

FEDERAL FUNDING FOR STEM CELL RESEARCH

HEARING BEFORE A SUBCOMMITTEE OF THE COMMITTEE ON APPROPRIATIONS UNITED STATES SENATE ONE HUNDRED EIGHTH CONGRESS FIRST SESSION

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THURSDAY, MAY 22, 2003

U.S. SENATE,
SUBCOMMITTEE ON LABOR, HEALTH AND HUMAN
SERVICES, AND EDUCATION, AND RELATED AGENCIES,
COMMITTEE ON APPROPRIATIONS,
Washington, DC.

The subcommittee met at 9:47 a.m., in room SR-418, Russell Senate Office Building, Hon. Arlen Specter (chairman) presiding.
Present: Senators Specter and Harkin.

OPENING STATEMENT OF SENATOR ARLEN SPECTER

Senator SPECTER. Good morning, ladies and gentlemen. The Appropriations Subcommittee on Labor, Health and Human Services, and Education will now proceed. This morning's hearing is on the subject of the determination as to whether the current stem cell policy is adequate on moving research toward cures of so many maladies, and we are going to be looking into the adequacy of the existing stem cell lines.

Until Monday of this week the conclusive information, consistent information, presented to this subcommittee and more broadly was that there were insufficient stem cell lines and that they were contaminated with mouse feeders. On Monday I was informed by staff that Dr. Zerhouni had just called to say that he believed there were stem cell lines which were not contaminated with mouse feeders, which was more than a surprise; it was a shock.

I sat down with Dr. Zerhouni on Tuesday afternoon to go into the matter in some greater detail. The reason that the issue was so startling was that this subcommittee has been consistently advised that the eligible stem cell lines have all been grown with mouse feeder cells. That information was given to us, by the Secretary of HHS Thompson; Dr. Alan Spiegel, the director of the National Institute on Diabetes, Digestive, and Kidney Diseases, which has principal responsibility on the stem cell issues; by Dr. James Battey, chairman of the NIH Stem Cell Task Force, all from NIH, and from other scientists as well—Dr. Roger Pederson from Cambridge, Dr. George Daley from MIT.

There is a considerable body of additional authority for the proposition that the existing cell lines, stem cell lines, are contaminated with mouse feeders, but I will not take the time to go into them now. We had a delay of about 15 minutes in commencing this hearing because the President addressed the Members of the Senate and the House on a meeting which was just scheduled late last

night, and he just concluded a few moments ago. I came directly from that meeting in the Capitol to this, to this hearing.

There is concern about the—putting this is somewhat delicate, but—the objectivity of the information which this subcommittee is getting and whether there is a politicization of the process. This subcommittee had a direct confrontation with Secretary Thompson when we sought information some time ago about stem cells and the information was requested from all of the directors and the information was edited before it came to us, which was out of line. And the subcommittee is concerned as to what is happening on a continuing basis.

The disparity between funding an NIH for human embryonic stem cells and adult stem cells raises real questions, to put it without hyperbole. Stem cell research has \$10.7 million in 2002 and \$17.1 in 2003. Adult stem cell research has \$147.6 million in 2002 and \$155.7 million in 2003.

There have been very strong reasons advanced for additional stem cell lines: first, genetic diversity; second, proper comparison of lines grown using mouse feeders and lines grown without mouse feeders; and third, the need for lines grown without feeders for use in treatment.

This is the 16th stem cell hearing which this subcommittee has had and we have focused a great deal of our time and energy on the subject because we think it is so important. It is very important that we get the scientific opinions without politicization, without shading. These issues are too important.

The President responded by allowing some 63 lines in his speech at 9 o'clock on August 9, 2001, after some 76 Senators had expressed themselves in favor of Federal funding on stem cells. The hands of the scientists should not be tied in any way. We ought to be finding out what the facts are and proceeding on them, and this subcommittee intends to do that. We have great respect for the new director, Dr. Zerhouni, but we intend to find out what the facts are.

STATEMENT OF ELIAS ADAM ZERHOUNI, M.D., DIRECTOR, NATIONAL INSTITUTES OF HEALTH, DEPARTMENT OF HEALTH AND HUMAN SERVICES

ACCOMPANIED BY JAMES BATTEY, M.D., DIRECTOR, NATIONAL INSTITUTE ON DEAFNESS AND OTHER COMMUNICATION DISORDERS, NATIONAL INSTITUTES OF HEALTH, DEPARTMENT OF HEALTH AND HUMAN SERVICES

Senator SPECTER. Our first witness is Dr. Elias Adam Zerhouni, the 15th Director of NIH. Prior to becoming director, Dr. Zerhouni has had an extraordinarily distinguished career: executive vice dean of Johns Hopkins, received his medical degree from the University of Algiers. Dr. Zerhouni is accompanied by Dr. James Battey.

Dr. Zerhouni, the floor is yours. We are looking forward to your testimony.

Dr. ZERHOUNI. Thank you, Mr. Chairman. I am pleased to appear before you and I will make my comments short as the full testimony is available for the record.

Senator SPECTER. Your full statement will be made a part of the record without objection.

Dr. ZERHOUNI. I am pleased to testify for you about the progress we have made over the past year and since the last hearing on human embryonic stem cell research. There are more than 60 investigators at 48 institutions that have received NIH awards and are working on embryonic stem cell research. There are 78 lines that are fully eligible for Federal funding in various stages of development.

In the last year alone, NIH support has helped increase the number of widely available lines to any researcher from one in April of 2002 to five at our last hearing in September to 11 today, and more are being developed and will be available in the near future.

I can report to you that we are as diligently as possible implementing the policy promulgated by the President August 9, 2001, which has enabled the field of embryonic stem cell research to advance. Prior to that date, no funding had been spent on the field of embryonic stem cell research and we are trying to accelerate funding as fast as we can over the past 18 months since we have developed our strategy at NIH to support this field.

So we continue to acquire new knowledge about human embryonic stem cells at a rapid pace. Recently, NIH-supported researchers have succeeded in replacing a stretch of DNA within a stem cell and this is a very important advance as it opens the door to scientists who want to study the function of specific genes and also provide a way to modify derived tissues for use in treating patients.

Scientists are currently working to identify those genes that are involved in the differentiation of human embryonic stem cells as well as those genes that permit embryonic stem cells to self-renew, and this is an important scientific prerequisite for progress to be made. Until recently, all human embryonic stem cells were expanded after derivation on mouse feeder layers.

Now scientists are discovering and establishing conditions that allow these cells to grow in the presence of human feeder cell layers. NIH-supported scientists in the United States, using approved NIH-available cell lines, tested the ability of human feeder cells to sustain these lines and are now learning as fast as they can the molecular signals that will allow us eventually to expand and grow human embryonic stem cells without any feeder layers.

Since I arrived at NIH about a year ago, I have been working hard to promote stem cell research based on recommendations received from the research community and by the NIH stem cell task force, which I established in August 2002, with scientists from within NIH and from the extramural community.

The newest effort, for example, is the establishment, after recommendation of the NIH stem cell task force, of an NIH characterization unit at NIH under the direction of Dr. Ron McKay. This unit will be located on our campus in Bethesda and this unit will provide what is missing right now in the field of embryonic stem cell research, reliable, standardized data derived from assays performed on human embryonic stem cell lines, so that we can make available for widespread distribution lines that are fully characterized so that scientists can compare results across experiments.

Again, I want to assure the committee of NIH's commitment to pursuing embryonic stem cell research vigorously, as well as continuing our advances in the field of adult stem cell research. The President's policy has provided us the opportunity to be at the forefront of new discoveries about stem cells and we are exploiting it as fully as we can.

As I said, over the past year I have been actively working on providing open discussions and open access to all the scientists who have ideas about how to promote the field. I have established processes to do so.

PREPARED STATEMENT

In echoing your own statement, Mr. Chairman, at my confirmation hearing I stated the fact that it was very important for the NIH director to be factual and provide accurate factual information to the maximum extent possible at the time needed to inform policy decisions, and this is what I will do.

Thank you, Mr. Chairman.

[The statement follows:]

PREPARED STATEMENT OF ELIAS ZERHOUNI

Mr. Chairman, Senator Harkin, and Members of the Subcommittee, I am pleased to appear before you today to testify about the progress of human embryonic stem cell research. In fiscal year 2002, NIH spent approximately \$11 million for human embryonic stem cell research to increase the availability of stem cell lines for federal research, train scientists how to use these technically-challenging cells, and conduct basic, pre-clinical research that represents the first steps toward understanding how stem cells might be used to treat injuries and diseases.

More than 60 investigators at 48 institutions have received NIH awards, including 14 investigator-initiated grants and 44 administrative supplements. The administrative supplements allow investigators to rapidly incorporate research on human embryonic stem cells into their ongoing work. As you know, there are 78 lines fully eligible for Federal funding, in various stages of development. NIH support has helped increase to 11 the number of human embryonic stem cell lines that are widely available for all researchers. More lines will become available in the future, as we help the scientific community capitalize on this opportunity. I can report to you today that NIH's implementation of the policy set by the President on August 9, 2001 has enabled the field of stem cell research to advance. We continue to acquire new knowledge about human embryonic stem cells (hESCs). Some of the significant discoveries include the following research findings.

- NIH-supported researchers at the University of Wisconsin recently succeeded in replacing a specific stretch of DNA in human embryonic stem cells. This technique, called homologous recombination, opens the door to scientists who want to study the function of specific genes within these cells and also provides a way to modify hESC-derived tissues in a very precise matter for use in treating patients.
- Scientists at NIH have been able to demonstrate that differentiated mouse embryonic stem cells can be directed to become specialized cells in order to repair damage when transplanted into the brain or spinal cord. This finding could lead to the development of replacement therapy for cells that are destroyed through injury or disease, such as stroke, Parkinson's disease or Alzheimer's disease.
- In vitro studies have produced more specialized cells from human embryonic cells that might be used for blood cell transplantation therapies for patients with blood malignancies such as leukemia or myeloma.
- Scientists are currently working to identify those genes that are involved in the differentiation of hESCs, as well as those genes that permit embryonic stem cells to self-renew. This knowledge, along with research involving gene transfer techniques, potentially will allow scientists to coax hESCs into becoming insulin-producing beta cells to treat insulin-dependent diabetes.
- Until recently, all hESCs were grown on mouse feeder layers. Now scientists are establishing conditions that allow hESCs to grow in the presence of human feeder cell layers. NIH-supported scientists in the United States, using stem

cells eligible for federal research, have tested the ability of human feeder cells derived from fetal or adult tissues to support the growth of human embryonic stem cell lines. Both fetal and adult human feeder cells were able to support and maintain the cells in an undifferentiated state. Also, we have seen published research on the existence of one cell line, developed in Singapore, that was created and developed using human feeder layers. However, the Food and Drug Administration has informed NIH that, given the complexity of this area of research, it is difficult to predict whether newly derived human embryonic stem cells grown exclusively on human feeder cells would result in clinical trials sooner than the existing eligible cell lines either grown exclusively on mouse feeder cells or adapted to human feeder cells.

At the same time, we continue to learn more about other types of stem cells, including adult and those derived from umbilical cord blood.

- An NIH-supported researcher at the University of Minnesota isolated multipotent adult progenitor cells from human bone marrow. These cells demonstrate the potential to differentiate beyond bone marrow stem cells into other cell types, including liver, neurons and blood vessels.
- In a laboratory of the National Institute of Dental and Craniofacial Research, NIH intramural scientists have recently characterized a population of stem cells found in the dental pulp of deciduous, or “baby” teeth. These stem cells have the potential to become cells expressing molecular markers characteristic of dentin, bone, fat and nerve cells and may provide an accessible source of stem cells to repair damaged teeth, regenerate bone, and treat nerve injury or disease.
- Scientists established a number of years ago that umbilical cord stem cells can repopulate the bone marrow of a small child. Umbilical stem cells can be used today to treat certain childhood disorders such as Fanconi’s anemia. With the current technology, however, these cord blood stem cells can only be harvested in small numbers, which limits their clinical utility. We are seeking methods to expand these cells in the laboratory to generate very large numbers of the cells needed for many other clinical applications.

Human embryonic stem cell research is still in its nascent stages, and there are many basic research studies that will be required before we can begin to plan clinical trials. NIH is supporting preliminary research to understand how to direct differentiation along specific pathways, to establish techniques for isolating specific cell types, to control cell proliferation, and to control interactions between the host immune system and transplanted cells that might mediate graft rejection.

Research using hESCs offers the potential to inform us about the earliest molecular and cellular processes that regulate normal development, and provides a tool to discover how a cell is able to be both pluripotent and indefinitely self-renewing. In addition, research using hESCs will help the scientific community to understand the molecular signals that specify differentiation into specific cell types, some of which may ultimately be useful for cell-based treatment of disorders, such as Type 1 diabetes or Parkinson’s disease, that involve loss of a specific cell type.

As we continue our work with the research community to fund new research and facilitate the availability of additional stem cell lines, the NIH Stem Cell Task Force is continuously and vigorously evaluating the state of the science to lead the implementation of a vigorous research program envisioned by the President. Attaining basic knowledge about the characteristics and potential use of stem cells remains the immediate challenge before the research community today. Until we understand the basics, we cannot know with certainty the future research requirements for advancing into clinical trials using embryonic stem cells. The NIH will continue to monitor the state of the science and assimilate the body of research evidence in order to make informed, evidence-based recommendations on this important issue.

We are working hard to promote stem cell research, based on recommendations received from the research community by the NIH Stem Cell Task Force. The newest effort is the establishment of the NIH Characterization Unit, located on our campus in Bethesda, Maryland. This unit will provide reliable and standardized data derived from assays performed on human embryonic stem cell lines available for shipment to the research community. The unit will provide a direct side-by-side comparison to be made among the cell lines, and will facilitate comparison with adult stem cells. These data will be publicly available and will arm the scientific community with state-of-the-art information, so scientists can make an informed choice when ordering one or more of the available cell lines. In response to additional recommendations from the research community, we continue our efforts to recruit new scientists to the field, to help mid-career investigators begin studies on embryonic stem cells, to monitor the state-of-the science through the NIH Stem Cell Task Force, to fund investigator-initiated grants, to disseminate information about

the science and initiatives via the NIH Stem Cell Task Force website and to plan for a symposium that will bring together two hundred stem cell researchers from all over the country and several foreign universities.

Again, I want to assure the committee of NIH's commitment to pursuing embryonic stem cell research, as well as continuing our advances in the field of adult stem cell research. The President's policy has provided us the opportunity to be at the forefront of the latest groundbreaking discoveries in the culturing, characterization and differentiation of stem cells, and I am confident that NIH will keep its premier place in this field for years to come.

Senator SPECTER. Dr. Zerhouni, this subcommittee has repeatedly requested that NIH recommend a non-NIH scientist to testify in support of your position that additional stem cell lines are not required. But NIH did not recommend a non-NIH scientist. Was that because you could not find one who would support you?

Dr. ZERHOUNI. Senator, I was not actually aware of this particular request.

Senator SPECTER. Dr. Battey, are you aware of the request?

Dr. BATTEY. Yes, I am.

Senator SPECTER. Why didn't NIH submit to this subcommittee a non-NIH scientist to back up the NIH position? Could you not find one?

Dr. BATTEY. We knew of no individual who was willing to testify.

Senator SPECTER. Dr. Zerhouni, why is it that so many authoritative spokesmen for NIH have told this subcommittee that all of the eligible stem cell lines were grown on mouse feeder cells, such as the Secretary himself, Dr. Spiegel, Dr. Battey, and on September 5, 2001 Secretary Thompson stated that categorically. On September 26, 2001, in response to my request NIH prepared a paper entitled "The Development of Embryonic Stem Cell Lines" and, among other things, concluded, quote, that "Although a major focus of their work at present"—this is referring to Goteburg—"is to develop a culture system that is free of mouse feeder layers, this has not yet been applied."

Why is it that the subcommittee was informed that all of the existing lines were on, developed on mouse feeders, until Monday of this week when you contacted staff and Tuesday when you had the meeting with me?

Dr. ZERHOUNI. Well, I was not there to know exactly what happened at NIH during those periods of time, but I take your point. There are inconsistencies in the responses that you have received over time. After I asked myself issues related to the new findings of human feeder cell layers being supportive of growth of human stem cells, that finding which was reported by Johns Hopkins scientists was important enough in my mind to make sure that we had a review of the field. Prior to that date, there was no other technique used to grow stem cells besides mouse feeder cells.

So it was very important, I thought, that we made sure we had a complete inventory. But I understand your point and I have reviewed those statements and your staff actually provided me with some of those. I cannot explain why. I can only tell you one thing, which is that I am absolutely committed to providing you with the most accurate information at the time it happens. This field is fast evolving. It is a new field. We have only been funding this field for less than 18 months, and this is the commitment I have for you. And I will be happy to be on the record reviewing the entire data

set and provide you information on the record, Senator. But I take your point.

Senator SPECTER. Dr. Zerhouni, they are not inconsistencies. It is a flat-out contradiction, from night to day, from black to white, from yes to no. It is not an inconsistency, just flat-out different.

Dr. Battey, how about it? You are one of those who said all the stem cell lines were grown with mouse feeders.

Dr. BATTEY. At the time I was asked the question, I knew of no cell line on the NIH registry that had not been grown on a mouse feeder cell line. I learned differently earlier this week, and I apologize for promulgating misinformation. It was not done deliberately on my part.

Senator SPECTER. Dr. Zerhouni, when we met on Tuesday you told me that you had a suspicion that there might be some stem cell lines not grown with mouse feeders and that it was that suspicion that led you to pursue the matter and led you to inform the subcommittee to the contrary. When did you first get that suspicion?

Dr. ZERHOUNI. Actually, Senator, as I told you, for those who have been involved in this field—and I should remind you that in my previous job I had been very involved in developing an Institute for Stem Cell Engineering, so I had a lot of contacts with scientists. And even at that time, many scientists were saying that they would freeze and preserve some of their lines until they learned more about optimal culture conditions beyond the mouse feeder cells.

Everybody wanted to discover, develop methods that would not require mouse feeder cells for future use. So from the contacts that I had with people, some informal conversations, for example from the Karolinska Institute, led me to believe that not everybody was growing cell lines, but they were preserving them prior to exposure perhaps. But I did not know that for a fact, Senator. I only focused on that issue—

Senator SPECTER. When did you suspect it?

Dr. ZERHOUNI. When the Johns Hopkins paper came out, it became an important scientific issue. You yourself raised the issue as well, which was appropriate, and we decided—I decided to, and the NIH staff decided, to have a laser focus on this issue to provide you with the best answer.

Senator SPECTER. My question, Dr. Zerhouni, is when you first had a suspicion and what happened in the interval between then and Monday?

Dr. ZERHOUNI. About 5 weeks ago, I believe. When the paper from Johns Hopkins was published, it became important for us to determine what were the conditions of growth. And we knew from descriptions of derivations versus growth that there were multiple steps there.

Senator SPECTER. Was that the first time you had a suspicion that there might be some stem cell lines not grown with mouse feeders?

Dr. ZERHOUNI. I knew before that there were some of the 14 private lines—and remember, these are private lines, so we do not always get access to the information. We are only provided voluntary information. Some had frozen them.

Senator SPECTER. I am trying to find out when.

Dr. ZERHOUNI. When did I know that—

Senator SPECTER. It seems to me that as soon as you had a suspicion I would ask why you did not make an inquiry then, or at least at the time you became director or perhaps prior to that time, when you and I discussed the matter. I am looking for the sequence of events as to why this was not determined earlier. That bears on the authenticity.

Dr. ZERHOUNI. I appreciate your point. Let me be very clear. We knew—I knew, I suspected, and I think we knew at various time points that people had frozen cell lines that they were keeping in reserve. I knew that over several months and before I even came to NIH.

Whether or not—the specific question, which is whether or not any of those frozen lines had or had not been exposed to mouse feeder cells, became relevant about 4 or 5 weeks ago when the Johns Hopkins paper was published, and this is when I started to question our knowledge about the specific growth conditions of these not yet de-frozen lines.

Senator SPECTER. Dr. Zerhouni, I do not agree with you. I think it became relevant before the Johns Hopkins paper about 5 weeks ago. If there is ever an inkling that there are some stem cell lines out there not grown on mouse feeders, that is a big deal, is it not?

Dr. ZERHOUNI. Yes and no—

Senator SPECTER. That is a major, a major matter.

Dr. ZERHOUNI. Yes and no, Senator, because at the time prior to that there was no known technique to grow these cells other than on mouse feeder cells. So the question becomes relevant when you have someone describing a viable technique. That is why, Senator.

Senator SPECTER. You are saying it only becomes relevant when there is some technique to grow them other than on mouse feeder cells? Well, Dr. Zerhouni, I do not—I do not agree with that, either, because techniques are developed and you never know when a technique is going to be developed if you have researchers and you have the wherewithal to develop techniques or new breakthroughs.

It is a major matter if stem cell lines are in existence which are not grown on mouse feeders to make that determination, you might say in anticipation, but not really in anticipation, because the scientists anticipate everything. You never know where science is going to lead. Every stone you turn over may lead to something else.

So you are saying that at some time before you came to NIH you had a suspicion, as you put it, that there might be some stem cell lines which were not grown or not contaminated with mouse feeders?

Dr. ZERHOUNI. No, no. Let me just be very specific. I knew that scientific groups were freezing their lines, waiting for better knowledge about how to grow their lines more effectively. At that point I did not focus my attention personally on mouse feeders or others because there was no other technique known.

Five weeks ago, with the report of a very specific method that was able to wean NIH-available lines from mouse feeder to human feeder, then it became very important to know. So I may have missed a turn, but frankly the point became of acute relevance

when finally a technique was publicly described that could do that. That is my approach to it.

Senator SPECTER. Well, I have your points and I have noted my disagreement as to the relevance of technique as a critical factor.

Senator Harkin says I should go ahead. I have quite a lot more to say, but I do not like to go over the time, even though I am the chairman. I am going to defer to Senator Harkin.

OPENING STATEMENT OF SENATOR TOM HARKIN

Senator HARKIN. This is very important, and I back you wholeheartedly on this thing. I did not want to interrupt you.

Senator SPECTER. Okay, you have talked me into it.

Senator HARKIN. But I do want to have a couple of questions, but I just did not want to interrupt you.

Mr. Chairman, first of all, thank you very much for having this hearing. I listened very closely to your line of questioning and we have talked about this. I think what we are finding out here is very upsetting. It is very upsetting as we try to get the correct information on which we can base our decisions here, because we are getting contradictory information.

Now, there may be reasons for that, but you can understand that when things like this come out it makes us question whether or not we are really getting correct information. It makes me wonder if the information process at NIH has been politicized.

Senator SPECTER. Permit me to interrupt you for just 1 second. I have to be at the Judiciary Committee markup for a moment or two and this is a good time for me to break, leaving the questioning with you, and I will return very shortly.

Senator HARKIN [presiding]. Okay.

So it just makes me wonder if the information process has been politicized at NIH, and I hope that that is not the case because we have to get to the science without political shading on this thing.

Now, I wanted to just talk a little bit about what has happened with this recent sort of revelation. I understand there is a lot of debate in the field about the best way to grow human embryonic stem cells. It is possible that these cells might grow differently depending on whether you use mouse feeder cells, human feeder cells, or no feeder cells at all.

Studying these differences is important, scientists tell me, because before you use stem cells in a human you have got to make sure they are safe. There is a contamination problem of those cells coming in contact with mouse feeder cells or other human feeder cells. But as I understand it, all 11 stem cell lines that are currently available to federally-funded researchers were grown using mouse feeder cells; is that correct?

Dr. ZERHOUNI. That is correct.

Senator HARKIN. Thank you. Now, apparently scientists in Sweden have grown four or five—I wish I knew; I have heard four and I have heard five—lines without feeder cells; is that correct?

Dr. ZERHOUNI. There is no published paper. This is a verbal communication of that fact. Yes, that is a statement, that is correct.

Senator HARKIN. So you do not know whether they really have or have not grown four or five cell lines without using feeders?

Dr. ZERHOUNI. They are asserting that they have. I have no peer-reviewed fully published method and paper to be categorical about it, but this is what they have told us.

Dr. BATTEY. Mr. Harkin, nor do we know how well characterized those cells are, growing in a feeder-free state. We do not know whether or not they can differentiate into all the major lineages. We do not know whether or not they can be continually self-renewed. We do not know whether or not they will remain karyotypically normal in their genome, in their karyotype, over long periods of time.

All those are issues that need to be addressed. So that is why Dr. Zerhouni refers to peer-reviewed papers and that is why that is the gold standard for the scientific community, and we need a gold standard because otherwise we will end up mired in controversy and contradictory information, which has been an issue that you and Senator Specter have raised.

Senator HARKIN. Okay. Accepting that, then would it not be helpful to scientists to be able to compare these four or five lines with the 11 lines that they have available? Would it be helpful to scientists to compare that or not?

Dr. ZERHOUNI. You know, the issue in stem cell lines is always to try to expand them reliably so they are available to the scientific community at large. We have funded studies by NIH of scientific groups to find and discover ways of growing cell lines without human feeder—without mouse feeder layers. We have funded that. We are currently funding—

Senator HARKIN. With human feeder cells?

Dr. ZERHOUNI. With human or even trying not to have any cell whatsoever to support the growth. So your statement is correct, we need to discover the molecular factors that control that growth and keeps those cells growing vigorously, but at the same time not differentiating into lines of cells that we desire or not desire.

So the answer is we are pushing the research. The question is, as you well know, it is difficult to grow cell lines. It took us a year to be able to expand them. We are working to expand those other lines so that we can have them.

Senator HARKIN. It does take time. It takes a year.

