UNITED STATES OF AMERICA

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

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BIOLOGICAL RESPONSE MODIFIERS

ADVISORY COMMITTEE

(BRMAC)

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33rd MEETING

+ + +

THURSDAY,

OCTOBER 10, 2002

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The Advisory Committee met in Salons ABCD, Hilton Hotel, Gaithersburg, Maryland, at 8:00 a.m., Dr. Daniel R. Salomon, Chairman, presiding.

PRESENT:

DANIEL R. SALOMON, M.D. Chairman

JONATHAN S. ALLAN, D.V.M. Member

BARBARA BALLARD Patient Representative

CHRISTOPHER BAUM, M.D. Guest

BRUCE R. BLAZAR, M.D.	Member
REBECCA BUCKLEY, M.D.	Guest

PRESENT (Continued):

JOHN COFFIN, Ph.D.	Temporary Voting Member
KENNETH CORNETTA, M.D.	Temporary Voting Member
JOHN M. CUNNINGHAM, M.D.	Guest
ALAIN FISCHER, M.D., Ph.D.	Guest (via teleconference)
DAVID M. HARLAN, M.D.	Member
KATHERINE A. HIGH, M.D.	Member
CHRISTOF KALLE, M.D.	Guest
KATHERINE E. KNOWLES	Temporary Voting Member
LORI P. KNOWLES, L.L.B., B.C.L., M.A., L.L.M.	Temporary Voting Member
DONALD B. KOHN, M.D.	Guest
JOANNE KURTZBERG, M.D.	Member
ALISON F. LAWTON `	Industry Representative
CRYSTAL MACKALL, M.D.	Guest
HARRY L. MALECH, M.D.	Guest
ABBEY S. MEYERS	Temporary Voting Member
RICHARD C. MULLIGAN, Ph.D.	Member
STUART H. ORKIN, M.D.	Guest
JENNIFER PUCK, M.D.	Guest
MAHENDRA S. RAO, M.D., Ph.D.	Member
BRIAN P. SORRENTINO, M.D.	Guest

PRESENT (Continued):

BRUCE E. TORBETT, Ph.D. Temporary Voting Member

ANASTASIOS A. TSIATIS, Ph.D. Member

LINDA WOLFF, Ph.D. Guest

GAIL DAPOLITO Executive Secretary

PRESENT FROM FDA:

NEIL GOLDMAN, Ph.D.
PHILIP NOGUCHI, M.D.
JAY P. SIEGEL, M.D.
RAJ K. PURI, M.D., Ph.D.
DAVID M. ESSAYAN, M.D.
AMY ROSENBERG, M.D.
MICHAEL A. NORCROSS, M.D.
CAROLYN WILSON, Ph.D.

PRESENT FROM NIH:

AMY PATTERSON

C O N T E N T S

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P R O C E E D I N G S

(8:10 a.m.)

CHAIRMAN SALOMON: Good morning, everybody. My name is Dan Salomon, and I am privileged to chair this session. You'll probably get to hear me a little bit better in a second when they get the speed back issue settled.

Today is the 33rd meeting of the BRMAC, and the topic is on retroviral gene therapy for the treatment of patients with severe combined immunodeficiency disease, specifically for safety issues. So if you thought you were at another BRMAC meeting, you're at the wrong one.

This is an unusual meeting in the sense that it is always an amazing amount of work for the FDA staff to put such a meeting together, and it's always an amazing commitment on the part of the expert staff, the expert panel that come from all over the country to join us.

But this particular meeting is noteworthy in that it was put together in a period of time that I imagine if there were records kept on these things at the FDA would be quite amazing since this whole thing was done in less than three weeks. Two weeks, yeah. I didn't want to exaggerate, but we've really basically just heard about it about two weeks ago before it hit the press, and it was coming from the top at the FDA from Phil and Kathy Zoon and Jay Siegel that this was something that the FDA wanted to step up and provide expert leadership to and

took on this amazing task.

And so normally whereas I would make my introductions and get on with it, I don't think I can just do that today, and so first give you that background.

Second, I'd like to especially recognize two major things here. First of all, the experts around the panel here have made an amazing effort that we really appreciate to rearrange their schedules. These are very busy people; you're all very busy, and to be here and just the amount of cooperation. The cooperation from Dr. Fischer who is on the line by teleconference link. Really I speak now for everyone at the FDA and for myself as Chair to say thank you to all of you for doing that. It's remarkable.

Second, I would like to read a list of names, and I don't ever do this, but, again, I think this is necessary, and that is of the people at the FDA who in a two-week period put this whole meeting together, and there's just not enough ways to thank them. I think they just need to be recognized.

From the Office of Cellular Tissue and Gene Therapies, obviously Phil Noguchi and Carolyn Wilson really took the lead in putting some of the initial issues together, developing the background, helped by Philippe Bishop, Joyce Frey Vasconcells, Tina Moulton, Cynthia Rask, who also took a lead on this; Stephanie Simek, and Daniel Takefman.

From the Office of Therapeutics Research and

Review, Jay Siegel, Karen Weiss, Michael Bazaral, Linda Forsyth, and Patricia Keegan. From the Scientific Advisors and consultant staff, Rosanna Harvey, who called me at 6:18 this morning to make sure that I knew to get here, which was very appreciated.

(Laughter.)

CHAIRMAN SALOMON: Seriously.

William Freas, Diana Widner, Jane Brown, Sheila Langford, and Gail Dapolito.

From CBER's Freedom of Information Office, Beth Ryan-Brockner and Joanne Binkley.

And from the FDA Dockets Office, Jennie Butler.

Again I just have to thank them and recognize publicly the incredible effort that it takes to do this so quickly. I mean just getting everybody's conflict of interest forms through and processed through the bureaucracies, you know, anyone who had dealt with our government knows that that's amazing. Actually anyone who has dealt with any government that's amazing.

Okay. Enough of that. Welcome here. I'd like to go around the table and have everybody introduce themselves. We have a lot of new people here as experts on the panel, and we'll get the people along the back line as well, and in the process I'd like to especially recognize three new members. As we get around to them we'll do that.

So if we can start, this is definitely not a new member here.

MS. MEYERS: Abbey Meyers. I'm the President of the National Organization for Rare Disorders, which is NORD, and I'm a former member of the committee.

DR. KATHY KNOWLES: Kathy Knowles, and I'm from Seattle, Washington, and this is my second or third time being here as a consumer representative.

DR. CORNETTA: Ken Cornetta from Indiana University, and my research interest is in retroviral gene transfer and also coordinate the National Gene Vector Lab for the NIH.

DR. LORI KNOWLES: I'm Lori Knowles from the Hastings Center and have a background in law and bioethics. It's nice to see you again.

CHAIRMAN SALOMON: And this is one of our first new members, Dr. Anastasios Tsiatis.

DR. TSIATIS: That's correct. Yeah, my name is Anastasios Tsiatis, and quite to my mom's horror, I go by "Butch."

(Laughter.)

DR. TSIATIS: Anyway, I'm from the Department of Statistics at North Carolina State University.

DR. HIGH: My name is Kathy High. I'm at the Children's Hospital of Philadelphia, and my research interests

are in gene transfer for hemophilia.

DR. LAWTON: I'm Alison Lawton, and I work for Genzyme Corporation, and I'm the industry rep. on the committee.

CHAIRMAN SALOMON: The second new member on the committee, this is Dr. Jon Allan.

DR. ALLAN: I don't feel like a new member, but I am a new members. I was ad hoc in this committee before. I'm a virologist from the Southwest Foundation for Biomedical Research, and I study retroviral pathogenesis mainly in nonhuman primate models.

CHAIRMAN SALOMON: As I said before, I'm Dr. Dan Salomon. I'm a scientist at the Scripps Research Institute, and I'm interested in cell transplantation and gene therapy.

Now, before we go this way, can we maybe start at the end of the back -- I don't want to call you the back row because that has a pejorative sense, but I think you'll have to get up and almost go to the mic, I hate to say.

DR. PUCK: Hello. I'm Jennifer Puck at the Genome Institute, and I do research on immunodeficiencies and retroviral gene therapy.

DR. MALECH: I'm Harry Malech. I'm at the National Institute of Allergy and Infectious Diseases, and I study immune deficiencies, and Jennifer and I are working together on a protocol for gene therapy for XSCID. That's why we're here.

DR. SORRENTINO: I'm Brian Sorrentino from St. Jude Children's Research Hospital, and my research interest is hematopoietic stem cell gene therapy.

DR. CUNNINGHAM: I'm John Cunningham from St. Jude Children's Research Hospital, and my interest is also hematopoietic stem cell gene therapy.

DR. KOHN: I'm Donald Kohn from Children's Hospital in Los Angeles, and I'm also interested in hematopoietic stem cell gene therapy.

DR. BAUM: I'm Chris Baum from Hanover Medical School, recently also joined Cincinnati Children's Hospital, and I have the same research interests as the other people here in the back row.

DR. KALLE: My name is Christof Kalle. I have the same research interest as the previous speakers. I recently joined Cincinnati Children's Hospital, coming from Freiburg University in Germany, and I'm also interested in retrovirus insertion analysis.

CHAIRMAN SALOMON: Thank you.

We've got you guys pegged. So that's easy to know what you do.

MS. DAPOLITO: Gail Dapolito from the Center for Biologics, FDA. I'm the Executive Secretary for the Committee.

And I'd also like to introduce the committee management specialist who is Rosanna Harvey.

Thank you.

DR. RAO: Mahendra Rao from the National Institute on Aging. My interests are in stem cells and retroviral therapy.

DR. KURTZBERG: Joanne Kurtzberg. I'm a pediatric hematologist/oncologist at Duke, and I run the Pediatric Pulmonary Transplant Program and have an interest in cord blood transplantation.

DR. TORBETT: I'm Bruce Torbett from the Scripps Research Institute, and I'm a basic research scientist. I have interest in HIV gene delivery and transcription.

DR. BLAZAR: Bruce Blazar, University of Minnesota, and I'm a pediatric pulmonary transplantation clinician and immunobiologist.

DR. MULLIGAN: I'm Richard Mulligan from Harvard and Children's Hospital, and I'm a gene transfer guy.

DR. PATTERSON: I'm Amy Patterson, Director of the Office of High Technology Activities in the Office of the Director at NIH. It's the office that, among other things, provides analytic and staff support for the NIH Recombinant DNA Advisory Committee.

DR. RASK: Cynthia Rask from Clinical Evaluation in the new Office of Cellular Tissue and Gene Therapy in CBER.

DR. WILSON: Carolyn Wilson from Division of Cellular and Gene Therapies in the Office of Cellular Tissues

and Gene Therapies.

DR. NOGUCHI: Phil Noguchi. I'm the Acting Director of the Office with a long name that covers tissue cells, gene therapies, and other related items.

CHAIRMAN SALOMON: If I can also have our guest experts.

DR. WILL: I'm Linda Will from the National Cancer Institute. My interest is in insertional mutagenesis of retroviruses in animal models and their role in cancer.

DR. BUCKLEY: I'm Rebecca Buckley from Duke University and my interest is in severe combined immune deficiency and the most effective way to treat it.

DR. MACKALL: I'm Crystal Mackall from the Pediatric Branch of the National Cancer Institute, and my interest is in T cell homeostasis and immune reconstitution.

CHAIRMAN SALOMON: Okay. So the one little piece of business here -- everybody did really well. When your red light is on, you're talking. When you're don, turn it off just by clicking it up. Otherwise you get the feedback loop on things.

Thanks for trying.

Okay, and the only thing I regret, and I'm sincere about this, is that there are equal sets of experts in our audience, and obviously we don't have time to introduce everybody sitting out there, but I think you will believe me,

please, when I say that as we go into the discussion phase this afternoon, I don't think that everything has to occur up here at this table, and I will do my best to recognize and facilitate people coming from the audience to communicate as well.

So I think I've covered everything that I have to do this morning. There are some other little issues that we'll get into in a few minutes, but with that, I'd like to introduce Gail Dapolito to read in the conflict of interest statement.

MS. DAPOLITO: Thank you, Dr. Salomon.

This announcement is made as part of the public record for the Biological Response Advisory Committee meeting on October 10, 2002.

Pursuant to the authority granted under the committee charter the Director of FDA's Center for Biologics Evaluation and Research has appointed Ms. Katherine Knowles, Ms. Abbey Meyers and Drs. John Coffin, Kenneth Cornetta, Lori Knowles and Bruce Torbett as temporary voting members.

Based on the agenda, it was determined that there are no products being approved at this meeting.

The committee participants were screened for their financial interest. To determine if any conflicts of interest existed, the agency reviewed the submitted agenda and all financial interests reported by the meeting participants.

As a result of this review, the following disclosures are being made. In accordance with 18 USC 208, Drs.

Bruce Blazar, John Coffin, Kenneth Cornetta, Daniel Salomon, and Anastasios Tsiatis were each granted a waiver that permits them to participate in the committee discussions.

Dr. Richard Mulligan was granted a limited waiver for Session 1 that permits him to participate in the discussions without a vote.

We also note for the record that Ms. Alison Lawton serves as a nonvoting industry representative member acting on behalf of regulated industry. She is employed by Genzyme and thus has interest in her employer and other similar firms.

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

Ms. Barbara Ballard will join us after a while. She's running a little late and serves today as the patient representative for this meeting. She's a member of the board of trustees of the Immune Deficiency Foundation and President of the SCIDs Alliance.

Dr. Christopher Baum is employed at the Hanover Medical School in Hanover, Germany.

Dr. Rebecca Buckley is employed at Duke University Medical School. She is involved in studies of retroviral gene

therapies to treat patients with SCID.

Dr. John Cunningham is employed at St. Jude Children's Research Hospital. He's involved in studies of retroviral gene therapies to treat patients with SCID.

Dr. Alain Fischer is employed at the Hospital Necker in Paris, France, and Dr. Fischer is joining us today by audio conference.

If you can hear me, good morning.

And he is involved in retroviral vector gene therapy studies to treat patients with SCID.

Dr. Christof Kalle is employed at the University of Cincinnati.

Dr. Donald Kohn is employed at the Children's Hospital, Los Angeles. He is involved in studies in retroviral gene therapy to treat patients with SCID.

Dr. Crystal Mackall is employed at the National Cancer Institute at the NIH. NCI is involved in retrovirus gene therapy research.

Dr. Harry Malech is employed at the National Institute of Allergy and Infectious Diseases, NIH. He is involved in studies of retroviral gene therapy to treat patients with SCID.

Dr. Stuart Orkin -- Dr. Orkin will also join us alter -- is employed at the Dana Farber Cancer Institute.

Dr. Amy Patterson is employed by the Recombinant

DNA Program, Office of Biotechnology Activities, NIH. NIH funds gene therapy research.

Dr. Jennifer Puck is employed at the National Human Genome Research Institute, NIH. She is involved in studies of retroviral gene therapies to treat patients with SCID.

Dr. Stephen Rose is employed with the Recombinant DNA Program Office of Biotechnology Activities, NIH, and again, NIH funds gene therapy research.

Dr. Brian Sorrentino is employed at St. Jude Children's Research Hospital. He is involved in retroviral gene therapy studies to treat patients with SCID.

Dr. Linda Wolff is employed at the National Cancer Institute, NIH. NCI is involved in retrovirus gene therapy research.

In the event that the discussions involve other products or firms not already on the agenda for which FDA's participants have a financial interest, the participants are aware of the need to exclude themselves from such involvement, and their exclusion will be noted for the public record.

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

I'm almost done.

A copy of the waivers addressed in this announcement is available by written request under the Freedom of Information Act.

And just as a little housekeeping request, we ask as a courtesy to the committee and to your neighbors in the audience if you'd put your cell phones and pagers on silent mode.

Thanks.

We may have broken the record for the longest conflict of interest reading.

Okay. Then I think it's time to get to the meat of this, and it's my pleasure to introduce Dr. Phil Noguchi, the Acting Director of the center with the long name.

DR. NOGUCHI: Thank you, Dan, and thank you, all of our guests at the Advisory Committee meeting.

Could I have the first slide?

I'll just sit here and make sure that everyone has appropriate time to discuss in more detail the very important issues we're discussing, but I'd like to frame it in this way. Why are we here?

We're here to acknowledge that there are extraordinarily difficult diseases that still remain to be treated. The purest recombinant insulin really doesn't matter if you're a brittle diabetic, and obviously for the thousands of

genetic diseases, therapies are really not necessarily a current reality but are on the horizon.

We're here to affirm that, in fact, rigorous clinical trials remain the societal imperative for us to move forward in these difficult areas. These are experimental products. We are asking human subjects to volunteer for this. We are asking them to participate fully knowing that the outcomes cannot be really truly and finally predicted, and it is only through the experimental process under a clinical trial regimen with all of the appropriate ethical, scientific, and regulatory controls that we can really understand and make rational decisions.

We're also here to learn that adverse events must be discussed, but they need to be discussed in the proper time, and I'll explain.

We actually learned about this adverse event back on Labor Day. Dr. Fischer has been -- and here we must give him absolute thanks -- totally open with this. His only request has been let me talk to my patients and my families in person, and they're all over the European continent, before we discuss it in public. And remarkably that has held.

Now, when we first learned about it, we went through at FDA identifying those trials that most closely resembled the trial in France, determined that there were three: two for SCID, one for ADA-SCID.

Two of those trials for XSCID actually were ready to begin, but did not because of this new event. The trial with ADA-SCID had treated four patients and, again, had been stopped. You'll hear more about those trials from the investigators today.

Literally at the time we learned of the adverse event, we just knew there was a lot of cells that were growing, and over the course of the last month -- and Dr. Kalle will be presenting a lot of this data which literally has only been generated within the last several weeks -- the initial notification has now turned to a better understanding of what may be going on here, and it is now the right time to discuss it.

And I think we should confirm that we're here because this is exactly where we should be. Several decades ago or more the prediction was made that if a person lacked a gene and you were able to replace it, maybe you would be able to treat or maybe even cure the disease.

And because you're putting a gene into the genome, you don't know exactly where it would go, and perhaps something untoward might happen at the same time.

So we're here to really review the fulfillment of those predictions and to deal with the issue, to really understand what the issues are, and then to hopefully -- we certainly hope -- advise the FDA on some very serious questions.

And if I can have the next slide, please.

The questions which will be asked at the end of the discussion and presentations today are: are there additional data or measures that clinical investigators need to provide before future and present clinical trials on SCID patients should proceed in the United States?

Please consider in your discussion each of the following as they pertain to X-linked form of several combined immunodeficiency disease and other forms, such as adenosine deaminase deficiency SCID.

As you go through your deliberations and as we seek advice, consideration of the risk-benefits of gene therapy in this particular disease, diseases, versus alternative therapies.

What revisions to the informed consent document and process should be imposed? What about alterations to the cell dose, alterations to the vector dose?

Mapping of vector insertion sites. We have to point out that Dr. Kalle has done a remarkable amount of work on this. It is not an easy thing to determine an insertion site by any means, and to be able to do it here really in a way was only possible because of the science and biology of the event.

What about alterations in vector design, for example, self-inactivating SIN vectors?

With that I will just stop, and I thank everybody,

again, for coming here. We look forward to a very intense, but a very enlightening and a very productive discussion.

CHAIRMAN SALOMON: Thank you, Phil.

So what we're going to do now is I'm going to need some help from the panel. The level of expertise here and the basic understanding of what's happened is such that I think we all realize we could all just forego everything, jump into quite an active debate and discussion of these questions, and I don't think anyone here thinks that we should do that.

So what I need help today is that you kind of make my job a little easier in terms of trying to do a two phase process. The first will be really a presentation of the case and what kind of information we have on it, which is going to immediately follow my comments.

And then scientific background on some of the issues that are directly pertinent, and as we roll through into the afternoon, very specific comments about some of the ongoing trials in gene therapy and SCID disease that are going to be potentially directly affected by our discussions, and then we will get to a discussion.

So what I'm saying is that we should try and hold back what could happen, you know, five minutes from now, and if you can help me, I would really appreciate that because I think that's going to be the hardest thing I have to do.

At the same time, certainly, you know, there

should be some key questions asked on these presentations to elucidate specific scientific elements that you think are going to be critical to the discussion later.

I also am not against someone saying, "Well, that is a really key theme and we've got to get back to it in the discussion," that sort of thing. So if you want to let a few things bubble to the surface and highlight them, I'll try and take note of those and come back to them, but if you'll forgive me in advance, I'm really going to try and hold us to a course so that most of the discussion, if not all of the discussion, occurs this afternoon in the question and answer period when everything has been done, we're all on the same page, and we start then, if that will be okay.

All right. So with that I would like to -- first of all, let me test our teleconference. Dr. Fischer, are you on?

DR. FISCHER: Yes, I am with you.

CHAIRMAN SALOMON: Thank you.

DR. FISCHER: Can you hear me?

CHAIRMAN SALOMON: Yes, we can. Thank you very much for joining us.

I'd also like to echo Dr. Noguchi's statements that, in simple terms, you're an example of an investigator in this field in the most positive way, and I'm proud to have you a part of this. Thank you.

DR. FISCHER: Thanks for your comments.

And good morning to everyone. I'm sorry I haven't been able to join physically for the meeting, but we will try to do our best.

CHAIRMAN SALOMON: My only request had been to do a high speed video conference line, but apparently that was asking too much to be arranged in two weeks.

DR. FISCHER: Okay.

CHAIRMAN SALOMON: So we can't see you, but we very much appreciate your being here.

DR. FISCHER: Okay.

CHAIRMAN SALOMON: So with that I'd like to bring Dr. -- is it Kalle or Kalle? -- Kalle, Dr. Kalle, and Dr. Kalle is helping us out here. He's obviously an expert in this area, but he is also going to be presenting Dr. Fischer's data. So I think he wants me to be very clear that he's not trying to plagiarize Dr. Fischer, and Dr. Fischer is obviously here by teleconference to answer questions.

Okay. We have a disconnect between the slides being projected and the slides on his computer.

MS. DAPOLITO: If there's a Dr. Candotti in the audience, would you please report to the registration desk?

Thank you.

CHAIRMAN SALOMON: There will be a two minute technical break here, but don't move.

DR. KALLE: That seems to be the right set of slides.

So I wanted to thank the organizers for the invitation and wish you all good morning.

Again, I want to stress that Dr. Fischer has requested that I would present part of his data, and I would kindly request your patience in referring to him for any further questions into the depth of the treatment of the patient.

I have been one of the members of the scientific group trying to work on the analysis on some of the samples of these patients, but again, I'm not the treating physician on this protocol.

SCIDX 1 gamma c deficiency is a disease that involves a genetic deficiency of the common gamma chain of the family of Interleukin-2, Interleukin-4, 7, 9, 15 and 21 receptors that blocks T cell differentiation in these patients leading to a severe and profound T and resulting B cell immunodeficiency.

And the only available treatment other than a genetic correction in experimental terms are haploidentical or HLA-identical stem cell transplantations.

Although this therapy has seen some success, and a very good success -- and I hope you can read this -- there is still some problems in this area that need to be solved.

By now more than 80 percent of the patients

treated by transplantation -- and we will hear more of this later on -- have good success with this transplantation. However, the long term outcome of these transplantations also have some problem areas that need to be solved.

There is poor T cell function resulting in some cases. There's some indication that the long term T cell function from these transplants tends to decline over time. The T cell counts tend to be low and the correction of the B cell immunity, which usually remains of recipient origin, requires intravenous immunoglobulin replacement for the lifetime of these recipients in most cases.

So here is some data that has already been published that Dr. Fischer wanted us to include. There is a decline in recent Thymic immigrants after stem cell transplantation in these patients in the long term.

The concept of ex vivo gene therapy of this condition by retroviral vectors does not need much explanation to this panel, where replication incompetent retrovirus vectors that carry the normal gamma chain, C, B, N, A, are exposed to stem cells and precursor cells of the bone marrow and basically lead, as has been shown in the mouse model, to functional expression of the gamma chain, then leading to interleukin responsiveness and development of T cell function.

The vector used in this trial is the MFG vector from Psi-Crip Packaging cell line, including the normal gamma c

CDNA in an anthiltropic (phonetic) envelope packaging.

And the preclinical studies that Dr. Fischer's group and other groups have conducted to prepare this kind of therapy first in vitro was conducted on gamma c deficient B lymphocytes to demonstrate the feasibility of a genetic correction and to measure the protein expression and function which was obtained.

In gamma c deficient CD34 cells in vitro again, expression in progenitor cells was detected, and there was evidence for a long term correction of the receptor defect in special types of cultures in K and T cell differentiation from these corrected progenitor cells has been observed.

In vivo studies in gamma c knockout mice showed that there was in t he mouse model up to an observation period of more than a year no toxicity of that type of treatment and the disease in the mouse model as far as several groups have reported stood corrected with introducing a strong selective advantage for the corrected cell population.

So the SCIDX-1 gene therapy trial was initiated. Eligible patients were, of course, only those with gc mutation and that did not have an HLA identical donor available at the time of treatment.

The protocol consisted on marrow harvesting, CD34 selection by immunomagnetic microbeads, a one-day preactivation step in the presence of stem cell factor of 52 ligands

(phonetic), MGDF, and Interleukin-3; three daily rounds of infection with the retrovirus vector supernatant in bags coated with fibronectin fragments to improve the construction efficiency.

The cells were then injected IV without additional therapy and no conditioning therapy was administered to these patients.

I'm afraid this is not very well legible.

There are so far 11 patients reported from the French group in different age ranges. The patient that we are going to talk about is patient number four on this trial. He has been treated at one month of age.

Most of the patients had preexisting serious infections and few of them, only two, had presence of maternal T cells or signs of their own endogenous partial gamma c chain production.

The trial was a success in nine out of these 11 patients, where you can see that the T lymphocyte recovery -- this is the time axis at month after gene therapy. After about three months after this treatment was observed normal numbers.

The patient that we are already going to discuss is outlined as patient number four again.

On analysis of the different subpopulations, you can see that basically the genetic correction which enables these cells to grow is, of course, because of the selective

advantage, 100 percent in the T cells and then K cells detectable in the peripheral blood of these patients.

