing that accounts for the success because then you might find there are better vehicles, there are better cellular vehicles to release those growth factors.

Therefore, I'd like to hear, as a non-expert at some point during the day, in any case where there is therapeutic effect, how much do we actually really know about whether connections were made, what cytokine is released, and whether you can get the same effect by poking the needle in another direction or just poking a cytokine.

DR. DRACHMAN: One other question. You mentioned that VEGF is produced by these cells. Will they grow as well without VEGF, do you know? Have you blocked it all? That's a question that one might really wonder, the need for endothelial growth factors in order to maintain this sort of function. Do you have any thoughts on that?

DR. VERFAILLIE: We haven't really looked at

trying to block that. So, this data is the last month's worth of data, that the VEGF is there. So, we haven't really been able to get around to trying to block whatever is being made. So, they have the receptor for Flk1 on the cell surface. Theoretically they can respond to the growth factor they make themselves.

We don't get endothelial differentiation unless we take away the other two growth factors, EGF and PDGF, and add large quantities of VEGF. But it might well be that they are maintained based on an autocrine route almost of lower levels of VEGF. I don't really have an answer.

DR. SAUSVILLE: So, one question I have -- and I guess this would be to Dr. Drachman. One clearly appreciates the concern that the microenvironment into which these cells are being introduced might be damaged as a basis for the original pathology. Nonetheless, I'd be interested in your thoughts and a comment as to one use of a safe product that we are called upon to advise the FDA in the definition of as actually defining some of these pathophysiologic states as a result of clinical trials. So, would you require that we actually know this microenvironment before we actually cut off the potential use of a tool to define the disease mechanism?

DR. DRACHMAN: No way you're going to know that. You're hoping that you will know about thousands of

guidance factors that might be critical for forming the circuitry. The answer is no. We're wondering here whether, if we put clay into this setting, we'll get a nice sculpture.

(Laughter.)

DR. DRACHMAN: That's one way of thinking about it. We're hoping the sculptor remains there to do the job. Otherwise, it's just clay.

DR. SAUSVILLE: I hope we could do that control somewhere.

(Laughter.)

DR. KOLIATSOS: Actually can I make a comment on that? This can have a little bit more specific answer. For example, there could be a legitimate concern that if you put cells in an Alzheimer's brain, you can have more amyloidosis either via the cells themselves or via the secondary inflammatory processes. You can use animal models to test that. Some of these questions can be tested. You can take the familial Alzheimer's disease transgenic mice. It would be a wonderful model to test the amyloidogenic potential, but many other issues of guidance and recapitulation of development probably will not be addressed.

DR. SALOMON: Can we have one brief question and then I think we have to go on to the break.

DR. AZIZI: I'd like to make a couple of comments. Ausim Azizi from MCP Hahnemann.

I sort of heard the fact of how much we don't know about these cells, and that's very true. But we don't know much about neural stem cells either. We can sit and take pot shots for things that experimentally have not been proved. But the thing that has been forgotten throughout the whole thing is an important issue of how useful these cells could be.

For example, these could be aspirated from the patient's own bone marrow. It can be grown, as Dr. Prockop pointed out, to multitudes of 10 to the 14 in 8 weeks. You don't have to go to pig, you don't have to go to transgenic animals, you don't have to go to embryos. And it comes from the patient's own marrow. If it could be even the slightest bit useful for treatment of any of the neurologic diseases, I think we still have positive things.

So, those are the two comments I would make. Thanks.

DR. SALOMON: Well, then my notes of this very interesting discussion, which I'm artificially cutting short just because I think it's time to go to a break and we've got some more time to talk about it, three sort of defining things came out that I got.

One was that defining the lineage and function

is going to be critical before concluding a cell is a neuronal cell or a neural stem cell for a clinical trial and that simple marker analysis may not be enough.

The second was that transplanting a cell that produces reparative factors, such as growth factors, but possibly what we consider now pro-inflammatory cytokines, could be another benefit in addition to just becoming neural stem cells. I think that came out quite clearly.

Third, I think this whole issue of microenvironment in the transplant site has been very clearly articulated. The idea I think stands that if there's enough injury in a site proposed to be a transplant site, due to the primary illness, you may not have the signals any longer. I think that is an important question that needs to be addressed perhaps for each setting and may have a lot to do with at what point in a disease progression you should be doing these sort of clinical trials and maybe points where you have gone too far and shouldn't be doing it. So, I thought that was really excellent.

There's one other theme here that I hope we'll come back to and that is if it's in the bone marrow, is it just the fact that, hey, it was easy, I could find it there and we tend to think about stem cells in the bone marrow, but there's no really overwhelming, total physiologic