Dr. ZERHOUNI. It takes a year to expand a line, yes, it does.

Senator HARKIN. So you have got 11 now?

Dr. ZERHOUNI. Right.

Senator HARKIN. They have all been contaminated—well, that is the word I use. They have come in contact with mouse feeder cells.

Dr. ZERHOUNI. Come in contact, right.

Senator HARKIN. To the best of our knowledge here at this committee, I know of no lines that have been developed in this country that have been developed without using either mouse feeder cells or human feeder cells. Is that correct, Dr. Battey?

Dr. ZERHOUNI. There is a study—there is a scientific group at Johns Hopkins who just reported about 5 weeks ago a validated technique, well-described technique, where they have been able to take human stem cells that had been initially grown on mouse feeder cells, these are NIH-available lines—

Senator HARKIN. And they separated them out.

Dr. ZERHOUNI [continuing]. And then they separated them out. But you know, Senator—

Senator HARKIN. I know that. But still, they separated them out, but we still do not know whether or not they might carry some contamination with them.

Dr. ZERHOUNI. Well, we do not know that. But the key, the key element here, is that until these papers appeared, no one had discovered the way to do it.

Senator HARKIN. I think, Dr. Zerhouni, the key element here is if in fact, which I do not know, but if in fact there are four or five lines in Sweden, lines that have been developed—I do not mean they are still in the frozen blastocyst stage, but have been developed—

Dr. ZERHOUNI. Right, right.

Senator HARKIN [continuing]. Without using any kind of feeder cells, it would seem to me that we would want to jump on that, get those lines out, get them to researchers in this country as fast as possible, to start comparing them and to see whether or not we can develop those even further on, because, as you just stated, it takes another year. It takes a year. And you know, when we have got people who—that is another year of time. Why not use those four or five lines that we have in Sweden? Why not?

Dr. ZERHOUNI. We do not have those lines. They are in very early stages of defining a technique. Even the Swedes themselves have not published their methods. They are asserting that they are in the early stages of thinking that they have made some advances that will allow them to define the technique.

At this point it is very preliminary and it is not scientifically established. So we want to encourage them. We have funded that group to find out what are the conditions. We have funded many groups, including the group at Hopkins and other grants, to try to accelerate our understanding of how you grow these cells without mouse feeder cell lines.

Senator HARKIN. But you do not know whether they have actually done that or not?

Dr. ZERHOUNI. We do not know that they have been successful. They claim that they are seeing early signs that they are able to do that, but with the caveats that Dr. Battey mentioned. They are not characterized, we do not know whether they are differentiated or not. There are lots of steps, Senator, that really—I can certainly provide for the record the steps that are necessary.

Senator HARKIN. Is it safe to say that they are further along than we are, though?

Dr. ZERHOUNI. No, I think not necessarily, because I do not know how—well, again, I think we should be factual and facts in science mean you publish the paper, it is peer-reviewed, the method is duplicated. The only two sources that we know have done that is Johns Hopkins with their recent paper and a source in Singapore that has claimed to have grown human feeder cells. But those cells have not been expanded and made available to the research community that we know of after a year of describing that advance.

Senator HARKIN. Again, my understanding—you can correct me if I am wrong—but that these lines that have been developed in Sweden, however far they have been developed—now you have

raised a question in my mind as to how far they have been developed, but it has been my information that they have been developed a lot further than anything in this country has ever been done without using feeder cells. That is what I am talking about, okay, that they have taken this process a lot further than what we have here.

Dr. ZERHOUNI. Right. Again, you are asking me to comment on a sentence, a verbal description, without really having the ability to have the scientific process look at it, Senator. So I think we need to really be very watchful. What I can tell you is that I and NIH are absolutely on every single piece of information that we can get to try to accelerate the field. But it is a difficult field. It is not easy.

Senator HARKIN. If those four or five lines were derived after August 9, 2001, does that limit you? I mean, I understand they were derived after August 9, 2001, Dr. Zerhouni.

Dr. ZERHOUNI. Right, and those would not be eligible for Federal funding.

Senator HARKIN. But you just told me that you had people investigating this.

Dr. ZERHOUNI. We are——

Senator HARKIN. But they are not eligible for Federal funding. Okay, now we are getting to where I want to get.

Dr. ZERHOUNI. Okay.

Senator HARKIN. You are right. These cell lines which have been developed much further than anything we have done here in terms of not using feeder cells——

Dr. ZERHOUNI. “Much further than anything we have done,” I would not be sure about the statement because we do not know what they have done. They have not published, they have not shared that information, publicly.

Senator HARKIN. Well, if it were true, if in fact they have been developed further, would you not want to compare them with what we have here?

Dr. ZERHOUNI. Right, and the strategy is multi-pronged. You cannot just rely on one technique or one source. My prodding of our own strategy is to really fund multiple groups of investigators. Now, what the Swedes are doing is they are trying to discover what we are trying to discover, a reliable technique to grow these cells without feeder layers. They say they have made some progress. We are looking forward for them making as much progress as possible, because we have heard about progress before that did not materialize.

Once that is done, the cells that are not yet expanded will hopefully be expanded with those new methods successfully at the same success rate that we have had with mouse feeder cells. That is the strategy, Senator.

Senator HARKIN. If it were true that they have developed these further and you wanted to federally fund researchers in this country to compare these cells, these cell lines, with what we have now, you would not be able to do it.

Dr. ZERHOUNI. No, not necessarily, Senator, because we have a characterization unit that I have just described in my oral statement, which compares all the lines, the ones that are available currently, the 11 lines. If they were to develop a reliable method,

which we are really seeking and actively promoting and pushing scientists to do, as soon as that method is available they will expand their frozen, 16 frozen lines that have not seen any—

Senator HARKIN. I am talking about the lines in Sweden, Dr. Zerhouni.

Dr. ZERHOUNI. Right.

Senator HARKIN. Listen, I think we are getting off on the 16 lines that are frozen here.

Dr. ZERHOUNI. You are asking me—

Senator HARKIN. I am not talking about those. Those were frozen before August 9, 2001.

Dr. ZERHOUNI. That is right.

Senator HARKIN. It is my understanding that the ones that have been developed not using feeder lines were developed from lines derived after August 9, 2001.

Dr. ZERHOUNI. Correct.

Senator HARKIN. And I am saying if you wanted to compare those with what we have done here, you would not be able to do it under the guidelines.

Dr. ZERHOUNI. We will be able to fund any of the eligible lines, including—

Senator HARKIN. Eligible lines.

Dr. ZERHOUNI. Can I finish? Including the ones that they could develop that were derived before August 9, 2001, with Federal funds. Any researcher can use other funds to compare, if those lines were available, compare those lines to the federally funded lines. We are not preventing that from happening.

Senator HARKIN. Okay, those were the 16 lines that you are talking about that were developed before August 9.

Dr. ZERHOUNI. Right. In other words, we will fund the Swedish researchers once we know that they have a reliable, scientifically-established technique to develop lines on human feeder cells that will then go to the registry that we have in the characterization unit to provide what you are asking for.

Senator HARKIN. Okay.

Dr. ZERHOUNI. And no scientist is prevented in this country from doing that, and we are not preventing anyone from using federally funded lines as well as non-federally funded lines and still be funded by NIH.

Senator HARKIN. What is the difference between the four or five cell lines that we have heard about, that you are saying you do not have good scientific data on, the four or five lines that we have heard about in Sweden, that have been developed to some point—I do not know where they are, at what point on the spectrum they are—the four or five lines that have been developed without using any feeder cells, what is the difference between those, using those, and taking the 16 that are still frozen and developing those?

Dr. ZERHOUNI. Well, first, again—

Senator HARKIN. What is the difference?

Dr. ZERHOUNI. The difference is that this is hearsay and we do not know that that information is valid, nor is it clear to us that the methods that they are exploring are going to be effective eventually. If proven effective and efficient, then we certainly would

want to see these methods transported to the 16 cell lines that are eligible for Federal funding and fund those.

But this is hearsay at this point. We cannot, as you mention yourself—I want to be factual and solid in terms of what we know, rather than—and avoid any—

Senator HARKIN. How long will it take you to find out whether or not those four or five lines have been developed and the status of them? How long will it take you to find that out?

Dr. ZERHOUNI. Obviously, researchers are to publish their results, to submit their methodology. It is really not in my hands. It is in the hands of the Swedish researchers to demonstrate the validity of their claim, the hearsay claim. But at this point I am looking forward and encouraging them to promote—to make those findings public so that we can exploit them as quickly as we can.

Senator HARKIN. Okay. I guess there is a hypothetical I am asking here and maybe that is not fair for me to do that. But I am asking a hypothetical, that if in fact—I will just make the point. I will not ask the question. I will make the point. If in fact those cell lines were derived and developed without using feeder cells and they are viable for further, for further differentiation, what I hear you saying is that, we will take that information and then we can apply that to the 16 that are still frozen, and then we can use Federal funds to take that information and apply it to those 16 lines.

Well, it seems to me that then what you are saying is you are basically wasting 1 year, maybe 1½ years or more, of time, because it is going to take you 1 year to develop those 16 lines. The only difference, Dr. Zerhouni, between those four or five lines that may be there and may be developed, ready for differentiation, and the 16 that are frozen, the only difference is August 9, 2001. That is the only difference.

I ask you as a scientist, is that a scientific basis?

Dr. ZERHOUNI. The Federal funding decision that the President made on August 9, 2001, is obviously a policy decision that is based on more than scientific considerations.

Senator HARKIN. Right.

Dr. ZERHOUNI. He made the decision based on moral and ethical considerations, allowing for the first time Federal funding from this line of research, which had never been funded before. At the same time, it does not preclude, preclude at all, the use of other funds for learning about cells derived after August 9, 2001.

Senator HARKIN. Well, I do not need to get into the weeds with you on that, Dr. Zerhouni. But I have often wondered why it is that it is moral and ethical to use these cell lines that were derived prior to 9 p.m.—I think that is the time—on August 9, 2001, but it is not moral and ethical to use it if they were derived at 9:01 p.m. on August 9.

That has always eluded me, why that is so. I make this statement only myself. You do not even have to comment on it. It is arbitrary and in this case, where it holds so much promise for really developing new cures and interventions, that we have handcuffed our scientists in this country.

Now, if the response is that other universities around the world can do it or that the private sector can do it and we do not have

to worry about it, well, then I am wondering what we are doing with NIH and why we are putting so much money into NIH. Maybe we ought to give the money to the private sector.

I am just—you know, I am expressing to you an extreme frustration. I will not speak for Senator Specter, but he and I have talked about this a lot and this is an area of research and development that commands us to move as aggressively as possible. Here we have NIH, we have doubled the funding for NIH in the last 5 years. You have got a lot of money, we have got a lot of good researchers in this country, and we could be moving rapidly.

Every time I see someone with Parkinson's or I see someone with spinal cord injury or I see someone with Alzheimer's, I mean, you just keep asking the question: Why are we not moving more aggressively on this? The only answer I can give them is because of August 9, 2001, which is an arbitrary something plucked out of the air someplace and not a scientific basis, nor do I think it is moral and ethical. I mean, we can discuss the moral and ethics, but to put it on a timeframe like that, I just disagree with that.

Well, anyway, I am not asking you to comment on that at all. But I have given you the sense of my frustration at least with this. Then when we find out that we have these in Sweden—I guess my question to you is, I know they have got to peer review and they have got to publish and stuff, but I would assume, Dr. Battey, since you are the head of the group at NIH, that you have dispatched some of our researchers to go over and take a look at it.

Dr. BATTEY. We are following the progress of this research very carefully, along with all the other breakthroughs that are taking place. If I could just reassure you a little bit about the progress that is being made in this field, I think availability of cell lines is a very important issue, and I think that this subcommittee is right to focus on the availability of cell lines. It is a key issue. It will continue to be a key issue as we move forward.

But equally key is developing a cadre of investigators that is ready to write research grant applications. The success rate for a human embryonic stem cell research grant application is the same as any other grant application. The reason why, as Senator Specter pointed out, we have so much less funding in human embryonic stem cells is that we are getting fewer grant applications.

We need to develop a pool of young and mid-career investigators that are able to write these grant applications and compete successfully for the funds. We are doing that with our hands-on training courses. I had the privilege of being at one just a couple of weeks ago at the University of Pittsburgh headed up by Dr. Gerald Schatten, who has testified before this subcommittee. There will be four others this year, each of whom will train 15 to 25 investigators in the hands-on techniques required to culture the cells.

In my looking at where we are with human embryonic stem cell research, I think that is really the rate-limiting resource for moving things forward in this country. Let me assure you that we are addressing that as vigorously as we possibly can.

Senator HARKIN. Thank you very much, Dr. Battey.

Dr. Zerhouni, do you have something?

Dr. ZERHOUNI. Well, I would like to echo also some of the statements that Dr. Battey made, but also your concerns about whether

or not we are doing everything we need to do in this field. Again, it is very important for us to be able to fund the teams, the training, the understanding of the very, very early milestones and steps that need to be overcome for any therapy to become real.

I do not want to impart in any way that we at NIH are not as vigorously and as enthusiastically pursuing all areas of opportunity in this field of science, and we are in every way possible pushing the field. Now, the field is very young. It is only 18 months since funding has been allowed in this field. It is, as you well know, a long process to go from a new technique to the development of a therapy. Even regular drugs take 12, 13 years to develop. Genetic therapy, gene therapy, is still a field that is evolving.

I think we need to enlarge the base of knowledge as much as we can. I will tell you, I am committed to doing that without any politicization whatsoever. At least you have my commitment of that, Senator.

Senator HARKIN. Thank you, Dr. Zerhouni.

Thank you, Senator Specter. I have no more questions.

Senator SPECTER [presiding]. Dr. Zerhouni, there were reports from Singapore last year that described methods to grow stem cells without mouse feeders. Why didn't that issue become relevant at that time?

Dr. ZERHOUNI. It is a very good question. I heard about those reports. The technique, however, was not shared openly. We could not understand exactly the source and the methods that, at least at my level—

Senator SPECTER. Did you ask about the technique?

Dr. ZERHOUNI. Yes, we did. But you know, again, these are private sources who are protecting intellectual property and not all private sources are forthcoming with the details of what they do.

Senator SPECTER. Well, did you need to know the details of the technique in order to pursue the question about the availability of stem cell lines not grown on mouse feeder?

Dr. ZERHOUNI. Well, again, if I have only one technique available, which was the only one we knew, and another supposed hearsay—I mean, technique where we do not have necessarily the ability to implement and use it, then the question becomes you cannot move until you have that in the public domain, that we can understand how they are doing it and why we would use it and expand it.

It is the same question Senator Harkin was asking about hearsay of new methods and new approaches. As long as we do not have public access to them, it is very hard for us to exploit that advance.

Senator SPECTER. Dr. Zerhouni, with respect to the reasons which I have articulated earlier and we discussed when we met informally 2 days ago, the reasons for additional lines, is it not important to have genetic diversity and is it not important to have a comparison of lines grown using mouse feeders and lines grown without mouse feeders as the second reason for additional lines; and the third reason the need for lines grown without mouse feeders for use in treatment? Are those not very strong reasons why there ought to be additional stem cell lines?

Dr. ZERHOUNI. We just discussed the issue between human feeders and mouse feeders. The real scientific goal is to understand the molecular signals that the feeder cells are sending to the stem cells to make them grow effectively and appropriately. That is the goal of that research, and we are pushing that research. We are funding grants to find out what are those molecular signals so we can have better growth conditions.

So that is very important, you are correct. The key thing there is to eventually make a breakthrough, which we will, in finding culture methods that do not rely on either mouse or human, although either one of those does not prevent therapeutic application. We can still proceed. But it would be in my mind better to not use animal feeders or human feeder layers.

Senator SPECTER. Well, are not these reasons to have more stem cell lines available sound?

Dr. ZERHOUNI. I think what is very important in the early stage of any research is to have very well characterized lines that all researchers can use and compare. Even in very developed fields of research, having too many lines early on where you do not understand all the mechanism is not necessarily the best strategy. And my colleagues across the stem cell task force and around the country will all tell you that when you want to make progress, for example, you do not want to have too much diversity early on. You want to understand the mechanisms and then go deeper into the understanding of the mechanism.

Example: the human genome. We know that no more than six individuals contributed to the human genome and now that we have completed the genome we are looking at genetic variation. Mouse stem cells research is done on a handful of cell lines, and Dr. McKay here could give you the exact number. We have, for example, at NIH funded a large project on human cell signaling, how to find out how cells signal, that a Nobel laureate is directing, Dr. Al Gilman. All of that project is focused on two types of cells.

In every early phase of science, you need to have first and foremost comparable, well-characterized material that a large number of scientists can use, and this is the strategy that I have been pushing since I became NIH director.

Senator SPECTER. Well, reluctant as I am to disagree with a man of your background, I do. It seems to me that if there were more lines, more research, we would get there faster.

Is it not fair to say, Dr. Zerhouni, that the vast majority of scientists disagree with your position and are in favor of—

Dr. ZERHOUNI. Again, I have to—

Senator SPECTER. You cannot even find one non-NIH scientist to come forward and back you up on this issue about whether there are other stem cell lines without mouse feeders? Isn't the official NIH position pretty much isolated?

Dr. ZERHOUNI. Well, Senator, I have put a process in place which is open and transparent, which is the stem cell task force. I have on it scientists who have recently published in Science magazine, and Dr. Battey can comment, people who have been very outspoken about different views on the policy: Dr. Weissman, Dr. Brigid Hogan, others who are on—Dr. Curt Civin. And I am—

Senator SPECTER. Are any of them saying that it is a disadvantage, that we should not pursue more stem cell lines?

Dr. ZERHOUNI. My discussion with them is this: Bring facts to the table that will inform us what is it we need to do today to accelerate this field. They are coming to that table. They are telling me what we need to do, and I am trying to implement every part of it through that process.

The issue of willing to testify, not willing to testify, is obviously more complex than just a scientific issue. I really have not focused on that particular aspect. But I have to say that all the scientists I talk to, all are welcoming of the openness of the process and their ability to come and state what is it that is needed. And I am welcoming any one of them to do so and do so transparently.

Senator SPECTER. I commend you on the process, but that does not focus on the narrow question which I have asked you, whether the vast majority of scientists think it would be desirable, NIH scientists, to have more stem cell lines available for research.

Dr. ZERHOUNI. Well, that is a question that I am willing to pose and ask. But I do not know that at this point one has enough knowledge. At the last hearing, for example, that you conducted, Senator, Dr. Roger Pederson stated that for the state of the science as we know it 10 to 12 cell lines might be sufficient, and then based on the knowledge that we accumulate from that we will know where to go.

I can only deal with facts, not whether or not someone could or could not express an opinion. I can only deal with providing the medium for those discussions to occur. I am doing it and that is my commitment to this field. I am not in any way, shape, or form trying to slow down or, as implied, not respond to the scientific community. I am.

Senator SPECTER. Okay, thank you very much, Dr. Zerhouni.

Would you care to add anything, Dr. Battey?

Dr. BATTEY. Only that the scientific community has articulated a number of needs to move the stem cell research area forward, including availability of cell lines, including enabling more investigators to become versed in the art of culturing cells, including providing standardized culture conditions under which all the cells could be grown. There are a whole list of things that the community has told us we need to do and we are vigorously going after all of those things.

I just want the subcommittee to understand that we share your enthusiasm for this area of research. I think it is an extraordinary breakthrough.

Senator SPECTER. But you are not vigorously going after the availability of more stem cell lines.

Dr. BATTEY. We are funding infrastructure awards to expand to distribution level cell lines that are available on the NIH registry. That will increase the diversity of cell lines that are available for the community.

Senator SPECTER. Well, perhaps I should not editorialize, but it is an occupational hazard of Senators. Senator Harkin told me he editorialized.

We do not have to tout what enthusiasm we have brought to the appropriations process, from \$12 billion to \$27.5 billion. And not to

have the full range of scientific freedom to use all that money is discouraging.

Thank you very much, gentlemen.

Dr. ZERHOUNI. Thank you, sir.

STATEMENT OF RONALD MCKAY, Ph.D., SENIOR INVESTIGATOR, NATIONAL INSTITUTE ON NEUROLOGICAL DISORDERS AND STROKE, NATIONAL INSTITUTES OF HEALTH, DEPARTMENT OF HEALTH AND HUMAN SERVICES

Senator SPECTER. We will now go to the second panel: Dr. Ronald McKay, Dr. John Kessler, Dr. Ron Ogle, Mr. James Cordy. Dr. Ronald McKay joined the National Institute on Neurological Disorders and Stroke as chief of the Laboratory for Molecular Biology in 1993, received his undergraduate and doctorate degrees from the University of Edinburgh.

Dr. McKay, thank you for joining us and we look forward to your testimony.

Dr. MCKAY. Mr. Chairman, Senator Harkin, it is an honor for me to have an opportunity to talk to you. I think, rather than just go through the document that I have provided, let me just summarize by just going to the last page the sort of major issues that I think are relevant. So in the statement that I provided I placed the specific issue of mouse feeder cells in a wider context of characterizing human ES cells, and I stated that there is new data coming from several groups, including our own, that confirms that human ES cells can differentiate to cells of great clinical interest.

I comment in the statement that there are many potential sources of problems as we move forward with this technology, and I specifically discuss the idea that in the cells that are available to us that the exposure to mouse feeder cells and the concern that we have with it should be thought of as one of a general class of problems where the cells have a history which is irreversible which makes them sub-optimal.

So if you are working with a restricted group of cells which has been generated at a particular point in time and these cells, for example, have been exposed to mouse feeder cells and the mouse feeder cells do something to these cells which you can no longer manipulate and that perturbation of the cells has serious consequences, then clearly this is an issue that we need to address if we are going to use this technology.

But what I say is that that is one of several kinds of change that could happen as you grow cells and when you grow cells you need to be very concerned with these changes. Another change that is a great concern in our day-to-day work is not the introduction of a genome from a pathogenic organism coming from the mouse cells, but some manipulation of the genome in the human genome itself in the cell which would make the cell no longer normal.

So I share your concern with the idea that these cells need to be—that their history, what happens to the cells, is of great interest and importance. But what I think is also important for me to say is that this is one of many issues that we will need to address as we move this technology forward. And I am not trying to diminish the importance of this issue at all. What I am trying to say is that as one develops these complex new technologies there are many occasions when it is important for the—let me put it this

way, where we have to exercise leadership. And this is one, but there will be many others as we develop these techniques.

I should say also that in my work on this issue the subject that you are raising today is of great personal concern to me and I think about it constantly, and I am confident that NIH is promoting work in this area which is at the edge of this field. And the idea that we will set up a unit at NIH to compare the human ES cells that are available under Federal funds, to study under Federal funds, at present is I believe a really important thing to do.

I think I will close by saying that I am in the lucky position that I do not have to speak policy for NIH. I can just speak to you as a scientist, which is I understand something that you seek. Both in my role as director of the characterization unit and also just as an individual who is interested in this area, that I am quite relaxed in giving you a completely candid view of where the technology stands at present.

If I thought that the mouse feeder cells posed a really serious inhibition of what I might do tomorrow in my research team, I would tell you so.

PREPARED STATEMENT

Senator Harkin, you used the word "handcuffed" and I just want to say that I have thought about this very precisely in my own professional career, and if I felt handcuffed I would no longer be a Federal employee. And I am quite confident that if a situation arises where I feel that our efforts in this area are seriously impinged by our availability to cells, that I can convey that opinion strongly to Dr. Battey and Dr. Zerhouni. And I am also very impressed by your specific interest in this area.

So I would be happy to expand on any of these points, and thank you for this opportunity.

[The statement follows:]

PREPARED STATEMENT OF DR. RONALD MCKAY

Mr. Chairman, Senator Harkin, and Members of the Subcommittee, I am pleased to appear before you today to testify about human embryonic stem cell research. Human Embryonic Stem (ES) cells have been proposed as a limitless source for the many specific cell types of the adult body. Cells obtained in this way will likely have many uses in the future, including the development of new cell therapies for degenerative diseases. There is wide agreement on the potential importance of knowledge about stem cells but much of this information comes from work on mouse ES cells. In the last few months, published reports have shown that mouse ES cells can generate cells of the skin, blood, brain and pancreas. Even in the mouse system there are technical questions we do not fully understand but there is no doubt that mouse ES cells can be used to generate many somatic cell types. There is clear evidence that human ES cells will form teratomas, complex mixtures of different cells, but much less is known about efficiently generating specific cell types. There are encouraging published reports of a preliminary nature but the research and biotech communities still needs to demonstrate that human ES cells can rapidly generate large numbers of a specific cell type of clinical interest. The recent wider access to human ES cells made possible by the President's decision of August 9, 2001 will accelerate progress on this question and I am confident that procedures for making some of the human cells that most interest us will be reported in detail in the next few months. As this area is new and rapidly developing, the major technical barriers that may slow our progress are not understood. However, some of the potential difficulties can be anticipated. The human ES cells may be difficult to grow and differentiate. Their genome may be unstable. The different cells may show very different properties resulting from their genetic origin. There may be unexpected difficulties in taking the cells to a point where they are clinically relevant. And once we have

obtained the differentiated cells, it may be difficult to integrate these cells with the other cells of the body. All of these possibilities may be influenced by the history of the cell line. There are several variables that differ when human ES cells were first placed in culture by different research teams around the world. But in the first wave of work most success was obtained by growing the human cells in the company of a supporting mouse cell. This procedure was derived from data showing that mouse ES cells grow well in the presence of another cell type, a fibroblast. We do not know the exact reason for the effects of this interaction. Workers in the field still actively discuss whether one or another type of mouse fibroblast is more effective. Recent work suggests that the beneficial effects of mouse cells can be replaced by human cells or by introducing specific chemicals into the environment that supports the human cells. There are many possible ways that differences in the growth conditions could influence the properties of the human ES cells. But there are two simple questions that must be asked: Can we accurately measure these effects of these different growth conditions and do they cause irreversible harm to the human ES cell lines? The answer to the first question is yes, but as we have discussed above, we are still developing the techniques to accurately measure all the interesting properties of human ES cells. So today, we cannot compare precisely the properties of cells grown under different conditions. A detailed answer to the second question depends on having access to these techniques. However, it is clear that any major irreversible change would immediately influence the use of an existing cell lines. The genome carries biological information through time so it is important to establish if the ES cells carry alterations in their own genes or harbor genes from other organisms that significantly affect their properties. Many cells carry pathogens that would have no practical consequences but we are explicitly concerned that the human ES cells have acquired significant genetic changes from any stage of their previous history.