You can see also clearly that although B cell immunity is reconstituted, that this is not happening as much through the genetic correction of the receptor. We're only around one to ten percent of the B cells carry indication of retrovirus integration, and the same is true for the myeloid cell compartment, which is not under the same type of selection and, therefore, shows a much lower prevalence of retrovirus vector integration.

The characteristic of the transduced T cells in this trial demonstrate that the patients develop normal counts at subset distribution of T cell function. The repertoire analysis of the T cell receptor beta shows a normal distribution. The cells have a normal phenotype with normal relations between naive and memory T cells, and there is evidence of thymic function as shown by the presence of recent thymic immigrants, as well as, I think, studies of thymus size.

The function of these T cells is normal, and they provide a normal functional immunity, basically enabling these patients to go home and start leading normal lives.

The summary analysis of the retrovirus as present in these cells shows that there is approximately one copy of retrovirus per corrected cell.

The follow-up of the patients on this trial as it

stands from October 1st, 2002, is indicated here. The two patients that have not received a full success of this therapy, patient number three, who went to allogeneic bone marrow transplantation after living more than half a year, and this is a patient who was older at the time of treatment and has not seen full success of generating functional T cells, probably due to the absence of thymic function and at his age.

All the other patients on the trial are alive and well, with as a notable exception of patient number four, which I'm now going to give you some more details.

Patient number four has been alive and well on this treatment for up to 30 months after transplantation. He observed, like the other patients, rapid T cell development, a fully clonal repertoire of T cell function.

The T and B cell immune responses developed normally. The patient has been capable of dealing with the usual type infections, including a VZV infection at month 30 after transplantation.

Integration site analysis conducted by my laboratory has indicated that there are more than 40 different integrational sites present in the T cells of the peripheral blood, again, at a frequency of one per cell zone, and the patient was doing well.

The patient then went on to develop an increased -- indications of an increased T cell count around month 30.

Most of these cells were gamma/delta T cells. About 7,000 cells of this phenotype were depicted at month 30 around the area of this episode of benign chicken pox.

But after the infection, these cells did not go down again. So from month 31 to 35, 50,000 to 80,000 white cells per microliter were depicted without the patient being symptomatic, where then he became what in clinical terms can be described as leukemic with 300,000 white cells per microliter in splenomegaly, at which point it was decided to treat him like a T-ALL patient at his home medical center where he has responded to chemotherapy, and currently the counts of the pathologic cells have dropped at or below the limit of detection.

The analysis that has been conducted so far by a panel of experts concludes that this has been a monoclonal gamma/delta T cell clone, both by immunoscope and T cell receptor sequence analysis.

The morphology of the cells is blast-like, with a mature T cell phenotype that is indicated here being positive for CD3, CD5, CD7, CD28, CD35R0 and expressing gc.

The negative for like one and two expression, CD34, CD10, CDT, CD1, CD4, CD8, and myeloid markers, as well as for CD16 and CD56.

Evidence for -- preliminary evidence for in vitro proliferation in the presence of Interleukin-7 and Interleukin-15 has been observed in culture. This depicts a picture of the

morphology of thee cells, and here is evidence of the cytogenetic analysis of these cells, where you can see an abnormality on the Chromosome 13 at the stage of the leukemic clone that could be identified as a partial price of the long arm (phonetic) at Chromosome 6 and in addition to one of the Chromosomes 13.

So the analysis that further has been conducted demonstrate that one provirus integration site could be detected by a procedure that I will later explain to you. The patient of that insertion locus is in Chromosome 11 at p1,3 within the first entrant of the LMO2 oncogene locus.

The cells show evidence aberrant expression compared to normal T cells of LMO2, where there is a transcription at least of the coding sequence of LMO2, and we have data on the follow-up of the integration site that is detectable starting in month 13 because not only does the integration site allow us to try to find out whether this interferes with any sort of genetic function of the cell, but it is also a molecular marker for the malignant clone that we can follow up at quantitative PCR, and I will give you some of that data later that we have just collected last week.

There has been an increase in the activity of this clone before any clinical symptoms were marked at months 17 and 24, and again, I will give you some more detail on that later.

And the analysis of the infusion, pre-infusion

product on 200 nanogram of that DNA has not shown presence for that integration site in the treated CD34 cells of that patient, which is not so surprising for us, but I can comment on that if you are interested.

Further, analysis has revealed that preliminary data of abnormal transcript has arisen from the viral LTR, although as we know and as we have seen, the receptor that is encoded by this vector is being made.

There's no indication so far for activity of replication of the retrovirus, and I've already told you about the translocation found at months 34 that has not been detected preexisting.

So I wanted to introduce to you briefly the concept on how we detect these integration sites, where a proviral site is integrated into the genomic DNA. It basically integrated so that it pushes apart the normal genomic locus with the introduction of a four base curve repeat just proximal to the proviral DNA.

The problem is that if we want to do this in a very high sensitivity you can't use the PCR technology because for the amplification of such integration locus you only know one of the flanks, whereas the other flank is at random or complete random in the whole cellular genome.

The method that we have developed has allowed us to detect such sequences now down to the single cell level where

we can do amplifications that both allow us -- and I will show some evidence later for that also -- to detect a parallel marker for different copies of integration sites, as well as single integration sites with very high sensitivity down to the single cell level.

It involves a preamplification and enrichment of the original locus, an immobilization on a magnetic bead system; double strand DNA synthesis; a restriction digest that basically gives each integration site a defined length; the ligation f a vast molecular excess of a polynucleotide precept that then adds a known stretch of DNA to the what we would call open flank of the integration site, and which allows us to amplify the sequence of these integration sites by conventional PCR methodology.

We have been involved in analyzing the patients from this trial for quite some time because we were interested as this is the first successful gene therapy correction what the number of transduced progenitor and stem cells may be in these patients.

And as you can see here, these are analysis panels of patients one, two, and four of the Paris trial. You can see that there is an abundance of PCR bands, and I can assure you that by sequencing analysis all of the bands that we have reamplified do actually represent integration sites in this type of analysis, shows an abundance of integration sites, more than

40 that we can count on this type of resolution.

Whereas you can see in samples of myeloid cells, there is some frequency of integration sites, and we tried to find out whether that was contamination or through integration sites.

So the group in Paris has been growing out the CIC colonies from bone marrow samples, and they were able to identify clones that were grown, CFU colonies that were grown in the absence of lymphoid growth factors which show evidence for single integration sites.

So we do know that myeloid cells were collected in these patients, and we also do know that the integration sites that we find in these myeloid cells 13 months after transplantation show up earlier in the CD3 fractions of these patients, indicating that at least in part collection has been achieved in progenitor cells that supply both the myeloid and the lymphoid cell system.

We see a similar type of analysis also on patient four. This is approximately 13 months after transplant where, again, you can see a fully clonal signal in the CD3 fraction.

You will hear a little later in one of the other talks about an analysis, what would this look like if it was not fully clonal. Here's an analysis of fully clonal (phonetic) and monoclonal patients that you will hear about in more detail, and the control on the site where we have a mixed monoclonal sample

with a fully clonal sample where you can see that we can detect both the mixed monoclonal band within the fully clonal sample, as well as are able to by PCR amplification from these products define the monoclonal integration site from the mixture for the single reamplification experiment.

You can see here this is rough, and unfortunately not yet ordered according to the time line, something that we thought was a sampling error on patient four, where you can see that at 24 months and 32 months after transplantation for the samples of patient one there still is a fully clonal sample, whereas the 17 month sample of patient four is still the fully clonal sample, but there is a preponderance of certain bands and the absence of detectable other bands in the 24 month sample of patient four.

These are very small children. The amounts of DNA that we usually obtain for this type of certainly scientific study are very small, and they are being sent around in Europe. So we were, of course, wondering whether this was an artifact of the method or of the DNA preparation rather than the true finding at the time.

Of course, the patient then developed the problems that have been previously described, and you can see here the LAM-PCR analysis on his peripheral blood where the sequence of his clones 7175, by our lab terminology, have been identified.

And independent of the DNA concentration, we have

only been able to find this one clone only at the leukemic state, and you can see here data that Man fred Schmidt and Manuela Wissler in my lab have done just last week, where you see a quantitative competitive PCR for this dominant clone.

Quantitative competitive PCR is a methodology that we use to get an indication of the quantity of these integration sites. We generate a PCR product that is a little smaller and a little different in sequence than what we're actually looking for in the integration site, but it does amplify with the same type of PCR primers.

And we spike that PCR probe at the defined copy number into defined DNA amounts of sample to get a semi-quantitative estimation of the presence of this clone.

And this data is remarkable for a number of things. First, you can see there is what you have to understand is that if the amount of DNA increases on a specific site, it concludes away the amplification of the internal control.

So what you can see here is along the time axis where there is no detection as compared to 50 copies of the internal standard in ten nanogram of wild type DNA, which would be indicative of about 1,500 cells here.

So you have to imagine these are about 1,500 T cells. You can see that there is about an equal amount at month 13, which means that out of the 1,500 lymphocytes here, approximately 50 are of this clone at month 13 already.

And there is an increase in the activity, a steady increase of this activity of this clone over time where at month 17 there's already probably between 100 and 200, maybe by the other dilutation step up to 500 copies within 1,500, and you have to keep in mind that the first time this clone showed up on FACS analysis with this type of percentage was at month 24.

We can basically see 11 months ahead of any indication in the FACS analysis that this clone has had already activity, and then of course, in the leukemic stage or in the state where there was the preponderance of the clone, it basically competes away the internal standard, and this is just a verification with the same type of technology with spiking 500 copies into that sample.

So Dr. Fischer has generated an estimation of the clonal growth of this cell clone, and you can see there's below ten to 100 copies early on where the clone starts picking up activity in almost linear fashion from month 13 through the various stages of sampling, through months 17 and 24, 30, 34, and 36.

You can see here indicated on the time axis the time when the chicken pox event that was sort of a clinical indication of the first stage where there was an abnormality in the T cell distribution, and then the clinical manifestations of the currently ongoing disease that is being treated.

The location of the integration site indicated

before was in the first entrant of the LMO2 gene. We will hear more about the function of the LMO2 gene later on. It is a transcription factor that is necessary for embryogenic blood formation. The knockout mouse of the LMO2 gene does not show any signs of blood formation and dies at an early embryonic stage.

The LMO2 gene has also been involved in childhood T-ALL, where it is over expressed in more than half of the diagnosed cases. You can see here the six different axons of the LMO2 gene and the location of the viral insertion site in the first entrant that spans about 10 kb, and it is about 2 kb into the first entrant.

Another interesting thing is that this retrovirus has inserted in reverse orientation with the sequence of the retrovirus promoter and transcription directed upstream of the genomic strain.

You can see here data that we have received from Bruce Areneau (phonetic) at Cincinnati Children's Hospital. This is a comparative genomic analysis of the LMO2 insertion locus, and you can see that the insertion has occurred in an area that is not conserved between man, the human, and the murine (phonetic) genome.

You can also see that the area has a high density of regulatory or potential regulatory elements of the DNA strain, for this curve here is indicating basically the number

of regulatory sites in the area.

Here data that the Paris group has also done on the LMO2 RT-PCR, where you can see in a negative control of normal peripheral blood cells there's no evidence for LMO2 messenger RNA, but there is in the clone of patient four, as well as in the positive control of CD34 cells.

The interpretation of all these data to us is that there is insertion mutagenesis in this patient cell clone in as far as there is compared to normal T cells in aberrant expression of LMO2.

There are additional factors that could be contributing to this. There may be you might want to call this aberrant gc signaling. However, there's no over expression of the gamma c chain.

There is a normal c for such a vector. The data on whether there's Step 3 and Step 5 activations currently pending.

Another open question is whether VZV has a role in precipitating the course of this event. The T cell clone is VZV positive by PCR, and there's further analysis conducted on this.

It is also a question of whether this T cell clone is involved in an immune response to that virus and whether there is genetic susceptibility in these patients and, of course, other contributing factors, including the chromosomal aberration on Chromosome 13.

There's a pedigree for medulloblastoma in the family of this patient with one sibling, as well as one distant relative on having this disease, and there's currently research going on also in terms of the transportations for these cancers.

Ongoing investigations, of course, include the mechanism of the LMO2 deregulation, the analysis of the LMO2 transcripts, whether the non-spliced RNA in fact contains evidence for vector sequence or whether the deregulation is happening rom the proximal promoter of LMO2 or from the other allele.

Of course, we want to complete the retrospective clone tracing of the integration site, as well as an overall profile of gene expression which is currently conducted.

And about the other features I've already talked.

The VZV replication and conscription in this clone is currently studied in the Cohen Lab here at the NIH, and also there is a question of the anti-VZV specificity of this clone.

There is a general screening for a genetic predisposition to cancer going on in the genome of this patient, as well as a comprehensive analysis of other integration sites that we are trying to find in this patient.

The other patients on these trials we are screening by integration site analysis for the presence of clones that show activity in terms of growing in their proportion, and we are planning to conduct a more comprehensive

analysis of all the integration sites we have sequenced from this trial so far and tried to find out what is the frequency that can be established at the current point in time of the human genome sequencing project as to at risk integration sites near cellular oncogene or other structures that could potentially be involved.

And this is something that may be reserved for the discussion where we would try to define a monitoring algorithm for the integration sites. We have clearly seen from other trials, and we will see that also that the presence of an oligoclonal or monoclonal situation as such may not be an indication or is clearly not an indication that problems need to develop.

And so we would have to look at clone integration site patterns over time repeatedly. Probably there could be a closer time interval if there was evidence of clonal deregulation, and as well, if there was evidence that one of the integration sites or one of the clones would become confunderant (phonetic) over other clones that showed a FACS analysis of T and pan leucocyte markers should have been conducted.

Keeping in mind that in this patient the first signs of activity that we could see by LAM analysis was around month 24 and could see the clinical disease by about ten months.

There should probably be item monitoring if the clone continues to grow, but probably this should only have

consequences in the clinical sense if you can clearly establish it and if a clone grows to more than 50 percent of the marked population within six months of observation.

So I would like to close by thanking the collaborators and, of course, Alain's group for being so kind to provide these samples throughout the study, as well as the magnificent work they have been conducting in trying to clarify the role of these events, and my collaborators at Freiburg University, Manfred Schmidt, who has conducted most of the integration site analysis, together with Manuela Wissler, and also Hanno Glimm, who is the post doc on our gene therapy analysis.

Thank you very much.

(Applause.)

CHAIRMAN SALOMON: Okay. Before we get started on the question and answer period, I would like to invite Dr. Fischer now. Dr. Fischer, do you have any additional comments you'd like to make before we even make any questions to you?

DR. FISCHER: Not really. I think Christof has really described everything that we have done and we are trying to do in collaboration with him and other groups throughout the world. So I don't think I have additional information to give.

Of course, I'm ready to answer questions.

CHAIRMAN SALOMON: Excellent. Okay. So we have 25, 30 minutes here, and my thinking about the focus now should

be any questions from the group on what's been presented and on some of the scientific issues here are all fair game, and the only place that, as I said earlier, I'll truncate things is when we get into discussions of, well, should we do this or should we do that. That will wait for this afternoon.

But certainly I suppose there are plenty of scientific issues here that I think are fair game for discussion in the next few minutes.

So to take the Chair's prerogative, I'd like to begin by asking: this is a gamma/delta T cell clone. Have you gone back into any of these other patients just by simple flow cytometry, for example, and looked for any evidence for an expansion of a CD4 negative, CD8 negative, CD3 positive subset?

DR. FISCHER: Maybe I will try to answer that question. Two things. One, there was one patient with number six in the trial who presented with severe meningoencephalitis caused by VZV infection at the time of diagnosis of XSCID, and this infection went on and on over weeks once the gene therapy was performed up to the time and that was approximately three months after gene therapy when T cells became detectable in the blood of that particular child.

And the very first T cell which became detectable were gamma/delta T cells, and they were V-gamma 9, V-delta 1 of phenotypes. And at the time, there was a fairly quick recovery of the VZV infection.

And then these T cells positively declined and became replaced very progressively by the different gamma/delta T cells one normally detects in the blood of a normal individual.

In the other patients so far we haven't seen any expansion of an abnormal gamma/delta T cell clone. At the very last screening that has been performed over the last month, all of the patients have been screened. They have almost normal or fully normal distribution of the different subsets of the gamma/delta T cells.

CHAIRMAN SALOMON: Okay. Katherine?

DR. HIGH: Christof, you showed one slide. I think it was an RT-PCR slide for LMO2 transcripts from the patient and from CD34 positive cells, and what time point was that from? And do you have data on other time points?

DR. KALLE: This was a part of the ongoing analysis. The RT-PCR on the patient cells was conducted at the leukemic state basically. The CD34 sample is not in this patient.

Looking at other time points is an analysis that we currently do, but that I don't have any data on.

CHAIRMAN SALOMON: Bruce.

DR. BLAZAR: Given the QC-PCR plot that you showed, with the expansion of cells over time beginning quite early, have such studies in other patients showed this kind of

expansion rate in other clones?

And would it have been predicted that regardless of VZV infection that this clone would have continued to grow above a threshold amount?

CHAIRMAN SALOMON: The question is do you want to direct that to Dr. Fischer or to Dr. Kalle?

DR. BLAZAR: I want to know whoever has studied clones, which is Dr. Kalle.

DR. KALLE: We have conducted those studies. Again, you will hear later on from other trials, yes, other clonal studies have been conducted. Other follow-up studies of clonal tracking have been conducted both in animal models and in this.

We have not seen an evidence of a clone that really continuously over grows or out grows other clones unless it had been established very early after transplantation and just continued to exist.

I have pulled up a slide again, and you have to see that at month 31 here is the situation when the VZV infection developed, where you can clearly see that there already was a preponderance of this clone at least in the inspection, and the patient was just bordering on elevating his leucocyte clones.

But within the T cell fraction and especially with the gamma/delta fraction, there was a preponderance of this

clone much earlier on. We would estimate between month 17 and month 24, and the growth of that clone was continuous throughout this time period, as you can see on the graph here.

Yes, that's the graph.

DR. BLAZAR: So that's unique to this patient. And how many have you studied?

DR. KALLE: Well, we have basically studied about six patients from the Paris trial. We have studied two patients from the Los Angeles trial. We're currently looking at the patients from the trial from London also.

DR. FISCHER: Maybe I should add that we have done quite a number of tests of the immunoscope so that the distribution of the use age of the different families and the length of the CDR3 in terms of frequency within each beta family has been tested in now almost all of the patients, and we never detected any abnormal pattern suggesting of expansion of a clone.

So we always detect what amounted to alpha/beta positive cells fairly fully polyclonal repertoire, which of course tells very much against any expansion of the clone so far, except, of course, this patient.

CHAIRMAN SALOMON: Dr. Mulligan.

DR. MULLIGAN: Christof, I have a question about the limit of sensitivity of the LAM-PCR approach. Eventually a question is: could we look at every integration in a CD34 plus

infected cell?

What do you know about the sensitivity?

So let's say that if you look at these patients, they're infected, and if infection goes as everyone would hope, with the best vector systems and you're infecting ten to the seventh or ten to the eighth CD34 plus cells, you would have many, many different integration sites. Let's say in the best case ten to the seventh or ten to eighth different integration sites.

What can you tell us as the potential for looking at all of them using your method? And tell us something about the sampling error.

One of the paradoxes of this is, of course, that you can't sample all of your sample or else you can't use your sample, and I know we're going to get into it at the end of it, you know, what can we do at the initial infection to try to see what the frequency of bad apples are after infection.

DR. KALLE: Yes. Of course, these are the key issues to go on with this type of studies from here. I can freely say that the sensitivity is very good up to I would estimate approximately 100 different integration sites in a given sample, but it would clearly be a humongous task to screen the integration events of, say, ten to the seventh cells were as you had rightfully indicated you would first have to make sure that they're all represented in what you are actually sampling.

And then also you would have to at least use some mode of visualization or screening them short of sequencing the integration site locus. So I think that was something that at the current point in time we could not do. We could not screen ten to the seventh integration sites in a reasonable time frame.

DR. MULLIGAN: Well, in fact, even in this particular case, I think you said it's not very surprising that you couldn't detect the LMO integration site in initial CD34 plus sample. You wouldn't have thought you could.

DR. KALLE: Oh, yes. That to us makes perfect sense because you have to keep in mind that this is basically two or three days after the infection happening when the retrovirus vector comes on, and if we consider that this insertion only happened in one cell at the time of reinfusion, this has grown at most to a four or eight cell stage where the sampling error of even if you were working up a third of that sample would still be considerable and we would probably not be speaking about it.

DR. MULLIGAN: The second technical issue is when you look at the effect of retroviral insertions on the modulation of cellular functions, obviously there are many mechanisms, and these things can occur at a distance.

So tell us about the capacity of the method to detect an insertion, say, outside the LMO2, you know, down stream. Let's say it's ten or 20 kilobases that nevertheless

would have the ability to activate LMO2.

What are the limits of your method in terms of being close? That is, I assume you have to be reasonably close to make this work in a representative fashion.

So what we need to know is how well would this detect things that are not, you know, right smack in the middle of a protooncogene.

CHAIRMAN SALOMON: Wouldn't the answer to that be just as good as your genetic data? I mean, if he finds the downstream sequence using his methodology, then you've basically used the available databases. You should be able to find the downstream genes.

DR. KALLE: yes, I would concur with that where we would say that the integration site that we usually -- the sequence that we usually obtain is anywhere between 50 and 500 base pairs, and then we basically go back to the human sequencing database to find out where the gene locus is.

We have done walking analysis with this. So even in an unknown segment of DNA, you could go on to do LAMs to walk that locus, but that, again, is a very tedious process.

DR. MULLIGAN: Can I ask one last question of Dr. Fischer? Because it's a distribution question of integration sites.

Alain, why is it that if, in fact, the CD34 infection was, let's say, however efficient, let's say one

percent efficient, one percent of cells or ten percent of cells infected, when you see the T cell counts returned at the first point where you've looked at the T cell counts returning, I think someone said there's 30 to 50 integration sites.

Again, is that a sampling error or does that tell you that there's a dramatic contraction of the number of clones of T cell precursors that are actually making contributions?

DR. FISCHER: Well, I think Christof is better placed to answer the questions about the number of integration sites. The only thing I can tell you they are obviously I would say a kind of oligoclonal efficient lymphopoietic ongoing deficient (phonetic), but actually we don't know how it works physiologically speaking, how many clones there are which generate all of our T cells at a given time point.

Maybe there are not so many in normal individuals. That's something we don't know about. There are some suggestions in mice that it could be the same.

So then, of course, we don't know how many different clones have been transduced during the ex vivo procedures, but I would guess that many of them, the large majority of them are fairly differentiated cells which still express CD34, but for instance are committed to the B cell lineage.

We know that among the CD34 positive cells in these patients there are many PD cells (phonetic), and I think

these cells are observed dying out by giving rise to a wave of B cells and are less than could be detected anymore.

CHAIRMAN SALOMON: And so I know Dr. Coffin is going to comment on integration, I think, and then we'll go to Dr. Rao, and I believe I've got two other people.

DR. COFFIN: Yes, I have a question, a follow-up question to that actually. And I apologize -- I just came in -- if I am asking something that's already been answered.

But the oligloclonality of what grew out may, in fact, not reflect a contraction, just a chance stochastic contraction and expansion of the clone so that it may, in fact, reflect the fact that all of those clones -- there's something else about them. For example, the integration site that gives them some sort of specific selective advantage over the others, and so the question I have is were those other integration sites characterized and located and were there any others?

If so, where there any others of interest as far as proximity to other known oncogenes?

DR. KALLE: We have so far from this patient sequence about 60 integration sites. We have not found anything that has drawn our immediate attention.

We are currently doing a mapping where we try to find loci of a preponderance of retroviral integration, but so far nothing.

CHAIRMAN SALOMON: The question I had was just as

a bioinformatics point, if you said you found 60 integration sites, how many of those, using the available bioinformatics you had, were you able to assign to a locus of a gene?

DR. KALLE: If you include the working five sequences that are still being edited, about 80 to 90 percent.

CHAIRMAN SALOMON: A follow-up on this point. I think the question is: does the fact that initially there is only around 50 or 80 integration sites, in fact, indicate that there is a selective advantage of something about those integration sites, or is it because the gene transfer efficiency intra-cell that gave rise to those or that you're really only getting 50 different infection events?

And how does that contrast with the T cell receptor variation?

I mean, I thought we heard that the repertoire was a very broad repertoire. Yet this would indicate that there's only 50 different T cell receptors unless obviously this occurred before you generated the repertoire.

DR. KALLE: Yes. There are both interesting questions. What I would like to state is that the number 40 is we say greater than 40. What we can reasonably differentiate on these type of high separation gels, we can separate in excess of 40 integration cites.

Cynthia Dunbar has done some additional work with gene scan analysis where you can see that on repeat sampling

these are about 100 nanogram samples of DNA reflective of about three microliters of peripheral blood if you so wish.

If you do repeat sampling there is a considerable sampling error so that the number tends to go up. The thing we do know is, of course, that all of these integration sites support expression of the receptor because that is a prerequisite of these cells growing in the patient.

And the other statement I would like to make is that the insertion site analysis is probably not a good tool for a pool situation preinfusion screening. However, we are fairly confident that a cell clone that after infusion starts to grow at the more than ten percent level we would pick up as a preponderance grown by this type of analysis and scheme.

CHAIRMAN SALOMON: Dr. Rao?

DR. FISCHER: To remind the previous work we did several years ago, which actually came as a rationale for gene therapy of XSCID, we had the opportunity in our laboratory to study a unication (phonetic) with a SCID X1 in whom we were able to determine that a reverse mutation occurred in a T cell precursor, and of course, the likelihood that it occurred in more than one cell is almost zero

And by assessing the peripheral T cell receptor repertoire of this child, it was shown that the child had at least 1,000 different T cell receptor beta chain among the different T cells, indicating that between the point at which

the reverse mutation event occurred and the time where the T cell receptor beta gene was rearranged in the time, at least ten or 11 division cycles occurred, which shows, I think -- and these kind of data have been reproduced in mice -- this shows that the potential for proliferation of T cell precursors prior to T cell receptor rearrangement is extremely large.