These problems have been clearly stated by the biomedical research community in discussions held by the NIH Stem Cell Task Force. The NIH response to these concerns is outlined in Dr. Zerhouni's statement but it might be useful for me to amplify on the resources and role of the Human ES Cell Characterization Unit that Dr. Zerhouni has asked me to direct. The ES Unit has been established to directly compare the cell lines that are available on the NIH stem cell registry. The groups holding intellectual property rights have agreed to allow the ES Cell Unit to compare the available cells and provide open access to this information. Space has been renovated, equipment is being purchased and we hope to have a core team of four scientists at work in 3 or 4 weeks. We are building strong contacts with scientists in this country and overseas to acquire additional eligible cells. This work is monitored by a committee that includes senior investigators at other medical research facilities. This project has been actively sponsored by Dr. Zerhouni, the Director of the Intramural Research Program, Dr. Gottesman and Dr. Battey. We will compare ES cells with adult stem cells that may be pluripotent and move quickly to analyze as many of the critical features of these cells as possible. The genetic composition of these cells will be one of several measures that we use to define the cells. Our immediate goal is to rapidly develop the Human ES Cell Unit as a source of high-quality information that will allow informed use of these cells. In this statement, I have placed the specific issue of mouse feeder in cells in the wider context of characterizing human ES cells. New data confirms that human ES cells can differentiate to cells of great clinical interest. We are all aware that there are many potential sources of problems as we move forward with this exciting technology. Should we find that the currently available cells carry irreversible changes that restrict their value, this information will be discussed openly without delay. But this specific issue is only one of many that we must address as we explore the potential of human ES cells. I am confident that the National Institutes of Health, here in Bethesda, will contribute both technical information and sound advice to the worldwide effort needed to harness the benefits of stem cells.

Senator SPECTER. Thank you very much, Dr. McKay.

**STATEMENT OF JOHN A. KESSLER, M.D., BOSHES PROFESSOR AND
CHAIRMAN, DAVEE DEPARTMENT OF NEUROLOGY, NORTH-
WESTERN UNIVERSITY MEDICAL SCHOOL**

Senator SPECTER. We now turn to Dr. John Kessler, Professor at Northwestern University Medical School. Thank you for joining us, Dr. Kessler, and the floor is yours.

Dr. KESSLER. Good morning, Chairman Specter and Senator Har-kin. I am a researcher who has devoted his entire professional life to developing techniques for regenerating the damaged nervous system. I am also the father of a 17-year-old daughter, Allison, who 2 years ago suffered a spinal cord injury that confined her to a wheelchair. So I am speaking to you today both as a scientist and a representative of the many families who want to see stem cell therapies reach their potential.

To avoid being redundant, since many of the issues I was going to discuss specifically were brought up already, I would like to not go through the prepared comments, but comment on some of the issues. The issue of the feeder layers has come up. That is very important. I think all scientists know that NIH-funded scientists should have cells available to them that were developed without the mouse feeder layers to be able to compare to them.

Dr. McKay has brought up an even more important issue, namely the history of the cells, the way they are derived, may change their behavior. That means that simply focusing on one way of deriving the cells, simply saying, gee, we have one way that works, now we are going to apply that to everything, will limit the field of science. We will perhaps be developing cells the one way that is not the optimal way. So we need to expand our ways of trying to develop the cells.

The issue of genetic diversity is not one that I think has received enough attention this morning. One of the things that we have learned as stem cell biologists is that the genetic background absolutely alters the behavior of the cells, and the genetic background of a very, very limited number of cell lines that is available to us may critically alter the properties of the cells, and there may be other genetic backgrounds that would make them vastly more helpful for clinical uses.

So I think it is very, very important to focus on that. You know, some individuals who oppose the derivation of new lines claim that all relevant experiments can be done with the existing lines and they overlook these two very critical facts, namely that the way they are derived and the genetic background, the history of the cells, will determine how they can be used.

We are all of us very grateful for NIH funding, myself included. I am the recipient of four grants and a recent supplement for human ES cell work. However, without question, NIH-funded researchers are going to competitively find themselves at a disadvantage with foreign scientists and with scientists in the private sector, and I think that is damaging to our mission. We simply will not be able to compete if we cannot use the best techniques.

One of the other things that the NIH really should be doing is developing a sponsored stem cell repository and registry, not just for those very specific 11 lines, but for all new lines and all appropriate lines. This is a policy which a recent article in *Science*, a large group of distinguished investigators, put forward as something that is really a necessity for the NIH, not just to deal with those lines, but be a repository and a registry.

All the things we have discussed today I think are compelling scientific reasons for me as a physician and a researcher. As a father of someone—that has a daughter who is paralyzed with this

kind of accident, I would really like to see all reasonable means pursued for finding a cure for her and for the millions of people suffering the diseases that Senator Harkin mentioned earlier.

PREPARED STATEMENT

I hope my comments today have helped to clarify both the social and the scientific reasons for allowing federally funded researchers to derive and study new lines. I thank you for your bipartisan and consistent support for the NIH that you have mentioned in the past. We are very grateful for it.

Thank you for allowing me to express my comments today.
[The statement follows:]

PREPARED STATEMENT OF DR. JOHN A. KESSLER

Good morning Chairman Specter, Senator Harkin, and other members of the Subcommittee. I am Dr. John Kessler, Boshes Professor and Chairman of the Davee Department of Neurology at Northwestern University's Feinberg School of Medicine. I am a researcher and physician who has devoted his entire professional life to trying to develop techniques for regenerating the damaged nervous system. I am also the father of a 17 year old daughter, Allison who two years ago suffered a spinal cord injury that confined her to a wheelchair. I therefore speak to you both as a scientist and as a representative of the many American families who wish to see stem cell therapies reach their full potential.

Although the potential for using human embryonic stem cells for regeneration of damaged or diseased organs is truly remarkable, it is clear that there are still significant technical and biological issues to be addressed before embryonic stem cell therapies can be instituted. Obstacles that delay the development of stem cell therapies are counterproductive for all Americans. Federally funded research is currently restricted to the study of an extremely small number of human embryonic stem cell lines, and this research may not involve the derivation or study of new lines. This policy is hindering the work of stem cell researchers, and these restrictions will become progressively more damaging to the field with the passage of time.

What is the specific basis for this statement? First, there are major issues regarding the techniques that were used to derive and maintain the cell lines that are currently approved under federal policy. All of these cell lines were developed using animal feeder layers of cells to support them. The possibility of contamination with mouse viruses or proteins poses unacceptable risks for use of these cells in patients, and it is unlikely that any of these cells could ever be used clinically. Recently it has become possible to grow embryonic stem cells without the need for animal feeder layers. Such cells should certainly be made available to federally funded researchers for their studies. More generally, the methods used to derive and maintain embryonic stem cell lines may alter their properties, and it is essential for American scientists to be able to utilize cell lines that were derived with the newest and best technologies. The importance of these seemingly technical issues should not be underestimated. To understand the point you need only look at how the supposed number of approved stem cell lines dwindled from the more than sixty announced initially to the mere dozen or so now reportedly available.

Studies of mouse embryonic stem cells have made it clear that the genetic background of stem cells exerts a very large but poorly understood effect on their biology. Every stem cell line has a different complement of DNA, and new cell lines with different genetic backgrounds may have different and important properties which may be critical for their clinical use. This issue alone makes it vitally important to be able to develop new lines. For example, although a myriad of mouse stem cell lines have been derived, only a precious few have been useful for the experiments involving homologous recombination that revolutionized the whole field of mouse genetics. Genetic diversity is a wonderful thing, and limiting stem cell research to a narrow and random source of cells is an extraordinary handicap for the study of human embryonic stem cells. Such restrictions would have crippled the field of mouse stem cell biology and genetics if they had been imposed on it. Those who oppose the derivation or use of new lines sometimes state that all relevant experiments can be done with the few existing lines. This overlooks the crucial point that they may be biased by the way the cells were derived or by their genetic background and may therefore give unhelpful or even misleading results. Further they will all have to be repeated with appropriate new lines before any clinical use could be con-

templated. The field of stem cell biology should be allowed to proceed in a parallel fashion on all fronts like every other field of biology. Past experience has made it abundantly clear that allowing broader access to breakthrough discoveries and new technologies greatly increases the likelihood of scientific innovation and of new breakthroughs.

These issues highlight the biologic imperative for changing federal policy and broadening NIH support for stem cell biology. Interestingly, the policy towards embryonic stem cell research runs counter to NIH policies and general philosophy regarding research involving humans and human materials. Although individual investigators who use cell lines may be exempt from guidelines regarding human subjects, the NIH has recognized that medical studies should, whenever possible, include subjects with a diversity of ethnic and racial backgrounds, and both sexes, and that there may be subtle but important differences among groups that ultimately are important for health care. What can be said in this regard about the 11 stem cell lines currently available to federally funded investigators? Can investigators examine the role of such differences in the biology of stem cells? Will there be the statistical power to study how different genotypes influence the phenotypes of cells that differentiate from embryonic stem cells? Will stem cell therapies be designed only for the genetic backgrounds of the Americans in Wisconsin and elsewhere who donated the embryos for these 11 lines? Thus, in addition to the scientific rationale for changing federal policy, there is also the social imperative to perform medical research that is applicable to all Americans.

Fortunately non-federally funded researchers and researchers from other nations have been developing new cell lines and have been advancing the field with new skills and techniques. However this raises the issue that the limitations imposed on federally-funded researchers will inevitably result in the most advanced work being done by industry or by scientists in other nations. Market forces and foreign governments may then dictate the course of science and medicine without regard to the overall social benefit of Americans. Moreover American scientists will eventually find that they can no longer compete with foreign scientists. Some states may find that their Universities are depleted of the best researchers who have chosen to go either to states that have legislatively endorsed stem cell research or to other nations. Federal funding is the best way to guarantee that stem cell therapies are developed with the greatest concern for the public welfare. It is also the best way to assure that the highest ethical standards are maintained with federal oversight. For example, an NIH sponsored stem cell repository and registry that includes all new and appropriate cell lines would serve both to maintain the highest scientific standards and to facilitate providing material to scientists.

Some individuals argue that multipotent stem cells that can be harvested from mature tissues ("adult" stem cells) can be used in place of embryonic stem cells for therapeutic purposes, and this is used as a political argument to limit studies of human embryonic stem cells. However while it is clear that the embryonic stem cell can generate virtually every adult type of tissue, it is unproven and highly debatable whether adult stem cells can produce tissues other than the organ from which they are derived. My own laboratory has studied "adult" stem cells for more than a decade, and most scientists encourage continued study of such cells. However such research cannot substitute for the study of human embryonic stem cells.

As a physician and a researcher these are compelling scientific reasons for allowing federally funded researchers to derive and work with new embryonic stem cell lines. As a father whose daughter suffered a devastating spinal cord injury, there are even more compelling reasons for pursuing every reasonable means of finding a cure for Allison, and for the millions of other Americans who suffer from incapacitating but potentially curable diseases. With regard to the ethical concerns about deriving stem cell lines from embryos slated for destruction, I question whether it is either moral or ethical to literally throw away a potential opportunity for treating human disease. Those of you whose families, like mine, have been touched by serious disease are best equipped to fully understand the issues. Those of you who have been more fortunate should carefully consider the overwhelming needs of Americans who have been devastated by diseases like the one afflicting my daughter.

This discussion has focused principally on policies governing federal funding of research on human embryonic stem cells. However I feel compelled to comment on another major political issue confronting stem cell biology, the issue regarding somatic cell nuclear transfer, often called therapeutic cloning. At the outset I want to emphasize that no responsible scientist wants to clone a human being, and that this is not what this debate is about. The scientific and medical communities overwhelmingly support a ban on such reproductive cloning. However the fear of abuse of the technology should not lead to repudiation or criminalization of the benefits that can be achieved. Nuclear transfer potentially offers the possibility of generating embry-

onic stem cell lines that have the patient's own DNA. Development of successful techniques for accomplishing this would bypass all of the concerns about immune rejection of transplanted cells or other problems that may ensue from genetic mismatch between donor cells and host tissues. What about concerns about potential abuse of the technology? We learned on Sept. 11 that airplanes can be used to bring down buildings. This does not mean that airplanes should be banned, but only that inappropriate uses should be outlawed. The same is true of the technology involved in somatic cell nuclear transfer. Irrational fears of this technology have even led to proposed legislation that would impose criminal penalties on doctors or patients who seek access to treatments developed in other countries using nuclear transfer methodologies. I find it difficult to believe that the United States would enact legislation that would prevent my daughter Allison from accessing a treatment that might enable her to walk again. I cannot believe that Americans with juvenile diabetes, Parkinson's disease, Alzheimer's disease, heart attack, or other such debilitating diseases might be prevented from seeking effective treatments. I implore you to distinguish between reproductive cloning, which can and should be banned, and nuclear transfer techniques which may ultimately lead to treatments for many dreaded disease.

I hope that my comments today have helped to clarify the scientific and social imperatives for allowing federally funded researchers to derive and study new human embryonic stem cell lines. I thank all of you for your bipartisan and consistent support for NIH funding, and for providing an opportunity for me to express my views.

Senator SPECTER. Thank you. Thank you very much, Dr. Kessler.

STATEMENT OF ROY OGLE, Ph.D., ASSOCIATE PROFESSOR OF NEURO-SURGERY AND CELL BIOLOGY, UNIVERSITY OF VIRGINIA MEDICAL SCHOOL

Senator SPECTER. Our next witness is Dr. Roy Ogle, Associate Professor at the University of Virginia. He received both his undergraduate and Ph.D. from the University of Virginia. We look forward to your testimony, Dr. Ogle.

Dr. OGLE. Thank you. It is an honor to be here.

I will try to focus on a couple of issues and reasons that I think we need more stem cell lines that have not been mentioned, and I would echo several of Dr. Kessler's comments. First, I just want to try to convey the excitement that those of us in this field have right now and the enthusiasm we have for this. This is a fun time. I love going to the lab in the morning during these days. This is the most exciting thing that I have seen in my 31-year career.

We will be able to repair and replace diseased and defective cells and tissues and deliver genes and drugs in ways that people could scarcely imagine. This is going to happen. I really believe that regenerative medicine therapies will happen. They will be standard practice within the lifetime of some of the people in this room today.

I want to reiterate the comment on our competitive disadvantage with other countries where they have more cell lines. It is clear that we are constrained in ways that scientists in Europe and Asia are not. I know for a fact that China is making embryonic stem cell research the cornerstone of their biotech industry from people that I have been recruiting to come join my lab. So we need to keep up in this area. We need to be the leaders.

As a scientific issue, clearly researchers need to be able to study many more embryonic cell lines than are currently available. The larger the number that we study, the better the statistical significance. We must study a large enough sample size to account for individual variation in genetic makeup or polymorphisms in genes that control the differentiation of the stem cells. We know this from birth defect studies, from population studies.

The United States is so diverse genetically that our heterogeneous genetic background is a serious confounding factor in studying gene expression and the interaction of genes and environment. So the genes that make stem cells differentiate are often the targets for birth defects. Although we do not yet know what variability exists among the genes governing developmental processes in the cells isolated from different embryos, it is reasonable to assume that such is the case.

It is gratifying that there has been excellent concordance in the results obtained so far at Wisconsin and at Johns Hopkins, but having so few cell lines is really of concern for other reasons. Each cell division carries some possibility of acquisition of genetic mutation. Cells in culture lack the protective mechanisms that those have in the body or in vivo. So culture of such rapidly growing, virtually immortal cells can rapidly amplify a genetic trait selected for by accident or that occurs.

So we are really running the risk of characterizing cells that no longer reflect the properties common to most embryos. We cannot use the mouse cells for many reasons, and it is important to note that it appears that the human mitotic apparatus is much more fragile than that of other animals. So it is probably a barrier right now, until we surmount it, to nuclear transfer.

There is a different complement of chromosomes. There are many differences in these cell lines. So this work has to be done with human lines.

I would like to give just a couple of examples of what we are doing in my laboratory that I think emphasize the fact that those of us who work more with adult stem cells than embryonic still learn a lot. These cell lines probably interact or will interact in their applications.

The most prudent approach to determining the optimal cells to use for anything is to cast a broad net. Therefore, we are comparing stem cells that we have isolated from human liposuction procedures, which are true adult stem cells. We are studying a cell line that my lab has discovered from the dura mater, the lining of the brain, that we will probably isolate in practice from fetuses, so these could be considered a fetal line. And we are looking at the human ES lines as well.

We are delivering undifferentiated stem cells along with those that we have coaxed to become precursors of bone, neuronal cells, and Schwann cells, and right now we are injecting them to try to regenerate the sciatic nerve of rats.

At every step of our work we have been helped tremendously by the advances that have been made in embryonic stem cell work. I do not think we would be anywhere near where we are without these.

But the last reason that I would really like to look at is that these tissues actually—or these types of cells appear to act in concert. Many of us know of the recent studies at Hopkins where they have injected into paralyzed rats the differentiated cells from their ES cell line. It is not those cells that are doing the repair. It appears that those cells are stimulating the cells lining the spinal cord, perhaps my dura mater cells, to actually do the repair.

So we have got to—no matter how great adult stem cells look, we are going to have to study the embryonic together with the adult to make sense of this whole thing.

PREPARED STATEMENT

I would like to finish by just, by being bold. I think we always need to be bold in science, and I think we need to set a goal to assemble an immunotype library of human stem cell types that would cover every histocompatibility set among our population, and that we need to release for use those cells that are frozen, those embryos that are frozen, that have been donated for these purposes.

Thank you very much.

[The statement follows:]

PREPARED STATEMENT OF DR. ROY OGLE

I am a developmental biologist and professor of Neurosurgery and Cell Biology at the University of Virginia Medical School, where I conduct basic and applied research into several types of stem cells including those from embryonic, fetal and adult sources. My major funding source is the National Institute of Dental and Craniofacial Research at NIH. The opinions expressed by me are those of a scientist and individual, and not official positions of the University of Virginia or the National Institutes of Health.

The rapid advances in stem cell science in recent years are the most exciting I have witnessed in my 31-year career as a biologist. The new science of regenerative medicine has been born from a convergence of stem cell biology, gene therapy, tissue engineering, and materials science. We will be able to repair and replace diseased and defective cells and tissues, and deliver genes and drugs in ways we could scarcely imagine 10 years ago. I believe regenerative medical therapies will be standard within the lifetimes of some of those present today.

The important studies that have fueled the progress were conducted with the support and review of the National Institutes of Health, with the exception of the pioneering human embryonic stem (ES) cell research. This work could not be done under federal support. Many scientists in this country, myself included, wanted to work with embryonic and fetal human tissues in the past, but simply could not find a way to do so without federal support. There is little doubt we would be much closer today to employing the technologies for repairing and replacing human tissues using stem cells had this not been the case. As we attempt to realize the great promise of regenerative medicine, we can accelerate the rate of discovery by making many more lines available and by increasing the funding available to study the new lines.

This area of science is attractive to many of the best students training for careers in medicine, engineering and scientific research. My four brightest students of the past few years have all chosen to pursue careers in stem cell research. As educators, we can train outstanding young scientists anxious to devote their careers to regenerative medicine, but it is critical that they have the tools—including adequate numbers of independently derived human ES lines—for their graduate and post-doctoral training as well as for establishing their own laboratories.

While scientists in this country are constrained by limited numbers of cell lines, it is clear that many scientists in European and Asian countries are not. China, for one, is making ES cell research the cornerstone of their biotechnology industry. We must maintain our position of leadership in biomedical research for educational and economic reasons as well as the scientific ones.

As a scientific issue, clearly researchers need to be able to study many more human embryonic stem cell lines than are currently available. The larger the number of individual lines studied, the greater the statistical significance of the results. We must study a large enough sample size to account for individual variation in genetic make-up or polymorphisms in genes that control differentiation of stem cells. The population of the United States is diverse genetically, and our heterogeneous genetic background is a serious confounding factor in studying gene expression and the interaction of genes and environment. We know from population studies of birth defects—many of which are caused by mutations in genes that are the same ones controlling differentiation in ES cells—these genes act differently in distinct genetic backgrounds. Although we do not yet know what variability exists among the genes

governing developmental processes in the cells isolated from different embryos, it is reasonable to assume such is the case.

While it is gratifying that to date, there has been excellent concordance in the results obtained with distinct human ES lines in the laboratories of Drs. Thomson at Wisconsin and Gearhart at Johns Hopkins, having so few lines under examination is of concern. Each cell division carries some possibility of acquisition of genetic mutation. Cells in culture lack some of the protective mechanisms afforded those in vivo. Culture of such rapidly growing, virtually immortal cells can rapidly amplify a genetic trait selected for by accident. Working with but a few lines carries the risk of characterizing cells that no longer reflect the properties common to most embryos.

We cannot use the many mouse ES cells available to compensate for the limited number of human ES cells. Human cells differ from other animal cells in important ways, thus there really is no substitute. Human ES cells cannot be cultured in the presence of antibiotics while mouse ES cells can. The cellular structures that move chromosomes during cell division are different and more "fragile" than those of animals—a fact that has been suggested to be a major barrier to nuclear transfer technology. There is a different complement of chromosomes in human and mouse cells, and undoubtedly other significant differences in human and other ES cells that we have yet to discover.

In my laboratory we seek methods to regenerate bone and nerve. I feel the most prudent approach to determine the optimal cells to use is casting a broad net, therefore, we are comparing stem cells isolated from human liposuction procedures—true adult stem cells; cells we have discovered in the dura mater, the lining of the brain and spine, which will probably be harvested from human fetal tissues; and human ES lines obtained from the University of Wisconsin. We are delivering undifferentiated stem cells along with those induced to become precursors of bone cells to rodent models to determine the optimal methodology to engineer new bone. In other studies we have succeeded in coaxing the fat-derived and dura mater stem cells to become true neurons and Schwann cells, critical cell types in the regeneration of nerve. We are currently testing the injection of both cell types to regenerate peripheral (sciatic) nerve, and hope to use a similar approach for regeneration of spinal nerve fibers in the future. Very preliminary studies suggest under some circumstances the cells may be able to "home" to the sites of tissue injury upon injection, which if true, will greatly facilitate this regenerative technology. We have drawn greatly on advances in culture and differentiation of ES cells in our study of the adult and fetal stem cells. Even though it appears likely that adult stem cells will find clinical applications before ES cells, progress in the ES research will clearly advance adult stem cell research. Advances in biology always come with surprises, so it would be foolish to not conduct rational experimentation, including comparisons of the stem cell types so there will be no doubt that the foundation of our new discipline is sound.

There are other reasons we must study all stem cell types including ES cells. Different types of stem cells may work in concert to repair tissues. As discussed above, we hope injected Schwann cells will release factors that signal nerve cells to extend new axons, thereby repairing severed nerves. One recent study using the Johns-Hopkins cell line showed that injection of neural cell progenitors derived from ES cells into the spinal canals of paralyzed rats restored motion. The actual cells effecting the repair were probably endogenous, "adult" stem cells—perhaps the dura mater cells discovered in my laboratory, which were stimulated to act by factors released from the injected cells. There are also preliminary reports in the past week of a European study in which similar cells were injected into animals with demyelination similar to that of humans with multiple sclerosis. The differentiated stem cells were reported to stimulate replacement of missing myelin of the nerve sheaths. These studies underscore the fact that we cannot assume that support of research using only or primarily adult stem cells will suffice to meet our goals in advancing basic science and regenerative medicine.

Looking to the near future, a reasonable goal might be to assemble an "immunotype library" of human ES cells. Such a cell library would contain at least one or more founder cell lines of each of the major human histocompatibility categories. Then the true advantages of the ES cells—unlimited potential to replicate and total developmental plasticity—might be realized. Perhaps advances in immunosuppression and transplantation will make this unnecessary. In any case, we stand to uncover many of the mysteries of early development by having a larger and more diverse set of cells, which are readily available to qualified researchers.

In summary, I believe that providing both increased funding and many more cell lines for human ES cell research as soon as possible is critical to the future of healthcare, science, education and the biotechnology industry in the United States. It is hoped that the federal government will be involved in contracting and estab-

lishing standards for the process of isolating and distributing additional ES lines. There are reported to be many human embryos in the United States, which are frozen and would be donated for research purposes if allowed or otherwise destroyed. While ethical debates continue on creation of embryos for research, can we not make use of those no longer needed for reproduction?

Senator SPECTER. Thank you very much, Dr. Ogle.

**STATEMENT OF JAMES CORDY, FOUNDER, PARKINSON'S ALLIANCE,
ON BEHALF OF THE COALITION FOR THE ADVANCEMENT OF
MEDICAL RESEARCH**

Senator SPECTER. We now turn to Mr. James Cordy, founder of the Parkinson's Alliance, a national group comprised and administered by individuals with Parkinson's disease. He served as president of the Pittsburgh chapter and is a member of the board of directors. Mr. Cordy testified before this subcommittee back in 1999. He has a great hourglass which he uses so effectively.