So that would fit with the concept that a rather small number of clones, transduced clones -- whether they are 100, it doesn't matter -- but a rather low number can give rise to a fully broad, diversified T cell receptor repertoire.

DR. RAO: I had a question on the LMO transcript. What do you know about it? I mean is it truncated? Does it look -- any data on the transcript?

DR. KALLE: Other than that it's made, everything else is too preliminary. We're looking at it.

CHAIRMAN SALOMON: Dr. Wolff and then Dr. Mackall.

DR. WOLFF: I had a comment on trying to map these initial insertion sites would be even more difficult by findings in mouse experiments where retroviruses have integrated as much as 100 or 250 kb away from the gene.

This is probably more rare, although we can't really say how rare it is because there are many insertion sites in the animal models that actually haven't been identified as to what gene they are activating, and so some of those unidentified ones may be working at a distance.

DR. MACKALL: I'm struck why on this slide here the multiplicity of integration sites in the T cell arm versus the relative paucity of integration sites in the myeloid repertoire. Now, do you think that that reflects simply the lack of sensitivity for the myeloid cells because so much fewer of those cells -- they don't have the competitive advantage -- or do you think this is because you're mainly getting your vector into the common lymphoid progenitor rather than the true pluripotent hematopoietic stem cell?

DR. KALLE: That is, of course, one of the questions that we have tried to answer with this experiment. So to summarize this data, we can say that there is integration in myeloid cells in LTCIC of integration sites that we also find in T cells.

So has there been integration in some precursors that support both myelopoiesis and lymphopoiesis? Clearly, yes.

Is this happening in the majority of cases? We don't know. The situation of the engraftment is such that 100 percent of the T cells are corrected, but the myeloid or common progenitors probably have a much lower seeding efficiency because that part of the bone marrow function stays completely recipient

DR. MACKALL: That would suggest that the lymphoid pool is going to be at much greater risk than the myeloid pool if it's simply a matter of statistics.

DR. KALLE: Yes, and also I would like to refer with regards to the lymphoid progenitors in that question, I would like to refer to Don Kohn's talk a little later where we've all seen some evidence from another study that that is ongoing.

DR. SORRENTINO: Okay. I have two questions. The first just addresses, I think, the general question how certain are we of the causality of this integration with this phenotype.

And you know, I'd subdivide that into two issues. One is do we know that the transcript as coming from the inserted allele. Are there polymorphisms present in the two allele that would allow you to discern whether this is really from the inserted allele?

And the second is if we were to look at gamma/delta monoclonal T cell proliferations not associated with gene therapy, would LMO2 be detected, LMO2 expression be detected?

And, you know, what's the relevant difference in expression level here versus what you would see in a normal case, quote, unquote, normal case?

Gamma/delta T cell proliferation not associated with gene therapy?

DR. KALLE: There's some data from the literature to answer the last question first, but there is gamma/delta T-ALL. It's very rare, and I think the frequency of LMO2

activation in these cases is less than in the other cases, although I think there have been descriptions where T-ALL of gamma/delta type has had LMO2 excavation.

About the causal nature, that is, of course, the question that we are trying to answer. I have tried to describe the data as it is, not invoking that we yet think that there is a true causal relationship.

Of course, there are methods where we can try and find out whether the allele is transcribed from the distal promoter and actually involves the site of the retroviruses as activated or whether there is another type of deregulation going on.

These are studies that we are currently trying to do.

DR. FISCHER: Excuse me. I should add that there are preliminary data on this, has to be confirmed that this is, indeed, the allele by polymorphic studies. This is the allele where there is the insertion which is expressed. I mean your LMO2 allele.

But this has to be confirmed.

CHAIRMAN SALOMON: Thank you, Dr. Fischer.

DR. SORRENTINO: My question is for Dr. Fischer.

I was interested actually in the patient 11 who has not had T cell reconstitution, and Christof indicated potentially due to a defective thymus. What information do you

have about the thymic defect in this patient, which I presume is independent of the gamma chain mutation?

DR. FISCHER: We don't have any direct information on the thymic function of these patient. This is just a guess based on what is known about thymic function on long term survivors with transplanted combined immunodeficiency patients. The only thing we know, that prior to the gene therapy when we were trying to look at the presence of a thymiclump by echography or by scan, by CT scan, we were not able to detect any thymic lump.

Of course, it doesn't tell us that there was not a small one, but we were hoping actually that there might be a small one.

So this is an indication, but of course, certainly not the demonstration that the failure in these patients was caused by the absence of a thymic lump. But I think this is a reasonable hypothesis.

CHAIRMAN SALOMON: A question I have is what -- well, actually there's two questions. The first is what promoter do you think is driving LMO2 then and its clone? Is it being driven by the promoter of the transgene, the C and B promoter or something?

DR. KALLE: Alain, please go ahead.

DR. FISCHER: There is no C and B promoter. It's just the LCR promoter there, but it doesn't seem, again, from

preliminary data that anything is driven from the LCR apart from the normal expected transcript from the vector itself.

But of course, we don't know more than that. Of course, these are ongoing studies to determine exactly what is driving LMO2 expression in these particular cytochromes.

CHAIRMAN SALOMON: I guess that's something I hope that we can have some more scientific discussion of because I'm trying to square that with what I've learned in the last week on LMO2.

Dr. Coffin.

DR. COFFIN: In retroviral leukemogenesis or oncogenesis by insertional activation, transcripts actually driven from the LTR promoter and containing LTR sequences are actually more the exception than the rule. Much more common is an enhancer type of activation that acts on either the normal gene promoter or on some cryptic promoter in the gene and not directly activated by insertion of the retroviral promoter itself, but more the enhancer elements.

CHAIRMAN SALOMON: I guess we'll get to it a little later, but again, there's the old saying about if you're ignorant of something it's better to stay quiet than open your mouth and remove all doubts, but let me keep going.

So what I understand about the typical splicing of the chromosome, the translocation is what ends up happening is that the promoter of the translocated gene drives LMO2 and you

get leukemogenesis.

DR. COFFIN: You probably know more actually about the activation of LMO2 than I do, but it's certainly clear that in cases of oncogene activation by retroviruses, you can have in different cases or sometimes in different species, you can have different modes of activation of which there are actually three or four different kinds of things.

Sometimes it leads to truncation of stabilization of the transcript, for example. Sometimes it's a direct LTR driven expression. Sometimes it's enhancement, insertion of an enhancer element which is how you get these, presumably how you get these activations from 250 kilobases away as was mentioned earlier.

And sometimes it's actually splicing into the gene from a cryptic splice, a donor or accepter in the pro virus. So there's a lot of different ways to do it, and even within a given gene that's activate, there are examples of different mechanism having been used in different cases.

Sometimes if you look at what happens in chickens, it's a slightly different case than what happens in a mouse, for example.

Certainly your point and the general point about having to get these well characterized and see what's really going on is clearly very important. Nothing would be incompatible with what we know from the history of looking at

retroviral activation.

CHAIRMAN SALOMON: Just so it's clear, sort of the agenda I have, and we'll get to it later, is, you know, clearly an issue is going to be design of the vector.

DR. COFFIN: Absolutely.

CHAIRMAN SALOMON: And if the conclusion here was that the design of the vector had nothing to do with it, that the insertional mutagenesis created by a random insertion, period, then you could discuss it till the cow come home, the design of the vector.

DR. COFFIN: It's going to be a real issue.

CHAIRMAN SALOMON: Okay. Dr. Cornetta, and then I know we have a couple of questions over here.

DR. CORNETTA: All right. You made a comment, I believe, that we had looked and there was no aberrant transcript from the LTR. How did you determine that?

DR. KALLE: So far this is basically also by looking at the RNA product of the vector that has been made, but we're currently looking more closely at that. We cannot exclude that other things are going on.

In fact, Christopher Baum is going to present some data from an animal model that is an example of how that could be working.

DR. CORNETTA: Is that a northern analysis or is it by PCR?

DR. KALLE: This is by PCR only so far.

CHAIRMAN SALOMON: Dr. Blazar, Torbett and Mulligan.

DR. BLAZAR: Dr. Fischer, have there been any studies to look at in vitro human T cell generation using fetal thymic organ culture or reaggregation assays or in vivo transfer into SCID mice to see if samples obtained prior to 30 months preferentially grew out of this clone?

DR. FISCHER: No, we have not done these experiments. Actually, I am afraid we will not be able to do it because the samples we have are too small to perform these kinds of experiments unfortunately.

CHAIRMAN SALOMON: It's also, I would add, unclear whether the gamma/delta T cells are derived in the thymic.

DR. BLAZAR: Yeah, yeah. Well --

DR. FISCHER: This is true. This is not easy to get gamma/delta T cell in fetal thymic organ culture from human cells, but anyway, we can't do the experiment.

DR. TORBETT: Perhaps this is still preliminary, but could you tell us a little bit more information from your work about LMO expression, LMO2 expression in these cells? Is it exceptionally high, comparable to an ALL patient?

And secondly, since LEM (phonetic) is a zinc domain type of arrangement, it works for other proteins to bring it together.

Secondly, have you looked at other kind of insertions around the area that may give some insight of what else could be hid in deregulating LMO2 if this is the case?

DR. KALLE: To answer the second part of your question first, and I might also refer to Dr. Fischer for the other part of the answer, we have looked at what is described in the literature where most of the translocations activate the gene from the distal promoter.

So the actual translocation hot spot is about three to five maybe upstream of where the retrovirus is, just outside of the first exit. Unfortunately because the promoter specter of the gene is so complicated and that is a hot spot, the regulatory influence of that first entrant, which is quite large at 10 kb, has not been studied in great detail. So there's very sketchy data about that also.

The expression level as to what we can see from this very preliminary analysis, as well as what Alain Fischer has from the gene array analysis indicates that the expression is somewhat higher compared to a, quote, unquote, normal expression level in CD34 cells, but height want to comment on that.

DR. FISCHER: No, no. This is what do we know about -- I mean if the order of magnitude is slightly higher than it is in leukemic cells expressed in LMO2, but more than that today we can't tell.

And about the potting (phonetic), this is ongoing in the lab of Terry rabbits in England, but we don't have the results yet.

DR. MULLIGAN: On this issue, how many different ways can a retrovirus insertion activate something?

I was interested by the vioinformatics approach that you're indicating there's something that looks like enhancers in that sequence, and so just to add to the pot, it could be absolutely simply the insertion of the sequence having nothing to do with any enhancement from the long term repeat or transcriptional activity; that you're just rearranging some regulatory element that now allows enhancement to occur.

CHAIRMAN SALOMON: That's essentially what Dr. Coffin was postulating.

The other question I had is LMO2 is on Chromosome 11, and you're talking about a 613 translocation. We never came back to that. What in the heck happened there?

DR. KALLE: There is a partial trisomy of Chromosome 6, of the long arm of Chromosome 6 added to one of the Chromosomes 13. It's not something that has been typically described for any form of leukemia, and it's currently, of course, being studied as to whether this can be pinpointed down to what is really happening in that location and whether there is any reference to the familiar cases of cancer that have been described in this family.

CHAIRMAN SALOMON: It actually wasn't present in the kid before. If you go back before the gene therapy, there was no 618 translocation?

DR. KALLE: As far as I understand the data, but Dr. Fischer may want to comment on that again. It hasn't been present in the peripheral blood cells that have been analyzed prior.

DR. FISCHER: Yeah, there was several cytogenic analyses performed between month plus 30 and now, and up to the last test which was performed one months ago, there was no abnormal cytogenetic -- I mean cytogenetic analysis was always normal. The mitoses were normal.

So it seems that the presence of these 6 to 13 translocations inducing partial Chromosome 6 trisomy comes as a secondary event which follows by at least month, and maybe more than a month, the proliferation induced by -- lightly induced by the aberrant LMO2 expression.

Now, what it means and does it induce a secondary event which is important in driving cell proliferation we don't know today. As long as we don't have characterized exactly the breaking point, I don't think we can tell more about it.

CHAIRMAN SALOMON: Yes, Dr. Torbett.

DR. TORBETT: Could you tell us a little bit -- I'm not familiar with the IL-7 receptor expression levels when it's regulated. Could you tell me a little bit about regulation

of IL-7 and gamma T cells? Since it's constantly being expressed, does IL-7 normally down regulated after activation? Does it become quiescent?

I'm trying to balance what we know with LMO2 with constant expression of the IL-7 receptor.

DR. KALLE: I'm certain not an immunologist. So I would like to refer this to Alain.

DR. FISCHER: Well, I don't know whether I have all of the information about it. What I can tell you is that the gamma c protein itself is constantly expressed by whatever, alpha, beta, or gamma/delta features. There is a baseline under which it never goes, and its expression is increased following T cell activation, and then it goes back to baseline after some time.

So there is no, as far as I know, no down regulation of the receptor, at least of the gamma c cytokine receptor subunit in any event, in any circumstances. It's always there.

About the alpha chain of the IL-7 receptor, I'm not so aware of data, but I don't think it does disappear at the surface, but I'm not sure gamma/delta T cell in any circumstance. But I'm not fully sure of that.

DR. MACKALL: I'd just make one comment on the IL-7 receptor alpha. I don't know that there have been detailed studies on gamma/delta cells per se, but in general it's also

ubiquitous. It's, you know, present throughout. As T cells become activated, they tend to down-regulate IL-7 receptor alpha, but I'm not aware that it disappears.

DR. KALLE: No, no.

DR. MACKALL: We do see with very high levels of IL-7, which can be present in T cell depleted hosts, that you also can down regulate it, but I kind of doubt that it completely goes away.

CHAIRMAN SALOMON: Dr. Rao.

DR. RAO: I had a quick question. With some vectors we often have hot spots because of either homologous or, you know, tied to a thing similar. Is there any evidence with this vector that there's any kind of hot spot for integration?

DR. KALLE: That is, of course, a very interesting question. We have no indication so far. We are currently very actively trying to map that both for murine and the human genome for retroviral vectors. As you know, it has been suggested in the Cell paper in August that that may be the case for retroviral vectors.

CHAIRMAN SALOMON: Dr. Baum and then Dr. Coffin.

DR. BAUM: I just want to comment that the retroviral LTR, which provides gamma c expression in the patient, also is expected to up regulate when the T cell is activated and to down regulate expression when the T cell --

DR. FISCHER: No, no.

DR. BAUM: That is at least the data with many marker genes that have been introduced into T cells.

DR. FISCHER: Well, I think what is biologically relevant is the expression of the protein at the surface, and the expression in association with the other cytokine receptors, say, the alpha chain of the Interleukin-7 receptor or the beta and the alpha chain of the Interleukin-2 receptors, for instance, and the level of functional gamma c protein at the surface is controlled by the core expression of the other cytokine receptors subunit.

Of course, there is no evidence in the treated patients that there is the kind of, quote, quote, physiologic regulation of the gamma c gene transcription, which is certainly not the case. But at the surface level, when one sees both in the patient four, the one with this malignant proliferation, as well as in the others, is that the level of gamma c expression is either exactly in the normal range or slightly below, and it goes up in, for instance, PHA blasts, but I think this is not, of course -- there is a physiological transcription control, but just the association with the other cytokine receptor at the surface which controls the level of gamma c expression.

CHAIRMAN SALOMON: Dr. Coffin.

DR. COFFIN: Just one clarification and a question. There is no evidence right now for any kind of targeted specificity for insertion of retroviral provirus. The

paper that was published this summer and alluded to showed a general correlation of regions where insertion was frequent with genes in a cell, identified one what they called a hot spot, but it was really a very large chunk of DNA over which there were far more integrations than was expected. It was like four integrations over 8 kb or something like that.

There's no evidence whatsoever that -- so there's clearly a non-random aspect both at that level and at a more local level to retrovirus integration, but nevertheless the numbers of possible target sites in a cell are truly huge, and the probability of insertion at identical sites with any given vector.

And also, there's no evidence that what's inside the retrovirus has any controlling aspect on where the integration occurs. It's possible that the specific aspect of the integrate that's used causes some minor level of selectivity, but there's no reason to believe that the sequence of the provirus itself actually has anything to do with direct integration.

The question I had, and again, forgive me if this was covered, but has this provirus been completely sequenced and shown to be exactly what was put in or have there been some changes in it?

DR. KALLE: At the preliminary level I would say, yes, that it has been sequenced once. Of course, this is

something that we would like to confirm.

DR. COFFIN: And at the preliminary level there are no obvious changes?

DR. KALLE: Yes.

DR. COFFIN: From what was put in?

DR. KALLE: And the functional receptor is also expressed.

CHAIRMAN SALOMON: Dr. Mulligan and then we're going to wrap it up.

DR. MULLIGAN: I know Dr. Fischer and I talked about this several days ago, but I think many people don't know about this VL-30, which in mouse cells is an RNH transcript that is well known to be packaged.

And so it in principle could be a secret player in this whole thing, that is, it's another vector that essentially transmits the cells out of all mouse cells, and therefore, obviously I would think it would be very, very important to do the same analysis on VL-30 insertions as on the vector because, again, it's very curious about these initial 50.

Is there something special? Not only is there the vector to think about, but there's also the VL-30 to think about, too.

DR. FISCHER: Well, as we discussed it earlier, we are, indeed, going to look at the presence of VL-30 sequences and, of course, if they are one, of course, one has to look

where they are.

DR. COFFIN: There are also other sequences that have been reported in addition to VL-30. So one has to look for all of these.

There's a defective kind of old NLV provirus that seems to give rise to a lot of contamination, genetic contamination of vector sequences. Actually that also has to be looked through.

CHAIRMAN SALOMON: Okay. Well, we've got -- one last one?

DR. KURTZBERG: This is a question for Dr. Fischer. What are the plans now for the treatment of this patient and what criteria are you going to use to continue chemotherapy or to go to transplantation?

DR. FISCHER: Well, for these patients so far received the chemotherapy, which is the one from the so-called EORTC for T cell leukemia because at this time we are not sure exactly which is this kind of disease. Of course, there are enough similarities to consider such a treatment.

So he went on for the first three or four weeks now with that treatment, and he's clinically well, and now his abnormal cell counts is down to a very, very low level.

There is a plan in about ten days from now to do a marrow examination and to do a minimal residual disease detection. Of course, we have many tools to do so and then to

discuss what to do next.

Of course, complete remission would be a good prognostic factor. There are several options, of course. An allogenic bone marrow transplantation can be considered, but by definition this child has no actually identical sibling. We are looking for potentially unrelated donors.

Another option we are considering might be to try to do an additional treatment with a monoclonal antibody targeting specifically either the V gamma 9 or the V delta 1 elements of the T cell receptor expressed by this particular clone. But these are still under discussion, and first we have to know whether the chemotherapy has induced full, complete remission or what is the level of the minimal residual disease.

So next options are further chemotherapy. That's not an idea we like very much, but we have to consider it; the usage of the monoclonal antibody and/or an allogenic stem cell transplantation from an unrelated donor.

CHAIRMAN SALOMON: Thank you.

We went a little bit longer, but there was no way I was going to truncate that important discussion.

Before we leave this, just because I really do see this first piece as central to what happens the rest of the day. Let me ask first, Dr. Fischer, do you feel like we've discussed most of the key points right now just to set the stage? I know you're not going anywhere, but is there any last comment you'd

like to make at this point?

DR. FISCHER: No, I don't think anything has been left. I mean, all of the as possible aspects have been discussed.

Well, maybe, I don't know. One small point. I would be interested to hear about possible ideas, further ideas to look at genetic predisposition in the family, given the occurrence of these two medulloblastomas, one in a sibling and one in a relative.

If someone has further ideas how to look at that, we would be extremely interested to hear about it.

CHAIRMAN SALOMON: And Phil, Carolyn, Cynthia, are there any key questions or ideas you want to get on the table?

DR. NOGUCHI: No. I would like to on behalf of the FDA personally thank Dr. Fischer for his openness. Obviously Dr. Fischer's trial is not under FDA jurisdiction. However, that has proven to be absolutely a nonexistent barrier, and we thank him for presenting here and for continuing to be here during this very important discussion.

CHAIRMAN SALOMON: Thank you.

Okay. Then what I'd like to do is go on to the next presentation of the morning, after which we will have the scheduled break.

And it's my pleasure to introduce Dr. Rebecca

Buckley from Duke University Medical Center in North Carolina. She's going to talk to us about her extensive experience doing bone marrow transplantation in human patients with SCID.

I've been reminded as long as we have a moment here let me introduce three other people that have arrived sine we went around.

Dr. Orkin from the Dana Farber Cancer Institute in Boston. Welcome, sir, and you'll hear from him later on LMO-1. He kindly didn't interrupt me in the middle of whatever stupidity I was saying about LMO-1, but I'm sure he'll correct me later.

Dr. Coffin, who's splitting his time between Boston and the Washington area, joins us.

And then Dr. David Harlan, who's the third new member of the panel, welcome to, I guess, the first meeting here. Dr. Harlan is at the NIH.

DR. BUCKLEY: Well, thank you very much.

I was asked to begin the discussion of alternative therapies for several combined immunodeficiency, and this is a condition that I've been involved with most of my career, along before there was any therapy available.

And having watched many of these patients die -- could I have the next slide? -- you have to remember that this is fatal syndrome. It was described about 50 years ago, and it's only been 32 years that there's been any treatment at all

that's been effective because most of these babies die by their first birthday.

And I'm making these remarks for the people in the room who are not aware of the findings about SCID because I think some people only know about it as "bubble boy" disease.

But this is not one disease. It's at least eight different genetic diseases, and it's always characterized by the absence of T cell function, and because there's no T cell function, there is usually no B cell function, and in some genetic types there is also no natural killer cell function.

Next slide.

And briefly this is a list of all the different types of SCID. This is the X-linked form that we've been talking about for the last hour, the gamma c deficiency, and these other are all autosomal recessive conditions. They have specific phenotypes that enables recognition of the possible genetic type just by looking at flow cytometry.

Next slide.

And just to give you an idea about the relative prevalence of the different genetic types, as Dr. Noguchi pointed out early on, the reason we're so interested in knowing all of the different genes that cause SCID is because one of these days we all hope for a gene therapy for all of the different genetic types of SCID.

The most common type is the one we've been

discussing, which is the X-linked type. These are all the SCIDs that I've seen in my career at Duke, and you can see that the next most frequent is ADA deficiency, which represents about 16 percent, and then right behind that is the IL-7 receptor alpha chain deficiency, which represents about ten percent; JAK-3 deficiency, about six percent. Then I think you can see up here at the top of the pie that there are still 30 babies in our group that we don't know what the molecular cause it is.

Next slide.

But relevant to this discussion is the fact that it's been known since 1968, the year after HLA was discovered, that all of these genetic types can be treated successfully by bone marrow transplantation without a need for pre-transplant chemotherapy because they don't have T cells, and therefore, they can't reject the graft.

However, despite the fact that we knew that HLA identical transplants in 1968 would cure this disease, it was not until the early 1980s that methods were developed that would allow treatment when there was no HLA identical donor.

And it would through the studies of people who worked with nice and rats that shows that if you could remove all post thymic T cells from the donor of bone marrow or in the case of the mice and rats, from their spleen cell suspensions, that you could get around the problem of graft versus host disease, which you would also have if you used any other than an

HLA identical donor, and it would be lethal.

However, there are now excellent T cell depletion techniques, and the fact that you can take out the T cells, which is the most effective way to prevent graft versus host disease, means that in these patients where the goal is to give them immunity, it's not correct their myeloid series or their erythroid series or their platelets. You want to give them immunity, and this allows the omission of drugs like cyclosporine and methotrexate that would interfere with the development of that immunity.

So what I plan to do in the next few minutes is just to talk about the experience that we've had at Duke, and then also I was asked by Dr. Wilson to try to give you an overview of what is known about the efficacy of bone marrow transplantation around the world.

So could I have the next slide, please?

This is the method that was developed by Yari Ricener initially in mice and then later in monkeys, where you start with approximately a leader of bone marrow. You get a buffy coat, and then by adding a protein from the soybean plant, you can clump some of the cells, and the other cells that are unclumped then still contain T cells.

So to get rid of the T cells, we take advantage of the fact that human T cells bind to sheep red blood cells, and by doing two radiant depletions, you can get rid of a large

majority, about four log depletion of T cells. So that you end up then with about five percent of the cells that you had up here.

And next slide.

This is a slide showing you what one of the final preps looks like. If you can see, there are essentially no lymphocytes in this final prep. These are immature myeloid cells, but we know that there are also CD34 positive cells. There are CD7 positive cells. There are dendritic cells. There are no CD3 positive cells that you can detect, except an extremely small number.

And this final preparation does not proliferate in response to PHA or Con A. So that when you put a cell suspension like that into the circulation of a patient with severe combined immune deficiency -- next slide -- you have to think about, well, you're not giving any lymphocytes. You're not giving any T cells. Is this child going to have any way of maturing these stem cells that you're giving so that they can become T cells?

And for many years, it had been considered that the thymus might be what was wrong in SCID. It's vestigially small. It doesn't have any lymphocytes in it, and it doesn't have any Hassell's corpuscles.x

This is a picture on the next slide showing you that this is essentially an epithelial organ, and there are no

lymphocytes present. So knowing this, and one of the first patients that we treated -- next slide -- and he's now 19 years old; he has JAK 3 deficiency. We put the cells in at this point in time, and then for about three months nothing happened, and so we were really concerned that maybe the child didn't have a way of having thymopoiesis.

But then all of a sudden CD3, CD4 and CD8 positive cells appear in the circulation.

Next slide.

At the same time T cell function appeared, and since these were divided in cells, we could do a karyotype. The child is a male who has JAK 3 deficiency. He had no siblings. His mother was a donor. These are 100 percent female cells.