Mr. Cordy, in welcoming you here I listened very closely to the statement of Dr. Ogle on everything, but especially when he said there would be regenerative medicine within the lifetimes of people who are in this room today. And I just hope you are one of those people.

Mr. CORDY. You and me both.

Senator SPECTER. I am sure of that. We look forward to your testimony, sir.

Mr. CORDY. Let me just add to the credentials a new one. I was present at the Pittsburgh course, a 3-week course of intensive stem cell work, and I would be glad to share my observations at the end of this presentation.

Mr. Chairman, ranking member, Senator Harkin, members of the subcommittee, I thank you for the opportunity to testify today. I am here representing the many millions who will benefit from the human embryonic stem cell technology made possible by the dollars you appropriate. I view my testimony here this morning as my 5 minutes to change the world. If I choose the right words and paint the right picture, I hope to influence your decisions.

I am here on behalf of the Coalition for the Advancement of Medical Research. My job today is to give you a view from the waiting room of biomedical science, what it is like for us waiting for the breakthroughs to happen. It is an awesome responsibility to represent over 100 million Americans who are likely to benefit—diseases such as Parkinson's and diabetes, heart disease, spinal cord injuries, liver disease, and many more.

I hope to give you a glimpse not only of what it is like to have a neurodegenerative disease, but also the staggering sense of despair and frustration and even anger when you first receive that diagnosis.

I use this hourglass I think fairly effectively to make two points: first, to help those who do not have Parkinson's appreciate the relentless and ruthless progression of this disease. Just as the grains of sand flow from this top chamber relentlessly, I lose dopamine-producing neurons relentlessly from my upper chamber, my brain. The result is a loss of functions, one after another after another. The worst case scenario, the one that everyone who has Parkinson's fears, is that which beset your colleague Mo Udall, who became trapped in a body, unable to speak or talk or move.

Second, this hourglass also reminds everyone that we who have Parkinson's as well as many other diseases are in a race against time. How do I feel about the need for increased stem cell lines? You need only look at this hourglass. Time is not neutral. The promise is so great for so many that we must have the scientific equivalent of a full-court press.

I think we have asked our gifted scientists to play this full-court press using only their left hand. As they are gifted scientists, they may do very well with that restriction. But could they do better if they did not have it?

Due to my advancing Parkinson's and the increasingly erratic and ineffective performance of my medication, my physical abilities are eroding. My hands and legs sometimes shake and my body is sometimes stiff. I can no longer tie my tie or tuck my shirt in. I cannot shuffle papers or drive my car. I have lost facial expression, sense of smell, and I now have a monotone voice.

But I consider myself fortunate for an individual who has had 15 years of Parkinson's. For the several hours a day of my on/off cycle when I get sufficient dopamine to my brain, I can function with some degree of normalcy, as you see me here today. Probably only my wife realizes the progression of my disease because I do not leave the house when I am off. I lie down and wait for the time to take the next pill and then wait some more for it to work.

But I would not be here today if that was the extent of the problems. Unfortunately, those are just a preview of the horrors to come if we do not cure this sinister disease.

By coincidence or perhaps serendipity, my invitation to testify came just as I spoke with senior scientists and beginning scientists from around the world at the 3-week symposium and course on stem cell technology in my home town, Pittsburgh. They are dedicated, brilliant, and enthralled with the potential of this new technology to dramatically improve the human condition.

They are also quite concerned about the legislative initiatives restricting embryonic stem cell research. Publicly the scientists are cautious about their predictions, but privately you can see the gleam in their eyes as they marvel at the possibilities of this new technology. If only a portion of this potential is realized, it will revolutionize medicine.

The development of the human embryonic stem cells technology may well be the most significant scientific initiative since we put a man on the moon. We need the same sense of urgency as when we did that. We are on the steep part of the learning curve of the technology. We know much, much more now than we knew when the President announced his policy, but we have much to learn.

Just a few years ago when I employed this hourglass, the situation was once my brain was depleted of most of its neurons my future was desperate. Now this technology offers the possibility of replenishing the upper chamber, just as I have done by turning this hourglass over. I have hope, as do others—I speak not just for myself, but for many others—that this technology may help.

But let me assure you, I am not going to sit back and wait for my body to stop working. I am determined to win this race against time. But I need your help and I appreciate your help. Please do

not let time run out on me and the millions of Americans who could almost certainly benefit from this technology.

PREPARED STATEMENT

I feel a tug on my heartstrings as I look at those in attendance today. Thank you all for coming. Missing are so many advocates that have been here at previous significant events with me. They are not here because of their advanced Parkinson's. Dale, Lupe, Peter, Jim Dandy, just to name a few, they are here in spirit even though they can no longer be by my side. We are going to beat this yet, and my message to them is: Hang in there.

Thank you for this opportunity and I really appreciate your support.

[The statement follows:]

PREPARED STATEMENT OF JAMES CORDY

Good morning Chairman Specter, Ranking Member Harkin, and Members of the Subcommittee. Thank you for the opportunity to testify today on the limitations of the current federal policy regarding embryonic stem cell research.

My name is Jim Cordy, and I am here on behalf of the Coalition for the Advancement of Medical Research.¹ The Coalition is comprised of more than 75 patient organizations, universities, scientific societies, foundations, and other entities advocating for the advancement of breakthrough research and technologies in regenerative medicine in order to cure disease and alleviate suffering.

I'm here to give you a view from the waiting room of biomedical science and what it's like to be a patient waiting for a breakthrough in medical science. I have Parkinson's disease and the promise of regenerative medicine is a significant part of my hope for a cure and a better, longer life. At this early stage, we must not overstate the science, but given the findings to date, there is no denying the hope stem cell research offers.

I am one of the many millions of Americans who will benefit from biomedical research, made possible by the dollars that you appropriate. I view this invitation to testify as my opportunity to change the world. If I choose the right words, paint the right picture, I hope to give you not only a glimpse of what it's like to have a neurodegenerative disease, but also a sense of the staggering utter despair, frustration, and anger that accompanies such a diagnosis. But the intensity of those emotions pale in comparison to my feelings as a potential cure is dangled in front of me only to see well-intentioned decision-makers limit our brilliant scientists and impede reaching that goal.

Parkinson's disease means that the neurons, the cells in the brain which control movement, continue to die day after day after day. I found this hourglass to be an effective aide to help those that don't have Parkinson's appreciate the relentless and ruthless nature of this disease. Just as the grains of sand flow from the upper chamber into the lower chamber, the neurons in the upper chamber of my brain relentlessly die. The result is the loss of one function after another after another. The worst-case scenario- the one everyone who has Parkinson's fears- is that which beset your colleague Mo Udall, who became trapped his body unable to move or speak as a result of his advanced case of Parkinson's.

You may ask how I feel about the need for increased stem cell lines. You need only look at my hourglass to know my answer. I'm in a race against time. Will the cure, which I hope for, come soon enough for me? We won't know until the scientists have the support of the federal government to fully explore this area. It's an unbelievable and horrible shock to hear the doctor say, "you have Parkinson's disease." I'm sure it's the same for MS, cancer, cardiovascular disease, or Alzheimer's. But it is incredibly frustrating to see potential breakthroughs on the horizon and not be able to reach them as fast as humanly possible.

¹The Coalition is comprised of nationally-recognized patient organizations, universities, scientific societies, foundations, and individuals with life-threatening illnesses and disorders, advocating for the advancement of breakthrough research and technologies in regenerative medicine—including stem cell research and somatic cell nuclear transfer—in order to cure disease and alleviate suffering.

Time is running out for the more than 100 million Americans with permanently disabling, and ultimately fatal, diseases and conditions such as Parkinson's, diabetes, and Huntington's. I am not a scientist, I am here today as the voice of all of us who may benefit from stem cell research. It is time to let the scientists work.

Leading scientists inform us that embryonic stem cells have significant potential to treat conditions like Parkinson's, Rett Syndrome, and autoimmune diseases; federal funding is integral to finding the promise behind the potential—it is imperative not just for my sake, but for the sake of so many Americans.

By coincidence, my invitation to testify here today came to me as I attended an intensive three-week course and international symposium on human embryonic stem cells. I've met and spoken with senior scientists and young scientists just beginning their careers. They are dedicated, brilliant, and enthralled with the potential of this new emerging technology to dramatically improve the human condition. I've seen and heard in detail the first steps taken to cure Parkinson's disease, Canavan disease, Kernicterus, liver disease, glaucoma, Tourette's Syndrome, urinary incontinence, and many more.

I had lunch with one of the world's premiere researchers who left the United States because of its prohibitive laws regarding embryonic stem cell research. Although I believe this to be highly unusual, it could be the beginning of a terrible trend. Typically we see the best and brightest scientists from other countries coming to the United States because of the great strength and capacity of our biomedical research initiatives. I have spoken with a senior NIH scientist who is actually placing embryonic stem cells into the brain of a rat that had the symptoms of Parkinson's disease. The stem cells recognize the damaged neurons, produced new ones to replace the damaged neurons, and stopped producing neurons when a sufficient number was achieved. As a result the Parkinson's symptoms of the rat were greatly reduced.

If we do not handcuff and shackle our scientists, the technology may be ready for clinical trials in the near future. Much of the embryonic stem cell debate has rightly focused on repair and replacement of damaged parts. But the unraveling of the secret of how these cells, which initially can produce any part of the human body, know to change into specific cells may be the Rosetta stone of human development and revolutionize medical science.

While I applaud President Bush for keeping the door open for federal funding of embryonic stem cells research, I believe that the current policy needs to be revisited.

It is my understanding that in 2001, when the President announced his embryonic stem cell research policy, there were thought to be at least 60 stem cell lines that qualified for federally-funded research. However, after first increasing that number to 78, the National Institutes of Health announced last month that there are just 11 lines. Furthermore, all 11 lines are contaminated by mouse "feeder" cells, which may disqualify them for human therapeutic use. Science has progressed, and now we have the technology to develop stem cell lines free of mouse cells.

In light of this situation, the President should broaden his stem cell policy—it could be a matter of life or death!

Debate on the current policy is not unwarranted, but please realize that every day that the debate continues and the current policy remains in place is one day less that patients spend with their families and friends as well as one day further from potential treatments—one day further from hope realized.

We need to prime the pump so that if the science reaches the point where clinical trials are appropriate we're not waiting and playing catch-up with other countries which have access to "clean" stem cell lines. The United States needs a comprehensive stem cell policy based on science and saving lives and not on politics. The scientists tell us that Parkinson's disease could be close to a breakthrough, but the benefits derived from progress will not benefit Parkinson's alone—since a rising tide raises all boats—cancer, juvenile diabetes, and others will benefit too.

You have the power to provide the scientists with the necessary resources to explore the promise of regenerative medicine and make it real in terms of better treatments, advanced therapies, and ideally, cures. As an individual forced to wait for the day this research advances enough to begin clinical trials, I look to the federal government to fund new stem cell lines, uncontaminated by mouse cells, in parallel with the current policy. Why should we ask our researchers to do their work with one hand tied behind their backs?

Due to my advancing Parkinson's, my physical abilities have eroded—my hands and legs shake and my body is stiff. I can no longer tie my tie, wash my hair, or tuck my shirt in. I can't shuffle papers or drive my car. I have lost my facial expression, sense of smell, and I now have a monotone voice. But I wouldn't be here today if that was the extent of my problems. Unfortunately those are just previews of the horrors to come if we don't cure this sinister disease.

But I consider myself fortunate for an individual who has had Parkinson's for over 15 years. For the several hours of the on/off cycle when I get sufficient dopamine to my brain I can function with some degree of normalcy as you see me here today. Many of my fellow Parkinson's advocates are in wheelchairs. One dear friend is, at this moment, in intensive care having fallen down 18 steps because of the balance problems associated with Parkinson's. I rarely express anger about my disease, except when I see my dear friends get progressively worse. Peter, Dale, Lupe, Jim Dandy, to name a few, I know are with me in spirit even though they're no longer able to be here by my side.

Probably only my wife realizes the progression of my disease because I don't leave the house when I'm off. I lie down and wait for the time to take my next pill and wait some more for it to work.

I have hope, as do others. I speak not just for me and my disease, but for the others, their families, friends, and caregivers who have hope as well. Let me assure you that I'm not going to sit back and wait for my body to stop working. I am determined to win this race against time, but I need your help. Before concluding, I will turn this hourglass over. Notice that the top chamber is replenished—just as a scientific breakthrough which cures Parkinson's will replenish my brain cells.

I believe we should leave the science to the scientists so the possibilities of the research can be uncovered. However, the potential reward is so great, it seems clear to me that we must pursue embryonic stem cell technology with all speed possible, which means developing new lines concurrently, and not sequentially.

Please, please don't let time run out for me and the over 1.5 million Americans with Parkinson's, and the over 100 million Americans with diseases and conditions who are almost certain to benefit from regenerative medicine, including embryonic stem cell research. It is unconscionable to let time run out—especially now that the scientists tell us the finish line might be within sight.

On behalf of the Coalition for the Advancement of Medical Research I again thank the Committee for its deliberations and for the opportunity to speak to this issue.

Senator SPECTER. Thank you very much for your very poignant testimony, Mr. Cordy, and for your hourglass. I quote you with frequency everywhere.

Mr. CORDY. Thank you.

Senator SPECTER. Dr. McKay, why is it that, notwithstanding repeated requests from the staff here for NIH to recommend one NIH scientist to testify in support of Dr. Zerhouni's position that additional stem cells are not required, that NIH could not make a single recommendation?

Dr. MCKAY. You mean just any scientist, right? Why aren't scientists prepared to come and support the NIH position, is the question you have asked?

Senator SPECTER. That is the question.

Dr. MCKAY. Yes. Scientists usually do not have any trouble expressing their opinions, so I can only imagine that they have reservations about the position that NIH is holding here. But it seems to me that the question that Jim Cordy's testimony poses to me in a very direct way, sitting next to him and knowing him and having visited his workshop where this scientific device was constructed, is whether I believe that right now we are moving in my group as fast as we possibly can to work on Parkinson's disease.

So I can say to you the answer is yes, I believe that is true. Now, if you ask me will there ever be a time when that is not the case, my answer is I can imagine that that would be true.

Senator SPECTER. Beyond Parkinson's disease, how about all the other diseases?

Dr. MCKAY. Well, sir, I suppose—

Senator SPECTER. Are we moving as fast as we could if we had more stem cell lines available?

Can I ask you that, Dr. Kessler. You have a 17-year-old daughter.

Dr. KESSLER. I would echo Dr. McKay's comments. I am moving as fast as I can. I am doing absolutely everything I can. Do I think the field as a whole could move faster? I know that the field as a whole could move faster. There is no question that when you get the very best scientists with the best tools the field moves faster than when you have scientists without the best tools, and I think there is a consensus among scientists that federally funded researchers are progressively not having access to the absolute best tools. That is why you are unable to get them to come and testify.

Again, this is not a comment about the NIH. I really hasten to add, the NIH is very supportive to all of this. This is a policy, as you stated, that was enunciated 2 years ago, not by the director of the NIH. But it is a policy that most scientists disagree with.

Senator SPECTER. It is the policy of the administration, but the administration does not have the last word under our Constitution. It is up to the Congress. Congress makes the laws for this country. The President can veto a law and the Congress has the option of overriding a veto. These decisions are up to the Congress, and they start right here. The buck starts right here.

Dr. Zerhouni, I would appreciate it—first, I appreciate your staying, but I would appreciate if you would comment on one of the statements by Dr. Ogle, that the larger the line we study the better statistical significance we have. What do you think?

Dr. ZERHOUNI. Well, statistical significance depends on the question you are asking at the time, the scientific question you are asking. I do not disagree with the notion that genetic diversity is an important issue that is an issue that needs to be considered in relationship to the specific strategies of therapy that anybody is proposing. At this point we do not have specific therapeutic strategies to consider that will be applied to the population at large, if that is the point that is being asked.

The second is that before you can really assess that, as the doctor pointed out, we need to completely understand the genetic stability of the cell lines and the mechanisms that lead to that, because it is very important to first have an understanding of that. So I do not disagree with the issue of genetic diversity, but this is not an issue that I think can be addressed without progress being made on the milestones that we have identified.

Now, the other statement I would like to make is that, you know, you are asking if the NIH Director has made a determination that the number of cell lines we have is sufficient. I do not recall having made that statement. I mean, my view is that we need to progress, we need to pass those milestones. And at this point I do not think anybody knows the answer to that question in terms of minimum or maximum for therapeutic applications, since at this point there is no therapeutic application that is being proposed in humans.

But we want to accelerate the discoveries that will create the cells that will provide dopamine, insulin, and so on as fast as possible. To do that, I need more researchers that are involved in very characterized cell systems, that understand genetic stability, that create as fast as possible the models that will help Mr. Cordy here.

So I want to be on the record to say that I agree with the questions that are posed. All of them are relevant at certain time points in the development of these therapies. But we cannot accelerate the therapy without understanding the basis of why the therapy will or will not work. That is my point, Senator.

Senator SPECTER. Are you saying, Dr. Zerhouni—I thought I heard you say it, but I want to confirm it—that you are not contending that we have a sufficient number of stem cell lines?

Dr. ZERHOUNI. If you ask me what is it we need to do today and again you are looking at is what we need to do today being done, the answer is yes.

Senator SPECTER. Now answer my question.

Dr. ZERHOUNI. With the number of cell lines that we have and the progress we are making in understanding all of the multiple aspects, that not only NIH scientists but also other scientists are providing us, we really, I believe, are doing what we need to do right now to advance the field. Whether or not in the future this will be sufficient is not known to me at this point and I am not making a statement that all the lines that we have today are sufficient forever in terms of being able to do the therapies that we need.

Senator SPECTER. But do we have a sufficient number of stem cell lines available today for what we need to do today?

Dr. ZERHOUNI. That is my statement.

Senator SPECTER. Senator Harkin.

Senator HARKIN. Well, I guess it is just a different assumption here. Dr. Zerhouni, I do not need to have you back. Just this is my statement. I do not need to ask you a question. It is just that you assume these 78 derivations, and what do we need to do with them to develop treatments. My position is, why do we assume 78 derivations? That is the difference. That is sort of, that is what it comes down to, Dr. McKay.

I can understand your points, that within that construct, within that construct, you are doing everything possible, and I have no doubt about that, that you are doing everything possible within that construct. We just keep getting back to the basic construct.

Dr. MCKAY. Could I add something to this, because I think there is a point here which may be subtle, but I think Dr. Zerhouni and I share, which is it is possible that I think both of us would be here today and tell you that we were dissatisfied with the cells, that we could not do what we felt we needed to do.

So the position we are holding, you might say, is constrained by the availability of cells and the policy, but we are not being—but I am in a position where I can quite easily tell you if I think that that is not true, that we cannot work with the cells that we have available. Do you see what I am saying?

Dr. Zerhouni is also being quite explicit with you about this point, too. So we are not saying to you that there is a situation here where we are essentially making it up. The reason I want to be so explicit about this with you is I think it is very important that you understand it because it is possible that this situation could arise very rapidly. This technology is developing very rapidly.

So what we are saying is quite explicit, because I get the impression that both of you think that in a way NIH is not being direct

with you. But I think we are being very direct, but we are being kind of subtle.

Senator HARKIN. I do agree with that.

But again, I make—Dr. McKay, I do not think I am wrong in this, in saying that, again if, in fact there are four or five lines that have been derived, not only derived but actually taken down the path, I do not know how far—I do not think to the point of differentiation, but I do not even know that—in Sweden, and these have been done without any—I use the word “contamination”; that is my lay term on it, with any kind of feeder cell layers—and since it takes a year from the derivation from the blastocyst to develop these lines, that if we do not utilize those now and we say, okay, we will let them go ahead, and then they find out that they can derive those, they can differentiate them, then we say, okay, now we are going to take what they did and go back to these 16 other cell lines that are still frozen, we are a year or more behind. That is my point.

Dr. MCKAY. Yes, but I think what—I mean, we are not arguing, I do not think anybody at NIH is arguing, that we want to limit what people learn on cells all over the world and with non-Federal dollars.

Senator HARKIN. You cannot do that.

Dr. MCKAY. But I think we are arguing very strongly, and I am personally arguing this to you and to Jim Cordy, that, the point that Dr. Zerhouni made, which is that there are going to be fundamental advances in our understanding of these cells, and so it is really in my view, the mouse feeder question is currently not the rate-limiting step in this area.

If I thought there was a rate-limiting step in the cells that I can work on, I would tell you. That is what I was saying a minute ago. I would tell you quite directly. And if it comes up in our work, we will make it publicly available quite directly and immediately.

Senator HARKIN. Dr. Kessler, isn't it true that scientists have taken certain stem cells and used these in rats, laboratory rats, that have actually honed in on, if the stem cells were derived from—I think this is either a nervous system or a spinal cord injury in a rat, that these actually honed in and there was some indication that the rat had movement after that? Has this not been done?

Dr. KESSLER. Yes, there is evidence that in fact embryonic stem cells are able to help in animal models of spinal cord injury, and that is the focus now of a lot of work, including my own laboratory.

With respect to what Dr. McKay said, it is very important to understand that the way the cells are derived and the constitution of the cells may make everything that is found on that specific cell line an artifact. And to put, as the expression goes, all your eggs in one basket or very few baskets is not really, I think, a valid scientific approach.

In all other fields of biology, we proceed in a parallel fashion on all fronts. In this particular field we are being told we cannot do things in parallel, they have to be done serially, and we cannot do them on all fronts. I think that is the fundamental error that we are dealing with, and that is really what you are saying, precisely

the same thing. We should not be doing them serially; we should be doing them in parallel.

With respect to the spinal cord injury, as a scientist, of course, it is frustrating to see these handcuffs being put on, I think is the phrase someone used earlier.

Senator HARKIN. It may not have been a good phrase.

Dr. KESSLER. As a father, it is infuriating to see the handcuffs being put on. Like Mr. Cordy, I want to see every possible thing that can be done being done for the field, and I would like to see scientists free to pursue all the various avenues.

Dr. McKay happens to be one of the most eminent stem cell researchers. Hopefully his research will lead to a cure for Parkinson's. But I think he would be the first to tell you that he does not know that his approach is going to be the right one. It may be somebody in another laboratory taking an entirely different approach. If we are constrained to have only one approach and not the whole diversity of approaches, it is going to slow the field.

Senator HARKIN. I think that is where I was headed, anyway.

Dr. KESSLER. I am sorry if I diverted it.

Senator HARKIN. Mr. Cordy, do you have something you wanted to add?

Mr. CORDY. I spent 3 weeks with these scientists in Pittsburgh.

Senator HARKIN. Say again?

Mr. CORDY. I spent 3 weeks with these scientists almost every day in Pittsburgh, and I do not believe I heard any of them say that their research was constrained right now because of the lack of additional stem cell lines. But I think the sentiment was that it will be at some point. I think the technology is so new, that there is so much to do, that they are busy doing the fundamental work.

But as Dr. Battey said, this program by NIH to train new scientists—I was at the first one of these. But as we get more scientists and are able to do more work, it seems obvious to me that the constraints are going to hurt, and I do not think that is inconsistent.

Senator HARKIN. Okay. Thank you.

Thank you, Mr. Chairman.

Senator SPECTER. Thank you very much, Senator Harkin.

Dr. Zerhouni, we would appreciate it if you would provide for the record: first, the total number of eligible stem cell line derivations; second, the total number of stem cell lines; and third, the total number of stem cell lines available to federally funded scientists.

[The information follows:]

QUESTIONS SUBMITTED TO DR. ZERHOUNI

Question. What is the status of each embryonic stem cell derivation?

Specifically, please give us the information requested below, and any additional information that you would feel would be significant for the Committee's understanding of the current implementation of the stem cell policy.

Also, contrast this information submitted by the NIH to the Subcommittee on September 27, 2001 and in Secretary's testimony before the Senate Health, Education, Labor and Pensions Committee on September 5, 2001. Explain all discrepancies.

Answer. To respond to your questions, in the attached table, NIH has summarized the status of human embryonic stem cells (hESCs) from information we compiled in 2001, as well as updated information we received in June 2003 and again on September 23, 2003 from each of the 14 providers listed on the NIH Human Embryonic Stem Cell Registry (<http://escr.nih.gov>) (Appendix A). In this response, we have also provided answers to additional questions from staff of the Senate Labor-HHS-Edu-

cation Appropriations Subcommittee regarding differences between the information provided in 2001 and information gathered in June 2003 (Appendix B). In addition, NIH is submitting additional information from the providers that were received in June 2003 and again in September 23, 2003. These tables are included as Appendix C and D, respectively.

It is important to note, those providers without NIH funding are not required to respond to NIH's questions. All providers on the Registry were sent an e-mail request outlining questions that your staff sent to the NIH Office of Legislative Policy and Analysis in June 2003. NIH staff met with hESC line providers at the annual meeting of NIH stem cell infrastructure grantees in June 2003, where the providers presented data on their approved cells and discussed issues on characterization, development and distribution of their cells, which is outlined in the summary table. As part of the process to update the NIH Stem Cell web site (stemcells.nih.gov) with the most recent data, the NIH queried the providers again during the summer of 2003 and received their latest information on September 2003.

Information provided to the Senate Labor-HHS-Education Appropriations Subcommittee in September 2001 was obtained in a similar manner, i.e., through e-mail, telephone contacts, and meetings with some of the providers in August 2001. The original 2001 report is attached as Appendix E, for your reference.