So even though we didn't put any lymphocytes in, his body was able to mature these stem cells, and we now -- I don't have time to go into this -- we now know that there is thymic enlargement, and there are tracks produced, and that this is caused by thymopoiesis from this very tiny organ.

Next slide.

Now, one of the things that's been alluded to is that bone marrow transplantation is not perfect; that B cell function often doesn't develop. It did in this particular child, but it took about two years, and he has never received IVIG in his lifetime, and he's, as I say, 19 years old and quite healthy.

Next slide.

So in summary then, we have been able to use this method to treat patients who would otherwise not have been able to receive any therapy, and this is since 1982. We've been able to treat now 128 patients with SCID. Only 15 of these had HLA identical siblings. The others had no HLA identical siblings, and we used HLA haploidentical donors.

And you can see that three quarters of these patients are going to survive it. The incidence of survival is about the same whether there are ADA deficient or not, with the caveat that some of these who did not accept the graft had been treated with PEG-ADA, and one of these has been treated by gene therapy in Italy, by the group there.

The next slide.

So just to go over the survival rates, I know that in Dr. Fischer's experience in Europe that the RAG patients there, that is, the T negative, B negative, NK positive patients tended to do less well with transplantation from haploidentical donors than the T negative, B positive SCIDs, but in our group here we've not really been able to see much of the difference in the survival rate among the different genetic types.

Okay. Next slide.

This is what they died from. They are 28 who have died, and you can see that most of these have died from viral infections that they were infected with when they presented. We

had one late death at 18 years of age from a child who was treated with PEG-ADA. This was an ADA deficient, but most of the deaths have occurred in the first year.

We've had a couple that occurred from CMV and adenovirus as late as three or four years out. None of the patients died of graft versus host disease even though only 15 of them had HLA.

Next slide.

So let me go back one slide if I could. I wanted to talk about this because Dr. Wilson asked me to comment on other problems in these patients, and that is that there is an increased incidence of malignancy. These are mainly lymphoid malignancies, and in our series you can see that three of our patients had EBV lymphoproliferative disease versus lymphoma. They were all EBV positive.

The next slide.

The article that came from Ellen Jaffe's group at the NCI is the most recent report that I could find on the incidence of malignancy and SCID, and this was a review article in which it was stated that the incidence was estimated at one to five percent. The mean age at diagnosis was a year and a half and mainly boys more than girls. Most of these were not Hodgkin's lymphoma. A few were the Hodgkin's type, and there was no mention about leukemia in this particular review. I know that there has been leukemia in SCID before.

Next slide.

Now, to talk about other centers and the experience with bone marrow transplantation, this was a slide that I put together for a chapter I wrote for Dick Steen's book on transplantation, and it was a survey I made by calling all the people I knew who were working in the field and getting an idea about how many transplants were being done.

And I think you can see that there were 576 SCIDs that I learned about, and these are the data here at the top. Since I can't calculate that fast -- next slide -- I have calculated it for you.

The HLA identicals, there were 125 and 105 were surviving or 84 percent, and this is consistent with the figure that you heard earlier.

For the haploidenticals, at that time 60 percent were surviving, and for the MUD donors, 71 percent. There have only been two cord blood transplants in SCID that I found out about as of 1997, and I'm aware of the data, the unpublished data, from the latest survey of the European immunodeficiency group where I think that less than one percent of the transplants have been of that type all told in SCID.

But all together the survival rate was 64 percent.

Next slide.

Now, these are publications since 1997, and they come, again, from Dr. Fischer's group and the European Society

for Immunodeficiency. The article by Haddad in 1998, I think, summarized the data through '96 or '95 or somewhere in the mid-1990s.

And at that time -- and this was 193 total SCIDs, this included HLA identical, as well as haploidentical and unrelated, and there was a 48 percent survival rate at that point.

Bertrand, again, from the European Society for Primary Immune Deficiency, published in 1999 178 that were just haploidentical, and as you can see, 52 percent were surviving there.

And I might point out that in many of the European centers, chemotherapy is used prior to bone marrow transplantation, and the thing that seems to be emerging from this is that the centers that use chemotherapy, the mortality rate appears to be higher, and I think this is because these patients present with lethal infections at the time they present, and when immunosuppressive drugs are given, it's not any surprise that there would be a higher mortality.

For example, this is the L.A. Children's group here, where I think they used chemo for all of their patients, and there were 37 that were hap-matched, and of those, there were only 17 surviving. Overall there was a 56 percent survival rate.

This is from UCSF, from Mort Talley's group, where

in the attabaskin (phonetic) SCIDs and the others who have the Artemis gene, the patients -- these are inbred groups here, and there are a series of 16 where only six were hap-matched. The rest were DR identical. There was a 75 percent survival rate.

And this is the current survival rate at our institution.

Next slide.

Okay. Now, I want to touch on something that is probably going to some up, and that is that since they die of infection, if you could diagnose them before they become infected, wouldn't the survival rate be better? And the answer is yes.

This is from Andy Cant's group, and the first author is Kane from Newcastle, where they did a retrospective review of 13 that they had transplanted in newborns because there was a family history, and these children were diagnosed early on, and they were all surviving.

In our series at Duke, and one of the references that was sent to the members of the committee as a pdf file is from our group here that was published in February, where we had I think at the time it was 21 or 22 neonates, and we had only lost one, and that was from a CMMV infection.

And included in this number 35 right here are these 24 newborns, but what the number 35 refers to is all of the patients that we have transplanted in the first three and a

half months of life, and we only have lost one patient, and that was from the CMV infection, from a mother who had no prenatal care, and the child was infected right after birth.

So survival rate is very good if you can diagnosis this at birth, but -- and I'll just say this briefly because I don't want to get into a long discussion of this -- but there's no screening in the United States or, for that matter, that I'm aware of anywhere in the world for this condition at birth. And I think that, you know, every baby in the country could be picked up if just a white count and a manual differential were done on the cord blood because then you could recognize leukopenia and transplant shortly after birth.

So next slide.

This is just a Kaplan-Meier plot showing you the neonates we transplanted.

The next slide. The next is the ones in the first three and a half months of life. I think you can see that we have several that are out in the teenage years, and the oldest child is a pediatrician's daughter who wants to be a physician, and she's in college now and very healthy.

The next slide.

This is just to show that this was a child who was transplanted at day 18 of life, and this was her thymus at four years of age. So by giving stem cells to a baby with a vestigial thymus with any of the types of SCID that we know

about, by putting the normal seed, the garden enlarges.

The next slide.

So now I wanted to talk about the other aspects of this, and so far I've only talked about survival, and survival, of course, is not everything, and just taking our 100 survivors currently, 90 of these patients are T cell chimeras. In other words, 100 percent of their T cells are their donors'.

Ten of the patients are not chimeras. Five out of 16 of the 88 efficient SCIDs, one has undergone successful gene therapy in Italy, and one is awaiting gene therapy there, and three of these patients are on PEG-ADA.

The other five are one X-linked; one JAK 3 deficient that you'll hear about this afternoon; three autosomal recessive.

Thirty out of 100 have donor B cells, but 58 of 100 are on IVIG. So even though giving this type of treatment can give you a high rate of survival, depending upon when you transplant, you end up with having poor or no B cell function in many of these patients, and in some cases there is resistance to engraftment so that you don't even have T cell chimeras.

The next slide.

So in conclusion, this is a pediatric emergency. The potential exists for diagnosis routinely at birth, and if a stem cell transplant from a relative can be done in the first three and a half months of life before infection has developed,

they would have probability of success.

T cell depleted hap-matched transplantation provides a life saving therapy for all forms of SCID, but it is not a perfect treatment, and the prospect of gene therapy offers hope, but the remaining defects in these chimeras will eventually be correctable.

And as you've already heard, most of Dr. Fischer's patients who received gene therapy, not only was their T cell defect corrected, but their B cell defect was as well.

So I'll close there, and I'd be glad to answer any questions.

(Applause.)

CHAIRMAN SALOMON: So this is open for some discussion. Dr. Torbett.

DR. TORBETT: Not following the literature and not knowing why there would be a difference between those that got gene delivery versus transplant, why is there such a difference in B cell? And are the B cells that are coming up in the patients that have been transplanted, are they completely functional across all isotypes?

DR. BUCKLEY: Okay. Remember that in many cases, in all cases at our institution, we do not give chemotherapy. So we don't get of the recipient's B cells.

But, again, the European group has looked at this. They've looked at the ones who did receive chemo versus the

ones that didn't receive chemo, and it was not statistically different as to whether or not they had chemo, whether or not they had B cell engraftment.

We don't know exactly why B cells don't engraft, but there are lots of theories about that. Some people would say it's a spacing problem, and you only engraft what you need.

So, for example, in the X-linked and the JAK 3 where they have a high number of B cells pre-transplantation, does that prevent the B cells from coming in?

We don't know really.

CHAIRMAN SALOMON: Can I address that because I am also -- I think this is really a potentially important point. One at some point could ask if one could address this issue when would balance the need for the gene therapy approach against using allogeneic transplantation.

And it's clear that this B cell issue isn't one that's totally out of the picture from the data Dr. Fischer has shown us today in the sense that it would appear that the paucity of insertion cites in B cell lineage suggests that, again, there's some sort of decrease in whatever selection pressure is that's positive for selecting the B.

It doesn't make a whole lot of sense to me. If you don't have a T cell pool, you shouldn't be driving more some of the later points in maturation and immunoglobulin switch. So it seems like you'd be missing a whole lot of your more mature B

cell population.

And I just don't follow this space argument either.

DR. BUCKLEY: Well, we have other data on B cell function in our patient population. In the JAK 3s and the gamma c's where their cytokine receptors don't work on their B cells, we postulated that the reason they don't have B cell function is because they still have abnormal cytokine receptors on their B cells.

However, in the IL-7 receptor alpha deficient patients who have all host B cells and all donor T cells, most of those had normal B cell function.

So I think it may have to do with the genetic type of SCID, and if you've got B cells that have nonfunctional cytokine receptors, then this may be the explanation.

And I should add that approximately one third of our X-linked patients do have normal B cell function, and they are the ones who have donor B cells. And we didn't do anything different in those patients than we did in the others.

So we don't know why in one third of the patients we do get donor B cells and in the others we don't.

MS. LORI KNOWLES: I wondered if you noticed any B cell function in the neonate group that differed from the other group.

DR. BUCKLEY: Well, no. We had hoped that. We

did see earlier T cell function and greater thymopoiesis by transplanting the newborn, period, but we didn't see any difference in the development of B cell function.

DR. ALLAN: I'm going to go out on a limb a little bit. The question I guess I have is that you show the neonates survived better, and do you know why the neonates survived better?

Because, I mean, one of the things that hits me is that one patient that had lymphoproliferative disease with gene therapy was the youngest patient, the one month old infant. So I'm wondering if you can tie these together in any way.

DR. BUCKLEY: Well, we have thought about that a lot. We've wondered whether the thymus might be more poised to be able to mature these cells at that age.

But my own feeling is that I think infection must have an enormous role in this because if you have a patient who comes in with CMV or adenovirus or VZV, those patients usually take longer for the graft to come in, and so I think the infection plays a major role.

But it could be that because the thymus is larger or more poised to mature these cells early on, that's the reason for the difference.

DR. TSIATIS: Some of these high survival rates that you show in the children of less than 25 months, does that seem in some of these other studies like what you were looking

at?

DR. BUCKLEY: No, because it all gets back to what I was saying, that the only reason it would be done early would be if there was a family history, and in most of our cases there is no family history.

So because there's no newborn screening program anywhere in the country and I think nowhere in the world, these babies are not picked up at that point.

I'm lobbying obviously for newborn screening for this condition.

DR. TSIATIS: The other question had to do with how these neonates were identified, and could they possibly have different prognostic factors than the other children and different rates?

DR. BUCKLEY: Well, the reference, I think there's a pdf file that was sent around with the paper in it. It contained a lot of the ADA deficients and the X-linked because those are the ones that you can screen easily for on amnion cells.

But there was no difference in the survival rate according to genetic type among that group.

CHAIRMAN SALOMON: Just for some more, Dr. Blazar, Kurtzberg and Coffin.

DR. BLAZAR: Rebecca, could you comment on the track decline that was shown earlier?

DR. BUCKLEY: Right, right. I would like to say there was a slide that was shown by the first speaker that had Batelle, et al., as a reference. I was the senior author on that paper, and we had very few data points out at 12 and 14 years of age.

We're going back now trying to get some of out teenagers who are now 18 or 20 years and see if, indeed, that is the pattern.

DR. BLAZAR: The second question is: have you seen a difference in B cell engraftment if you transplant neonates versus older?

DR. BUCKLEY: That was what she asked.

DR. BLAZAR: Oh, I'm sorry. I missed that.

DR. BUCKLEY: Right, and in that paper that we published in Blood in February you can see that's what we had hoped for, but we didn't see any difference in B cell function in the neonates versus the older group.

DR. KURTZBERG: I just wanted to make a comment about neonatal transplantation. It's not only in SCID, but in any disease that you transplant. The younger the child the better they do, and whether you use chemotherapy or not, the outcomes are much better.

So we have transplanted a larger group of kids or neonates with inborn error where we used belated chemotherapy and have 100 percent survival in ten kids.

So I think regardless of the age or the disease, the younger the child the better the outcome.

CHAIRMAN SALOMON: Just to follow up on that, Jon, in general those children have complete reconstitution of B cells.

DR. KURTZBERG: Yes, they do. And as an aside because the experience has grown over the past two or three years, we had 20 babies with immune deficiency syndromes of varying types and with chemotherapy in the first two years of life and have an 85 percent survival in that group. They do have a full B and T cell function, and the median follow-up in that group was over two years.

So I think there's still a controversy about whether or not chemotherapy helps or not. I think in the very youngest kids, regardless of whether you use chemotherapy or not and regardless of the diagnosis that survival is much better because of age.

DR. BUCKLEY: I would just like to comment that I think you can't lump all immune deficiencies with the others. You know, most of the ones -- all of those patients in not the SCID group, but the others, and I clearly didn't get to point this out on the overall slide, but except for SCID one does have to use chemoablation prior to transplantation in order to get the graft to come up.

CHAIRMAN SALOMON: Christof.

DR. KALLE: I may have missed this, so please excuse. There's data from John Vick's group that transplantation of human progenitor cells to the not SCID mouse model allows B cell proliferation independent of progenitor cell function. So I was wondering whether that data had been looked at as to the dosage of B cells that have been co-transplanted or whether any attempts have been made to transplant B cells in an isolated fashion.

DR. BUCKLEY: That's a very good point because, as I showed you the smear there, there were essentially no lymphocytes in the final prep of most of these T cell depletions. Occasionally we will by monoclonal antibodies pick up a few B cells, but we certainly do not give them many B cells.

CHAIRMAN SALOMON: Dr. Mulligan.

DR. MULLIGAN: Rebecca, I have a question about enzyme replacement. You know, about a decade ago when there was the original ADA clinical trial, actually there was a little bit of a controversy about how the enzyme replacement at that point influenced the risk-benefit ratio with gene therapy, and since we may be getting back to this issue of how you reset the risk-benefit ratio, how does the current point, the PEG-ADA -- how is looked upon with patients? Should it influence this whole thing?

DR. BUCKLEY: Well, in our hands, when we have a

new patient with adenosine deaminase deficient SCID, we transplant before we give PEG-ADA because of the first patient, the one that was written up in the New England Journal. We had had just the opposite thought.

We had thought that we had to detoxify the micro environment by giving red blood cell transfusions repeatedly to lower the de-oxyadeneonucleotides (phonetic).

And what happened was we empowered her T cells to reject the graft, and she was the person I mentioned that at age 18 died of pulmonary hypertension.

The patients who have been on PEG-ADA have not fared as well as those who have been transplanted.

CHAIRMAN SALOMON: Dr. Blazar.

DR. BLAZAR: Would you make a comment on the repertoire diversity since that's relevant to the discussion?

DR. BUCKLEY: Right, right. We have, of course, looked a that. We're looking now at B cell immunoscope, T cell immunoscope, and in the patients who have been successfully engrafted, we have very good T cell diversity, but in the ones who have resistance to engraftment, of course, there's either oligoclonality or very few T cells.

DR. HIGH: I wanted to ask two questions. One is you mentioned several factors that appear to influence the outcome of bone marrow transplantation: age at presentation, presence of infection at presentation, whether they have an

identical versus haploidentical donor.

So could you essentially define a set of criteria that would predict people who are not going to do well at transplant?

DR. BUCKLEY: I would think the average baby who comes in at age even months because nobody has diagnosed him before that time, who comes in with an adenovirus infection or with CMV, varicella, that that patient is destined to probably not survive.

DR. HIGH: And the second question is that for those of us who aren't immunologists, can you comment a little bit about the cost and the trouble of IVIG therapy through life?

DR. BUCKLEY: I can't really tell you how much it costs a year, but it's a lot. I think -- is it $50 a gram now for IVIG? Well, I think it depends on which brand and whether they use Home Health to do it, a lot of different things, but it's very expensive to give IVIG.

It's also very expensive to give different types of transplants. For example, for a chemoablated transplant, perhaps Dr. Kurtzberg can tell you what the average cost of that is, but I would say it's well over a couple hundred thousand dollars, whereas in the neonates that we've transplanted, we've transplanted them as out-patients, and we've kept them in apartments in our community, and the average cost is around $50,000.

So there are all sorts of cost factors in the various treatments for these patients.

CHAIRMAN SALOMON: Abbey.

MS. MEYERS: Going back to a comment about the controversy about PEG-ADA, I was on the gene therapy subcommittee at that time when French Anderson came to us with the first human trial and wanted to do SCID, but about two weeks before PEG-ADA had been approved by the FDA, and so we said this used to be a fatal disease, but now it's treatable, and so there are other ethical questions that have to be addressed.

And most of the patients or families that I speak to have been on for years, are still doing very well, and have grown up and are full grown adults now.

So I think that there's not so much controversy. What we have to face with this issue is if we find that other children are at risk of leukemia because of gene therapy, whether for SCID or anything else, we really have to look at the question is this an untreatable disease.

And if there is treatment and maybe treatment that is not as drastic as bone marrow transplant, but you know, other kinds of normal treatments, then maybe they shouldn't be exposed to that type of risk.

CHAIRMAN SALOMON: Well, this is a good example of allowing something that's a key discussion point to bubble up at this point, but we won't go anywhere on that line right now.

But we'll definitely come back to that because it is a key point.

I'd like to go to the break. Richard, is this short?

DR. MULLIGAN: Is there any attempts to look in the reconstituted patients on whether there is any sort of genomic or genetic instability that's not associated with tumor formation, but just may eventually lead us to think that there's another chance for another kind of hit?

DR. BUCKLEY: The only thing that I can tell you is what we've not seen any evidence of any malignancy in any of the long term survivors. Prior to development of T cell function, we will often see myeloma-like proteins develop in the serum of these patients. We've had several that have had like IgA myeloma proteins or IgG myeloma proteins, and we didn't treat them with chemotherapy.

When the T cells came in, they down regulated these, and these clones went away.

DR. FISCHER: Excuse me. Can I add something from Paris?

DR. BUCKLEY: Yes.

DR. FISCHER: I just became aware yesterday -- this is just by chance I don't know -- but that a patient was transplanted 27 years ago. He's a SCID in France, has how a diagnosis of lymphoma in his stomach. I know it's a B cell

lymphoma, and I don't know more today, whatever is occurring on donor or host B cells, but I think this is important to know. This is the first time I've been aware of late malignancy in a c patient who was transplanted.

DR. BUCKLEY: Alain, was that an HLA identical?

DR. FISCHER: I don't know yet, but it is very likely because it occurred in 1975. We are working how to get the data.

CHAIRMAN SALOMON: That's interesting. So I can't resist a last question. You guys can beat me up later on this one.

But the question that came to me a few minutes ago is you guys, either you, Dr. Fischer, but what percentage of the B cells post transplant in these kids are donor derived?

DR. BUCKLEY: Well, it varies. When I said that there were 39 -- I forget what was on the slide -- 39 of the 100 have donor B cells. By that I mean it ranged from anywhere between three percent and 100 percent. So it can really vary.

CHAIRMAN SALOMON: Do you have a sense of what -- is it very low, suggesting that there's an engraftment/survival issue?

DR. BUCKLEY: It's really a spectrum, and I think we have a lot that have like 20 to 30 percent donor B cells who have good B cell function.

CHAIRMAN SALOMON: Dr. Fischer, do you have any

comments on that?

DR. FISCHER: Yeah, well, in our experience in those patients who were not myeloablated and this is the majority, there is not more than ten to 20 percent of them who have donor B cells at a significant level, and as Becky Buckley just said, we have some data that those patients do develop B cell functions whereas others don't.

CHAIRMAN SALOMON: Okay. Thank you.

A break. If I say ten minutes, it will be 15. So be back in ten.

(Whereupon, the foregoing matter went off the record at 10:37 a.m. and went back on the record at 11:01 a.m.)

CHAIRMAN SALOMON: Okay. Thank you all for coming back.

I would like to introduce one additional member of the panel who arrived just recently, and that's Barbara Ballard, and she represents the Immune Deficiency Foundation, which represents patients and families of children with SCID disease.

Okay. Well, I think we'll go right on with the next presentation, which is from Dr. Linda Wolff, National Cancer Institute, on retroviral insertional mutagenesis and cancer in animal models.

DR. WOLFF: I'm very pleased to be invited today to be able to share information from my own research, and I hope

that this will contribute a lot to the insight of the discussion that occurs this afternoon.

My research over the last 20 years has really been focused on leukemia in mice and in cats. In my graduate work I did some work with feline leukemia virus, and then ever since I've been at the NCI I have worked on mouse retroviruses with an emphasis on insertional mutagenesis.

What I'd like to cover today, shown in the first slide, I would first like to give a brief historical overview of insertional mutagenesis and cancer, but then I'm going to talk about an example of a model where we used information to promote leukemia progression in conjunction with retroviral mutagenesis.

And inflammation, of course, might be considered to mimic a chronic infection.

Next I'm going to talk about how we've used collaboration or combination of genetic events to induce leukemia in a more rapid way, and I'll take examples from our current studies where we use retroviral insertional mutagenesis in transgenic mice carrying oncogenes or in knockout mice in which a tumor suppressor has been deleted.

And then finally I'm going to give an example. This is a quite historical example from the '80s where we induced cancer with a nonreplicating retrovirus vector.

Retroviruses were first discovered in association with cancer around the turn of the century. So people took

extracts from leukemias in the chickens and they made cell-free extracts and transferred the disease to other chickens, and then these extracts, they used them to clone out viruses in cells and then finally molecularly cloned viruses, and similar things were occurring also in the rodents.

Now, just for historical background again, many of these cancer causing retrovirus isolates from mice and chickens were composed of two different viruses. The virus on the right is a replication competent genome we've also called many times "helper virus."

I don't think this is really working, is it? I can see it over there. Anyway, I'll go on anyway.

This replication competent genome is composed of -- has genes that we call the structural proteins, as well as enzymes that are crucial for replication, such as the gag, pol, and env genes.

Also, I just want to point out about the long terminal repeats, which we've heard mentioned already here. The long terminal repeats contain an important aspect. They contain transcriptional promoters and enhancers. Of course, the purpose originally is for transcription of the viral genome.

Now, the other retrovirus on the left was a typical defective genome that was found in these isolets in which many of the essential replicating genes were replaced by an oncogene. To varying degrees this replacement occurred.

This is just simplified. And these also had the long terminal repeats on the end.

Now, if these retrovirus complexes, they call them, composed of a couple of different virus isolets on the one, are put into animals, they get a rapid disease mainly because of the oncogenic gene that has been picked up into this defective genome.

It is believed that these were transduced from normal cellular sequences. I'm not going to talk about this aspect anymore, these defective genomes, but I'm going to concentrate now on these replication competent genomes because they can cause disease by insertional mutagenesis by themselves.

To explain insertional mutagenesis I just briefly want to mention how the virus replicates in its life cycle. The virus has a membrane on the outside, and it binds to receptors on the cell, and then the membrane fuses with the cell membrane so that the virus can be introduced into the cytoplasm.

At this point the RNA in the virus is reverse transcribed into DNA, and then that DNA is formed into a double stranded DNA, and this DNA goes into the cell, and the important part I want to point out, of course, as we've already been talking about is that the virus, called the provirus now, becomes integrated into the cellular genome, as John Coffin had mentioned, is essentially random throughout the genome.

John himself has done many studies along this line

to determine where these integration sites are, but within local regions there may be some specificity, but overall the integration is fairly random, and cell division is required for efficient integration.

Now, of course, these proviruses can integrate next to proto-oncogenes. I just want to clarify the definition of a proto-oncogene is in my definition any kind of a gene that can stimulate accumulation of cells during the normal process of cell growth, whereas an oncogene is an activated proto-oncogene having increased capacity to cause continued inappropriate growth.

You may see these terms, and I wanted you to understand what they were.

Now, there are many, many mechanisms for activation of oncogenes by the viruses, but I've tried to point out here the most common type.

First of all, the virus can integrate the five prime end of the gene, and its promoter here can be used to drive transcription. But actually what occurs more commonly, as John mentioned, is that the enhancer elements of the virus LTR can enhance transcription from other promoters so that it may just enhance the transcription downstream of this gene.

This is a more versatile way of activating a gene because the provirus can also be at the three prime end, and its enhancers can have an effect on the five prime end. And as I

mentioned earlier, these proviruses have been shown in some instances to be integrated as far as 100, 200 kb away, kilobases, away from the gene that they're activating.

This is a list of many of the current genes that have been shown to be activated by retroviruses by insertional mutagenesis, and I put it up here not for you to read every detail in it, but just to show you that there are a lot of them, and we're still accumulating more of them as the research goes one.

But to simplify this table, I've summarized some information here. Typical types of genes that are activated by insertional mutagenesis include growth factors, growth factor receptors, cytoplasmic kinases which are involved in signal transdiction, which can change the program of a cell, and transcription factors themselves that regulate the transcription of genes. This is not all inclusive, but these are the major groups.