In August 2001, the NIH identified 11 entities or organizations that derived and/or were capable of distributing hESCs that were derived from 64 unique embryos. In September 2001, the NIH reported that cells were in various stages of characterization; some were fully characterized and some were in the very earliest stages of characterization. In all cases, the providers agreed to work with the NIH to find ways to make cells available for research consonant with relevant national policies and depending upon their self-renewal capabilities, undifferentiated state, characterization, scalability, as well as the resolution of intellectual property issues. The process for characterizing and scaling up hESCs is lengthy and difficult, where success in generating a well-characterized hESC cell line ready for widespread distribution is by no means a certainty. Thus, some of the derivations identified in 2001 have not been able to be further developed; some are still in various stages of development, while still others are now available to researchers. Some of the derivations are still frozen, while providers explore more advanced culturing techniques. In still other cases, international policies have been developed which prohibit, hinder, or present barriers to the export of stem cell lines outside the country policies that were not in place in 2001.

THERAPEUTIC APPLICATIONS

Question. Do you think the 12 NIH approved human ES cell lines will prove sufficient for use of human ES cells in therapeutics in the future? Will more lines eventually be needed?

Answer. Currently, cells from the NIH Human Embryonic Stem Cell Registry are being used to understand the basic principles of "stemness," e.g., factors involved in maintaining stem cells' undifferentiated and pluripotent states. In addition, these cells are being used by scientists to discover the molecular mechanisms that regulate differentiation into various adult cell types. NIH Infrastructure awards to the hESC providers listed on the Registry may result in additional hESC lines becoming available to researchers later this year. Until scientists fully understand the basics of hESCs, it is impossible to say with certainty whether or not more lines will eventually be needed. But any future experiments needed for therapeutics that require the derivation of new embryonic stem cell lines would not be eligible for Federal funds.

Question. Do you believe that the mouse feeder component of the NIH approved human ES cell lines will affect clinical research in this area? If human ES cells were grown without mouse feeder material, would you think that they would be a better option for human clinical research? In your view, would it be ethical to use human ES cell lines made with mouse feeder cells if cell lines without mouse feeders were available?

Answer. Representatives from the Food and Drug Administration (FDA) discussed with members of the NIH Stem Cell Task Force and with the hESC infrastructure awardees whether or not hESCs grown on human feeder layers could be used more readily and with greater safety than hESCs grown on mouse feeder layers. As with any proposed therapy, FDA safety requirements stipulate that risks must be balanced with the potential benefit achieved by the intervention. In the case of hESCs, the FDA would like to know certain facts before the cells can be used in clinical trials: the characteristics of the stem cells, how the stem cells were derived, the properties of any feeder layer used to propagate the cells, potential contaminants

introduced through the media or serum used in culture, and the presence of infectious agents transmitted from feeder layer cells to cultured hESCs.

One important point made by FDA representatives was that cell lines grown on human feeder layers are not necessarily safer for clinical trials than stem cells grown on mouse feeder layers. Both mouse and human feeder layers may harbor pathogens that could be transmitted to the hESCs grown on them. This said, there are presently therapies in clinical trials that have been developed using contact with animal cells. Thus, if the safety and effectiveness of hESC lines grown on mouse feeders can be demonstrated, these cells would be a viable option for therapy. Contact with feeder cells is one of many safety considerations that need to be assessed before clinical application of this technology.

NIH appreciates the opportunity to respond to these questions and has made a good faith effort to present information obtained directly from human embryonic stem cell providers which outlines the progress made since 2001. In addition, NIH believes this information illustrates how quickly the science evolves in this exciting field of research.

QUESTIONS SUBMITTED TO DR. VON ESCHENBACH

Question. This subcommittee has heard from many researchers over the past few years about the unique potential that stem cells could have for understanding the basic biology and treatment of cancer.

- On September 14, 2000, Dr. Gerald Fischbach, now the Dean of Columbia School of Medicine told the subcommittee that there is evidence for stem cells becoming tumors to deliver toxins to tumors cells.
- On September 14, 2000, Dr. Lawrence Goldstein, professor of Cellular & Molecular Medicine, an investigator with the Howard Hughes Medical Institute at the University of California, San Diego told the subcommittee that human ES cells could help produce bone marrow to treat cancer.
- On September 14, 2000, Dr. Richard Hynes, Director of the Center for Cancer Research at MIT told the subcommittee that human ES cells and adult stem cells are needed for cancer research.
- On June 21, 2001, former NCI Director Richard Klausner told the subcommittee that stem cell research could be helpful to replace tissues in patients that were damaged by cancer.
- On July 18, 2001, the hearing record includes a statement by former NCI Director Richard Klausner noting that “stem cell research is critical to cancer research” and expressed the need for side by side comparisons of embryonic and adult stem cells.
- On July 18, 2001, Dr. Mary Hendrix, FASEB President from University of Iowa College of Medicine stated that human ES cells might allow us to engineer cells and tissues that are resistant to the most effective, but most toxic, cancer therapies.
- On September 25, 2002, Dr. Curt Civin, Professor of Cancer Research, Johns Hopkins University told the subcommittee that studying Human ES cells will help discover the molecular pathways by which they can proliferate without differentiating and then figure out how this applies to adult stem cells. He said this research would help develop new treatments for his cancer patients.

Do you agree with these views from leading cancer researchers that human ES cell research will be critical for cancer? Given these views, why did NCI spend only \$48,000 of its nearly \$5 billion budget on human ES cell research in fiscal year 2002 and why are you projecting no funding for fiscal year 2003? Isn't this woefully inadequate? How much do you think that NCI should spend on human ES research?

Answer. The National Cancer Institute is fully supportive of cancer-related research on human embryonic stem cells within the current federal guidelines. We agree that stem cells will be an important tool in basic cancer research and, in the long term, offer potential new pathways to cancer treatment. This response to your questions will detail our current activities and our plans for near-term initiatives, putting human embryonic stem cell research in the broader context of cancer-related stem cell research.

Stem cells of several different types are relevant to cancer research. For many years we have funded a large share of the research on hematopoietic stem cells, because of the frequent use of bone marrow transplants in cancer patients. There is also growing support for the idea that tissue-specific adult stem cells are prominent targets of malignant transformation. If this is true, then prevention strategies should focus on protecting stem cells, and treatment strategies must be designed to eliminate not just the many more differentiated cells within a tumor, but the trans-

formed stem cells that are responsible for continued cell growth. The immediate applicability of questions related to tissue-specific adult stem cells and cancer has given them high priority within the NCI, and we plan to organize a "Think Tank" on this subject in the coming year.

Research on human embryonic stem cells will complement the research on adult stem cells, although direct application to cancer treatment is likely to take longer because so little is known about the biology of these cells. The research community has not submitted any new grant applications to NCI proposing work on human embryonic stem cells, so we have not had the opportunity to support new investigator-initiated research in this area. Last year was the final year for the grant that was reported for this area in fiscal year 2002. We are devoting resources to human embryonic stem cells through a contract mechanism as part of the NCI supported Cancer Genome Anatomy Project (CGAP). CGAP has been characterizing the genetic transcripts present in normal and transformed cells of many different types for several years, producing a great deal of information valuable to the research community. Because so little is known about human embryonic stem cells, NCI has recently added them to the set of cells characterized by CGAP. The expression profiles determined for these cells are unique, and this information is freely available to the research community through the NCI website.

The fiscal year 2003 funding projections for human embryonic stem cell were based on the fiscal year 2002 research portfolio and confirmed fiscal year 2003 initiatives. This is an evolving area of research, and NCI continues to review the portfolio to identify other ways to learn more about human embryonic stem cells. The addition of human embryonic stem cells to the set of cells characterized by CGAP is one such initiative. As other initiatives are identified, they will of course become part of the research portfolio. In an effort to address the lack of investigator-initiated applications, the NCI has announced at a number of meetings its willingness to support investigator-initiated applications proposing cancer-related studies of human embryonic stem cells, but this has been insufficient to attract applications. We are currently discussing the best strategy to attract new research grants in this area, and expect to begin work on one or more fiscal year 2004 initiatives shortly.

Appendix A
 Summary of Human Embryonic Stem Cell (hESC) Information from Individual Providers
 9/2001 and 6/2003 and 9/23/2003

<u>Source</u>	<u>September 27, 2001</u>	<u>June 2003</u>	<u>September 23, 2003</u>
BresaGen, Inc.	4 derivations, 4 characterized, 0 available for distribution	4 derivations, 4 characterized, 2 available for distribution.	4 derivations, 4 characterized, 2 available for distribution.
CyThera, Inc.	9 derivations, 0 characterized, 0 available for distribution.	9 derivations, 0 characterized, 0 available for distribution.	9 derivations, 0 characterized, 0 available for distribution.
ES Cell International, Ltd. (Australia and Singapore)	6 derivations, 5 characterized, 0 available for distribution.	6 derivations, 5 characterized, 5 available for distribution.	6 derivations, 5 characterized, 5 available for distribution.
Geron Corporation*	7 derivations with 2 subclones (5 from Wisconsin and, 2 from UCSF).	7 derivations, 7 characterized, 0 available for distribution.	7 derivations, 7 characterized, 0 available for distribution.
Cell Therapeutics Skandnavia AB, Göteborg (Sweden)**	19 derivations, 3 characterized (16 under development. Of those under development: 4 partially assessed, and 12 in the early passages beginning characterization), 0 available for distribution	19 derivations, 2 characterized (1 derivation lost, 16 derivations not developed until new xeno-free feeder techniques are perfected), 0 available for distribution. †	Cell Therapeutics has asked that they be represented separately from Göteborg Univ. This resulted in two separate charts for CTS and Göteborg. CTS holds 3 derivations, 1 withdrawn by donor; Göteborg Univ. holds 16 derivations.

Appendix A
 Summary of Human Embryonic Stem Cell (hESC) Information from Individual Providers
 9/2001 and 6/2003 and 9/23/2003

Source	September 27, 2001	June 2003	September 23, 2003
Karolinska Institute (Sweden)	5 derivations, 10 lines characterized, 0 available for distribution (Note: each derivation produced 2 lines).	5 derivations (6 in cryopreservation, 2 of which were cultured on mouse feeders and are partially characterized, 4 of which were cultured on human feeders and have not been characterized, are viable, but they are not proliferating), 0 available for distribution.	5 derivations (6 in cryopreservation, 2 of which were cultured on mouse feeders and are partially characterized, 4 of which were cultured on human feeders and have not been characterized, are presumed to be viable, but are not currently being cultured, 0 available for distribution.
Maria Biotech (Korea)	Existence of eligible derivations not known at this time.	3 derivations, 3 characterized, 0 available for distribution	3 derivations, 3 characterized, 0 available for distribution, new characterization data.
MizMedi Hospital (Korea)	Existence of eligible derivations not known at this time.	1 derivation, 1 characterized, 1 available for distribution.	1 derivation, 1 characterized, 1 available for distribution.
National Centre for Biological Sciences (India)	3 derivations, 0 characterized, 0 available for distribution.	3 derivations, 0 characterized, 0 available for distribution	3 derivations, 0 characterized, 0 available for distribution
Pochon CHA University (Korea)	Existence of eligible derivations not known at this time.	2 derivations (in cryopreservation), 2 partially characterized (may not be viable), 0 available for distribution. Source did not respond to NIH request for additional information.	2 derivations (in cryopreservation), 2 partially characterized (may not be viable), 0 available for distribution. Source did not respond to NIH request for additional information.

Appendix A
Summary of Human Embryonic Stem Cell (hESC) Information from Individual Providers
9/2001 and 6/2003 and 9/23/2003

Source	September 27, 2001	June 2003	September 23, 2003
Reliance Life Sciences (India)	7 derivations, 0 characterized, 0 available for distribution.	7 derivations. Source did not respond to NIH request for additional information.	7 derivations. Source did not respond to NIH request for additional information.
Technion R&D Foundation, Ltd. (Israel)	4 derivations, 4 characterized, 0 available for distribution.	4 derivations, 4 characterized, 2 available to NIH supported researchers.	4 derivations, 4 characterized, 2 available for distribution in the near future. (Note: Notice of Infrastructure Grant Award sent to Technion).
University of California at San Francisco	2 derivations, 2 characterized, 0 available for distribution.	2 derivations, 2 characterized, 1 available for distribution.	2 derivations, 2 characterized, 1 available for distribution, new characterization data.
WiCell Research Institute	5 derivations, 5 characterized, 0 available for distribution.	5 derivations, 3 characterized, 3 available for distribution.	5 derivations, 5 characterized, 3 available for distribution
Summary	64 derivations, 24 characterized, 0 available for distribution. ††	71 derivations, 24 characterized, 12 available for distribution. ††	71 derivations, 26 characterized, 12 available for distribution. ††

*Geron supported the derivation of the Univ. of Wisconsin and UCSF cells, but are listed on the Registry because they may be a source of these cells through collaborative agreements with scientists.

**Cell Therapeutics, Skandnavia, is a biotech company that began operations after 2001 and is the provider of the established cell lines, however, Göteborg University is the organization that derived the cells and currently appears on the NIH Stem Cell Registry.

†Cell Therapeutics/Göteborg University informed the NIH that 16 derivations remain frozen in human serum. The investigators indicated that their strategy has been to perfect methods for developing lines in feeder-free conditions and then try to develop some of the 16 derivations as cell lines. All 16 derivations were established by August 9, 2001.

††Geron has seven cell lines listed on the NIH Human Embryonic Stem Cell Registry. Five of them were derived at the University of Wisconsin; two are clones of one of the Wisconsin lines. The seven Geron lines are available for distribution but are not counted in this summary total.

APPENDIX B.—EXPLANATION OF CHANGES IN DATA BETWEEN 2001 AND 2003

Question. Why the differences between September 2001 and June 2003? What are the differences between June 2003 and the present (September 2003)?

Answer. NIH is pleased to respond to your questions about three specific differences between information from 2001 and June 2003 on the summary chart (Attachment A). We would like to underscore that all information provided in the charts was collected from the providers in 2001, in June 2003, and then again in September 2003. Given the rapid pace of development in the area of human embryonic stem cell research, additional scientific information regarding the cell lines has emerged since 2001. In addition, the term “characterization” is applied differently over time as scientists discover new technologies to study the properties of these cells.

As for your specific questions about three differences:

Cell Therapeutics, Skandania AB (CTS), Göteborg, Sweden

9/2001: NIH reported the following: “[19 or 18] stem cell lines in various stages of development. Of these, 3 are fully characterized cell lines, and 15 are under development. Of the lines under development, four are partially assessed, and 12 are in the early passages and beginning to undergo characterization.”

6/2003: 19 derivations, 2 characterized, 1 derived characterization lost, 16 derivations not developed until new feeder techniques are perfected.

9/2003: 19 derivations, 2 characterized, 1 derivation withdrawn by donor, 16 derivations not developed until new feeder techniques are perfected). Cell Therapeutics, Skandania (CTS), a biotechnology company founded out of University, requested that NIH report CTS and Göteborg University as separate entities, with SA-01 and SA-02 licensed from Göteborg and SA-04-SA-19 property of Göteborg University.

Explanation.—In 2001, a representative of Göteborg University reported to NIH that it had “fully characterized” 3 derivations, “four are partially assessed, and 12 are in the early passages and beginning to undergo characterization.” In June 2003, Göteborg University informed NIH that “16 derivations were not developed until new xeno-free feeder techniques are perfected.” In a licensing agreement between Göteborg University and CTS, three established cell lines have been transferred to CTS while Göteborg University retains the remaining derivations until cell lines are established. Also, in September 2003, CTS reports that 1 of the derived and characterized lines was withdrawn by the donor.

In 2001, a representative of Göteborg University reported to NIH that four derivations were “partially assessed and 12 are in the early passages and beginning to undergo characterization.” On November 4, 2003, a representative of Göteborg reported that this information is incorrect. Göteborg informed NIH that these same derivations remain frozen after minimal expansion and have not yet been developed further. Göteborg University told NIH that the cells had been through minimal expansion post-immunosurgery in order to have the critical mass of cells for cryopreservation, but there are no immediate plans to characterize these derivations. Göteborg also informed NIH that the derivations may have been exposed to fetal calf serum during the derivation process, but have not been exposed to mouse feeder cells.

The Karolinska Institute, Sweden

9/2001: 5 derivations, 10 characterized

6/2003: 5 derivations, (6 in cryopreservation, 2 of which were cultured on mouse feeders and are partially characterized, 4 of which were cultured on human feeders and have not been characterized, are viable but not proliferating . . .).

9/2003: Same information as 6/2003, but the cells, “. . . . are presumed to be viable, but are not currently being cultured.”

Explanation.—Although the Karolinska Institute described these cells as characterized in 2001, since that time, given what they have learned about these cells, they no longer refer to them as characterized. The Karolinska Institute has been awarded an NIH Infrastructure grant which allows them to determine the viability and properties of their frozen cells.

WiCell Research Institute

9/2001: 5 derivations, 5 characterized, available for distribution

6/2003: 5 derivations, 3 characterized, 3 available for distribution

9/2003: 5 derivations, 5 characterized, 3 available for distribution

Explanation.—In June 2003, WiCell submitted a chart to NIH outlining characteristics of 3 cell lines, but omitted characterization information for two other lines that are undergoing further quality control testing and are not yet available for distribution. Based on that information, NIH reported 3 of 5 cell lines as characterized,

which was different than what was reported in 2001. To verify this information, NIH contacted WiCell. WiCell confirmed that all 5 of their eligible human embryonic stem cell lines are fully characterized, but they did not include the specific characteristics for two lines in June 2003 because these lines are not yet available for distribution. NIH updated this on the September 23, 2003 summary information on the attached table.

Update on additional information since June 2003.

Maria Biotech, Korea

9/2003: Provided additional information about cell characteristics.

Technion, Israel

9/2003: NIH Notice of Grant Award made for Infrastructure grant.

Univ. of California at San Francisco

9/2003: Provided additional information about cell characteristics.

APPENDIX C.—INDIVIDUAL PROVIDER TABLES ON HUMAN EMBRYONIC STEM CELL DERIVATIONS

BRESAGEN PHS REGISTERED CELL LINE (UNITED STATES)				
R24 DK 63689 0				
4-30-2004 (1)				
	hESBGN.01 (BG01)	hESBGN.02 (BG02)	hESBGN.03 (BG03)	hESBGN.04 (BG04)
Embryo				
Source	IVF Clinic	IVF Clinic	IVF Clinic	IVF Clinic
Category	Fresh	Fresh	Fresh	Fresh
IVF Status	Discarded – unacceptable for further use	Discarded – unacceptable for further use	Discarded – unacceptable for further use	Discarded – unacceptable for further use
Characteristics				
Passage #	>70	>40	<5	<5
Mouse Feeder Cells used in Isolation	Yes	Yes	Yes	Yes
Karyotype	Normal	Normal	TBD	TBD
Stem cell Immuno markers:	+	+		
SSEA-1	-	-	-	-
SSEA-3	+	+	+	+
SSEA-4	+	+	+	+
TRA 1-60	+	+	TBD	TBD
TRA 1-81	+	+	TBD	TBD
Oct-4	+	+	TBD	TBD
Alkaline Phosphatase	+	+	+	+
Gender	Male	Male	TBD	TBD
Frozen/thawed:				
Short-term	yes	yes	yes	yes
Long-term	yes	yes	TBD	TBD
Pluripotent	yes	yes	TBD	TBD
Commercialization				
available	Yes	Yes	No (schedule mid-2003, presently recovering and expanding)	No (schedule end-2003, soon to be recovered and expanded)
Price	\$5,000 + actual shipping cost	\$5,000 + actual shipping cost	NA	NA
# shipped	8	3	NA	NA
Customer Base			NA	NA
United Kingdom	1	0	NA	NA
Israel	2	0	NA	NA
US	11	5	NA	NA
Australia	1	0	NA	NA
NIH Investigators	9	5	<i>Not shipped yet</i>	<i>Not shipped yet</i>
Non NIH	6		<i>Not shipped yet</i>	<i>Not shipped yet</i>

- TBD = To be Determined; NA = Not Applicable
- (1) End Date of NIH Grant

CELLTHERAPEUTICS REGISTERED CELL LINES (SWEDEN) (UNIVERSITY OF GÖTEBORG) R24 RR 15914 END DATE OF 07-06-2005 (1)				
	SA01 (Salgrenska 1)	SA02 (Salgrenska 2)	SA03 (Salgrenska 3)	SA04-SA16 (Salgrenska 4 – Salgrenska 16)
Embryo				
Source	IVF Clinic	IVF Clinic	IVF Clinic	IVF Clinic
Category	Fresh	Fresh	Fresh	Fresh
IVF Status	Surplus, to be discarded			
Characteristics				
Passage #	TBD	TBD	Cell line lost	2 - 3
Mouse Feeder Cells used in Isolation	Yes	Yes	Yes	No
Karyotype	TBD	46XX	N/A	TBD
Stem cell Immuno markers:	TBD		N/A	TBD
SSEA-1	-	-		
SSEA-3	+	+		
SSEA-4	+	+		
TRA 1-60	+	+		
TRA 1-81	+	+		
Oct-4	+	+		
Alkaline Phosphatase	+	+		
Gender	TBD	Female	N/A	TBD
Frozen/thawed:	TBD		N/A	TBD
Short-term		Yes		
Long-term		Yes		
Pluripotent	TBD	Yes	N/A	TBD
Commercialization available	N/A at this time	N/A at this time	N/A	N/A at this time
Price				
# shipped				
Customer Base for all hESC lines	N/A	N/A	N/A	N/A
International				
US				
<i>NIH Investigators</i>				
<i>Non NIH</i>				

- TBD = To be Determined; NA = Not Applicable
- (1) End date of NIH Grant

Emailed questions 6-4-2003 No response received	Cell & Gene Therapy Research Institute Pochon CHA University College of Medicine		
	CHA-hES-1 (CH01)	CHA-hES-2 (CH02)	
Embryo			
Source			
Category			
IVF Status			
Characteristics			
Passage #			
Mouse Feeder Cells used in Isolation			
Karyotype			
Telomerase activity			
SSEA-1			
SSEA-3			
SSEA-4			
TRA 1-60			
TRA 1-81			
Oct-4			
Alkaline Phosphatase			
Gender			
Frozen/thawed:			
In vitro differentiation			
Pluripotent			
Commercialization			
available			
Price			
# shipped			
Customer Base limited to at this time			
<i>NIH Investigators</i>			
<i>Non NIH</i>			

CYTHERA REGISTERED CELL LINES R24 DK 63689-01 05/01/2004 END DATE (1)				
	CY12 (hES-1-2)	CY30 (hES-3-0)	CY40 (hES-4-0)	CY51 (hES-5-1)
Embryo				
Source	IVF Clinic	IVF Clinic	IVF Clinic	IVF Clinic
Category	Frozen embryo	Frozen embryo	Frozen embryo	Frozen embryo
IVF Status	Surplus, to be discarded			
Characteristics				
Passage #	2	3	4	5
Mouse Feeder Cells used in Isolation	Yes	Yes	Yes	Yes
Karyotype	TBD	TBD		
Stem cell Immuno markers:				
SSEA-1	TBD	TBD	TBD	TBD
SSEA-3	TBD	TBD	TBD	TBD
SSEA-4	TBD	TBD	TBD	TBD
TRA 1-60	TBD	TBD	TBD	TBD
TRA 1-81	TBD	TBD	TBD	TBD
Oct-4	TBD	TBD	TBD	TBD
Alkaline Phosphatase	+	+	+	+
Gender	TBD	TBD	TBD	TBD
Frozen/thawed:	TBD	TBD	TBD	TBD
Short-term				
Long-term				
Pluripotent	TBD	TBD	TBD	TBD
Commercialization	N/A at this time			
available				
Price				
# shipped				
Customer Base	N/A	N/A	N/A	N/A
<i>NIH Investigators</i>				
<i>Non NIH</i>				

- TBD = To be Determined; NA = Not Applicable (1) End date of Infrastructure Award

CYTHERA REGISTERED CELL LINES				
R24 DK 63689-01				
05/01/2004 END DATE				
	CY81 (hES-8-1)	CY82 (hES-8-2)	CY91 (hES-9-1)	CY92 (hES-9-2)
Embryo				
Source	IVF Clinic	IVF Clinic	IVF Clinic	IVF Clinic
Category	Frozen embryo	Frozen embryo	Frozen embryo	Frozen embryo
IVF Status	Surplus, to be discarded			
Characteristics				
Passage #	4	3	5	2
Mouse Feeder Cells used in Isolation	Yes	Yes	Yes	Yes
Karyotype	TBD	TBD		
Stem cell Immuno markers:				
SSEA-1	TBD	TBD	TBD	TBD
SSEA-3	TBD	TBD	TBD	TBD
SSEA-4	TBD	TBD	TBD	TBD
TRA 1-60	TBD	TBD	TBD	TBD
TRA 1-81	TBD	TBD	TBD	TBD
Oct-4	TBD	TBD	TBD	TBD
Alkaline Phosphatase	+	+	+	+
Gender	TBD	TBD	TBD	TBD
Frozen/thawed:	TBD	TBD	TBD	TBD
Short-term				
Long-term				
Pluripotent	TBD	TBD		
Commercialization	N/A at this time			
available				
Price				
# shipped				
Customer Base	N/A	N/A	N/A	N/A
<i>NIH Investigators</i>				
<i>Non NIH</i>				

- TBD = To be Determined; NA = Not Applicable
- (1) End Date of Infrastructure Award

CYTHERA REGISTERED CELL LINES R24 DK 63689-01 05/01/2004 END DATE				
CY10 (hES-101)				
Embryo				
Source	IVF Clinic			
Category	Frozen embryo			
IVF Status	Surplus, to be discarded			
Characteristics				
Passage #	2			
Mouse Feeder Cells used in Isolation	Yes			
Karyotype	TBD			
Stem cell Immuno markers:				
SSEA-1	TBD			
SSEA-3	TBD			
SSEA-4	TBD			
TRA 1-60	TBD			
TRA 1-81	TBD			
Oct-4	TBD			
Alkaline Phosphatase	+			
Gender	TBD			
Frozen/thawed:	TBD			
Short-term				
Long-term				
Pluripotent	TBD			
Commercialization available	N/A at this time			
Price # shipped				
Customer Base	N/A			
United Kingdom				
Israel				
US				
Australia				
<i>NIH Investigators</i>				
<i>Non NIH</i>				