Now, the viruses that have been shown to be involved in insertional mutagenesis, including the avian system, aviam mucosis virus in the rodents, murine leukemia virus, mouse mammary tumor virus, and IAP articles. These IAPs are endogenous retroprosone type of elements that have been implicated in moving within the genome, and in the feline system, the feline leukemia virus.

Now, these are not single viruses. There are many

in each group of subtypes of these viruses, and they have been shown to cause myeloid leukemia, lymphoid leukemia, erythroid leukemia, and mammary carcinomas.

Now, I'd like to give you an idea of how many of us look at the way in which insertional mutagenesis leads to cancer. If you have the normal progenitor cells in the blood and they become infected by a virus eventually you may get an insertional mutagenic event, and during the pre-leukemic phase, you get a progression such that additional oncogenic events may occur.

And finally you reach a point where you get a clonal event that has a -- it can rapidly expand its population, and so now you have more of a leukemia which has a malignant transformation.

I want to emphasize that this expansion is often more of an accumulation. These cells don't necessarily grow faster, but sometimes they're blocked in differentiation and do not terminally differentiate.

So what other types of cooperating events that may enhance cancer when there's already been an insertional mutagenic event? One we found in our studies was inflammation, which is a kind of immunological response. It could also have activation of another oncogene by translocation, mutation, deletion.

In transgenic mice this may just be a transgene

that's being expressed.

Also, you can have inactivation of the tumor suppressor by deletion, mutation, or hypermethylation which causes decreased expression.

Now, for those people who are not working on cancer regularly, I wanted to just go over how these events, various oncogenic events may affect the cell biologically. It may cause loss of cell cycle control. They may block terminal differentiation, which is normally associated with growth arrest. This is a very common kind of situation that is found in leukemia.

It may inhibit apoptosis. Cells normally undergo apoptosis or programmed cell death as part of the normal process when the cells are not needed anymore, and inhibition of this apoptosis is a very common oncogenic event.

Another thing is altered adhesion of the stromal cells allowing metastases.

Now, I want to talk some about some previous work of ours in which we had a model in which insertional mutagenesis collaborated with inflammation leading to acute myeloid leukemia. The way this model worked is you give Pristane, which is an oil in the peritoneal cavity that causes a chronic granuloma, and if you give MuLV to these mice intravenously, in an average of three months you get a leukemic outgrowth in the peritonea cavity.

So the retrovirus, what we've found over the years, causes mutagenesis in many cases in the c-Myb locus, which is a proto-oncogene involved proliferation and anti-apoptosis, and also in a number of other loci which are still being characterized.

And the Pristane allows for this chronic inflammation, which you may think of or could be similar to mimicking a chronic infection.

Now, the interesting thing that we found was that, of course, without Pristane, without the inflammation we got no disease at all. This is shown here.

We could give Pristane either before we gave virus or after we got virus, and of course, it's a chronic situation. It just continues to precipitate as an inflammation over a long period of time.

But interestingly, if we gave the Pristane longer and longer times after we gave virus, we found that the latency after Pristane remained the same. So this suggests that the progressive events leading to the leukemia all were precipitated after the start of the inflammation, and so in a way this insertional mutagenic event was laying rather dormant.

I just want to summarize the findings that we learned from this inflammation experiment. The first one is what I just told you. The effects of the provirus at the site of the oncogene can remain dormant until these cells are

affected by other cancer promoting events, such as an inflammatory response.

Now, the other thing we found, which to us was rather amazing, was that we could find proviruses integrated next to the c-Myb locus as early as three weeks in the bone marrow following virus injection, and we did this using a very sensitive RT-PCR assay.

So it doesn't take a long time for the virus to spread to hit the oncogenic site. It may happen right away.

Another thing we found is that a minimum of one provirus can be found in many neoplasms. This is just examples where we digested DNA and probed with a viral LTR probe, and you can see these three are just endogenous background bands, but these tumors have just single integrations as I've tried to note by putting a little red dot by them. That's in na Mml locus, and this is in the Myb locus. We had similar kinds of things.

In some cases there are two bands, but they represent the two LTRs from one virus.

Okay. Now I want to talk a little bit about some of our recent work in which we try to get a collaboration of events to get a more rapid leukemia and understand how thee events collaborate with each other.

So we're using insertional mutagenesis in engineered mice. For example, we give retrovirus to transgenic mice that might be carrying an activated oncogene or we inject

mice -- I'm sorry -- inject virus into mice that have a deleted tumor suppressor.

Now, the reasons that we do this are to provide proof that the genetic alteration in the mouse is, indeed, oncogenic in the case that it has no effect by itself.

And the other reason we did it is we can use the cooperating genetic events at the insertional mutagenesis to actually tag the site of integration and determine what the cooperating event is.

The first example I'm going to show you here, we had a human oncogene involved in acute myeloid leukemia, and by itself it really didn't have much effect on the mice except after 12 months, and so we gave it retrovirus, and then we could accelerate the disease.

Okay. So the gene here is the CBF beta mice and heavy chain gene, fusion gene in codes for an aberrant transcription factor found on inversion 16 in acute myeloid leukemia, and this occurs in 12 percent of acute myeloid leukemia in man.

Now, Paul Liu at the NIH, who I'm collaborating with in this study, a while ago, in 1996, he generated a mouse which he put the human MYH11 sequences next to the mouse CBF beta. So you have a potential oncogene here that's expressed from the endogenous mouse promoter.

So he didn't really make a lot of alterations,

except to change the gene itself.

This turned out to be lethal, embryonically lethal because it brought differentiation into the myeloid and lymphoid lineages.

However, if you took embryo stem cells that had this potential oncogene and he put it in blastocysts so that they got chimeras, after 12 months, he started to see some leukemia.

So we used a virus, 4070A virus, to accelerate this disease. So with virus alone up to 12 months, this particular virus in this system is not very oncogenic. So you would only see the effects of the virus alone at least after a year, and effects of the oncogene alone were not seen until after a year, but the combination, he got about 40 percent incidence, 40 or 50 percent incidence of leukemia in about three to five months.

This is another example from our current research. We have used the retrovirus to provide a second hit in validation of a proposed human tumor suppressor p15INK4b in leukemia. The p15INK4b is a cycle independent kinase inhibitor, and it's a tumor suppressor, a proposed tumor suppressor which is hypermethylated in 80 percent of human AML, and in secondary AML I believe it's at the 100 percent.

Mariano Barbasett's lab in Spain had made a knockout of the p15, and they didn't see any myeloid leukemia or

even lymphoid leukemia, but they did se some extramedullary myelopoiessiand lymphoid hyperplasia.

So we got interested in generating our own knockout, which we did in NCI, and see if we could accelerate the disease using retroviruses. So when we injected wild type mice, we didn't see any myeloid disease up to a year in time, but in the mice that had one allele knocked out or two alleles knocked out, we had 15 to 18 percent myeloid leukemia. Most of these leukemias were of the myelomoncytic lineage.

Okay, and then the last part, I'd like to talk about some quite old data where we used a nonreplicating virus to induce disease. Because I know that's part of the issues of what we'll talk about today.

What we did is we were able to induce erythroid leukemia without the helper virus. So this was a paper in Science in which we were able to get a proliferative disease in erythroid cells, and then later we showed that these were malignant, actually malignant cells, and even after that it was found that they had insertional mutagenesis in a very specific site, and I'm going to show you a little bit more detail about this study.

So Friend erythroleukemia traditionally was induced by a combination of viruses, one, a replication competent helper Friend virus. I describe basically what that would be like, and a defective virus in which there had been

recombination from endogenous sequences in the mouse cells, deletion, and insertion to produce an aberrant protein called gp52 and often called gp55.

This combination of viruses, when it's injected into mice, causes a rapid expansion of the erythroblast in the spleen, but this is due to this gp52, which affected the proliferation of the cells.

But within that population, there are malignant -- there's malignant transformation that can go on in these erythroblasts, and it blocks the differentiation, and there's further expansion.

But the question early on was whether this was due to helper virus or not, and so we did some experiments where we -- let me back up.

So we demonstrate this malignant transformation stage of the cells by doing transplantation into other mice, and we could see an outgrowth of these cells, the transformed cells in the omentum, which is a fibrous connective tissue near the spleen, but it shows that they don't have to grow any more in the spleen in a normal environment, but they are autonomous and grow other places.

So we wanted to know if the first or second stage disease could be carried out with helper free virus, and these studies were done a long time ago, right after Richard Mulligan and his colleagues made the Psi-2 packaging cell line.

And now there are more sophisticated packaging cell lines, of course, but this again, was done quite a while ago. We transfected the SFFV genome, the plasma into these cells. These cells have a murine leukemia virus, which has all of the genes, except that it cannot be packaged because it has a deletion in the packaging sequences.

SFFV that was produced from these cells, but not helper virus, and we tested to make sure that there wasn't any helper virus by infecting NIH3T3 cells for five days and then looking for envelope proteins from helper, which is gp85, which is produced by the helper virus, the replication competent virus, and then of course, this defective gene which we wanted to transfer.

Now, this shows the positivity of both of these proteins in the Psi-2 cells, and over here after infection of the NIH3T3 cells we had only the gp52 and no helper virus.

So this helper free vector was injected into mice and got very large spleens due to erythroblast hyperplasia, and thee were also tested for lack of replicating virus by taking the spleen and making cell-free extracts and injecting them into mice to see if it got disease, which it did not, and put them on NIH3T3 cells to test, again, for whether there was replicating virus.

Then in a subsequent study we looked to see if we had evidence of malignant transformed cells within this

erythroblast population. This shows the results of such a study. This is the results of the primary disease in NFS and BALB/c mice.

We took these spleen cells and passed them on to other mouse recipients and looked for growth in the omentum, and you can see that we had a very high positivity even in the primary spleen, primary transplant.

We developed cell lines from these same transformed cells, and again looked to make sure that there wasn't any replicating virus, evidence of the envelope protein that would be produced by the helper virus. And you can see that it was negative.

Now, these studies were completed in 1986, and it was a couple of years after that that it was published that erythroleukemia is induced by the Friend virus complex, usually had integrations in the Spi-1 gene, which is also known now as PU-2.

And this was done in Pierre Tamboran's lab in France, and they sent us a letter. They looked at our we called them nonproducing cell lines, and this is just a letter showing that, indeed, I think about 50 percent of our nonproducer cell lines had integrations in this locus.

This data was confirmed also later by David Cabott, who also developed helper free system and induction of SFFV disease and also had integrations in Spi-1 and PU-1.

I'm just going to summarize now what I presented to you today. Retroviruses are capable of activating oncogenes by integrating next to or near these genes and activating them transcriptionally so that they are expressed.

These activating events can collaborate with previous or future oncogenic events in the cell to induce lymphoid, myeloid, or erythroid leukemia.

Chronic inflammation in a mouse model is shown to promote neoplastic progression in conjunction with retroviral mutagenesis, and evidence I've provided in a mouse model that replication defective viruses can integrate into DNA, activate an oncogene leading to overt leukemia.

And I think this last issue will also be addressed by Dr. Baum later.

Thank you.

(Applause.)

CHAIRMAN SALOMON: Okay. So some discussion of this. Dr. Cornetta and then Dr. Coffin.

DR. CORNETTA: Dr. Wolff, two points I think are very relevant about the Pristane model that you showed. I remember reading those and being somewhat concerned because it showed two things:

One, that you were getting disease in an adult, and in general, for Maloney disease you're injecting into newborn mice because as you showed today, that you don't get

disease generally when you put it into an adult mouse.

And, two, it seemed to change the tropism of the disease where Maloney type is often caused T cell, and relevant to our discussion today is that when Maloney is put into newborn mice, it's usually the T cells that become malignant. When it's been injected into monkeys, they develop T cell lymphoma. And the case we're talking about today, again, is a malignancy of the T cells.

So maybe could you give us your thoughts about, one, how age of this in a sense newborn child who's been affected might be playing into the role here and, two, about the general tropism of LTRs and how that might be affected in regard to the case we're discussing today?

DR. WOLFF: I think part of what you're saying in how the viruses -- you get different diseases or either you don't get disease and then you get disease depending on the situation, and it's very, very complex, and I think a lot of it has to do with what the collaborating events are, for example, Maloney giving disease in the adults and causing myeloid disease instead of lymphoid.

What was the other question? I'm not addressing it directly.

DR. CORNETTA: I think it was just both age and then the tropism of the --

DR. WOLFF: Well, I don't know how it plays in

human, but in mouse the age factor has to do with the immune response, is my belief. Usually if you -- usually we inject mice as newborns. I do for most of my experiments because the mouse doesn't have a chance to mount as good an immune response against the virus, and I don't know how that would play a role here.

DR. CORNETTA: One of those reasons to ask, because probably the discussion later today will not only be on this application to SCID, but we're really talking about all the folks who might be getting retroviral gene transfer experiments, and so I just wondered if --

DR. WOLFF: Of course, I think that in the mouse that immune response that the virus first plays more of a role when we have replication competent viruses that are expressing envelope genes.

DR. COFFIN: I'm curious about the numerology in your last experiment. A back-of-the-envelope calculation would suggest that you have to infect primarily a few million cells to have a reasonable probability of coming anywhere near the oncogene that you identifies.

Was the multiplicity that high or do you think actually there has been, even though you can't see replication competent virus, that there might be some motion of the virus in these cells due to complementation with endogenous envelope genes and things of that sort?

DR. WOLFF: Well, let me explain in a little bit more detail about the experiment. That might help answer the question for you. These mice were given phenylhydrazine. I didn't give all of these details, which causes the erythroblasts to proliferate a lot and go out into the blood stream.

So what we think we were doing was infecting these cells in the blood stream. You might say that's artificial, but, I mean, to compare that to putting cells in the culture vision (phonetic) and putting virus right on them, then --

DR. COFFIN: So what was the actual multiplicity of infection of the animal, of the cells? Do you know?

DR. WOLFF: I can't tell you unless --

DR. COFFIN: I mean, do you think it was high enough? Do you think it was high enough to have put a provirus down with that frequency next to your Spi-1?

DR. WOLFF: I have to say I was amazed by it when it happened, but I think so, and I think if there was replicating virus there, it would have showed up in all of those different tests we did.

DR. COFFIN: But there is the possibility of just complementation by endogenous envelopes, not that there's much of that stuff known in this particular mouse, but --

DR. WOLFF: Confrontation to spread it, you mean?

DR. COFFIN: Well, just if you have a cell which has started to proliferate because it's being driven by gp55, if

that cell is then -- also can express in an envelope, I forget. The gag and pol gene is good in Friend or is there another deletion in there? Can they make particles?

DR. WOLFF: No.

DR. COFFIN: Okay. But you could still have enough scatter genes that could give rise to some complementation and allow that cell to be reinfected basically.

DR. WOLFF: Well, in theory, and then you could go back and look at those cells and see if there's any other virus components.

DR. COFFIN: That was the question I was leading up to. Well, no, the other virus components. I mean, there are endogenous proviruses of all kinds in those cells, most of which are defective. Some may not be as defective.

I mean, the reason I ask that is because this is maybe a little bit different from the human situation where it's much less likely that that endogenous sequences would exist that would allow that kind of an amplification, but it might happen in that mouse model.

DR. WOLFF: I think the fact that we got disease that readily would argue against it, but it's just hypothetical at this point. We'd have to look at the cells in more detail to see.

CHAIRMAN SALOMON: Well, actually your experiments where you took the spleen and made cell free extracts and

adoptively transferred entities that were in your area, but anyway, to mice or to NIH3T3 cells, those would argue against essentially a viral pseudotyping, wouldn't it, John?

DR. COFFIN: Well, there you'll give a secretion of replication competent virus very well. That's fine, but I don't think they would argue against sort of intracellular complementation which might allow some local movement.

CHAIRMAN SALOMON: Ms. Ballard?

MS. BALLARD: Am I correct in assuming then all of these models were done on mice that had fully functional immune systems as opposed to any of the SCID mice models?

DR. WOLFF: They were essentially what?

MS. BALLARD: Fully functional immune systems in the mice on all of these models.

DR. WOLFF: Yes, yes.

MS. BALLARD: None of the models were done on a SCID -- the SCID mouse?

DR. WOLFF: No.

Can I just make another comment that in relationship to information and its role, I got interest in doing those experiments because I worked in a laboratory of Michael Potter, who just by giving Pristane to mice? It caused the translocations of the Myc locus in plasmocytomas. So it's just another example how inflammation can contribute to the disease, cancer.

MS. MEYERS: I'm trying to understand this as a layman. So let me tell you what I got out of it and tell me if I'm wrong and correct me in anything that I say.

In this series of experiments, you and other investigators have administered viruses to animals, and they have come up with a wide variety of diseases, not just leukemias, because I see there's mammary cancers and so forth in here, but I think it's mostly leukemias.

DR. WOLFF: Mostly hematopoietic.

MS. MEYERS: So since gene therapy is mostly done by using engineered viruses, is it possible that other people who don't have severe immune deficiency may come down with leukemias or other cancers because of the engineered viruses that were given to them?

DR. WOLFF: It's always possible, especially if you're putting them into hematopoietic cells. I think there's a difference, too, there. They're not always used for hematopoietic cells.

MS. MEYERS: So there's no reason to believe that what we see in this experiment in France with severe combined immunodeficiency jut applies to SCID, right? I mean --

DR. WOLFF: Right.

MS. MEYERS: -- there are people with all kinds of diseases getting gene therapy.

CHAIRMAN SALOMON: Well, okay. So right there we

could go off into a very important discussion. So we'll get to this, Abbey, later.

MS. MEYERS: Yeah.

CHAIRMAN SALOMON: Because the whole question now is what's the target for your gene therapy, and depending on what your target for the gene therapy is what happens to that target in terms of its life cycle in the patient who gets the gene therapy, and that will have everything to do with assessing the risk, and we'll discuss that.

MS. MEYERS: Right. I just want to say that I feel that we owe an extra measure of regard to all of the people who are alive who have volunteered for gene therapy throughout the years, and they should all be told about this risk and checked for it because there may be ways to avoid it or there may be ways to treat it if they come down with some kind of cancer.

CHAIRMAN SALOMON: That, again, is something that we need to get to, and conceptually, of course, that's absolutely correct. In reality we'll have to discuss how practical something like that -- I mean, how we could really do something like that.

MS. MEYERS: Years ago, FDA was supposed to create a registry for all gene therapy patients. That was eight years ago they were told to do it by Congress. They haven't done it.

Do you want to say something?

DR. NOGUCHI: Specifically, we think there's roughly 150 other trials that use retroviral vectors not for SCID. The initial evaluation was for the trials that most closely resemble the trials in France, and we put the active ones on clinical hold.

We have gone back, and we are actively reviewing not just the reports that had come in, but we're re-reviewing all of the reports for the other trials. We are not complete, but we are, I think, pretty complete on the active trials. We're now going back to the inactive trials based on our records, and I'll get to your point that you're going to bring up.

So far we have not seen anything yet of a leukemia or a lymphoma that was not already present in the person being treated. That could change obviously as time goes on.

We will, in fact, be contacting our sponsors to tell them of this event. We're working on the language for that and having the sponsors contact all of the survivors as well as any family that may have been entered in these trials.

So in the short term we are doing that part of it in terms of notifying the community, especially of those who have been treated with the retroviral vectors.

The separate question of a registry without regard to the timing of it, we do understand, and I think that this discussion clearly illustrates that the concept of being able to

not only address the immediate, but also past recipients of a product that may have consequences not predicted originally is absolutely solid. We don't have it yet.

CHAIRMAN SALOMON: I'd just make the additional comment that within a week of this going public, we are having this meeting, and that's another good way of making it very clear the kinds of questions that are being asked at the highest level in the FDA. So that's the point I tried to make earlier this morning.

Okay. Dr. Cornetta and then --

DR. CORNETTA: Just a quick follow-up, too, since I was in French's lab when their first patients were done with the TIL (phonetic) trial and have been involved with some other trials, too. This is not something that necessarily is unanticipated. This is something which we have known is a concern and have continued to monitor in informed consent of all the trials that I've known and just talking to other investigators around the country. This is included in the informed consent.

So as we talk to the patients or subjects about participating in retroviral trials, this concern is something that has been communicated to them. So it's not something that I think is new to them, but obviously, it's something we've always been concerned about. We just didn't know what the frequency might be.

CHAIRMAN SALOMON: Dr. Sorrentino.

DR. SORRENTINO: Yes. I would just also like to respond to Ms. Meyers' comments by just pointing out that the data that was just shown in the animal models had some very important differences with what we do in patients. Many of the tumors were seen with wall type replicating Maloney viruses. My understanding is that was done in the inflammation model.

And secondly, some of the tumors were seen in mice that had been engineered to have pre-oncogenic lesions.

So I think, you know, that distinction is important to keep in mind here.

MS. LORI KNOWLES: I just want to change the topic just a bit and put on the table something for later, which it seems to me that this experiment and Alain Fischer's question about the importance of pedigree; it seems to me that this experiment brings that again out to the forefront, and we need to think about issues of eligibility and informed consent with respect to the role of family pedigrees.

CHAIRMAN SALOMON: That's a good point.

Dr. Fischer, are you still on the line with us?

(No response.)

DR. FISCHER: Well, then what I'd like to do is just, because I feel like we need to keep on going here, to thank you very much and go on to the next talk, which is from Dr. Christopher Baum.

Have I got this right? I was going to attribute you to the Hanover Medical School. Yeah?

And he's going to talk about his experience with myeloid leukemia following retroviral transfer of the dLNGFR gene into murine hematopoietic cells.

So you got one page in Science. I guess we're doing you a favor by letting you have a bunch of slides. That's good.

DR. BAUM: I'm going to talk about a case report of leukemia that we observed in mice following a preclinical gene marking study, and on behalf of all the people involved in the work, I would like to thank the committee for being able to share the data with you in more detail than we could publish.

And I should say that this is not just another group of German opponents against current U.S. policy, but rather this is a group of people who really have a vivid interest in promoting gene therapy, and that's why I'm quite happy to be able to join all the Cincinnati Children's as an adjunct professor recently, to work with Christof Kalle and David Williams, promoting also the field.

Okay. So, however, we have to be aware in promoting gene therapy that there are risks associated with this technology, and we are talking about today about oncogenic progression related to insertion mutagenesis, and I tried to summarize the data briefly, and this just summarizes actually

what Linda Wolff has presented to you.

The risk of an insertion to promote oncogenic development has been determined to be in the range of about ten to the minus seventh per insertion in human TF1 leukemia cells, but note these are already malignant cells.

It has also been shown that insertion mutagenesis as just seen from manifestation in numerous animal models, but never ever has a single insertion sufficiently explained malignancy, and accordingly no disease induction was reported using replication defective vectors designed for gene therapy in numerous preclinical and clinical trials probably -- and this is a rough estimate, maybe far higher the number -- involving manipulation of more than ten to the 12th hematopoietic lymphoid cells.

That says probably more than 100,000 cells with activated oncogenes have been put into patients and animals without ever giving rise to manifest side effects.

And I would simply come up with the hypothesis that either a side effect of the transgene or, of course, active vector replication or maybe a kind of too strong cell replication is required for inducing pathogenesis.

So we have an interest in selection marker technologies. So we set up an animal experiment where we looked at effects and side effects of different marker genes proposed for human gene therapy for various purposes.

And so what is of interest here is primarily the results obtained with one of those vectors that expressed the truncated lower nerve growth factor receptor gene. I'll explain to you in a minute what that is.

We designed a mouse experiment with long-term follow-up where we manipulated only about ten to 30 percent of the input cells to have one integration or two per manipulated cell only. Then we follow up the mice for about seven months, and in order to have a more long-term observation we pooled the marrow of the initial recipients and distributed this to secondary recipients so that we have an amplification step of the cells through secondary bone marrow transplantation.

And note that the cells have been pooled. So an event happening in one of those animals is now distributed to all subsequent animals, and there was either, you know, affinity selection involved or not, and then we observed the mice for another five months until final analysis, and by the year after gene transfer, some of the animals in this group, they were obviously sick and -- oops, sorry. I jumped to the end. It's not this short, the talk.

(Laughter.)

DR. BAUM: And they were obviously sick and came down with large spleens, and there was disturbance of spleen architecture, infiltration of blasts in the peripheral blood. They had what looked like and was confirmed to be a monocytic

leukemia.

And actually all of the recipients, secondary recipients of this LNGFR marker group came down with leukemia at various stages. Six had an overt disease. Three had dysplastics or pre-leukemic alterations, and one only microscopic blast islands in the spleen.

In summary, what has been published, it's important to note that this disease had a very long latency. No disease was observed in the first cohort, which was observed for seven months.

All secondary recipients developed in the disease. The leukemia has been shown to be transplantable to a third cohort without lethal radiation, but it ends up in a lethal disease soon, after three to four months.

It is monoclonal, as you will see in a minute, in origin, but has a heterogeneous kinetics in secondary recipients, and however, always we get the same identical entity, AML, of the monocytics, subtype and trial.

It has been shown that the clone has a single vector insertion. The vector has been intact and continues to express LGNFR. We had insertional activation of an oncogene. You'll see that in V1, and the PCR -- the several data like PCR and functional cell biology data and Southern Plot data excluded the activation of exogenous or endogenous mouse leukemia viruses as far as possible.

So the vector integration, EV-1, has been confirmed by the -- found by the labor of Christof Kalle to be in this case in the first noncoating axon. Upstream activation use is translational, start codon, and the clone originated from one of the primary recipients and then was found in all diseased animals.

Oh, sorry again.

So what is EV-1? It's a conscription factor, a known oncogene which has some endogenous expression of limited stem cells, but when it's ectopically expressed, it blocks granulocytic and erythro differentiation and promotes megakaryocyte, not typically monocytic differentiation.

The activation has been implicated in human myelodysplastic syndrome and AML. Usually this is often immature and not often DM5 phenotype, and transgenic mice exist that are at increased risk for leukemia, kind of dysplastic hemopoiesis, but require further events to develop complete leukemia. So this is not sufficient to explain AML M5.