• TBD = To be Determined; NA = Not Applicable (1) End Date of Infrastructure Award

ES CELL INTERNATIONAL REGISTERED CELL LINES R24 RR 17499-02 04/17/2004 END DATE (1)				
	ES01 (HES-1)	ES02 (HES-2)	ES03 (HES-3)	ES04 (HES-4)
Embryo				
Source	IVF Clinic	IVF Clinic	IVF Clinic	IVF Clinic
Category	Frozen	Frozen	Frozen	Frozen
IVF Status	Surplus, to be discarded			
Characteristics				
Passage #	TBD	TBD	TBD	TBD
Mouse Feeder Cells used in Isolation	Yes	Yes	Yes	Yes
Karyotype	46XX	46XX	46XX	46XY
Stem cell Immuno markers:				
SSEA-1	-	-	-	-
SSEA-3	+	+	+	+
SSEA-4	+	+	+	+
TRA 1-60	+	+	+	+
TRA 1-81	+	+	+	+
Oct-4	+	+	+	+
Alkaline Phosphatase	+	+	+	+
Gender	Female	Female	Female	Male
Frozen/thawed:				
Short-term	Yes	Yes	Yes	Yes
Long-term	Yes	Yes	Yes	Yes
Pluripotent	Yes	Yes	Yes	Yes
Commercialization				
available	>100	>100	>100	>100
Price	\$5000	\$5000	\$5000	\$5000
# shipped	3	8	46	37
Customer Base for all hESC lines	see page 2 for summary			
<i>NIH Investigators</i>	<i>not known</i>			
<i>Non NIH</i>	<i>not known</i>			

• TBD = To be Determined; NA = Not Applicable
(1) End Date of Infrastructure Award

ES CELL INTERNATIONAL REGISTERED CELL LINES R24 RR 17499-02 04/17/2004 END DATE (1)			
	ES05 (HES-5)	ES06 (HES-6)	
Embryo			
Date derived	September 2000	September 2000	
Racial origin	Caucasian	Caucasian	
Source	IVF Clinic	IVF Clinic	
Category	Frozen	Frozen	
IVF Status	Surplus, to be discarded	Surplus, to be discarded	
Characteristics			
Passage #	TBD	TBD	
Mouse Feeder Cells used in Isolation	Yes	Yes	
Karyotype	TBD	TBD	
Stem cell Immuno markers:			
SSEA-1	-	-	
SSEA-3	+	+	
SSEA-4	+	+	
TRA 1-60	+	+	
TRA 1-81	+	+	
Oct-4	+	+	
Alkaline Phosphatase	+	+	
Gender	TBD	TBD	
Frozen/thawed:			
Short-term	Yes	Yes	
Long-term	Yes	Yes	
Pluripotent	Yes	Yes	
Commercialization			
available	undergoing final characterization	>100	
Price	\$5000	\$5000	
# shipped	0	0	
Customer Base for all hESC lines			
International	33		
US	16		
NIH Investigators	not known		
Non NIH	not known		

* TBD = To be Determined; NA = Not Applicable
(1) End Date of Infrastructure Award

Geron Corporation, Menlo Park, CA							
Emailed 5-06-2003 Response received 6-12-2003, Please see attached text.	GE01 (same as WICELL H1)	GE07 (Same as WICELL H7)	GE09 (Same as WICELL H9)	GE13 (Same as WiCell H13)	GE14 (Same as WiCell H14)	GE91 (clone)	GE92 (clone)
Source							
Category							
IVF Status							
Characteristics							
Passage #							
Mouse Feeder Cells used in Isolation							
Karyotype							
Telomerase activity							
SSEA-1							
SSEA-3							
SSEA-4							
TRA 1-60							
TRA 1-81							
Oct-4							
Alkaline Phosphatase							
Gender							
Frozen/thawed:							
In vitro differentiation							
Pluripotent							
Commercialization							
available							
Price							
# shipped							
Customer Base limited to at this time							
<i>NIH Investigators</i>							
<i>Non NIH</i>							

RESPONSE RECEIVED FROM GERON 6-12-2003

All of the embryonic stem cell lines in Geron's possession that are on the NIH Stem Cell Registry were derived elsewhere. Five of them were derived at the University of Wisconsin-Madison; two are clones of one of the Wisconsin lines; and two others were derived at the University of California, San Francisco. Under Geron's agreement with Wisconsin Alumni Research Foundation, we are not permitted to transfer the undifferentiated Wisconsin cell lines to third parties, except for Geron collaborators for work on projects described and directed by Geron. WiCell Research Institute does distribute the Wisconsin lines to researchers, however, and UCSF distributes the UCSF lines.

As to your specific questions: None of the lines is a frozen inner cell mass; they are all cell lines.

They are all proliferating.

The H1, H7 and H9 lines have been extensively characterized. We have recently submitted manuscripts on the detailed characterization of these three lines. We can provide you a copy of the manuscripts in confidence, or send them to you once they have been published. The lines have the expected markers to show undifferentiated status, and we have demonstrated differentiation in all three germ layers for all 3 of the lines.

The H1, H7 and H9 lines have been passaged extensively. In most cases, over 70 passages have been achieved.

The lines are all useful for research. Two of the lines, H1 and H7 have been tested quite extensively for the presence of potential pathogens of human and animal origin, based on the specified by FDA in its Points to Consider and other guidance.

Please let me know if you need additional information, and if you would like to arrange to review the manuscripts confidentially.

William D. Stempel, vice President and General Counsel, Geron corporation, 230 Constitution Drive, Menlo Park, CA Email: bstempel@geron.com.

KARLOLINSKA REGISTERED CELL LINES R21 RR 18177-01 07/14/2003 END DATE (1)				
	KA08 (hICM8)	KA09 (hICM9)		
Embryo				
Source	IVF Clinic	IVF Clinic		
Category	Fresh	Fresh		
IVF Status	Surplus, to be discarded	Surplus, to be discarded		
Characteristics				
Passage #	Frozen , awaiting characterization	Frozen , awaiting characterization		
Mouse Feeder Cells used in Isolation	Yes	Yes		
Karyotype	TBD	TBD		
Stem cell Immuno markers:				
SSEA-1	TBD	weak		
SSEA-3	TBD	TBD		
SSEA-4	TBD	++		
TRA 1-60	TBD	TBD		
TRA 1-81	TBD	TBD		
Oct-4	TBD	TBD		
Alkaline Phosphatase	+	+		
Gender	TBD	TBD		
Frozen/thawed:	TBD	TBD		
Short-term	3 weeks	3 weeks		
Long-term	3 months	3 months		
Pluripotent	TBD	TBD		
Commercialization available	N/A at this time	N/A at this time		
Price				
# shipped				
Customer Base	N/A	N/A		
United Kingdom				
Israel				
US				
Australia				
NIH Investigators				
Non NIH				

• TBD = To be Determined; NA = Not Applicable (1) End Date of Infrastructure Award

KARLOLINSKA REGISTERED CELL LINES R21 RR 18177-01 07/14/2003 END DATE (1)				
	KA40 (hICM40)	KA41 (hICM41)	KA42 (hICM42)	KA43 (hICM43)
Embryo				
Source	IVF Clinic	IVF Clinic	IVF Clinic	IVF Clinic
Category	Fresh	Fresh	Fresh	Fresh
IVF Status	Surplus, to be discarded			
Characteristics				
Passage #	Frozen , awaiting characterization			
Mouse Feeder Cells used in Isolation	No	No	No	No
Karyotype	TBD	TBD	TBD	TBD
Stern cell Immuno markers:	TBD	TBD	TBD	TBD
SSEA-1				
SSEA-3				
SSEA-4				
TRA 1-60				
TRA 1-81				
Oct-4				
Alkaline Phosphatase				
Gender	TBD	TBD	TBD	TBD
Frozen/thawed:	TBD	TBD	TBD	TBD
Short-term				
Long-term				
Pluripotent	TBD	TBD	TBD	TBD
Commercialization available	N/A at this time			
Price				
# shipped				
Customer Base	N/A	N/A	N/A	N/A
United Kingdom				
Israel				
US				
Australia				
NIH Investigators				
Non NIH				

• TBD = To be Determined; NA = Not Applicable, (1) End Date of Infrastructure Award

MARIA INFERTILITY HOSPITAL, MARIA BIOTECH, KOREA			
	MBO1	MB02	MBO3
Embryo			
Source	IVF Clinic	IVF Clinic	IVF Clinic
Category	Cryopreservation	Cryopreservation (27 passages) In vitro (>30 passages)	Cryopreservation (27 passages) In vitro culture (>50 passages)
IVF Status	Were to be discarded 5 years after IVF-ET program	Were to be discarded 5 years after IVF-ET program	Were to be discarded 5 years after IVF-ET program
Characteristics			
Passage #	40	>30	>50
Mouse Feeder Cells used in Isolation	STO cell feeder (After immunosurgery, isolated ICM cell was cocultured with mouse STO cell and hES cell line was established)	STO cell --feeder free (After immunosurgery, isolated ICM cells were cocultured with mouse STO cell. To grow hES cells without a feeder layer, aged MB02 and MB03 cells that had been passaged 10 times were cultured on Matrigel coated plates, respectively.)	STO cell --feeder free (After immunosurgery, isolated ICM cells were cocultured with mouse STO cell. To grow hES cells without a feeder layer, aged MB02 and MB03 cells that had been passaged 10 times were cultured on Matrigel coated plates, respectively.)
Karyotype	46 XY, Normal	46, XX, Normal	46, XX
Telomerase activity	+	+	+
SSEA-1	-	-	-
SSEA-3	+	+	+
SSEA-4	+	+	+
TRA 1-60	+	+	+
TRA 1-81	+	+	+
Oct-4	+	+	+
Alkaline Phosphatase	+	+	+
Gender	Male	Female	Female
Frozen/thawed:	40 passages frozen	27 passages frozen	>50 passages in

		>30 passages cultured	vitro culture
In vitro differentiation	Neuron, muscle, Cardiomyocytes, AFP	Neuron, muscle, Cardiomyocytes, AFP	Neuron, muscle, Cardiomyocytes, AFP
Pluripotent	yes	yes	yes
Commercialization			
available	To scientists in Korea	To scientists in Korea	To scientists in Korea
Price	Unknown	Unknown	Unknown
# shipped	Unknown	Unknown	Unknown
Customer Base limited to Korea at this time			
<i>NIH Investigators</i>	0	0	
<i>Non NIH</i>	unknown	0	

Maria Biotech, Korea

Question. What is the status of each of your embryonic stem cell derivation listed on the NIH Embryonic Stem Cell Registry?

—Is it a frozen inner cell mass?

Answer. We established hES cells derived from ICMs of frozen-thawed blastocysts that were destined to be discarded 5 years after human IVF-ET program.

Question. Have these derivations been shipped to any NIH-funded researchers?

Answer. Our cells are available to collaborators in our country.

Question. If the derivation has not been shipped, are there any plans to develop this derivation to research quality?

Answer. We have some plans to study for major chronic degenerative diseases (Parkinson's disease & Alzheimer's disease and Diabetes). Especially, as a preliminary study of PD animal model, our group submitted as Title of Genetically modified human embryonic stem cells relieve symptomatic motor behavior in a rat model of Parkinson's Disease in international journal.

Question. When will they be readily available to NIH researchers?

Answer. Our cells are all set available to collaborators in our country, no plans to ship cells abroad at present.

Question. Have any restrictions been placed on the use of these derivation by your national laws?

Answer. There is no our governmental guideline to ship our cell lines abroad at present.

ADDITIONAL E-MAIL RECEIVED JUNE 22, 2003

(I) MB01 cell line;

After immunosurgery, isolated ICM cell was cocultured with mouse STO cell and hES cell line was established.

(II) MB02 and MB03 cell lines;

After immunosurgery, isolated ICM cells were cocultured with mouse STO cell. To grow hES cells without a feeder layer, aged MB02 and MB03 cells that had been passaged 10 times were cultured on Matrigel coated plates, respectively.

Thank you for your consideration.

Jinho Lim, MD, President/CEO, Maria Hospital/Maria Biotech, Co.,
lim@mariababy.com.

MIZMEDI REGISTERED CELL LINES R24 RR 118406-01 09/29/2004 END DATE (1)			
MI01 (Miz-hES1)			
Embryo			
Source	IVF Clinic		
Category	Frozen embryo		
IVF Status	Surplus, to be discarded		
Characteristics			
Passage #	TBD		
Mouse Feeder Cells used in Isolation	Yes		
Karyotype	46 XY		
Stem cell Immuno markers:			
SSEA-1	-		
SSEA-3	+		
SSEA-4	+		
TRA 1-60	TBD		
TRA 1-81	TBD		
Oct-4	TBD		
Alkaline Phosphatase	+		
Gender	Male		
Frozen/thawed:			
Short-term	Yes		
Long-term	Yes		
Pluripotent	Yes		
Commercialization			
available	Yes		
Price	\$5000		
# shipped	11		
Customer Base			
Korea	8		
Singapore	1		
US	1		
Australia	1		
NIH Investigators			
	TBD		
Non NIH			
	TBD		

* TBD = To be Determined; NA = Not Applicable (1) End Date of Infrastructure Award

NATIONAL CENTRE FOR BIOLOGICAL SCIENCES/TATA INSTITUTE OF FUNDAMENTAL RESEARCH (INDIA)			
Embryo	NC01	NC02	NC03
Source			
Category			
IVF Status			
Characteristics			
Passage #			
Mouse Feeder Cells used in Isolation			
Karyotype			
Telomerase activity			
SSEA-1			
SSEA-3			
SSEA-4			
TRA 1-60			
TRA 1-81			
Oct-4			
Alkaline Phosphatase			
Gender			
Frozen/thawed:			
In vitro differentiation			
Pluripotent			
Commercialization			
available			
Price			
# shipped			
Customer Base limited to Korea at this time			
<i>NIH Investigators</i>			
<i>Non NIH</i>			

NATIONAL CENTRE FOR BIOLOGICAL SCIENCE—RESPONSE TO E-MAIL

Question. What is the status of each of your embryonic stem cell derivation listed on the NIH Embryonic Stem Cell Registry?

—Is it a frozen inner cell mass?

Answer. Yes

Question. Is it proliferating?

Answer. It was frozen after 7 days in culture. We have not characterised them. We are obtaining experience in generating new cell lines and will thaw these when we decide we have sufficient data and experience.

Question. Has it undergone any characterization? If so, are there markers identified to show undifferentiated status? Is their evidence the cells can differentiate into any of the three germ layers (ectoderm, mesoderm, endoderm)?

Answer. No evidence for that—see above.

Question. If the lines are proliferating, how many passages have been achieved?

Answer. See above.

Question. Have these derivations been shipped to any NIH-funded researchers?

Answer. No

Question. If the derivation has not been shipped, are there any plans to develop this derivation to research quality?

Answer. Yes, we hope to develop these and other lines.

Question. When will they be readily available to NIH researchers?

Answer. I hope that once they are derived and characterized they would be available to the academic community.

Question. Have any restrictions been placed on the use of these derivation by your national laws?

Answer. The guidelines that are being discussed at this point suggest that cells may not be shipped outside of India. My understanding is that this may be allowed in the future only on approval by a National Committee on a case by case basis for research purposes. The guidelines as available now place restrictions on their export.

Reliance Life Sciences Mumbai, India							
Emailed 5-06-2003 No response received	RLS ES 05 (RL05)	RLS ES07 (RL07)	RLS ES 10 (RL10)	RLS ES 13 (RL13)	RLS ES 15 (RL15)	RLS ES 20 (RL20)	RLS ES 21 (RL21)
Source	IVF Clinic	IVF Clinic	IVF Clinic	IVF clinic	IVF clinic	IVF clinic	IVF clinic
Category							
IVF Status							
Characteristics							
Passage #							
Mouse Feeder Cells used in Isolation							
Karyotype							
Telomerase activity							
SSEA-1							
SSEA-3							
SSEA-4							
TRA 1-60							
TRA 1-81							
Oct-4							
Alkaline Phosphatase							
Gender							
Frozen/thawed:							
In vitro differentiation							
Pluripotent							
Commercialization							
available							
Price							
# shipped							
Customer Base limited to at this time							
<i>NIH Investigators</i>							
<i>Non NIH</i>							

TECHNION REGISTERED CELL LINES R24 RR 18405-01 POSSIBLE END DATE OF AUGUST 2005 (1)				
	TE-03 (I.3)	TE-32 (I.3.2)	TE-33 (I.3.3)	TE-04 (I.4)
Embryo				
Source	IVF Clinic	IVF Clinic	IVF Clinic	IVF Clinic
Category	Frozen embryo	Frozen embryo	Frozen embryo	Frozen embryo
IVF Status	Surplus, to be discarded	Surplus, to be discarded	Surplus, to be discarded	Surplus, to be discarded
Characteristics				
Passage #	TBD	TBD	TBD	TBD
Mouse Feeder Cells used in Isolation	Yes	Yes	Yes	Yes
Karyotype	TBD	TBD	TBD	TBD
Stem cell Immuno markers:				
SSEA-1	-	TBD	TBD	TBD
SSEA-3	+	TBD	TBD	TBD
SSEA-4	+	TBD	TBD	TBD
TRA 1-60	+	TBD	TBD	TBD
TRA 1-81	+	TBD	TBD	TBD
Oct-4	+	TBD	TBD	TBD
Alkaline Phosphatase	+	TBD	TBD	TBD
Gender	TBD	TBD	TBD	TBD
Frozen/thawed:		TBD	TBD	TBD
Short-term	Yes			
Long-term	Yes			
Pluripotent	Yes	TBD	TBD	TBD
Commercialization	Waiting on NIH MTA agreement	N/A at this time	N/A at this time	N/A at this time
available				
Price				
# shipped				
Customer Base	N/A	N/A	N/A	N/A
NIH Investigators				
Non NIH				

• TBD = To be Determined; NA = Not Applicable
(1) End date of Infrastructure Award

TECHNION REGISTERED CELL LINES R24 RR 18405-01 POSSIBLE END DATE OF AUGUST 2005 (1)				
	TE-06 (I 6)	TE-62 (I 6.2)	TE-07 (J 3)	TE-72 (J 3.2)
Embryo				
Source	IVF Clinic	IVF Clinic	IVF Clinic	IVF Clinic
Category	Frozen embryo	Frozen embryo	Frozen embryo	Frozen embryo
IVF Status	Surplus, to be discarded	Surplus, to be discarded	Surplus, to be discarded	Surplus, to be discarded
Characteristics				
Passage #	TBD	TBD	TBD	TBD
Mouse Feeder Cells used in Isolation	Yes	Yes	Yes	Yes
Karyotype	TBD	TBD	TBD	TBD
Stem cell Immuno markers:				
SSEA-1	-	TBD	TBD	TBD
SSEA-3	+	TBD	TBD	TBD
SSEA-4	+	TBD	TBD	TBD
TRA 1-60	+	TBD	TBD	TBD
TRA 1-81	+	TBD	TBD	TBD
Oct-4	+	TBD	TBD	TBD
Alkaline Phosphatase	+	TBD	TBD	TBD
Gender	TBD	TBD	TBD	TBD
Frozen/thawed:		TBD	TBD	TBD
Short-term	Yes			
Long-term	Yes			
Pluripotent	Yes	TBD	TBD	TBD
Commercialization	Waiting on NIH MTA agreement	N/A at this time	N/A at this time	N/A at this time
available				
Price				
# shipped				
Customer Base	N/A	N/A	N/A	N/A
NIH Investigators				
Non NIH				

• TBD = To be Determined; NA = Not Applicable
(1) End of Infrastructure Award

UCSF REGISTERED CELL LINES R24 RR 17498-02 04/30/2004 END DATE (1)					
		UC01 (HSF-1)	UC06 (HSF-6)		
Embryo					
Source	IVF Clinic	IVF Clinic			
Category	Frozen	Frozen			
IVF Status	Surplus, to be discarded	Surplus, to be discarded			
Characteristics					
Passage #	TBD	TBD			
Mouse Feeder Cells used in Isolation	Yes	Yes			
Karyotype	TBD	46XX			
Stem cell Immuno markers:					
SSEA-1	-	-			
SSEA-3	+	+			
SSEA-4	+	+			
TRA 1-60	+	+			
TRA 1-81	+	+			
Oct-4	+	+			
Alkaline Phosphatase	+	+			
Gender	TBD	Female			
Frozen/thawed:					
Short-term	Yes	Yes			
Long-term	Yes	Yes			
Pluripotent	Yes	Yes			
Commercialization	N/A at this time				
available		>100			
Price		\$5000			
# shipped		20			
Customer Base for all hESC lines	N/A	TBD			
International					
US					
NIH Investigators					
Non NIH					

- TBD = To be Determined; NA = Not Applicable
- (1) End date of Infrastructure Award

WiCELL REGISTERED CELL LINES R24 RR 17721-02 04/30/2004 END DATE (1)				
	WA01 (H1)	WA07 (H7)	WA09 (H9)	
Embryo				
Source	IVF Clinic	IVF Clinic	IVF Clinic	
Category	Frozen	Frozen	Frozen	
IVF Status	Surplus, to be discarded	Surplus, to be discarded	Surplus, to be discarded	
Characteristics				
Passage #	22	23	22	
Mouse Feeder Cells used in Isolation	Yes	Yes	Yes	
Karyotype	TBD	TBD	TBD	
Stem cell Immuno markers:				
SSEA-1	-	-	-	
SSEA-3	+	+	+	
SSEA-4	+	+	+	
TRA 1-60	+	+	+	
TRA 1-81	+	+	+	
Oct-4	+	+	+	
Alkaline Phosphatase	+	+	+	
Gender	TBD	TBD	TBD	
Frozen/thawed:				
Short-term	Yes	Yes	Yes	
Long-term	Yes	Yes	Yes	
Pluripotent	Yes	Yes	Yes	
Commercialization available				
Price	273	100	125	
# shipped	\$5000	\$5000	\$5000	N/A
	127	6	0	
Customer Base for hESC lines	TBD	TBD	TBD	
NIH Investigators	<i>not known</i>			
Non NIH	<i>not known</i>			

• TBD = To be Determined; NA = Not Applicable (1) End Date of Infrastructure Award

Provider: Wisconsin Alumni Research Foundation

Wisconsin Alumni Research Foundation Registered Cell Lines (United States)					
NIH Code	WAF1	WAF2	WAF3	WAF4	WAF5
Accession #	24	25	26	27	28
Embryo					
Source	IVF Clinic				
Storage	Frozen	Frozen	Frozen	Frozen	Frozen
IVF Status	Surplus, to be discarded				
Characteristics					
Passage #	22	23	22	TBD	TBD
Autologous	Yes	Yes	Yes	Yes	Yes
Measurability	Yes	Yes	Yes	Yes	Yes
Karyotype	TBD	TBD	TBD	TBD	TBD
Chromosomes	TBD	TBD	TBD	TBD	TBD
Alkaline Phosphatase	+	+	+	+	+
SSEA-1	-	-	-	-	-
SSEA-3	-	-	-	-	-
SSEA-4	+	+	+	+	+
TBA 283	+	+	+	+	+
TBA 1-81	+	+	+	+	+
TGSC	+	+	+	+	+
Frozen/Thawed	TBD	TBD	TBD	TBD	TBD
Short-term	yes	yes	yes	yes	yes
Long-term	yes	yes	yes	yes	yes
Commercialization	no	no	no	no	no
Available	273	100	125	NA	NA
Price	\$5,000	\$5,000	\$5,000	NA	NA
# Shipped	127	6	0	NA	NA
Customer Base	TBD	TBD	TBD	NA	NA
NIH investigators	Not known				
NIH applications	Not known				

TBD = To be Determined; NA = Not Applicable

This cell line meets the criteria for the use of human embryonic stem cells by federally funded researchers.

In your grant applications please use the NIH codes, rather than the providers' codes, to identify the cell lines.

Information about the cell line comes from the provider and has not been independently verified by the NIH.

APPENDIX D.—INDIVIDUAL PROVIDER TABLES ON HUMAN EMBRYONIC STEM CELL DERIVATIONS

Provider: BresaGen, Inc. (9-23-03)

BresaGen PHS Registered Cell Lines (United States)				
IVF Group	Retri- (R01)	Retri- (R02)	Retri- (R03)	Retri- (R04)
Embryo Source	IVF Clinic	IVF Clinic	IVF Clinic	IVF Clinic
Embryo Category	Fresh	Fresh	Fresh	Fresh
IVF Status	Discarded— unacceptable for further use	Discarded— unacceptable for further use	Discarded— unacceptable for further use	Discarded— unacceptable for further use
Characteristics				
Passage #	>70	>40	<5	<5
Mouse Feeder Cells Used in Culture	Yes	Yes	Yes	Yes
Karyotype	Normal	Normal	TBD	TBD
Open Cell Immune Markers	+	+		
SSEA-1	-	-	-	-
SSEA-3	+	+	+	+
SSEA-4	+	+	+	+
TRA-1-80	+	+	TBD	TBD
TRA-1-81	+	+	TBD	TBD
Oct-4	+	+	TBD	TBD
Alkaline Phosphatase	+	+	+	+
Gender	Male	Male	TBD	TBD
Frozen/Thawed:				
Short-term	Yes	Yes	Yes	Yes
Long-term	Yes	Yes	TBD	TBD
Therapeutic	Yes	Yes	TBD	TBD
Commercialization				
Available	Yes	Yes	No	No
			(schedule end- 2003, presently recovering and expanding)	(schedule mid- 2004, soon to be recovered and expanded)
Price	\$5,000 + actual shipping cost	\$5,000 + actual shipping cost	NA	NA
# Shipped	0	3	NA	NA
Customer Base				
Australia	1	0	NA	NA
Ireland	2	0	NA	NA
United Kingdom	1	0	NA	NA
USA	12	5	NA	NA
Total registered customers	10	5	Not shipped yet	Not shipped yet
USA only	6		Not shipped yet	Not shipped yet

TBD = To Be Determined; NA = Not Applicable

Provider: Cell Therapeutics (Göteborg University) (9-23-03)

Cell Therapeutics Scandinavia Registered Cell Lines (Sweden)			
NIH Code	SAC1	SAC2	SAC3
Provider's Code	(Subject 1)	(Subject 2)	(Subject 3)
Source	IVF Clinic	IVF Clinic	IVF Clinic
Category	Frozen	Frozen	Frozen
IVF Status	Surplus, to be discarded	Surplus, to be discarded	Surplus, to be discarded
Passage #	TBD	TBD	Donation withdrawn
Mouse Feeder Cells Used in Isolation	Yes	Yes	
Karyotype	TBD	46XX, 47XX +13	
Stem Cell Immersal Markers:			
SSEA-1	TBD	-	
SSEA-3	TBD	+	
SSEA-4	TBD	+	
TRA 1-60	TBD	+	
TRA 1-61	TBD	+	
Oct4	TBD	+	
Alkaline Phosphatase	TBD	+	
Gender	TBD	Female	
Frozen/Thawed	TBD		
	Short-term	Yes	
	Long-term	Yes	
Pluripotent	TBD	Yes	
Commercialization	NA	NA	
Customer Base	NA	NA	

- TBD = To be Determined; NA = Not Applicable
- (1) End Date of NIH Grant

Provider: CyThera, Inc. (9-23-03)

CyThera, Inc. Registered Cell Lines (United States)										
Cell Code	CY12	CY33	CY48	CY51	CY61	CY82	CY91	CY92	CY10	
Provider's Code	(HES-1-2)	(HES-3-0)	(HES-4-0)	(HES-5-1)	(HES-5-1)	(HES-6-2)	(HES-6-1)	(HES-7-2)	(HES-7-1)	(HES-8-0)
Embryo										
Source	IVF Clinic									
Category	Frozen embryo									
IVF Status	Surplus, to be discarded									
Characteristics										
Passage #	2	3	4	5	4	3	5	2	2	
Media Feeder Cells	Yes									
Used in Location										
Karyotype	TBD									
Stem Cell Immuno Markers										
SSEA-1	TBD									
SSEA-3	TBD									
SSEA-4	TBD									
TRA-1-80	TBD									
TRA-1-81	TBD									
Oct-4	TBD									
Alkaline Phosphatase	TBD									
Gender	TBD									
Frozen/Thawed	TBD									
Short-Term Long-Term										
Pluripotent	TBD									
Commercialization	NA									
Customer Base	NA									

TBD = To be Determined; NA = Not Applicable

Provider: ES Cell International (9-23-03)

ES Cell International Registered Cell Lines (Australia)						
NH Code	ES01	ES02	ES03	ES04	ES05	ES06
Provider's Code	(HEB-1)	(HEB-2)	(HEB-3)	(HEB-4)	(HEB-5)	(HEB-6)
Embryo						
Source	IVF Clinic	IVF Clinic				
Category	Frozen	Frozen	Frozen	Frozen	Frozen	Frozen
IVF Status	Surplus, to be discarded	Surplus, to be discarded				
Characteristics						
Passage #	TBD	TBD	TBD	TBD	TBD	TBD
Mouse Feeder Cells Used in isolation	Yes	Yes	Yes	Yes	Yes	Yes
Karyotype	46XX	46XX	46XX	46XY	TBD	TBD
Stem Cell Immune Markers:						
SSEA-1	-	-	-	-	-	-
SSEA-3	+	+	+	+	+	+
SSEA-4	+	+	+	+	+	+
TRA-1-60	+	+	+	+	+	+
TRA-1-81	+	+	+	+	+	+
Oct-4	+	+	+	+	+	+
Alkaline Phosphatase	+	+	+	+	+	+
Gender	Female	Female	Female	Male	TBD	TBD
Frozen/Thawed:						
Short-Term	Yes	Yes	Yes	Yes	Yes	Yes
Long-Term	Yes	Yes	Yes	Yes	Yes	Yes
Pluripotent	Yes	Yes	Yes	Yes	Yes	Yes
Commercialization						
Available	>100	>100	>100	>100	Undergoing final characterization	>100
Price	\$5,000	\$5,000	\$5,000	\$5,000	\$5,000	\$5,000
# Shipped	3	8	45	37	0	0
Customer Base						
International					33	
U.S.					18	
NH Investigator	Not known				Not known	
Non NH	Not known				Not known	

TBD = To be Determined; NA = Not Applicable

Provider: Geron Corporation (9-23-03)

Geron Corporation Registered Cell Lines (United States)							
NIH Code	GE01	GE07	GE09	GE13	GE14	GE91 (clone)	GE32 (clone)
Provider's Code	(WiCell H1)	(WiCell H7)	(WiCell H9)	(WiCell H12)	(WiCell H14)	(WiCell H9.1)	(WiCell H9.2)

Geron responses to the NIH's request for information on cell characteristics, 6/12/2003

All of the embryonic stem cell lines in Geron's possession that are on the NIH Stem Cell Registry were derived elsewhere. Five of them were derived at the University of Wisconsin-Madison; two are clones of one of the Wisconsin lines; and two others were derived at the University of California, San Francisco. Under Geron's agreement with Wisconsin Alumni Research Foundation, we are not permitted to transfer the undifferentiated Wisconsin cell lines to third parties, except for Geron collaborators for work on projects described and directed by Geron. WiCell Research Institute does distribute the Wisconsin lines to researchers, however, and UCSF distributes the UCSF lines.

As to your specific questions: None of the lines is a frozen inner cell mass; they are all cell lines. They are all proliferating.

* The H1, H7 and H9 lines have been extensively characterized. We have recently submitted manuscripts on the detailed characterization of these three lines. We can provide you a copy of the manuscripts in confidence, or send them to you once they have been published. The lines have the expected markers to show undifferentiated status, and we have demonstrated differentiation in all three germ layers for all three of the lines.

* The H1, H7 and H9 lines have been passaged extensively. In most cases, over 70 passages have been achieved.

* The lines are all useful for research. Two of the lines, H1 and H7 have been tested quite extensively for the presence of potential pathogens of human and animal origin, based on the specified by FDA in its Points to Consider and other guidance.

Provider: Göteborg University (9-23-03)

Göteborg University Registered Cell Lines (Sweden)	
NH Code	SA04-SA10
Provider's Code	(See page 4-10)
Source	IVF Clinic
Category	Frozen
IVF Status	Surplus, to be discarded
Passage #	2-3
Mouse Feeder Cells	No
Used in isolation	
Karyotype	TBD
Stem Cell Immuno Markers:	
SSCA-1	TBD
SSCA-2	TBD
SSCA-4	TBD
TRA 1-80	TBD
TRA 1-81	TBD
Oct4	TBD
Alkaline Phosphatase	TBD
Gender	TBD
Frozen/Thawed:	TBD
	Short-term
	Long-term
Pluripotent	TBD
Commercialization	NA
Customer Base	NA

- TBD = To be Determined, NA = Not Applicable
- (1) End Date of NH Grant

Provider: Maria Biotech Co. Ltd.-Maria Infertility Hospital Medical Institute (9-23-03)

Maria Biotech Co. Ltd.-Maria Infertility Hospital Medical Institute Registered Cell Lines (Korea)			
WH Code	M801	M802	M803
Provider's Code	(M801)	(M802)	(M803)
Embryo			
Source	IVF Clinic	IVF Clinic	IVF Clinic
Category	Cryopreservation	Cryopreservation (27 passages) In vitro (+30 passages)	Cryopreservation (27 passages) In vitro (+30 passages)
IVF Status	Were to be discarded 5 years after IVF-ET program	Were to be discarded 5 years after IVF-ET program	Were to be discarded 5 years after IVF-ET program
Characteristics			
Passage #	43	+30	+50
Mouse Feeder Cells Used in Isolation	STO cell feeder (After microsurgery, isolated ICM cell were cocultured with mouse STO cell and HES cell line was established)	STO cell-feeder free (After microsurgery, isolated ICM cells were cocultured with mouse STO cell. To grow HES cells without a feeder layer, aged M802 and M803 cells that had been passaged 10 times were cultured on Matrigel coated plates, coated plates, respectively.)	STO cell-feeder free (After microsurgery, isolated ICM cells were cocultured with mouse STO cell. To grow HES cells without a feeder layer, aged M802 and M803 cells that had been passaged 10 times were cultured on Matrigel coated plates, respectively.)
Karyotype	46, XY, Normal	46, XX, Normal	46, XX, Normal
Stem Cell Immuno Markers			
SSA-1	+	+	+
SSA-3	+	+	+
SSA-4	+	+	+
TRA 1-60	+	+	+
TRA 1-81	+	+	+

Or3-4	+	-	+
Alkaline	+	-	+
Phospholipase			
Gender	Male	Female	Female
Frozen/Thawed:	40 passages frozen	27 passages frozen >30 passages cultured	>50 passages in vitro culture
Platypus:	Yes	Yes	Yes
Commercialization			
Available:	To scientists in Korea	To scientists in Korea	To scientists in Korea
Prior:	Unknown	Unknown	Unknown
# Shipped:	Unknown	Unknown	Unknown
Customer Base:	Limited to Korea	Limited to Korea	Limited to Korea
NIH investigators:	N/A	N/A	N/A
Non NIH:	N/A	N/A	N/A

Provider: MizMedi Hospital—Seoul National University (9-23-03)

MizMedi Hospital—Seoul National University Registered Cell Lines (Korea)		
Cell Line Name	Year	Notes
Embryo		
Patient's Consent		Yes
IRB Approval		Yes
Source		IVF Clinic
Category		Frozen Embryo
IVF Status		Surplus, to be discarded
Characteristics		
Passages		125
Teratoma Foundation		Three germ layer
Embryoid Body		Three germ layer
DNA Fingerprinting		TBD
Karyotype		Normal 46 XY
Stem Cell Immuno Markers		
	SSEA-1	-
	SSEA-3	Partially +
	SSEA-4	+
	TRA-1-81	+
	TRA-1-81	+
	Oct4	+
	Alkaline Phosphatase	+
	Oct3/4	MSX
Frozen/Thawed:		
	Short-Term	Yes
	Long-Term	Yes
Pluripotent		Yes
Commercialization		Yes
Available		Yes
# Shipped		11
Customer Base		
	Australia	1
	Korea	8
	Singapore	1
	U.S.	1

TBD = To be Determined; NA = Not Applicable

Provider: National Centre for Biological Sciences/Tata Institute of Fundamental Research

National Centre for Biological Sciences (INDIA)

NCBS responses to the NIH's request for information on cell characteristics:

1. What is the status of each of your embryonic stem cell derivation listed on the NIH Embryonic Stem Cell Registry?

Is it a frozen inner cell mass?

Yes

* Is it proliferating?

It was frozen after 7 days in culture. We have not characterised them. We are obtaining experience in generating new cell lines and will thaw these when we decide we have sufficient data and experience.

* Has it undergone any characterization? If so, are there markers identified to show undifferentiated status? Is their evidence the cells can differentiate into any of the three germ layers (ectoderm, mesoderm, endoderm)?

No evidence for that - see above.

* If the lines are proliferating, how many passages have been achieved?

See above.

2. Have these derivations been shipped to any NIH-funded researchers?

No

3. If the derivation has not been shipped, are there any plans to develop this derivation to research quality?

Yes, we hope to develop these and other lines.

4. When will they be readily available to NIH researchers?

I hope that once they are derived and characterized they would be available to the academic community.

5. Have any restrictions been placed on the use of these derivation by your national laws?

The guidelines that are being discussed at this point suggest that cells may not be shipped outside of India. My understanding is that this may be allowed in the future only on approval by a National Committee on a case by case basis for research purposes.

Provider: Cell & Gene Therapy Research Institute (Pochon CHA University) (9-23-03)

Pochon PHS Registered Cell Lines (KOREA)		
Cell Line	Country	Source
SKNSH-1	Korea	SKNSH-1

No response from Provider.

Provider: Reliance Life Sciences (9-23-03)

Reliance Life Sciences PHS Registered Cell Lines (INDIA)							
Cell Line	Country	Source	Cell Line	Country	Source	Cell Line	Country
SKNSH-1	India	SKNSH-1	SKNSH-1	India	SKNSH-1	SKNSH-1	India

No response from Provider.

Provider: Technion University (9-23-03)

Technion University Registered Cell Lines (Israel)									
Cell Line	TBD								
Embryo	Yes								
Source	IVF Clinic								
Category	Frozen embryo								
IVF Status	Surplus, to be discarded								
Characteristics									
Passage #	TBD								
Mouse Tumor Cells	Yes								
Karyotype	TBD								
Genetic Markers									
SSCA-1	-	TBD	TBD	TBD	-	TBD	TBD	TBD	TBD
SSCA-3	+	TBD	TBD	TBD	+	TBD	TBD	TBD	TBD
SSCA-4	+	TBD	TBD	TBD	+	TBD	TBD	TBD	TBD
TRA 1-60	+	TBD	TBD	TBD	+	TBD	TBD	TBD	TBD
TRA 1-61	+	TBD	TBD	TBD	+	TBD	TBD	TBD	TBD
Oct4	+	TBD	TBD	TBD	+	TBD	TBD	TBD	TBD
Alkaline Phosphatase	+	TBD	TBD	TBD	+	TBD	TBD	TBD	TBD
Gender	TBD								
Frozen/Thawed	Yes	TBD	TBD	TBD	Yes	TBD	TBD	TBD	TBD
Short-Term	Yes								
Long-Term	Yes								
Autologous	Yes	TBD	TBD	TBD	Yes	TBD	TBD	TBD	TBD
Genetic Stabilization	Yes	NA	NA	NA	Yes	NA	NA	NA	NA
Other									
Customer Data	TBD	NA	NA	NA	TBD	NA	NA	NA	NA

TBD = To be Determined; NA = Not Applicable

Provider: University of California at San Francisco (9-23-03)

University of California at San Francisco Registered Cell Lines (United States)			
Cell Line	DOB	Sex	Age
Source	Test		
Source	IVF Clinic	IVF Clinic	
Category	Fresh	Fresh	
IVF Status	Surplus, to be discarded	Surplus, to be discarded	
Characteristics			
Passage #	TBD	TBD	
Mount Feeder Cells	Yes		
Substratum			
Karyotype	46XY	46XX	
Genetic Marker Methods			
SSEA-1	-	-	
SSEA-3	+	+	
SSEA-4	+	+	
TRA-1-90	+	+	
TRA-1-81	+	+	
Oct-4	+	+	
Alkaline Phosphatase	+	+	
Gender	Male	Female	
Frozen/Thawed:			
Short-Term	Yes	Yes	
Long-Term	Yes	Yes	
Replated	Yes	Yes	
Specialization	NA		
Available	NO	Yes	
Price	\$5,000	\$5,000	
# Shipped		>35	
Customer Base	NA	TBD	

TBD = To be Determined, NA = Not Applicable

Provider: WICell Research Institute, Inc. (9-23-03)

Embryos	Male	Female	Male	Female	Male
Embryo	20Y	20Y	20Y	20Y	20Y
Embryo					
Source	IVF Clinic				
Category	NA	NA	NA	NA	NA
IVF Status	Surplus, to be discarded				
Characteristics					
Passage #	20 - 24	20 - 24	20 - 24	Not Provided	20-24
WUSA Recipient Cells Used	Yes	Yes	Yes	Yes	Yes
Used in Research					
Karyotype	46XY	46XX	46XX	46XY	46XY
STR Cell Type					
Markers					
SSEA-1	-	-	-	-	-
SSEA-3	+	+	+	+	+
SSEA-4	+	+	+	+	+
TRA 1-60	+	+	+	+	+
TRA 1-61	+	+	+	+	+
Oct4	+	+	+	+	+
Alkaline Phosphatase	+	+	+	+	+
Gender	Male	Female	Female	Male	Male
Frozen/Thawed					
Short-term	Yes	Yes	Yes	Yes	Yes
Long-term	Yes	Yes	Yes	Yes	Yes
Pluripotent	Yes	Yes	Yes	Yes	Yes
Commercialization					
Available	500	200	500	No	No
Price (in US)	\$1,000	\$5,500	\$5,000	\$5,000	\$5,000
Outside US (\$20,000)					
# Shipped	135	3	22	0	0
Cellular Cases	TBD	TBD	TBD	NA	NA
Available for Research	Available	Available	Available	Available	Available
Available for Commercialization	Available	Available	Available	Available	Available

TBD = To be Determined; NA = Not Applicable

APPENDIX E.—THE DEVELOPMENT OF HUMAN EMBRYONIC STEM CELL LINES

(September 26, 2001)

I. BACKGROUND

The emergence of a policy for Federal funding of biomedical research using human embryonic stem cells has prompted many questions from scientists, Congress, volunteer health agencies, and the lay public regarding the establishment of cell lines. In this regard, NIH has provided the scientific evidence in the form of scientific reports, memoranda, and letters regarding existing human embryonic stem cells <http://www.nih.gov/news/stemcell/082701list.htm>. This paper provides additional information on the development of human embryonic stem cell lines.

On August 9, 2001, President Bush opened the door for Federal funding of research using human embryonic stem cells by allowing such research to be conducted under certain criteria <http://www.whitehouse.gov/news/releases/2001/08/print/20010809-1.html>. Prior to the President's announcement, Secretary of Health and Human Services Tommy G. Thompson, instructed the NIH to prepare a report on the broad area of stem cell research, including stem cells from adult tissues, fetal tissue, and human embryos. In developing the report, NIH was asked to obtain information from all sources of research—private and public sector, United States and abroad. In June 2001, the NIH transmitted the report, *Stem Cells: Scientific Progress and Future Research Directions* (NIH Stem Cell Report) <http://www.nih.gov/news/stemcell/scireport.htm> to Secretary Thompson indicating that in developing its report, the NIH had identified approximately 30 cell lines which were either fully characterized or under development. Upon presenting the report to Secretary Thompson, NIH noted that there was some preliminary evidence that other laboratories had been conducting research with the intent of developing human embryonic stem cell lines, but that this evidence had not been confirmed.

In early July, Secretary Thompson asked that the NIH pursue additional information on other human embryonic stem cell lines, including those that were under development. In late July, Secretary Thompson was informed that NIH had identified additional stem cell lines, some of which were in varying stages of development. On August 27, 2001, NIH released a list of ten laboratories in the United States and around the world who reported that they had derived human embryonic stem cells from 64 individual, genetically diverse blastocysts. All of the existing cell lines, some of which in varying stages of development, reported to the NIH meet the President's criteria—that is, the derivation process (which begins with the destruction of the embryo) was initiated prior to 9:00 p.m. EDT on August 9, 2001; the stem cells were derived from an embryo that was created for reproductive purposes and was no longer needed for this purpose; informed consent had been obtained for the donation of the embryo and the donation did not involve financial inducements. NIH also acknowledged and continues to anticipate that other human embryonic stem cell lines are under development and will be disclosed in the future and that they may also be eligible for use in Federally funded research under the President's criteria.

This report addresses technical issues related to the laboratory processes involved in the development of human embryonic stem cell lines. The report does not provide information on the following issues: background or health status of the embryo donors, intellectual property, patenting or licensing or material transfer conditions, availability for distribution or research collaboration, informed consent, directed differentiation studies, distribution practices, research costs or financing of cell line developments, ethical, legal or social aspects of stem cell research, or development of public policies and oversight of such research. This report does not address scientific information about adult stem cells, embryonic germ cells, or stem cells of other species, unless designated. Finally, although the NIH reviewed information regarding human embryonic stem cells prepared using somatic cell nuclear transfer or from embryos that were created for research purposes through the use of gamete donation, these cells would not be eligible for Federally funded research according to the President's criteria and are, therefore, not discussed in this report.

Data provided in this report are accurate as reported to the NIH and as of the state of the science on or about August 1, 2001. Given the rapid pace of development of this area of science, additional scientific evidence regarding the cell lines may have emerged since this data was first gathered. In developing this report, NIH did not review the data with the sources of the information, conduct further interviews, or obtain additional information that had not been previously reported to the Agency.

II. KEY DEFINITIONS

As an aid in understanding the details of the descriptions of techniques and cells described in this report, a glossary of commonly used terms is provided. The definitions are taken from the NIH Stem Cell Report, Dorland's Medical Dictionary (25th edition), or other scientific resources.

Blastocyst—a preimplantation embryo of 30–150 cells. The blastocyst consists of a sphere made up of an outer layer of cells (the trophoctoderm), a fluid filled cavity (blastocoel), and a cluster of cells on the interior (the inner cell mass).

Cell line—a group of cells derived from a primary culture at the time of first subculture, it is considered to be an established cell line when it demonstrates the potential for indefinite subculture in vitro.

Characterization—the description of the biological properties of the undifferentiated human embryonic stem cell.

Derivation—the process of removing the cellular contents of the inner cell mass from the blastocyst and the initial plating of the cells as a primary cell culture.

Differentiation—the process whereby an unspecialized early embryonic cell acquires the features of a specialized cell such as a heart, liver, or muscle cell.

Embryonic stem cell—undifferentiated cells from the embryo that have the potential to become a wide variety of specialized cells.

Embryoid body—clumps of cellular structures that arise when embryonic stem cells are cultured. Embryoid bodies contain tissue from all three of the germ layers: endoderm, mesoderm, and ectoderm. Embryoid bodies are not part of normal development and occur only in in vitro culture conditions.

Inner cell mass—the cluster of cells inside the blastocyst.

Proliferation—the reproduction or multiplication of cells.

III. SOURCES OF INFORMATION

The information used in the development of this report originates from multiple sources. Included are the NIH Stem Cell Report, scientific publications (including peer reviewed manuscripts and abstracts), presentations at meetings and conferences, and notes and personal communications with scientists who have conducted the research discussed in the report. The report does not include information from lay press publications. As of September 20, 2001, there are 11 known publications on research using human embryonic stem cells (1–11).

IV. OVERVIEW OF THE DEVELOPMENT OF HUMAN EMBRYONIC STEM CELLS LINES

In 1998, James Thomson et al. described for the first time the creation of embryonic stem cell lines from cells removed from the inner cell mass of human embryos (1). This paper and research preceding it on mouse embryonic stem cells has provided the research community with a framework for the description of human embryonic stem cells. However, other scientists have since developed their own techniques and augmented the studies of Thomson by providing alternative approaches to describing these cells. Therefore, while there is no question about the basic properties of these cells from the standpoint of the two essential features: the ability to proliferate and having the potential to develop into many different cell types, *there are at this time no uniform standards or mutually agreed upon scientific criteria or parameters to describe the features of these cells*. Thus, it is important to note that this report describes the procedures and features of stem cells from multiple perspectives, but does not make qualitative statements about the varying approaches to defining them.

An overview of the procedures used in the creation of human embryonic stem cells is presented graphically in Figure 1, and details are provided in the following sections of the report. The concepts shown in this figure and the terms applied in the following sections were developed internally by NIH in 1999 to help distinguish the time point at which NIH funded investigators could use cells in their laboratory. Several broad steps are considered here. First, the derivation step is the use of a fresh or frozen human embryo (usually takes place around day 5 after fertilization) and the subsequent removal of the inner cell mass. It is the cells of the inner cell mass that will ultimately give rise to human embryonic stem cells. This process requires the destruction of the embryo. At the time of this report, there have been no human embryonic stem cell lines that have been established from a single cell from the inner cell mass. The reasons for this are not fully known, but are believed to be related to the requirement for cell-cell contact, which is thought to provide necessary nutrients or growth factors enabling cells to be maintained in the undifferentiated state.

The cells are plated on a petri dish and cultured on so-called “feeder layers” of cells. Feeder layers are live cells that have been treated with irradiation so they are alive but do not divide or grow in culture. These cells provide nutrients to the newly plated embryonic cells, hence the name “feeder” cells. These primary cultures take several days to grow into colonies of cells. Colonies that divide and grow in a characteristic pattern are selected for subsequent culturing. Each cycle of growing, selecting colonies, and culturing is referred to as a “passage.” With each cycle, the nutrient conditions may be adjusted and appropriate cell density is maintained so as to optimize cell growth and to help ensure they continue to divide in an undifferentiated state. This is sometimes referred to as the “proliferative” phase in the development of a stem cell line, where the goal is to expand the number of undifferentiated cells in culture. At various time points in this process, researchers may examine a variety of factors that distinguish whether or not the cells retain their properties as unspecialized cells. This is often referred to as the “characterization” phase in the process. It is important to note that throughout each of these steps, there is a natural tendency for the cells to clump together and begin to specialize into defined cells and tissues.

The typical process from derivation to the establishment of a cell line that retains the properties of embryonic stem cells is time consuming (6 to 8 months), labor intensive, requires special facilities, and is expensive. Some experts in tissue culture often refer to the culturing of these cells as more of an art than a science in that the techniques for maintaining and growing these cells are sometimes subtle and learned through trial and error. Generally speaking, many laboratories want to perform between 35 to 50 cell passages before beginning directed differentiation studies—that is, studies to direct these undifferentiated cells to become specialized. On the other hand, some laboratories employ strategies that attempt to partially differentiate cells early in the cell line development process as an intentional effort to direct the cell line development from a very early step. To date, there is no evidence that one approach has an advantage over another.