Of course, the gene had been really activated. This is a Northern Blot showing the activation by RT-PCR. It was found that several kinds of activations took place either from the three prime LTR or from the five prime LTR involving a spliced event, including the retroviral spliced donor.

So EV-1 is not sufficient to explain the leukemia. The M5 subtype is unusual. We did exclude RCR involvement. So

what may have been the second teat here, and we are still puzzled concerning this story.

We thought what about the transgene involved. This is a gene involved in human gene therapy with lymphocytes so far. It's been derived from the neurotrophen, lower continual neurotrophen receptor P75, and to make this an inert cell surface market people clipped off the intracellular signal transduction domain before its function was known.

It turns out later then by the work of other people that this is a pro apoptotic domain, and in fact, the artificially generated LNG of our marker gene looks like human endogenous genes of the same receptor family, the TRAIL family. There are pro apoptotic TNF receptor related genes, and there are some human endogenous genes that look like LNGFR where this cytoplasmic domain is deleted, and they have an anti-apoptotic decoy function.

And also it's interesting to note that LNGFR may be shattered because its anchorage in the membrane is not very strong, and there is another member of this same receptor family known as osteoprotegerin, which acts as a soluble decoy receptor for other cytokines.

So this may still bind the ligand since the ligand binding domain has not been deleted.

What is known about the role of this receptor? This associates at the heterodimer with Trk receptors. Trk

receptors are turacin kinase receptors for a neurotrophin, such as nerve growth factors and related cytokines, and these as typical turacin kinase receptors confer a signal that this provides the survival of proliferation.

Now, the association with the wild type form of P75 neurotrophen receptor from which our marker was developed leads to an increased binding of neurotrophen and increased signaling for this pathway here, but in addition, at the same time it promotes a signal that gives more rise to differentiation or even can be pro apoptotic.

And this receptor can bind all known neurotrophens and cooperate with the different, more specific high affinity receptors.

Now, we would expect balanced troph (phonetic) as the outcome of the subject configuration, and then a paper in 1994 has shown that when this LNGFR marker was co-expressed with the wild type drug receptor and found neurotrophens, that in this configuration this gave rise to transformation of fibroblasts.

So this data has never been reproduced by anybody so far, but also never challenged by experiments. We're going to try to reproduce the data, but as far as the literature says, this is a partial oncogene we would say and not just an inert marker.

How is the configuration now looking at cells?

Well, they do express LNGFR, expressed from the retroviral vector. We have evidence by Northern Blot for TrkA expression, and now it's important to know that TrkA is typically expressed in monocytes.

Somebody has a monocytic leukemia here. So this may be endogenous expression of TrkA, and now we end up with the same configuration that was shown in pretransformed fibroblasts to give rise to their real transformation then.

And what about neurotrophens in hemopoiesis? The growth factors are expressed in the bone marrow microenvironment, also in the lymphatic system. There are many serotypes that can produce them, and here, again, it's to show that TrkA, the receptor is typically found on monocytic cells, and we think this created the bias for developing this unusual monocytic leukemia in conjunction with EV1 activation.

Although the data have to be concerned, this is all hypothesis right now, what I tell you. Drug receptors are known to play a positive role in human leukemia also. They can be found in many human leukemic cell lines.

Signaling has not been investigated in great detail here, and there are these two papers and one paper that ha an indirect evidence that human leukemia drug receptors can be involved in leukemia progression, one involving a translocation or mutation of TrkA receptor that makes it constitutively (phonetic) active.

So these data suggest the possible role of this signaling system and the mutant forms of those receptors in human leukemia development, but I should note that for applications in lymphocyte cells, so far there's no concern because this receptor system has never been shown to be able to transform lymphocytes.

So all of these oncogenes, of course, act in a context dependent form.

Now, when we put this together, how can we interpret our finding here? The insertion site, I think, has a very highly likely causal role, but of course, it's not sufficient. The role of the transgene, I would suggest that this is really cooperating here in the manifestation of the disease. Others challenge this hypothesis.

The role of the vector architecture need to be discussed. This is a retroviral vector that has absolutely got follow and function (phonetic), only the LNG for our marker gene, but we designed it in a way that only the splice donor was present and it was accepted because these records have the highest titers in our hands, and we know that there is a splice event going on from this splice donor to a downstream receptor in EV-1.

So when you would have a strong splice acceptor up front here, this may reduce the risk, and this leads me to two general remarks regarding vector design.

First of all, there's no integrating vector system known that has a lower risk than retroviral vectors for inducing insertional mutagenesis. This should be emphasized. We don't have any alternative technology in our hands that is as well characterized as retroviral vectors.

But they have a certain risk, of course. So how can we improve the retroviral vectors? We could try to alter their configuration. We will discuss this maybe in the afternoon, and the perfect vector would look like this set. Instead of having enhancer and promote elements at the end of the long term and repeats, there should be a strong insulator signal that does not allow any influence on neighboring tromatin (phonetic).

However, so far nobody has shown that from all the type of insulator sequences that have been described that these are able to plot the effect of an enhancer sitting in a trans-stream (phonetic) on a downstream or upstream sequences has to be tested.

So I think currently when we talk about using self-inactivating vectors, this is not the solution for lack of data to show that these are really safer. We always end up with a residual risk of vector insertion that could destroy an endogenous stream irrespective of vector configuration, and it remains to be tested how alternative vector designs would be safer.

The safest vector, of course, is one where you can regulate conscription by chemical inducers, but these are currently not available for clinical work.

So what about other risk factors? Five-FU exposure of donor cells by retroviral gene transfer has to be considered, and this specific experiment, this is not is not a strong mutagen. It's a common procedure, has never been shown to promote leukemia manifestation, but there may be one or two somatic mutations introduced into our leukemic clone -- who knows? -- by this procedure.

And I think important is to discuss first expansion in our case through serial bone marrow transplantation. This is the same within the work that's shown with chronic inflammation. Whatever you do to force expulsion of a cell that has a mutation will increase the risk that this will end up supporting malignant clone.

And we have to consider our clinical setting relates to this problem.

People say observations from rodent cells have no meaning for human cells because the pathways are a bit more complicated, but lead to consummation of human cells.

We would be cautious here because the data that are on basically from one group and others are primarily from epithelial cells and not from transformation experiments with stem cells, and specifically the role of the telomerase system

may be different between rodent and human cells when it comes to mature cells, but we don't know whether it's similar in stem cells between these two organisms.

Implications for other cell types are another important discussion, of course.

So more general views, I think that it remains to be said really that not every insertion mutagenesis is expected to result in tumor formation. This is just a model, what may have been going on in the mouse or also in the patient.

Initially a clone is introduced that has two hits, an activation by insertional activation of an inborn oncogene and a second hit that is somehow related to a side effect of the transgene, if it is only to lock a bit apoptosis of the cell in the engraftment period.

Because we would expect that in the first two to three months or so we would anyway lose more than 99 percent of the input cells because they have a limited life span, but also that we, with our current technology, we manipulate far too many cells. It would be much better if you would have technologies to really enrich the pure stem cell fraction, come down to manipulate only 10,000 cells per patient, and then the risk would be minute in terms of insertion mutagenesis.

Now, if this combination of events allows a cell with a limited proliferation potential to have a more unlimited potential, then the risk increases, and if you then have a

selective expansion pressure on the cell, the risk increases to accumulate more mutations, and this will reduce the probability of extinction of this clone, and so on, until you have a manifest problem in the patient.

So the implication is try to define the stem cell population better that we manipulate and try to define the minimum dose of cells needed for each patient and try to define safe expansion profiles.

Conclusions. Again, to be repeated, insertion mutagenesis is not sufficient to induce malignant transformation, but there is a potential for cooperation with side effects of transgene products.

High proliferative activity of the target cells may promote manifestation, and on top of this, conditional endogenous or exogenous oncogenic factors are probably required. So this has some consequences regarding point one.

We should keep vector in cell dose as low as possible and as high as required. This is easily said and remains to be worked out in appropriate models.

Vector improvements may reduce the risk somewhat, maybe 80 percent or so, but never 100 percent.

We need to develop risk classification of transgene. I think this is an important step. To what extent does a transgene cooperate with insertional activation events? There may be low risk transgenes and no risk transgenes, like

purely metabolic genes, or others that have a higher risk that are active in different signaling cascades.

And we, of course, still have to come down with a risk-benefit evaluation for each application of interest. So that says that this is really asking for multi-center research which takes a few more years, but still, clinical studies are required to date, and we have to define simply the risk-benefit evaluation with the available technologies.

Thank you for your attention.

(Applause.)

CHAIRMAN SALOMON: Thank you.

Dr. Coffin?

DR. COFFIN: Yes, very nice talk. Actually with regard to your last point, I think there's a substantial amount that can be done in preclinical mouse models to get a much better handle on what kinds of vectors, self-inactivating or whatever, might, in fact, have lower probabilities of doing this.

I think sort of taking the lead from your experiments, it should be possible to develop quite attractive mouse models I think that will tell us quite a bit.

DR. BAUM: Oh, yes. Also one could combine it with the models that you developed to look on the inference of insertion sites for targeting of retroviruses, activation of transcription, but this is just, as I said, kind of long term

research goal.

DR. COFFIN: Right, exactly. I have actually a similar question to what I asked Linda Wolff, and that is the numerology. How many hits were actually administered to the original five mice? How many proviruses were there in the cells that were put back in?

DR. BAUM: We transplanted the mice with a million cells per mouse, and that's says the first core just got five million cells, and we know that after this manipulation under the culture conditions that we had there were maybe only 50 stem cells.

That's why I think that our clone did not arise, in fact, from a mutagenized stem cell. The likelihood then to hit EV-1 is very small here, but rather from a more mature progenitor, and that, in fact, this combination of events that I suggest to happen here at this otherwise short-lived progenitor to become long lived and establish --

DR. COFFIN: So your initial multiplicity of infection in those million cells was how much? Do you know?

DR. BAUM: Multiplicity was just two, and we ended up with one vector insertion. We --

DR. COFFIN: So you put in two million proviruses do you think?

DR. BAUM: Yeah.

DR. COFFIN: In the total cell population you put

in?

DR. BAUM: Yeah. This was a mixture of anthiltropic (phonetic) and VSV pseudotypes, and these were the infection conditions that end up in one or two insertions per target cells.

I should mention that the vectors were produced from Phoenix human 293T cell based packaging cell. So there is no way that endogenous mouse retroviruses have been transported by the vector system.

DR. MACKALL: So you've obviously done a lot of thinking about what the nature of the second hit can be, and assuming you've got a patient with severe combined immunodeficiency, who we know has high circulating levels of Interleukin-7 and now has been given the gamma c gene, which can very efficiently transduce this signal. As best you can tell, would this be sufficient for a second hit, this "on" signal from this growth factor?

DR. BAUM: It certainly helps the manipulated cell to survive, of course, but I cannot say whether this could somehow promote translocations or mutations.

I think it would be good if you put the final safe away (phonetic) of most low expansion of the cells, like in the ADA SCID patients. You could give an exogenous ADA, limiting levels, and this way somehow prevent a tool and force proliferation of the corrected cells, but this is pure

speculation. We need experimental data to see which approach is best.

DR. TORBETT: I know you said that in the transformed cells the NGFR receptor was transducing signals. Was that true in cells that weren't transduced? Did you do studies showing that the truncated receptor can transduce signals in primary cells which have not been transduced?

DR. BAUM: Where the signals were different in the malignant versus nonmalignant cells?

DR. TORBETT: Right. So you had to have a transformation before it was acceptable --

DR. BAUM: Right.

DR. TORBETT: -- to be able to transduce the signals. So a nonmalignant hit would make the cell nonresponsive to signals via the reporter gene product.

DR. BAUM: Well, we are currently investigating this area. We didn't have the reagents, and we tried to address this issue.

Also in cell culture based models there is specific cooperation of the oncogene with the signaling pathway. This is completely unknown right now.

DR. TORBETT: I see, and the second question is on vector design. Since many times insertions are in active genes that might or might not close later, what is your feeling that putting insulator element or other kinds of elements that will

keep a chromatin area open way past when it should have on the bearing of vector design?

DR. BAUM: Well, the data, some people are sitting here that are working with those vectors. We don't work with them. The data that are owned are somewhat controversial. They show some potential of the insulator sequences, but it's still insertion site dependent, whether they are really dominant, acting as insulators.

And so far people only looked on whether neighboring sequences could modulate vector transcription. It's unclear whether the other way around. Vector transcription is no longer affecting the neighboring sequence, and this is more difficult to investigate.

CHAIRMAN SALOMON: The insertion in this particular truncated NGFR construct within this Evi-1, which is ecotropic viral insertion site one --

DR. BAUM: Yeah.

CHAIRMAN SALOMON: -- I mean, you kind of passed over that. Is that still a random event that depicts an ecotropic viral insertion site or is that not a random event?

If it's not a random event, if there's not a homologue of Evi-1 in the human, is that relevant at all then to thinking about human risk?

DR. BAUM: So what we are confronted here is a cross-match with the literature, not with experimental data in

our hands. It's completely unclear whether this is the locus that has an increased risk of retrovirus vector insertion in the mouse, and if so, whether this would also be true for the human locus.

There is a recent very elegant study of the Bushman lab where they studied HIV vector insertion. It's published in August in Cell, and they found that this is also only semi-random and affects certain loci at increased likelihood.

Again, it needs to be studied whether these loci are more dangerous or less dangerous loci compared to the average locus. So these data still have to be accumulated.

DR. COFFIN: Let me just expand on that a little bit. Once again, in the paper in Cell the effect actually wasn't that great. It was a factor of about two enhancement into transcribed regions as compared to random events, and it wasn't in the specific site. It was occurring in very large regions of the genome.

So for all practical purposes it's much safer and more reasonable to consider the effects to be random, and you will be correct to within a factor of two or so as far as that goes.

CHAIRMAN SALOMON: So th fact that this is an ecotropic viral insertion site --

DR. COFFIN: That's just its name.

CHAIRMAN SALOMON: -- a different kind of ecotropic virus? It's not a retro?

DR. COFFIN: That's just its name. It was identified in -- I believe I mentioned Jenkins and Neil Copeland originally.

DR. BAUM: It might be, as far as I remember, Germali's (phonetic) lab.

DR. COFFIN: Well, it might have been a collaboration between the two actually, but that's just the name of it. It's basically just one of a long list of oncogenes that has been identified by virus insertion, and as far as we know in many cases the same gene has been identified in different species in mice and cats and chickens and the same genes in many cases have been found to be activated by chromosomal translocation or other means in humans.

So there's no reason not to believe that this particular gene -- there's no reason to believe that this particular gene is a specific target for integration, and there's no reason not to believe that this gene is also present in humans. I'm sure it is, but I just don't know for sure and could not also be an activation target in humans.

CHAIRMAN SALOMON: Dr. Kalle.

DR. KALLE: I briefly wanted to comment that the original locus that they described picking this up was farther away from the Evi gene than what we have described. So in terms

of the DNA, it's actually not the same integration locus at all.

CHAIRMAN SALOMON: You didn't find the human homologue to Evi-1 in any of your 60 or 80.

Okay. It's my pleasure to introduce Dr. Stuart Orkin from Harvard Medical School and Dana Farber, who is going to talk about the role of LMO2 gene in hematopoiesis and Leukemia.

And no pressure, sir, but then we go to lunch.

DR. ORKIN: Thank you.

It's good to be here, and this for me is another chapter in the gene therapy story. A previous chapter is shown here on the slide.

I apologize. It looks better on the computer here than it does on the screen, but this summarizes a comment, a small paragraph in the 1995 report that I co-chaired with Arno Motulsky on the assessment of gene therapy at that time.

And for those who can't read it, it says, "Because clinical experience is so limited, it is not possible to exclude long term adverse effects of gene transfer therapy such as might arise from mutations when viral sequences randomly integrate at critical sites in the genome of somatic cells. It must be noted that multiple integration events resulting from repeated administration of large doses of retroviruses theoretically pose a risk for leukemic transformation. Only long-term clinical follow-up of treated patients can provide data on the long-term

safety of gene therapy protocols."

I don't think we could have guessed seven years ago that we'd end up today in this discussion. At least I didn't think it would come at this point.

I want to make one aside and say that what we advocated in this report was careful study and follow-up, and I would applaud the FDA and Dr. Fischer and all of the colleagues for the detailed investigation that's going into the case that's being discussed today. I think it really is precisely what was advocated back even years ago.

Next slide. Do I control the slides? Oops. Am I in the right -- ah, good, thank you.

So what we're discussing is leukemia, and I want to emphasize that leukemia is a derangement in blood cell development, and this is a standard cartoon of what adult blood cell development looks like, and the critical cell is the hematopoietic stem cell, which self-renews and gives rise to progenitor cells, and then all of the mature blood cells.

And over the past decade a tremendous amount has been learned about the basic workings of cells largely in the mouse, but I'll argue that the workings are identical in the human because there has been a question as to differences between mouse and human perhaps, but I'd argue that they're much the same.

And there's been a convergence of work on the

basic underpinnings of blood cell development and leukemia which will be obvious in my remarks.

There are a number of genes which are all transcription factors involved in gene expression, which I've listed here as members of the stem cell class. These are genes that are essential in mouse studies for development or function of hematopoietic stem cells.

And the critical point I want to emphasize here is the genes with the asterisks are all genes that were identified through the study of human leukemias, indicating this close convergence between leukemia and normal blood cell development and the mechanics of blood cell development.

So these genes were all discovered at chromosomal break points, translocations, and LMO2 is right there in the middle. These genes are either deregulated or expressed as fusion proteins in these translocation events, and curiously, some of these genes are present together or have interactions with one another which you might not have predicted, such as some are present in fusion proteins together. So two of the genes, tel and AML1 OR runx, are found together in fusion protein in childhood B cell leukemia.

What would be the chance of that randomly? Probably rather low if it didn't mean something functionally.

In addition, some of the proteins interact as proteins themselves physically, and it's very important in terms

of today's discussion that one of the T cell leukemia genes, SCL or tal, sometimes called interacts physically with LMO2, and they are very tight partners in the cell.

These genes are all required in some way for formation, survival or maintenance of hematopoietic stem cells.

I mentioned there are two different ways that translocations can lead to leukemia in humans. One is through deregulated expression, and the paradigm for that is the abnormal expression of c-myc in Burkitt's lymphoma, but we also have the examples of activation of SCL and the LMO2 genes in acute T cell leukemia.

In addition, a number of the fusion -- the more common translocations activate expression of fusion proteins, such as the bcr able in chronic biologics leukemia, which is really the hallmark of PML-RAR and APML, and then a number of the genes have many different partners.

Now, what is LMO2? And I'll just give you some bullet facts. LMO2 is called a transcription factor. It was formerly called Rbtn2. A number of these genes have multiple names in the literature. It was discovered by Terry Rabbits' lab, but also by Roya Picora (phonetic) more than ten years ago, I believe, or just about ten years ago at breakpoints of 11-14 chromosomal translocation in patients with T cell leukemia.

The translocation brings the LMO2 gene under the control of the TCR delta locus on chromosome 14, and thus, the

regulation of LMO2 is deranged as it's under the expression of other regulatory elements, but it's important to emphasize that the normal protein product is produced in that translocation.

LMO2 is a LIM-only protein. A LIM is a form of zinc finger, but this LIM domain mediates protein-protein interaction. Although LMO2 participates in transcription, it does not bind DNA. So it doesn't act on DNA directly by itself. It needs to do that through physical interaction.

It participates in gene regulation through physical interaction with other proteins, and one of the most critical proteins or the best studied in that regard is SCL, another leukemia oncoprotein involves in translocations in leukemia.

LMO2 is normally expressed in hematopoietic stem cells, red cell, and vascular cells. It's normally extinguished in lymphoid development. So the translocation maintains expression where it ought not to be.

The LMO2 gene through gene knockout studies from Terry Rabbits' lab is essential for all hematopoietic formation and for some aspects of angiogenesis.

The phenotype of the LMO2 loss of function, that is, the null mouse, is identical to the loss of its partner protein SCL-PAL, and in fact,, studies in my own group suggest that the function of these two proteins is only to interact and to transmit a signal one to another.

Transgenic expression of LMO2 and another protein, LMO1, in T cells leads to T cell leukemia in the mouse, but only after a very long latency. Prior to the leukemia, there's an inhibition of differentiation with accumulation of double negative T cells, and leukemia can be enhanced or the latency can be shortened by co-expression of the partner gene SCL/tal, again indicating the cooperation of these two genes.

But I think this point emphasizes the expression of LMO2 is not sufficient for leukemia. They have been cooperating in additional genetic events.

Finally, LMO2 is often expressed in human T cell leukemia, importantly even in the absence of recognizable chromosomal translocations. Indeed, it's the infrequent situation in which the translocation activates the gene. The gene is activated by other mechanisms presumably through a regulatory network, and one of the forms of leukemia in which it's -- and I'll show a slide later about this -- that where it's frequently activated is in a leukemia that's called lyl positive. Lyl is a gene that resembles SCL. It's a close relative, and the T cell leukemias that are identified as expressing lyl often express LMO2 as well, and those do not have a translocation involving LMO2.

So this is where LMO2 is required. It's required actually in the earliest part of blood cell development to form the hematopoietic stem cells, and it acts, again, in a protein

complex, and the initial identification of this complex goes to Terry Rabbits as ell in Cambridge, England, and this complex curiously involves a number of other players in hematopoiesis or leukemia. It involves the SCL gene, which is essential also for hematopoiesis and which is deranged in T cell leukemia.

It involves the LMO2 gene that's being discussed today. It also involves a GATA protein, and in fact, one of these, GATA-1, can be mutated in megakaryocytic leukemias.

It involves a partner of SCL which is also mutated in a number of lymphomas. So this is a hematopoietic complex that is central for maintaining the homeostasis in proper regulation during hematopoiesis.

Now, leukemogenesis by LMO2 has been studied and seen in two situations. One in the top is that in the transgenic mouse where the gene is expressed under T cell regulatory elements, and one gets a block of development that the double negative cell stage, eventually leading to tumors with presumably second hits of some sort.

And then in human patients when there's a chromosomal translocation mediated at the stage that the RAG gene is expressed, one then also gets T cell leukemia.

I want to emphasize what I said before, and that is that misexpression of LMO2 is sometimes due to chromosomal translocation involving LMO2 itself, but perhaps more frequently LMO2 and other T cell oncoproteins are misexpressed in the

absence of recognizable translocations.

This is a recent paper by Ferrando in Cancer Cell, which just shows a number of samples of T cell leukemia, just sort of all comers, and it was from a gene expression profiling study in which they show that a number of the T cell tumors in this case express either LMO1 or LMO2, and they're not expressed in normal thymus.

And this brings up another point I made, is that some subsets of T cell leukemia express LMO2 abundantly. This is a gene expression profiling or microarray chip, and this is the lyl-1 positive form if T cell ALL, and one of the genes that's consistently expressed in all sample is LMO2. It's sporadically expressed in some of the other subtypes.

Now, this is a less sophisticated version of what we've heard in probably the two previous talks, but I just want to make a couple of points. First is that the insertion of the retrovirus in mouse and presumably as well in human hematopoietic cells leads to its consequences by deranging or abnormal expression of neighboring genes, which can be due to dominant influences of the regulatory sequences contributed by the retrovirus or by interference with the regulatory elements of the gene itself.

And I think one could imagine almost any scenario whereby an integration event could alter the expression of the resident gene in which the retrovirus is integrated.

Two recent studies that have been published using the susceptible mouse strain so that they're sort of pre-engineered or pre-chosen, to look at genome line susceptibility. Two studies have recently been published, both in Nature and Genetics, and what they indicate is that in one study at least 150 different loci were tagged or identified as potentially collaborating to give leukemia in an animal.

And in the second study there are many unique sites that were identified, and specifically 17 previously identified common integration sites were seen and 37 at least characterized.

I think the only message I want to transmit from this is there are potentially numerous sites within the genome which can contribute or cooperate with some other event to then give leukemia. And I think this kind of cooperation is certainly being dissected in a number of laboratories, and I think we'll see quite a bit more of this.

But I think the number of genes that contribute are probably more than we might have guessed a number of years ago, and certainly more than I would have guessed seven years ago when we wrote the gene therapy report.

So I just want to make a few concluding comments. LMO2 is a bona fide target for T cell leukemogenesis either through chromosomal translocation or secondary to changes in the regulatory network. So I think relevant to the case today, I

think it's the insertion of the retrovirus into that site I would think is highly likely to be related in some way to the outcome in phenotype.

The long latency of LMO2 mediated leukemia in the mouse suggests that additional genetic events are required for the onset of leukemia, and in the leukemia field, it's quite clear now that many somatic mutations which are found in leukemia arrest development of cells, the normal maturation, and then second events often lead to a proliferative signal within those cells to cooperate to generate leukemia.

So I wonder whether a block to differentiation perhaps by expression of LMO2 might be complemented by a gene that converts proliferative or survival advantage to the cells, and obviously in this case a gene has been introduced into the patient which gives a selective advantage to the rescued cells.

And I wonder whether that isn't a double edged sword in this particular instance.

Finally, LMO2 is representative of genes that are required for hematopoietic stem cell formation and maybe function, and it is certainly enriched in hematopoietic stem cells, at least their expression.

So one wonders whether loci of stem cell expressed genes might be somewhat more accessible than the average gene to retroviral integrations.

We've already heard of discussion about how random

is integration, but maybe a few fold difference in accessibility might actually be important in thinking about these kinds of events.

I'll stop at this point and take any questions. I think people probably want to eat lunch.

Thank you.

(Applause.)

CHAIRMAN SALOMON: Thank you for a very nice presentation.

Questions? Dr. Coffin.