Although much of the research performed on directed differentiation of embryonic stem cells is conducted only after the cell line is developed, many researchers consider cells in the earliest phases of the cell line development process to be a highly desirable for investigating many aspects of cellular regulation. As scientists begin their search for improved ways to control the specialization of cells for reparative or restorative functions, it is likely that much attention will be directed to these earlier phases of cell line development which occur long before the cells might be considered to be fully characterized.

V. METHODS OF DERIVATION OF HUMAN EMBRYONIC STEM CELLS FROM THE BLASTOCYST

In this report, the term “derivation” means the process of removing the cellular contents of the inner cell mass from the blastocyst and the initial plating of the cells as a primary cell culture. In this process the blastocyst (the early embryo) is destroyed. From the point of establishing the initial cultures, the cells are considered to have been derived and are eligible for Federal support.

Thomson et al. (1) described the derivation of their cell lines in the following manner: “Thirty-six fresh or frozen-thawed donated human embryos produced by IVF were cultured to the blastocyst stage in G1.2 and G2.2 medium. Fourteen of the 20 blastocysts that developed were selected for ES cell isolation, as described for rhesus monkey ES cells. The inner cell masses were isolated by immunosurgery, with a rabbit antiserum to BeWO cells, and plated on irradiated (35 grays gamma irradiation) mouse embryonic fibroblasts. Culture medium consisted of 80 percent Dulbecco’s modified Eagle’s medium (no pyruvate, high glucose formulation; Gibco-BRL) supplemented with 20 percent fetal bovine serum (Hyclone), 1 mM glutamine, 0.1mM β -mercaptoethanol (Sigma) and 1 percent nonessential amino acid stock (Gibco-BRL). After 9 to 15 days, inner cell mass-derived outgrowths were disassociated into clumps either by exposure to $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free phosphate-buffered saline with 1 mM EDTA (cell line H1), by exposure to dispase (10 mg/ml; Sigma; cell line H7) or by mechanical dissociation with a micropipette (cell lines H9, H13, and H14) and replated on irradiated mouse embryonic fibroblasts in fresh medium.”

The technical details are presented here for several reasons. First, to demonstrate that within one laboratory, multiple procedures were used in the first steps of establishing the early cultures of the cells. Second, to point out that the outer cell layers of the blastocyst were peeled away from the inner cell mass by using antibodies, so-called “immunosurgery.” This is important to consider as there are at least three other methods that other researchers have used in the removal of the inner cell

mass cells from the blastocyst, including laser ablation, mechanical disruption, or digestion in culture medium.

The cells growing on the culture plate are examined under a microscope and those that have the shape consistent with unspecialized cells are placed back into culture again. The unspecialized cells double in number (replicate themselves) about every two days, after which they are separated and cultured again to grow an even larger numbers of cells. This is called the “proliferation” process, where cells undergo new “passages” to be expanded into cultures with large numbers of cells. After 10–20 passages, researchers perform a series of tests to ensure that the cells are stable and retain stem cell properties. For example, the chromosomes are checked and tests are done for certain “markers” on the cells to make sure they haven’t yet become specialized. This process is commonly referred to as the characterization of the cells. It is important to note that because research on human embryonic stem cells is in its earliest days and because so few investigators have had access to these cells, there are no agreed upon uniform scientific standards or protocol for the determination that a stem cell is “fully characterized.” If after six to eight months or between 30 to 50 passages the testing shows that the cells have been determined to (1) grow properly, i.e., continue to proliferate, and (2) have characteristics of stem cells, researchers determine them to be a stable cell line. Scientists can then begin their work at trying to make specialized cells (e.g., neurons, insulin-secreting cells, etc.) from them. An important aspect that is often overlooked is that researchers can now conduct valuable research during the earlier stages of the cell development to better understand the molecular and cellular mechanisms that account for the unique properties of embryonic stem cells.

Individual investigators have described variable success rates in establishing viable embryonic stem cells from the initial derivations. Success in achieving a stable culture of undifferentiated cells ranges from 20–50 percent.

VI. METHODS OF ASSESSING PROLIFERATIVE CAPACITY OF EMBRYONIC STEM CELLS IN CULTURE

One of the defining points about embryonic stem cells is their ability to replicate indefinitely. One aspect of their viability is determined by testing to ensure that after they are frozen and thawed, they retain their ability to divide in culture. Embryonic stem cells in culture have a characteristic doubling time of approximately 36 hours. Another important feature that many researchers examine is the length of telomeres and the enzyme that maintains their length. Telomeres are repeating sequences of DNA at the end of chromosomes (8–15 kilobases); as cells age, telomeres become shorter. Telomerase is a ribonuclease enzyme that adds the repeated sequences to the end of chromosomes maintaining their length and presumably extending the lifespan of the cell. Thus, telomerase expression is highly correlated with immortality of human cell lines and the restoration of it in some human diploid cells has been shown to extend the cell’s lifespan. Therefore, researchers developing human embryonic stem cell lines expect to observe high levels of telomerase activity in these cells as an indication that they may propagate for a long time. However, not all embryonic stem cells show high levels of this enzyme.

VII. METHODS OF ASSESSING PROPERTIES OF HUMAN EMBRYONIC STEM CELLS

Characterization of stem cells is typically refers to the biological properties that define cells as being undifferentiated (1). In the original publication by Thomson, the term “characterization” was used specifically to refer to the presence or absence of certain cell surface markers. Among the various investigators who have derived stem cells, there is no precise time in the developmental process when such marker studies are conducted. The characterization phase includes many steps, assays, and approaches and varies substantially from laboratory to laboratory. Described here are some of the approaches used.

A. Cell Surface Markers

All cells have proteins on their surface membrane to which antibodies can be made to attach. Each type of cell has different types of proteins on their surface and the antibodies to them can be used to help identify particular cell types. From embryonic research in other species, it was recognized that embryonic stem cells have unique proteins called stage specific embryonic antigens (SSEA) of different types. Although they vary slightly from species to species, the detection of SSEA–3 and SSEA–4 on the surface of cells using antibodies to these proteins has been a cornerstone for detecting which cells retain an undifferentiated state. Cells that have become specialized do not express these proteins. While other species express SSEA–1, humans do not. Other proteins are also thought to be unique to embryonic cells

that are unspecialized. These include tumor rejection antigens (TRA) 1–60 and 1–81. Some laboratories use other cell surface markers to detect undifferentiated cells.

B. Alkaline Phosphatase Activity

Alkaline phosphatase is an intracellular enzyme that is expressed in high levels in undifferentiated cells and its presence or activity is commonly used to describe embryonic stem cells.

C. Other Markers of the Undifferentiated State

Researchers are making steady progress at developing other techniques to assess the state of the embryonic stem cell. The transcription factor Oct-4 is highly expressed in undifferentiated cells. Many studies have shown that the decline in the level of Oct-4 signals processes that are under way in the cell for it to become specialized (e.g., begin expressing genes and other markers of differentiated cells). Some controversy exists about the degree of expression among various cell lines and its value of reflecting cells with undifferentiated characteristics.

D. Markers of the Differentiated State

Another approach to assess embryonic stem cells in culture during the development of a stem cell line is to assess cellular markers that indicate that cells are becoming specialized. For instance, one approach is to measure the culture media for the presence of human chorionic gonadotrophin and alpha fetoprotein. More commonly scientists use antibody studies or gene expression methods to assess for the production of cell surface proteins or genes that are activated when cells begin to take on characteristics of specialized cells—such as neurons, muscle, bone, or epithelium.

It should also be noted that embryonic stem cells when growing on feeder layers take on characteristic morphology, particularly at the interfaces of the two cells. Researchers continually inspect the cells for subtle features that may reflect that the cell is beginning to differentiate. During the course of the cell line development, researchers use any number of combinations of these methods to assess the characteristics of the cells. Many laboratories repeat these studies at various passages, but no standard protocol exists for when or which of these studies are conducted. Nor is there a standard frequency (passages) at which these tests are performed.

VIII. METHODS OF DEMONSTRATING THAT EMBRYONIC STEM CELLS ARE PLURIPOTENT

The NIH Stem Cell Report and many scientific publications refer to human embryonic stem cells as being pluripotent—or capable of developing into nearly all cells of the human body. Historically, the major feature or evidence supporting the notion that these cells are pluripotent is the demonstration that embryonic stem cells can give rise to differentiated cells that are characteristic of cells that normally develop from all three germ layers—ectoderm, endoderm, and mesoderm. It is known that over 200 cell types exist in the human body, and obviously, given the brief history of the existence of human embryonic stem cell cultures, no one has yet demonstrated that human embryonic stem cells do develop into all of these various cell types (although there is no evidence to suggest that they cannot do this). In scientific reports, the term multipotent is sometimes used to describe that multiple cell types can be shown to develop from embryonic stem cells.

There are three techniques that have been used to establish the multipotency or pluripotency of embryonic stem cells:

- In mice, the undifferentiated cells are injected into the blastocyst cavity and the resultant embryos implanted into pseudopregnant mice. The embryonic stem cells contribute to all cell types in a chimeric mouse, including the germ layer (12). The mice of the subsequent generation contain the genotype of the embryonic stem cells thereby providing evidence of their pluripotency.
- Subcutaneous injection of the embryonic stem cells into syngeneic mice induces teratomas—a tumor that may include cells of endodermal, ectodermal, or mesodermal origin (13). Laboratories use different approaches to performing such studies, and they require several million cells, therefore, they are usually performed late in the development of the cell line when larger amounts of cells are available.
- In vitro* assessment of embryoid bodies formed by the aggregation of embryonic stem cells that develop into cells of distinct endodermal, ectodermal, or mesodermal origin (5). In these studies, researchers test for cellular markers of differentiated cell lineages, such as noggin, nestin, gamma-globin, neurofilament-68 KD, albumin, neurotubulin, brachyury, Pax-6, PDX-1, among others.

Although the pluripotency of a mouse embryonic stem cell is usually determined using the first of the methods described above, research described in this report with regard to the pluripotency of human embryonic stem cells is limited to the latter two methods. The creation of chimeric mice by injecting human embryonic stem cells into mice embryos would not be deemed acceptable. Given this limitation, it is not yet possible to demonstrate the pluripotent capabilities of human embryonic stem cells to the same extent as pluripotency is established with mouse embryonic stem cells.

IX. OTHER METHODS USED TO ASSESS HUMAN EMBRYONIC STEM CELLS

Researchers use other tests to demonstrate that embryonic stem cells retain properties that make them useful for research. These include:

- karyotype analysis (determination of the number and structure of the chromosomes) using either standard G banding or spectral karyotyping (SKY);
- other genetic analysis including fluorescent in situ hybridization (FISH) for certain genes or proteins;
- culturing and testing for growth of pathogens known to infect laboratory cell cultures such as *Mycoplasma* species; and
- development of subclonal lines from colonies grown in primary cultures.

X. DESCRIPTIONS OF INDIVIDUAL CELL LINES

Provided here are descriptions of what has been reported to the NIH regarding the development of stem cell lines from the ten sources that are known to fulfill the President's criteria. It is important to reiterate that there is no standard definition of what is a fully characterized line. Therefore, a designation of fully or partially characterized is at the discretion of the source and differs among the entities. It is also worth noting that a fully characterized line may or may not be ready for distribution. With regard to stem cells that are not fully characterized, entities may choose to collaborate with NIH funded investigators for the purposes of characterization research or developmental studies.

WiCell/University of Wisconsin

There are five cell lines that were derived, and all are fully characterized according to the methods that were originally published in 1998 (1). Fresh and frozen blastocysts were used in the research leading to the establishment of the cell lines and the sources were from IVF clinics in Israel (as part of a collaboration with Dr. Joseph Itskovitz-Eldor) or Wisconsin. Of the blastocysts that yielded human embryonic cell lines, H1 originated from a blastocyst from Wisconsin and H7, H9, H13, and H14 from blastocysts that were from Israel.

Cellular characterization of the undifferentiated state consisted of data on the presence of cell surface markers SSEA-3, SSEA-4, TRA-1-60, TRA-1-81; the lack of expression of SSEA-1; and presence of the intracellular enzyme, alkaline phosphatase. This paper also provided data on a marker of the proliferative capacity of the cells by measuring levels of the cellular enzyme, telomerase. High levels of telomerase expression were shown in all five cell lines. Measurement of Oct-4 transcription factor levels was not reported in the original work, however, other investigators using these cells have confirmed the retention of Oct 4 expression.

The tests for the capability to differentiate into specialized cells was conducted using the injection of cells from passages 14 to 16 into rear leg muscles of four week old male SCID-beige mice. Seven to eight weeks after the injection, the resulting teratomas were examined histologically. The results of those tests included the demonstration of gut-like cells (H9), neural epithelium (H14), bone (H14), cartilage (H9), striated muscle (H13), tubular structures resembling fetal glomeruli (H13). Additional research showing multipotent characteristics of the H9 line has been shown using the approach of characterizing markers specific to cellular lineages in embryoid bodies (5). These cells showed the presence of markers for gamma globin, neurofilament 68Kd, alpha-cardiac actin, and alpha fetoprotein. Normal karyotyping has been reported at multiple passage levels and three cell lines are XY (H1, H13, and H14) and two are XX (H7 and H9).

Several of the H lines have been used extensively in studies for directed differentiation in a variety of laboratories and a complete review of those studies is beyond the scope of this report. Subsequent studies with these cells have shown them to be stable in phenotype and karyotype for several years and over 400 population doublings.

Researchers involved in the development of these initial cell lines have also developed approximately ten subclonal lines from the original five lines. A substantial amount of research is being conducted with several of the subclones, H9.1 and H9.2.

ESI/Monash University

There are presently six human embryonic stem cell lines that have been reported to the NIH as being fully characterized and were developed in collaborations with researchers from the National University Hospital of Singapore and Monash University. These lines are designated as HES-1 through HES-6. The method used in the derivation used an immunosurgery technique but differs somewhat from the approach used by Thomson (1). The details of preparation of HES-1 and -2 lines have been described in detail (11). The steps involved in the initial culturing of the cells differs substantially from those described by Thomson (1). In initial cultures, cells were cultured in the presence of the growth factor LIF (leukemia inhibitory factor), but in subsequent cultures they were not.

Marker expression studies in these cell lines have been done at multiple passage levels. All the cell lines test positive for alkaline phosphatase activity, have immunostaining present for SSEA-4, TRA 1-60 epitopes, and are labeled with the antibody for GCTM-2 which detects keratan sulfate/chondroitin sulfate proteoglycans. They did not express SSEA-1. HES-1 and HES-2 have normal karyotype (HES-1 and HES-2 are XX). HES-1 through HES-4 have had xenograft differentiation studies done. In this model, cells from early and late passages were inoculated beneath the testis capsule of SCID mice. After five weeks, all mice developed teratomas that were resected and examined histologically. Differentiated tissues were observed to include cartilage, squamous epithelium, primitive neuroectoderm, ganglionic structures, muscle, bone, and glandular epithelium. One notable distinguishing feature is that these cell lines do not appear to develop embryoid bodies in culture and neural progenitor cells may be isolated from differentiating ES cell and lead to neuron formation (11). The proliferative capability of these cell lines has been demonstrated by their continued growth after multiple freeze/thaw cycles and the expression of Oct-4.

Technion University

In addition to the cell lines developed as a part of the collaboration with Thomson et al., researchers led by Dr. Joseph Itskovitz at the Technion University and Rambam Medical Center in Haifa, Israel have established four additional cell lines, which have been reported to the NIH as fully characterized. These are characterized using several methods as described in the original paper (1). In addition, subclones of the primary embryonic stem cell cultures have been developed. At this time, the Itskovitz laboratory has approximately 19 cell lines that are being used for research (including subclones). The laboratory has collaborated in several studies demonstrating the first functional evidence of directed differentiation of human embryonic stem cells yielding muscle cells that contract and have features of cardiac muscle and cells that secrete insulin (2, 7).

BresaGen, Inc.

Scientists at BresaGen in Athens, Georgia, have developed four human embryonic stem cell lines designated HES#4896, HES#7226, HES#6510c, and HES#7283a, which have been reported to the NIH as fully characterized. The derivations were conducted using fresh blastocysts from an IVF clinic in Georgia. The cell lines have been characterized by the immunostaining of the following markers: SSEA-3, SSEA-4, TRA 1-60, TRA 1-81, and the lack of staining for SSEA-1. The cells have undergone multiple freeze/thaw cycles, demonstrate histologic characteristics of human embryonic stem cells, and have been shown to develop embryoid bodies when allowed to differentiate in culture. Each of the lines has undergone more than 30 passages. Expression levels of Oct-4 have been determined and alkaline phosphatase activity is present. Telomerase activity has also been assessed. Karyotype analysis has been conducted on each line and at multiple passages and shown to have normal number. Xenograft studies in mice are being completed for histologic analysis of tissue differentiation. Other studies are being conducted to evaluate markers of differentiation.

University of California, San Francisco

Researchers at the University of California, San Francisco, have successfully established two lines of human embryonic stem cells, one of which has been reported to the NIH as fully characterized and the other as nearing completion. Drs. Roger Pedersen and Meri Firpo were developed them from fresh blastocysts donated at a California IVF facility and they are designated HSF-1 and HSF-6. The method of derivation is similar to that used by Thomson et al. (1). The stem cells have typical histologic morphology and possess the characteristic markers of human embryonic stem cells: SSEA-3, SSEA-4, Oct-4 expression, and lack of SSEA-1. Karyotype analysis of HSF-6 has been completed multiple times and is XY and the analysis

of HSF-1 was being completed. The capability to develop into specialized cells has been demonstrated using studies conducted on embryoid bodies in assessing the presence of specific markers for differentiated cells.

University of Goteborg

Researchers at the University of Goteborg, Goteborg, Sweden, have reported to the NIH that they have [19 or 18] stem cell lines in various stages of development. Of these, three are fully characterized cell lines, and 15 lines are under development. Of the lines under development, four are partially assessed, and 12 are in the early passages and beginning to undergo characterization. successfully established cultures of embryonic stem cells cell lines These were derived from 19 individual blastocysts. The derivations were conducted using fresh blastocysts from IVF clinics in Uppsala and Goteborg. The derivation technique used in these is substantially different from that described by Thomson et al. (1) and uses a culture digestion method over the course of several days that allows for the dispersion of the cells of the inner cell mass. Regulations in Sweden allow for the blastocyst to be maintained in culture for up to 14 days. Researchers report that three of the cell lines have completed their characterization assessment, three are partially assessed, and 12 are in the early passages and under going characterization. Marker expression studies include presence of SSEA-3 and SSEA-4, alkaline phosphatase activity, and Oct-4 expression in those that have undergone characterization. Karyotype studies were normal on those that have completed characterization. The investigators are using multiple new approaches (proprietary) for developing conditioned media for the expansion of their cultures. Although a major focus of their work at present is to develop a culture system that is free of mouse feeder layers, this has not yet been applied. The emphasis for this group is being directed to establishing cell lines for chondrocyte, neuron, insulin producing cell, and cardiomyocyte replacement strategies.

Reliance Life Sciences

Scientists at Reliance Life Sciences in Mumbai, India, are in the process of establishing have reported to the NIH that they have seven human embryonic stem cell lines in various stages of under development. The project is headed by Dr. K.V. Subramaniam with his colleagues Drs. Fizuza Parekh and Satish Totey. They conducted their derivations using frozen embryos donated from an IVF clinic in India. They used a modification of the method described by Thomson (1). Their scientists described the cells as having characteristic morphology of human embryonic stem cells and can form embryoid bodies in culture when allowed to differentiate. Characterization studies have been completed on one cell line and three additional cell lines are nearing completion. Three additional cell lines are in the early phases of proliferation, have undergone freeze/thaw testing, and retain characteristics of human embryonic stem cells. The laboratory assesses the cells as being undifferentiated by the positive immunohistochemical detection of SSEA-3, SSEA-4, the lack of detection of SSEA-1, and the measurement of alkaline phosphatase activity.

National Centre for Biological Sciences

The National Centre for Biological Sciences at the University of Agricultural Sciences in Bangalore, India, collaboration headed by Dr. Mitradas Panicker with an IVF clinic in India, has have reported to the NIH that they have three lines under development, which conducted successful derivations of are in the earliest phases of expansion. As of mid-August, they were being retained in a frozen state and characterization studies had not yet been initiated. The derivation was conducted using frozen blastocysts with a laser ablation technique modified from that of Thomson et al. (1). Morphological characteristics of the cells are consistent with those of human embryonic stem cells.

Karolinska Institute

The research on stem cells is being conducted between the research groups at the Huddinge University Hospital at the Karolinska Institute, Stockholm, Sweden. As of August, the researchers reported to the NIH that they have 10 stem cell lines in various stages of development. These lines developing ten separate cell lines that were established from the derivation from the inner cell mass of five individual blastocysts; these lines and are partially characterized. The blastocysts were obtained from a local IVF clinic using a method similar to Thomson et al. (1). The cell lines are noted to have positive detection of cell surface markers SSEA-3 and SSEA-4, and lack detection of SSEA-1. Karyotype results were completed in several cell lines and under way in others. Studies to demonstrate pluripotency using xenografts were reported as ongoing in multiple cell lines.

Cythera, Inc.

Cythera, Inc. reported to the NIH that they have nine cells cell lines in various stages of development under development and that the ongoing characterization studies are being conducted with a focus on gene expression data. The derivations were conducted using fresh and frozen blastocysts that were donated at a California IVF facility. The derivation procedures and culture are custom designed. Laser surgery approaches are used for the removal of the inner cell mass, and proprietary methods are used to develop embryonic stem cells that proliferate, but express certain markers that are found with particular cell lineages. In this regard, the characterization of these cells will reflect the intent of directing them toward a particular germ line. Cells are noted to have undergone freeze/thaw testing, and expand with characteristics in culture similar to undifferentiated human embryonic stem cells. Characterization studies are being conducted with a focus on gene expression data.

XI. SUMMARY

There are at least 64 individual human embryonic stem cell lines that are either fully characterized or under development in various stages of development, all of which qualify for Federal funding under the criteria announced by the President on August 9, 2001. In addition to those listed above, NIH is aware of other such cell lines that are under development that may meet the eligibility criteria, but technical details have not been shared by those researchers. In nearly all cases, researchers in the various laboratories use techniques that are different from those which were originally described by Thomson et al. (1). The process of developing a human embryonic stem cell line is long, tedious, and successful development requires substantial skill and experience in cell culture techniques.

It is worth noting that in all the derivations stem cell lines identified by the NIH, including those under development, the primary cultures were conducted on mouse feeder layers. At the present time, no investigators have been successful in deriving stem cells absent mouse feeder layers in the initial cell culture. It is also important to understand that, in most cases, animal proteins are also used in other stages in the development of an embryonic stem cell line. Some laboratories report, however, that they have been successful in removing animal proteins from these later phases in the development of a cell line.

Nearly all scientists have reported that maintaining cell growth in the undifferentiated state is the major challenge in the development of the cell line. The cultivation of these stem cell lines continues to evolve as researchers test for improved methods and materials to work with these cells.

Among the key features to assess during the cell line development process is the description of the key characteristics of the cells and their ability to proliferate. There are many approaches to this, and there does not exist any standard method or protocol to ascribe that a cell line has attained the status of a human embryonic stem cell line. To date, characterization of these cells usually consists of the demonstration of certain cell surface markers, alkaline phosphatase activity, and characteristic morphology. Proliferative capacity is also assessed by determining growth patterns, particularly after freeze/thaw cycles, and the measurement of telomeres and telomerase activity. There are several approaches to assess pluripotent capabilities of the stem cells, including the use of certain genetic markers and the formation of teratomas in animals.

Of the 64 human embryonic stem cell lines in various stages of development that have been described to the NIH by the scientists who have prepared them, investigators have reported to the NIH that 24 have been fully characterized and are being prepared for use in laboratory experiments on directed differentiation or are having additional experiments conducted with them. Some of the other lines under development are partially characterized and are undergoing additional passages to expand their numbers and to reconfirm that their undifferentiated state has been retained. The remaining lines are in earlier stages of development. It is also noteworthy that in several laboratories, subclones of the original lines were developed in the primary culture stage and these are showing interesting laboratory findings in that they behave differently from each other and from the primary culture from which they were derived. This suggests that such subclones should be investigated as independent lines.

In conclusion, there are heterogeneous approaches to the development of a human embryonic stem cell line. Given the various techniques used in establishing these cells, and the lack of an embryonic stem cell line which has been derived from a single cell of the inner cell mass of the blastocyst, it is likely that research using existing stem cells will reveal many functional differences among them. Such differences must be fully explored to determine the optimal characteristics of embry-

onic stem cells for their differentiation into particular specialized cells for the purposes of tissue repair or replacement.

XII. REFERENCES

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Senator SPECTER. This is an evolving issue. Mr. Cordy represents more than 100 million Americans. The figure has been put at 128 million Americans who have diseases like Parkinson's or Alzheimer's or heart disease or cancer or others. The subcommittee held its first hearing about 10 days after stem cells came on the scene in November 1998 and we are up to 16.

We agree with the testimony that this could be the most remarkable breakthrough since man walked on the moon, and we intend to pursue it, to see to it if we can find a breakthrough with this remarkable method that is described of replacing defective cells.

CONCLUSION OF HEARING

Thank you all very much for being here. That concludes our hearing.

[Whereupon, at 11:26 a.m., Thursday, May 22, the hearing was concluded, and the subcommittee was recessed, to reconvene subject to the call of the Chair.]