DR. COFFIN: Let me make a comment about multi-hits. It's very clear in many cases for retroviral activation of oncogenes that a second hit need not be either simultaneous or preexisting; that often the first hit -- what the retrovirus integration at a specific site, for example, an exocemic (phonetic) dose alone, must be sufficient to initiate the process to the extent where second hits and subsequent hits become inevitable.

So, in other words, it must be sufficient to if not induce malignant transformation, at least lead to expansion of that cell clone to the point where random mutation events will now inevitably cause some sort of relevant second hit to occur.

That may not be true. That's clearly not true in all cases because in the cases of preactivated animals that were

shown, for example, where you can have much more rapid oncogenesis, and so clearly there are more sites available and the cell can expand more rapidly if there's a preexisting hit.

But the hit need not be preexisting or simultaneous. It could be the cell could probably be completely normal in that respect and still be inevitably subject to activation by hits at least at certain oncogenes.

CHAIRMAN SALOMON: Yes. So consistent with that, the LMO2 knockout animals that Terrance Rabbits did, they had a really long latency period, like nine months.

DR. ORKIN: The transgenics. Yeah, the transgenics have a very long latency period.

CHAIRMAN SALOMON: So that would go along with the idea that there was a discrete point in time where there was a hit, and then there was a long time after that that selective survival of the hit cell allowed another. Now, whether that's inevitable --

DR. COFFIN: Exactly. That's certainly the most straightforward way to explain that observation

DR. ORKIN: I think there's also another point. At least with respect to the SCL gene, which is for all intents and purposes much the same, people have been trying to generate transgenic models to show T cell leukemia in a mouse, and a number of laboratories failed until one laboratory chose the correct promoter.

So it's very sensitive to the time of expression, presumably the cell window in which the gene expression is activated.

CHAIRMAN SALOMON: So I guess you guys need to help me through one thing that came up earlier today, and then I think, you know, we will have accomplished almost everything I thought we could possibly have gotten up till this point.

And that is what's driving transcription of LMO2 in this patient, and I'm not convinced by what I heard, but maybe I'm just being dumb. So help me through why it isn't the LTR of the transgene. That's the only thing that makes sense to me from everything I know about LMO2 and the way this should work.

DR. KALLE: Maybe I can comment to this. One simple reason is that the retrovirus is oriented reversely to the structure of the LMO2. So the retroviral transcript is actually going upstream of the original gene locus and would even if there were a fusion transfer of which we haven't seen any evidence yet go basically through XM-2 and then out

DR. ORKIN: Yeah, but presumably the retroviral enhancer, the LTR enhancer could act at a distance on endogenous and I guess there's also the possibility that an anti-singe transcript might change the regulation of LMO2 transcription given recent work showing that anti-singe transcripts at least do seem to affect gene regulation.

DR. KALLE: It could be enhancer or interruption of regulatory sequences in that interim, of course, could very well.

DR. ORKIN: It's consistent with the most common, I think, as Linda said, year or was about to say, is consistent with the most common models.

DR. WOLFF: Yeah, the most common models enhancers, and actually in most of the cases where the enhancers are working, the virus is integrated in the opposite direction from the gene, at least especially when it's at the five prime end.

So it's not surprising at all to me.

CHAIRMAN SALOMON: Well, good. Because, I mean, what was bothering me was this morning I -- again, maybe I got it wrong -- my impression was that it might have been knocking out an endogenous regulatory sequence, but that it wasn't due to the virus in the --

DR. WOLFF: That can't be ruled out.

CHAIRMAN SALOMON: No, I know you can't rule it out, but I didn't like the idea that it couldn't be due to the virus. That didn't make sense to me.

DR. PUCK: I'm wondering if you could help me think about the possibility that varicella infection could have provided another hit in this patient, and I'm wondering -- I realize LMO2 itself is not expressed in lymphocytes except in

this one abnormal integration cell clone, but perhaps some of the interacting proteins that act in concert with LMO2 could be induced in a lymphocyte by an infection such as this.

DR. ORKIN: I think that is a question, whether the other things have been looked at. I don't know whether Lyl and SCL have been looked at. I mean that would be very important. If they were activated, I think that would be additional evidence that something happened.

I don't know enough about varicella infections, you know, myself and immune responses, but I would imagine that the response could lead to the proliferation of a clone, which I imagine would have preexisting hits in it, and that might predispose to another event.

But I would be reluctant to pin it on varicella per se because I would imagine that this child would, you know, over his or her lifetime come in contact with other viruses which can stimulate the immune system.

So I think I wouldn't rest on that.

DR. PUCK: this is one of those perhaps bubbling up issues. Varicella could be treatable, could be preventable, and so if we know that's one bad actor, which we might learn from microarray data in other patients undergoing varicella infection, that could be helpful.

DR. ORKIN: It might be helpful, but I would imagine there would be a whole host of viruses that you would

not be able to look for a priori.

CHAIRMAN SALOMON: And the other point here is that when you've had consistent up regulation of LMO2, we know that you get T cell ALL. So that I don't really follow a direct reasoning that varicella per se created a hit because we know that these other cases of T cell ALL that's been associated with LMO2 expression, this endogenous leukemia is due to the translocation, not associated with viral infections.

Okay. Lunch. It's a little after 12:30. It's 12:35. At least I did change my watch, right? This isn't California time. Okay.

So one o'clock, a few minutes after one o'clock?

PARTICIPANTS: That's 25 minutes.

CHAIRMAN SALOMON: That's 25 minutes. that's too fast? Well, that's why we have discussions.

Thirty minutes? Eat as fast as you can.

MS. DAPOLITO: I believe there is space reserved for the panel so that they can get a quick lunch in the restaurant.

(Whereupon, at 12:40 p.m., the meeting was recessed for lunch, to reconvene at 1:30 p.m., the same day.)

AFTERNOON SESSION

(1:34 p.m.)

CHAIRMAN SALOMON: Okay. Given the fact that usually my double time rule, you know. So I said to be back at one o'clock and it's 1:30. That's about right.

Anyway, I'd like to get into the afternoon session, and obviously my interest, I think the whole point of this is to get to the discussion of the questions, and so I think you guys did a fantastic job this morning and I really appreciate it. It make my job actually very easy.

Today we now have three talks from a group of expert investigators who have direct interest in this whole area of gene therapy trials, and so it's very important to put the design of their trials and choice of patients, et cetera, on the same page now as the stuff we've discussed up till now.

These three talks will go one after another without any question or discussion, and then we'll have a discussion, sort of the general characteristics of these three areas.

After that we'll have the open public hearing. We'll talk about that in a moment, and then go into the committee's questions and discussions.

So the first speaker of this after lunch session is Don Kohn from the USC Children's Hospital, Keck School of Medicine: gene transfer for ADA deficient and X-linked SCIDs at

the Children's Hospital of L.A.

Don.

DR. KOHN: Thank you, Dan.

I'm going to tell you about three clinical trials. The first one was performed under Mike Blaze's original gene transfer trial between our group at Children's Hospital and the NIH for ADA deficient SCID, and working with Christof Kalle, we have some new findings that are relevant to the discussion of this morning.

Then I'll tell you about the second trial, which is a second collaboration between our group and people at the NIH, Fabio Candotti, Cindy Dunbar, with ADA gene transfer, and that's one of the trials that's in process that's now on hold.

And then the third trial I'll just talk about briefly is done by one of my colleagues, Kenneth Weinberg, and that's for X-linked SCID, and that is also on hold with no patients enrolled.

So just a little more background in ADA deficient SCID, and we've heard some of this this morning already.

SCID is the causae of ADA deficiencies that cause about 20 percent of SCID, and like other forms of SCID, without early treatment, there's mortality, and bone marrow transplant can be curative with a higher success rate with a matched sibling donor than with a T depleted haplo or a match unrelated donor.

And I'll talk a couple of comments about enzyme replacement therapy. PEG-ADA is an enzyme replacement therapy that can restore and sustain immunity, and I'll give you some of the data on patients on that.

It certainly can sustain immunity, but it's expensive, in the range of 200,000 to $500,000 per year per patient, and it requires twice weekly intramuscular injections.

And just some data. Again, Dr. Buckley really covered this this morning.

The outcome for ADA deficient SCID is not as good, I think, as a subset, and this is data that Dr. Buckley provided to us during our discussions with the RAC about the risk to benefit ratios, and this was the data as of about, I think, early 2000 or 1999 at Duke.

At that point there were 12 patients that underwent haplo BMT for SCID. Two had died from CMV infection they presented with. Three had been nonengrafted and went on PEG-ADA, and seven were surviving.

And so, again, to put it into context, this represents a 16 percent mortality for these patients, although it probably wasn't transplant caused, 25 percent nonengraftment. So of 58, 68 percent cure in this subset. I think the updated numbers that she showed today were in line with this.

And then to summarize the PEG-ADA data, Mike Hershfield, who is the guru of this, published a review article

of this in 2000, and to that point 95 patients had been treated at some point with PEG-ADA between March or April of '86 and 1999.

And at the time that this article was written, 59 patients were under treatment. Two adults with late onset of ADA deficiency diagnosed sort of late in life had stopped PEG-ADA, and 15 patients taking PEG-ADA had died, again 16 percent mortality.

Of these 11 were infants who presented critically ill with SCID, who died shortly after starting PEG-ADA, and again, this makes the point that for many of these patients the infection that leads to their diagnosis may be something that will be fatal before any therapy can get them good immunity.

Three patients though on PEG-ADA died some years later. Two had preexisting lung disease from years of immune deficiency, and one from an unrelated cause.

And then 19 patients actually stopped PEG-ADA to undergo bone marrow transplants. I know, for example, in Canada, there's been some push to get patients off this very expensive drug and to have them have a bone marrow transplant.

So the first trial that we did in 1993 used umbilical cord blood as the source of CD34 cells for three ADA deficient SCID neonates. CD34s were isolated and using the technology of that year, they were cultured for three days with the vector in growth factors that we now know are suboptimal for

stimulating stem cells, and there was no support layer like fibronectin like we would now use.

And the cells were given IV back to the patients without cytoablation, and the patients were started on PEG-ADA in the infant period.

And this is data from one of the patients. This has been published, showing the gene marking. So this is looking over the first four and a half years. We're now over nine and a half years out from this procedure.

And what you see is that over time the frequency of PBMCs, the circles containing the gene rose up to around one to ten percent, and if we split those out by FACS sorting from the PBMCs, T cells showed the high marking, whereas monocytes, the Ms that are in here, were not increased similar to the level that we saw in the granulocytes.

So we saw a preferential increase in T cells.

Over this time, we were slowing weaning them off their enzyme therapy at this point. At four years their PEG-ADA in this child was held for a two month period. And during that time, once we stopped the enzyme, we saw basically that biochemically he became as he was as a neonate. In other words, he was still generally ADA deficient. So his plasma ADA and red blood cell nucleotides rose showing there was not a large effect from the gene transfer.

However, during this brief window at least the T

cell numbers and proliferative responses to PHA were stable, but the T cell response to tetanus antigen and the numbers of B cells and then K cells declined more than tenfold.

So it appeared that PEG-ADA was what was keeping the B cell function going and not the gene transfer.

The gene marking the T cells remained at the same level, but then two months afterwards the patient developed an upper respiratory infection and thrush and was placed back of PEG-ADA and has been on PEG-ADA since that time.

And so the data that has been referred to a few times, and Christof showed another colored version of this slide earlier, this is data where he has now gone back and looked through this patient as well as the second one in the trial for their clonal integrations. So this the LAM-PCR method that you heard about this morning.

What we see in patient one in their PBMCs, although there are multiple integrants, there's a predominant single integrant seen in the PBMCs, as well as in the T cells, and at late times also in the myeloid cells.

Patient number two has a few bands, but different ones, and so it's not just that this is a consistent artifact of the method, and in fact, what this suggests is that this patient had just a few gene containing stem cells transduced, and has this almost monoclonal pattern.

And to summarize more data analyzing this patient,

as I just showed you, the LAM-PCR revealed the stable presence of a predominant vector integrant in T and myeloid cells over the past eight years, with no changes in the patient's lymphocyte counts. There hasn't been any rise in lymphocytes associated with this.

And T cell clones were grown from the peripheral blood of the patient eight years after the procedure, and looking at the clones, 13 out of 220, or six percent, contain the vector, which is about the level of marking we had seen on PCR of the bulk cells, and of those 13 gene containing clones, ten had that same predominant integrant.

But looking at those clones, there were different T cell receptor rearrangement patterns seen among the clones with the predominant integrant. So all of these data together are different than what you heard this morning about a single mature T cell proliferator or a single immature T cell proliferating.

Here it appears that there was a single pre-thymic stem or progenitor cell that accounted for the majority of the gene marking, and so this is another example of a relatively monoclonal pattern that doesn't go along with the lymphoproliferation.

And the patients all remain well on full dose PEG-ADA at the present time with gene marking persisting down to eight years, and as I just showed you there's oligo and

monoclonal markings seen.

And we also observed in this trial that there was no detectable vector expression when we just took PBMCs fresh from the patient, but there was a higher level of expression from the PBMCs after they were stimulated ex vivo with PHA and IL-2, suggesting that the vector was off in the mature circulating T cells.

So that work then led to a second trial seven years later done, again, in collaboration with the NIH. Fabio Candotti is the PI on the study for the NIH, and I'm the PI at Children's Hospital.

And in this trial that opened last year, it's a Phase 1 study for ten patients, and the way the protocol was written was to be conservative and keep the patients on PEG-ADA. And so an entry criteria is that the patients have to be on PEG-ADA at the time of enrollment, and it's for ADA deficient SCID neonates or children, and we target CD34 cells from either cord blood in neonates that was available or from their bone marrow in children.

Our gene transfer methods have evolved as the field has to using a retroviral vector in a GALV pseudotype using growth factors that we think are better for transducing early stem cells on retronectin serum-free.

And then written into the protocol is a phased withdrawal of PEG-ADA after one year if gene marking is present,

and the protocol calls for a two year active phase follow-up.

And the study is using actually two retroviral vectors. They're relatively similar. The nuances are more for the aficionados in the field. They're both basically Maloney based retroviral vectors driving the human ADA gene. Ours has a couple of minor modifications in the LTR that seem to help expression in the mouse, and it's not clear whether those make a difference in the human.

And we had developed this vector, and Fabio's group had developed that vector, and so both have been produced at GMP grade, and each patient gets both vectors. So the CD34 cells from each patient are split, and half of them get our vector. Half get the other vector to see if either affords a better result in the patients.

So the objectives of the trial are, first of all, to assess the safety, the toxicities from the procedures, the bone marrow harvest, the reinfusion of the cells. We look for replication competent retrovirus and for other toxicities.

Then we would document progenitor transduction engraftment by DNA PCR, gene expression by ADA enzyme and RT-PCR. As I mentioned, we compare the relative mark of the two different vectors, and then look at immune function as PEG-ADA is withdrawn.

This is an outline of the study diagram, and again, I think for most of the trials it's relatively similar.

When ADA deficient SCID patients are identified and they're diagnosed as a confirmed, first of all, they would be excluded if they had a medically eligible HLA-matched sibling. Since the results are so good with matched transplants, those patients are excluded from the study.

And then the patients would start on PEG-ADA or continue it if they are already on it, and then there would be the informed consent process, screening test to determine whether the patient is eligible. Then because there is a bone or a harvest involved, there's an assessment of the preoperative status.

Then there's a bone marrow harvest for the children, cord blood for the newborns, and then isolation of CD34 cells, transducing with the two vectors, half the cells with one, half with the other.

Then the cells are mixed together. The final cell product is characterized and infused IV into the patients, after which we then assay peripheral blood samples for safety and toxicity endpoints and for the frequency of transduced cells.

So this is the demographics on the patients so far. Four patients have been enrolled and undergone the procedure, the first in September of '01, the last in January of '02. They are significantly older than the patients in the Fischer study, ranging from four years up to 20 years of age. And these are the numbers of cells that we obtained from their

bone marrow per kilo, and I'll come back to that.

And in the first patient right before we were ready to do the transduction, there was a question of one of the tests, and so the other vectors to that first patient only got one vector.

These are the data of the gene marking in the patients that we have analyzed so far so that the patients are displayed. Two, oh, one was done first. C was done at Children's Hospital. N means done at the NIH.

And what you can see is that the level of gene marking in all of the subjects is relatively low, in the range of one cell in 10,000 roughly, and in fact, in the patient 201C, who we have now followed out to a year, the blood samples at ten months and 12 months were negative by TaqMan analysis, so below the limit of detection for gene containing vectors.

The other patients still have marking at the time points we've analyzed, and the patients are all still on full dose of PEG-ADA which would blunt any selective advantage.

And so, you know, at this point we would say that there appears to be some stable level of marking and potentially if PEG-ADA was withdrawn, we would see an increase in the marking frequency in the T cells.

And so the findings in the study so far is that four subjects have been enrolled and underwent the procedure

without significant adverse event. There have been low levels of ADA gene containing peripheral blood in mononuclear (phonetic) cells present in all the subjects in the first three to eight months, but it has become undetectable in the first subject at ten and 12 months.

There have been no significant changes in either direction in lymphocyte numbers or function other than sort of their normal fluctuations.

And there's a planned PEG-ADA withdrawal after one year if gene marking persists, and so in the first patient where gene marking is not detected at the present time, she's remaining on her full dosages of PEG-ADA.

So then I just want to tell you briefly about a third study that's at Children's Hospital, Los Angeles, although this has not yet been opened, and as I said earlier, the PI for this study is Kenneth Weinberg, and I'm a co-investigator on it.

And it's similar in design to our current ADA trial. It's a Phase 1 study, up to 12 patients for X-SCID either neonates or children, targeting either umbilical cord blood for the neonates or bone marrow for children and using the same gene transfer methods that I described earlier with two years of active follow-up and lifelong follow-up.

And, again, similar kind of endpoints and objectives. The Phase 1 endpoints obviously to look for toxicities. The efficacy endpoints are to look for engraftment,

to look for expression, to evaluate immune function, and Ken also plans after the patients -- if they demonstrate stabilized engraftment, to assess their ability to respond to a neoantigen by immunization with PhiX174, which is a neoantigen that we have used as a test of the ability to make new antibodies.

And the vector is a similar kind of retroviral vector with slightly modified LTRs, but just the CDNA in a basically NLV backbone.

And the protocol, again, is similar to the one I showed you. X-SCID infants with no matched siblings would be begun on appropriate antimicrobial prophylaxis. Parents would be met with to discuss informed consent. Patients would be screened for eligibility to assess the preoperative status of bone marrow harvest would be done. The cells would be transduced, infused, and the endpoints would be followed.

And the exclusion criteria, first of all, is other forms of SCID. So they have to demonstrate a gene defect in the gamma c gene. They would be excluded if they had a medically eligible HLA identical sibling for transplant.

Some patients with X-linked SCID can present with maternal graft versus host disease, where they've acquired some maternal lymphocytes at the time of birth, and if that's severe, that would complicate the analysis, and it would probably be an exclusion and in our center would probably be an indication for giving them at least monoclonal antibodies again for T cells or

ATG in a transplant. And then other sort of organ function exclusions.

And then where the status of this trial is that the vector has been produced and certified at the National Gene Vector Laboratories. The cytokines have been purchased and certified. All of the regulatory oversights and approvals basically are approved and in place, but the trial is not yet open due to the clinical hold.

So if I just may have one minute for my philosophical weighing. Why in this one subject in France has this occurred?

We have talked about a number of these. Is it just a stochastic integration event and just a bad luck event in this patient at some frequency, either genetic predisposition, redisposing factors? Is it related to the disease of a gene?

And the point I really want to make is I think an important consideration is the age of the subject. This is the youngest of the subjects in the current trials. It was one month old, and that correlates with getting a very high CD34 dose.

And so over here I plotted, and it's probably hard to read the graph in the back. The age of patients in gene therapy studies that have been published recently and our study as a log function, if that's possible, versus the doses of CD34 cells that were obtained.

And what you can see, for example, in our two older ADA patients, the 16 year old and the 20 year old, we got very low doses of CD34 cells per kilo, whereas the French patient number four and five who were under six months of age, I believe, both of them, the cell doses were a log or more higher.

And I think this is a correct finding that younger kids have more CD34s in their marrow. They are also more actively dividing, and therefore, the deficiency of transduction is higher, and then, of course, young children have very active thymopoiesis.

And so I think that one of the factors that needs to be considered is that things may be very different for a one month old or six month old than for a five year old or a 15 year old.

And I guess the questions with these large cell doses available, would a lower dosage of cells decrease the risk and do we need to think about dose limiting, how many cells we give?

And then the other question that's raised by the Milan study for ADA deficiency where they gave chemotherapy beforehand is does marrow cytoreductive conditioning reduce the risk by allowing effective immune recovery with the lower cell dosage or does it increase the risk by increasing the proliferative demands on transduced stem cells?

I'll stop there. Thank you.

(Applause.)

CHAIRMAN SALOMON: Thank you, Don.

As I said, we'll give all three talks, and then questions will be address to sort of the whole group.

So the next talk is from Dr. Brian Sorrentino and Dr. John Cunningham. This is Dr. John Cunningham, and this is gene transfer for JAK 3 deficient SCID.

DR. CUNNINGHAM: Okay. Thank you.

I'd like to thank everybody for inviting us. My name is John Cunningham. I'm from St. Jude Children's Research Hospital, and what I'm going to talk to you about today is a joint protocol between ourselves at St. Jude Children's Research Hospital and Rebecca Buckley's group at Duke University Medical Center.

And one of the first issues that has come up at this meeting this morning is what is the risk of gene transfer, retroviral gene transfer, when it comes to hematopoietic cells. So as many of you are aware, we at St. Jude have been interested in gene transfer for over ten years now, and I thought I would just briefly summarize in one slide our experience with gene transfer in 133 subjects.

Of those, 64 are still alive, and 69 are deceased. In the 133 subjects, 49 received hematopoietic stem cell gene transfer, and of those, 32 are still alive and 17 are deceased, with a follow-up of two to ten years. And we have not observed

any retroviral associated adverse events.

So although many of these subjects were transduced with retroviruses in an era when retroviral genes transduction wasn't as efficient as perhaps it is today, I think this provides some information about the potential denominator for the risk-benefit ratio for gene transfer in human subjects.

CHAIRMAN SALOMON: Can you clarify one thing? I'm guessing these are not all JAK 3 deficient.

DR. CUNNINGHAM: No. I'm sorry. These are all our gene transfer studies, and most of them are gene marking studies. Excuse me.

Most of these patients had malignant diseases. There were some with genetic disease where marking was used as well.

So on to JAK 3 deficiency, and as you heard this morning, the common gamma chain -- I'm not sure if this is working -- but the common gamma chain deficiency represents about 45 percent of children with SCID deficiency.

However, another disease which we have become interested in is JAK 3 deficiency where seven percent of children with SCIDs have a deficiency in this gene.

This gene is involved in modulating the response to the IL-2, IL-7, and IL-4 receptors as shown in this diagram.

So Brian Sorrentino's group at St. Jude has studied a genetic model of JAK 3 deficiency generated by Jim

Eiley (phonetic) at St. Jude and has shown three things. The first thing is he's shown that in a paper published in Medicine in 1998 that in a myeloablated host, mouse host, when the JAK 3 hematopoietic cells were transduced with the retrovirus, he could correct the immunophenotypic defect.

He subsequently showed that this protection was translated into protection against infectious agents, specifically influenza.

More recently he's shown that in a non-myeloablated mouse host, he can also show protection. So this stimulated us to generate a clinical protocol, and it's very similar to the one that Dr. Kohn has described for a common gamma chain deficiency in which we plan to enroll approximately six patients, and the follow-up is exactly the same. So I'm not going to go into the details.

And we were just about to submit this study when Dr. Buckley at Duke identified a patient with JAK 3 deficiency who had undergone two unsuccessful T cell depleted haploidentical maternal bone marrow transplants, and so it was felt at this stage that since the maternal bone marrow transplant wasn't working that we should consider gene transfer.

So let me tell you a little bit about this child. When we became involved at St. Jude with Dr. Buckley on this case, it was a 14 month old male infant who had been admitted to Duke University Medical Center in April 2001. The clinical

course was characteristic of SCIDs with recurrent infections, including severe oral moniliasis, and the patient also had a vaccine derived varicella infection.

There was no family history of immunodeficiencies. The parents were related. The child had no full matched sibling. So it wasn't possible to use one of them as a transplant donor, and unfortunately only the mother was available as a family donor.

So these were the findings at diagnosis: an ALC of 700 per millimeter cubed -- cubic millimeter; serum immunoglobulins which were deficient; and flow cytometry was characteristic of JAK 3 deficient SCIDs.

Of specific note, there was functional deficiency in responses to PHA, CON Kaplan A and poke week mitogen (phonetic). And molecular diagnosis and immunoblotting confirmed that this child had JAK 3 deficient SCID.

So the child was commenced on intravenous acyclovir and alpha turacin B. However it had persistent Candida esophagitis and intermittent varicella lesions, and over the next few months, starting in May 2001 and subsequently in October 2001, the child received two haploidentical maternal transplants in exactly the same fashion as Dr. Buckley described this morning, and the dosage of mononuclear cells received in both doses was approximately 1.5 and four times ten to the eight mononuclear cells per kilo.

The patient, like all the other patients described by Dr. Buckley this morning, received no conditioning and had no graft versus host disease prophylaxis.

So what was the outcome of both of these transplants? Well, unfortunately, the child had no T cell function, did not reconstitute T cell function, and did not reconstitute B cell function, requiring monthly IVIG infusions.

And interestingly, just one side point that has come up this morning. In fact, this child did develop an oligoclonal T cell alpha-beta proliferation which was transient in nature, and this has been seen, and Dr. Buckley can address this further, in many children, that they do develop these oligoclonal transient proliferations.

So at this stage, because there was no availability of other donors and because we had begun our collaboration related to JAK 3 deficiency, we considered the risk-benefit ratio of going forward with a treatment with hematopoietic stem cell transfer with a certified JAK 3 vector that we had produced in collaboration with Ken Cornetta.

So we went to the FDA, the agency, in January 2002, and on February 12th, 2002, we infused the first transduction of autologous bone marrow stem cells transduced with the JAK 3 expressing retrovirus.

This product was selected on the Isolex device, and unfortunately we got a very poor yield with this device, and

we only infused two times ten to the five CD34s per kilo.

However, our transduction efficiency was quite highly effective at 55 percent CFU-C.

Over the next three months we observed the patient and observed no adverse clinical reactions. However, we were not able to detect the retrovirus in peripheral blood mononuclear cells.

So in May of this year, we went back to the agency and suggested that we could go forward with a second procedure where we would do a more extensive harvest and also use the CliniMACS device, which in our hands has been highly effective in selecting large numbers of CD34 cells.

I should point out that Dr. Fischer's group has used this device for CD34 selection.

The patient received a second infusion in early June of this year of approximately 3.2 times ten to six CD34s per kilo. I should point out that the transaction efficiency in this approach was even higher at 85 percent.

The transduction conditions were very similar to those that were described by Dr. Kohn for the common gamma chain and ADA trials.

Since that time we've seen no adverse clinical reactions related to the gene transfer event, and we're now approximately 120 days from treatment, and unfortunately at this stage we're still not seeing any T cell function, and we have

several speculations about why that is and we can discuss that perhaps at the end of this period.

Just to point out before I turn it over to Dr. Sorrentino that this is what the T cell subsets look like in this patient after the first transplant. There was a modest increase in CD3 cells, but nothing very significant. The axis here is only 400, and this is the transient rise that was seen in the alpha-beta cells.

Unfortunately, as I say, after the two gene therapy procedures we still haven't seen a response. However, I should point out that 120 days is still within the time frame that has been seen for reconstitution in transplanted patients with allogeneic bone marrow.

I'll just turn it over to Brian.

DR. SORRENTINO: I just have four slides here, but I want to talk a little about the vector and the marking data. The vector that we use is based on Bob Holey's murine stem cell virus vector, and it's a very simple design where we're driving a human JAK 3 CDNA from the promoter within the LTR.

We did this for several reasons really. We have extensive experience with this vector in preclinical models, and it's clear that reporter genes are expressed in mice and in rhesus monkeys, both in stem cells, TMB lymphocytes, and in myeloid cells.

And since the gamma chain needs to be expressed

both in early cells and pre-thymic cells, as well as in the mature progeny, this seemed like a logical choice.

Furthermore, the MSCV based JAK 3 vectors we've shown are clearly therapeutic in the JAK 3 knockout model. So that gave us further rationale for choosing this design.

Together with Ken Cornetta at IU National Vector Lab, we derived a PG13 clone that uses the GALV pseudotype, using their standard procedure at the vector laboratory, and this clone had a relatively high titer of five times ten to the sixth particles on HeLa cells, and it also leads to efficient transduction of CFUC derived from human CD34 cells.

This is a test of the vector, the clinical vector supernatant that was used in the patient, on the patient's EBV cells, an experiment that we did together with Joe Roberts and Rebecca at Duke. You can see these are the EBV immortalized, and this is a JAK 3 Western Blot. Here are the cells prior to transduction. Here's a mock transduced group. Here are vector transduced EBV cells, and here are normal EBV cells.

Now, while it appears that the amount of JAK 3 is significantly less than in control, it's important to know that there's no selectable marker in this vector and really only in about five percent of the EBV pool was transduced; that when you normalize this degree of expression, the transduction based on Southern Blot analysis, it's approximately in the range of normal.

This is the gene copy number data in the patient at three points after the second transplant, day 34, day 69, and day 111.

This is a PCR reaction that was run on a gel and then blotted with a JAK 3 specific probe using primers that are specific for the vector. One of the primers is in the vector sequence, the other in the three prime part of the JAK 3 CDNA.

These are dilutions of the producer cells. So this represents one copy in 100, one in 1,000, one in 10,000, and one in 100,000.

For controls, this is normal peripheral blood from a volunteer, normal PG13 HeLa in water.

And what we've been seeing is about one in 10,000 copies per cell that have been really quite stable since the second gene transfer procedure. It's interesting to note that these are approximately the same copy numbers that Dr. Kohn just showed in his ADA patients, and also this is approximately the copy number that's been seen in the French study in myeloid cells.

And this probably represents about how many transduced autologous stem cells are present in an unablated host and is quite consistent, I think, with these other studies.

So we are now at about 110 days post transplant, and obviously disappointed that we haven't had immune reconstitution in this patient, and we're considering what are

the possible explanations for this.

One possibility is simply that not enough time has passed, and if you look at the published gene therapy data, some of these patients do reconstitute at 120, at 130 days post procedure. So there's some possibility we'll yet see reconstitution.

The second possibility is that the patient may have a thymic defect that's leading to failure of T cell maturation, and it's important to note that this patient did fail to prior haploallogeneic transplants. So that would potentially be consistent with the allogeneic results as well as the inability to establish immune reconstitution with genetically corrected autologous cells.

Potential mechanisms would include the viral infection, the drugs that have been used in this patient, and it has to be considered a possibility.

A third possibility would be inadequate expression of the JAK 3 vector due to an unfavorable integration site position. Now, while I showed you were confident that this vector expresses the protein in EBV cells, it will be important to establish whether or not the vector as it is integrated in the dominant clone in this patient is leading to expression.

Now, our preliminary results, it's actually been difficult to detect the MRNA in RT-PCR reactions. However, it's not clear if this is due to the sensitivity, which we're now

going back and looking at quantitatively, or whether, in fact, there is no expression.

So we should be able to resolve this. I would consider it unknown at this point.

And one last possibility, I think, that was alluded to in this morning's general discussion of vectors is while we know that the sequence of the JAK 3 CDNA is normal and the plasma used to make the producer clone, is there any possibility that some type of inadvertent inactivating mutation occurred in this specific clone during the reverse transcription phase?

So we're doing several experiments to prove that the JAK 3 in this clone is wild type and functional. We should have that information shortly and list that as a possibility.

And you know, the questions I'd like to leave open, perhaps during the discussion period if anyone would like to offer some suggestions, we've obviously been discussing in detail amongst ourselves as, you know, what is the options for this patient, which really are quite limited at this point or none.

For instance, if the problem is due to a thymic defect, would a thymic transplant be useful? Or conversely, if the problem is due to not enough transduced cells, would another transplant be useful? Should both be done simultaneously in the absence of the known mechanism for failure to reconstitution.

And I will note this patient, despite his ongoing viral and fungal infections, is reasonably stable and growing and developing, but has been an in patient for a significant period of time at Duke.

CHAIRMAN SALOMON: Thank you, and regardless of how this comes out, I'm sure I speak for everyone here that we hope your patient does well.

The third talk today is from Dr. Harry Malech and represents work by he and Jennifer Puck, representing the National Institute of Allergy and Infectious Diseases and the National Human Genome Research Institute and, again, is discussion about gene therapy protocol with patients with XSCID.

DR. MALECH: I'd like to thank the committee for the opportunity to speak today.

What I'm going to discuss is our protocol, ex vivo retroviral gene transfer for treatment of X-linked severe combined immune deficiency. Jennifer Puck and I are partners in this protocol, and what distinguishes this protocol is that it's for treatment of patients with persistent immune defects despite allogeneic bone marrow transplantation.

I'm going to go through this very quickly because this just reminds the group that bone marrow transplantation is the current standard of therapy for SCID. There's 60 to 90 percent survival, obviously better than this, the upper end of this with HJA matched sibling donors, which is really available

to only 25 percent of patients; less success with haploidentical from a parent or matched unrelated donor; and as Dr. Buckley pointed out, better outcome with diagnosis and transplant by I found it to three months of age, and her data was 3.5 months.

Limitations of haploidentical bone marrow transplant patients are sun graft versus host disease incomplete immune reconstitution or graph loss, in some patients poor B cell function with IVIG dependence, immune disregulation and autoimmunity, recurrent infections, growth retardation, nutritional problems, and lung disease in a subset of these patients.

I just would like to very, very briefly outline the design of the NIH XSCID trial, and then I'll return to this after I talk a bit about the potential patient population that we're focused on for this trial.

So our design is treatment of up to six XSCID patients who would be two to 20 years old with persistent immune defects despite bone marrow transplantation. This would be an ex vivo retrovirus gene transfer to cytokine immobilized autologous CD34 positive peripheral blood hematopoietic cells. It would be a single infusion of gene corrected CD34 cells with no marrow conditioning to enhance engraftment, where there's no radiation, no chemotherapy, similar to the other trials.

And then, of course, this really truncates a lot of things we're going to do with long-term follow-up, with

immune reconstitution, vector marking, and changes in clinical status.

I'd like to focus a bit on the subjects part of this protocol design. So an important feature of our study is that all subject have already received one or more allogeneic bone marrow transplants, but demonstrate persistent immune defects which result in IVIG dependence, recurrent infections, growth failure, chronic gastrointestinal problems, chronic inflammatory skin conditions, and chronic lung disease.

No patients have yet enrolled in this gene therapy protocol, but we have studied eight post bone marrow transplant ex-CgD patients dependent on IVIG who have a variety of persistent clinical problems. So keep in mind as I talk about these patients that these are a highly selected group. They were people who sought out coming to the NIH because they weren't satisfied with the outcome of the standard of care in these patients. So they represent fortunately a small subset of patients, but an important set of patients who we hoped we could do something for in addition to what had already been done.

Just a quick outline. Lining them up by age, we find that they've received from one to in one case four attempts at haploidentical bone marrow transplants from either one or both parents in some cases. All of these patients were at least three years, with a range of three to 11 years from the last bone marrow transplant.

You can see that as the patients get older, if we take this as a kind of snapshot, the ones who are younger appear to be growing and so on, but as they get older, they lose their growth curve and are in the very low percentile for weight and height.

This is a somewhat unreadable slide, but it's real meant to sort of quickly outline the patients have a variety of things. Not all patients have all problems. Many of them have all sorts of skin problems, alopecia, eczema, recurrent infections, sometimes molluscum and warts. Many of them have recurrent ear infections. Some have had hearing loss. Almost all of them have recurrent and chronic sinusitis.

Some do and some don't get pneumonias. Many of them get recurrent bronchitis and have bronchiectasis and many of them have decreases which is progressive over time in their pulmonary function.

All of them interestingly, except one, have elevated liver enzymes. What's very interesting is that a number of them have required gastric tube feeding to keep up nutrition. They have probably because of the chronic GI problems that they have, and so this has been something that's been done for them at their home institutions to help them out.

So have diarrhea. I didn't include it on here. Many have recurrent infections with cryptosporidium and other infections of that sort.

Also, we often don't pay attention to the psychosocial factors. Many of them have delayed development probably because of their chronic illness. Many need speech therapy, have delayed speech, require special education.

One child troubles even entering school. So we're talking about kids who are getting by. They've certainly survived. We're pointing out that one of them here is 11 and one is 19, but they're not having a great time, and I think that's important to emphasize.

The immunologic data from these patients, you can see that they kind of vary from normal to subnormal amounts of T cells. B cells are for the most part in the normal range, very few NK cells. They don't make IgA for the most part. Particularly as they get to the older patients they really don't make IgA, and they continue to have very low IgM.

Even though many of them are living either on small numbers of their own T cells or the transplant T cells, they're not functioning that well, and again, there's a gradation from youngest to oldest, indicating what happens to the graft if they have it.

We have performed microsatellite PCR chimerism assay of blood cells, and what's very interesting is that of the, again, very select subgroup of patients referred to us at NIH, we find that two of the patients don't even have T cell grafts from the donor, but of the others, the only lineage

that's engrafted are T cells.

And if we look at B cells and granulocytes, there's no engraftment, and we don't even show in K cells because we can't isolate enough DNA to do that kind of analysis.

Now, what's interesting is in the context of another protocol where we've collected mobilized CD34 cells for the development of our studies and analysis of CD34 cells, what's very interesting is that this just sort of shows that they actually mobilize okay. So these were the peak mobilization that we saw in the peripheral blood.

But when we collected cells and analyzed them, 100 percent of the CD34 cells, meaning the progenitor cells, are of recipient origin. In these patients, we could not find any evidence of CD34 cells any more from the donor.

So conclusion about post transplant XSCID patients, of that subset that didn't derive all that many of them do derive from their transplants, we find that some XSCID patients have persistent immune deficiency despite one or more prior haploidentical T depleted bone marrow transplants.

These patients have immune defects, poor growth, and chronic medical conditions. Engraftment of donor T cells was detected in six to eight patients, but no patient had any donor B cells, granulocytes or monocytes.

Six of six patients in whom we actually collected and analyzed CD34 cells. No patient had any donor CD34 cells.

Just to return to our protocol design, I'll just briefly outline and then finish up quickly where we are just to remind you of what our protocol is about.

The vector is a GALV pseudotyped MGFS gamma chain vector. If you remember the trial in France, it used MFG. Both MFG and MFGS were developed in Dr. Mulligan's laboratory and have the feature of producing a lot of protein from these constructs, and the MSGS vector really is very similar. Just in our case we've only put in the open reading frame of the gamma c, but MSGS differs from MSG really by only three nucleotides in the truncated gag region, further reducing the potential for production of gag peptide through recombinational events, but basically they're the same exact vector.

Replication incompetent vector was packaged by the PG13 cell line and supernatant for clinical use was collected from confluent cultures of the stable, highly characterized producer clone in a GMP facility under contract to BioReliance.

Our protocol calls for using one to ten times ten to the sixth autologous mobilized peripheral blood CD34 cells, which will be subjected to four daily transductions ex vivo with our vector. Transductions will occur in the same exact system as has been used in the French study, which is in these gas permeable containers with serum free medium, coated with retronectin.

In our case, we're using six rather than five

vectors. So it's really the same as the French study, except we've also added IL6 to this, and based on our tests of the clinical vector, we expect transduction efficiency to be 40 to 60 percent with actually most times that we've done this at the 60 or higher percent.

So subjects will receive a single infusion, as I noted, and subjects, of course, will be monitored for G marking and blood cell lineages, changes in numbers of these cells, changes in function, changes in clinical status.

And, in particular, we developed a rather extensive sort of quality of life assessment that we're going to be doing because we think those are the things that are important, is whether we find something at the molecular level.

The first three subjects will be treated at least one month apart, and the protocol calls for the appearance of gene marked T cells in at least one of these three patients before we would enroll any other patients.

Keep in mind that it is quite possible that the patients who fail transplants have something about them that may make them also not as receptive to gene therapy as well, and we feel that if we failed in three patients, we probably should not proceed.

Safety studies include monitoring for replication competent virus in blood cells and, of course, evaluation of other safety studies.

So how might we modify our current protocol if we were to go forward, and obviously we're extraordinarily interested in the input from what's been learned today and what people advise us, but some of our proposals are that a further limited enrollment to patients with immune defects, growth impairment, recurrent infections who are -- our protocol actually didn't call for them to be post haplo transplant. It could be any post transplant.

But I think what's showing up in our clinic are all of the patients who all of the patients have haplo transplants. But I think we would probably limit it to that since the patients who do poorly have no engraftment and BNK cells, myeloid cells and 34 cells. I think we can safely say that that should probably be a criteria of the study.

In light of the family history of a tumor, of a medulloblastoma in the French study, we would propose to exclude subjects with a history of leukemia or any childhood cancers in first degree relatives. We don't know what role that played, but why not do that from the start?

Of course, informed consent. People haven't addressed it today, but it's so obvious, but it obviously needs to be said and full disclosure in the informed consent document of everything we know about the severe adverse event in the French study needs to be communicated at many levels, both verbally and in language that parents can understand, not in

language that we've used here today at the meeting.

Well, this is a bit rambling, but I anticipated some of the things that were going to be talked about, and this really is a kind of truncated way of saying we'd like to look at inversion sites. We're obviously going to talk with people who are experts at this and try to line up, sort of get in the same queue as others who are having this done.

And I won't read the whole thing because I want to move along, but basically the one thing I would say is one of the questions that was proposed at the committee is should we do some kind of screening of the ex vivo transduced cells before they go in the patient, and I think there's a lot of reasons to believe that this is probably not a reasonable idea, and I'm not proposing it as it's highly unlikely to reveal the predominant clones and the rare cells which actually graft long term and would give rise to the cells that we'd later look at in the patients.

We obviously want to analyze T cell subsets. This was already part of the protocol, but I think we would probably modify exactly what we're going to study and how we're going to look at it and the frequency with which we look at it based on the experience in the French study.

And finally, one of the things that comes up is we don't know what role the chicken pox infection, varicella infection, played, but at the very least it may have egged

things along by pushing cells to stimulate them in some way.

And I think that we probably are going to err on the side of early interventional treatment of all infections, particularly virus infections with specific therapies. We're, of course, any approved therapies that are available, antibiotics, anti-viral agents and/or specific immune globulin where appropriate.

And with my last slide, I just want to remind everybody that the subset of patients with XSCID who have persistent immune defects and chronic medical problems, despite allogeneic bone marrow transplantation, do not have other reasonable treatment options and may benefit from ex vivo gene therapy.

The risk-benefit assessment for gene therapy treatment of these patients should take into account the degree of impairment of their immune function and quality of life and the lack of alternative therapies.

And I'll stop here. Thank you.

(Applause.)

CHAIRMAN SALOMON: Yes. Well, thank you.

And this has served as a good reality check on the darker side of this whole thing.

John.

DR. COFFIN: One thing you didn't suggest which comes to mind as a modification is reducing the number of cells

that are treated and introduced. Perhaps in a stage fashion, starting with small numbers and then increasing until one sees some successful engraftment.

Is there some reason that drives -- some solid reason for using the number of cells that you do in this protocol?

DR. MALECH: I think a lot of us have tried to learn where we can from experience in transplantation, and although there are many people sitting around this table who are more expert at transplant than I am, I'll make some statements. Maybe I'll have some bricks thrown, but basically I think from the transplant setting in the ablative or subablative transplant setting, one probably needs at least the equivalent of one times ten to the sixth per kilo CD34 cells to get reasonable rates of engraftment and reconstitution, and most people aim in the range of five times ten to the sixth.

Our protocol, actually it turns out we probably aren't going to get much more than that, and realize that the gene transfer is much fewer of those cells.

And while we're very proud of our bulk transduction efficiency rate, the actual rate in true repopulating stem cells may be far lower than that, perhaps even only anywhere from one to 20 percent of that number.

So I think if anything, we're still at the margins of what may be necessary to do this job reproducibly, and keep

in mind that even in the French study two of their patients didn't get full correction. One of them lost a graft, required a transplant, and they gave a lot more cells overall than we're likely to get from these older patients.

It's an argument for saying that I don't think we're anywhere near what I think is the optimum number, let alone saying let's hold back on what we have.

DR. COFFIN: It's perhaps unfortunate, of course because if one could reduce the number of cells by a factor of, say, ten, one could almost certainly greatly reduce the risk of insertion, you know, next to some oncogene or another just on a numbers basis.

If there was some way to sort of investigate that as you went along, it would certainly be --

DR. MALECH: In the case of XSCID, of course, you have this tremendous magnifying effect of the selection for T lymphocytes, and I was very interested to see the data that Dr. Kohn presented showing the great expansion of a very small number of cells and the persistence of them, and yet they did, in fact, have the receptor diversity that one would hope for.

So while at the one time arguing for not reducing, one could also take the other side and say if you have enough, you probably don't need more than that enough. I don't know what that enough is.

DR. COFFIN: But perhaps there would be some say

to do that in the context of these trials, to investigate that by staging or something.

CHAIRMAN SALOMON: Yeah, I think, John, you've got a good point, and I don't think that's the end of it either. I think we should come back to that a little bit later and talk about it.

David.

DR. HARLAN: I wonder, Harry, if in calculating the risk-benefit analysis, which we all want to do at the end of the day, if you could tell us how these patients that you describe, and maybe, Dr. Buckley, you could comment, too -- is this the far end of the bell shaped curve of these haploidentical transplants, or how common is this syndrome that he described?

DR. BUCKLEY: Well, I'll tell you at our institution I would say it's the far end of the curve, but you know, again, it depends on how the transplant was done. If it was done with chemotherapy and if there was graft versus host involved and the patient has chronic GVH, you know, I don't really know what the incidence of that is in the other groups, but in our institution I would say it is the far end of the curve.

DR. MALECH: And that was the point of the referral here. They were the far end of the group.

CHAIRMAN SALOMON: Dr. Orkin.

DR. PUCK: Could I just add? The patients who have been referred to NIH have come from multiple centers. So there are at least four different centers that have referred patients, although all the ones we've seen so far have received nonablated transplants.

DR. HARLAN: Well, one way to look at the question is what's the denominator. You describe eight patients here. Out of how many nationwide have received these haploidentical transplants?

DR. PUCK: Of course no one knows what the incidence of SCID is. We presume that it's on the order of one in 50,000 to one in 100,000 births, but we also recognize it's under diagnosed because babies die of infections before being recognized.

CHAIRMAN SALOMON: What Dr. Harlan is asking is how many patients have been transplanted to draw these eight patients, and I think Dr. Buckley can answer that, right?

DR. BUCKLEY: I have some figures that I wrote down. I think all together in the whole world there probably are around 800 SCIDs that have been transplanted. About 500 of these are in Europe, and I know of at least 300 in the United States, and if you take 75 percent of that figure, that would tell you how many haplos roughly

DR. HARLAN: So then I could conclude this isn't way on the far of the bell shaped curve because then it's eight

out of maybe 200 that have this syndrome.

DR. BUCKLEY: Or eight out of 250 or something like that, yes.

DR. MALECH: And it didn't take forever to accumulate these patients. There are others who actually want to visit us. CHAIRMAN SALOMON: There's a lot of people who want to talk here. So at some point here we're going to truncate this because I want to get on to the discussion.

I know, Dr. Orkin, you're waiting to make a comment.

DR. KOHN: I have just one comment on this point.

CHAIRMAN SALOMON: Okay. So Kohn, Orkin, and Dr. Kurtzberg.

DR. KOHN: I think in general this is not seen as commonly with transplants with ablation. However, you do have chemotherapy complications. So there's no free lunch either way.

DR. ORKIN: I just want to make one comment. Obviously we'd all like to figure out what is the denominator in terms of risk to individual patients. I think John Cunningham or it was Brian Sorrentino mentioned the data on gene marking, how many patients had been entered into gene marking studies and the fact there were no adverse events.

But I think what has plagued the field is the low frequency of transduction. So if you have no transduction, you

can erase those cases. So I think the real question is: what is the denominator in terms of transduced cells?

And I think one thing that at least concerns me is in the set of experiments which is the most conclusive in terms of demonstrating efficacy, those of Fischer, there is an event in a limited number of patients, but we don't know what the denominator is, but I think we just can't add up numbers of patients who have been enrolled in other trials.

CHAIRMAN SALOMON: So that's a good example. That's a key point. I'm glad you made it, and we're not going to discuss that yet, but we've definitely got to go back to that.

Dr. Kurtzberg.

DR. KURTZBERG: I was going to say two things. One, you know, these are very heterogeneous diseases, and even though you may have the same mutation, we all know that in genetic disease there is variable clinical scenarios with the same mutation. So it's very hard to just automatically assume that every gamma SCID is going to look the same.

Second, I wonder in some of these patients if they don't have GVHD. I mean, if somebody just gave me that table with that set of symptoms and didn't tell me what it was about, I would say that's GVH.

And I think there's a huge spectrum of immune deficiency all the way to GVH that relates to this mismatched

transplant syndrome, and I don't really care to put a name on it, but I think it's related.

And so that may be what we're seeing in some of these kids.

CHAIRMAN SALOMON: Alex.

MS. BALLARD: Yes, the point I wanted to make also in conjunction to the topic on, you cow, eight patients out of how many, these are eight patients who have sought out this treatment at this time, and that doesn't mean that's all of the ones who could benefit from it.

CHAIRMAN SALOMON: Yeah, but I mean, these kids clearly are not doing well, and that's enough to do the trial. So I think Dr. Malech's point is well taken.

Okay. Then what I'd like to do here is what we're doing is getting closer and closer to all of the sensitive issues. So I think that the best way to deal with it is to stop here, go to the public comments, which I think will raise the temperature in the room a bit, and then I can relax, and we can get into it finally.

So with that, I would like to request the people who have requested time for public comment to step up at this point.

Basically there's five minutes allotted for each of these. No one will be upset if it's shorter, but if you need the five minutes, you've got it.

The first one is -- I'm just asking Gail if there's any point in the order here.

MS. DAPOLITO: No.

CHAIRMAN SALOMON: Okay. So, Mr. Gelsinger, you're welcome to be first.

MR. GELSINGER: Hello. I'm Paul Gelsinger. You may know me as the father of Jesse Gelsinger, the boy that died in the gene therapy protocol at the University of Pennsylvania in September 1999.

The first thing I'd like to do is commend the FDA on the swiftness with which they brought about this meeting. It's an area I found very lacking when we tried to uncover what happened, why Jesse died, is that adverse event reporting was not being done properly, and if the scientists had gotten together and been openly discussing what was going on, they could have avoided his death. Okay?

My observation of this XSCID clinical trial or this clinical work that's going on is that it's the only real positive thing you have going in gene therapy to show that it's working in human beings, and you're in an quandary now because you have an ethical concern that you may be creating a problem in these kids that they didn't have before.

But you're also giving them a viable treatment, and I'm supportive of that. And yet I've heard from the other side on the bone marrow transplant side that if we can diagnose

these kids early and get them into a bone marrow treatment program, they have an excellent chance of survival also.

So you have a lot of things to consider here, and I think you need to go to the ethical guidelines that have been established for reviewing why you even do your work. You have the Declaration of Helsinki. You have the common rule here in the United States, respect for persons, justice.

And you need to take into consideration that these are people first. The advancement of science and society is secondary to their welfare.

There are a lot of scientists in this room that want to see their work proceed, and it may not be in the best interests of these patients for your science to be used in them at this point, and you may need to do more preclinical work.

It's going to be a tough decision for a lot of you. I don't know how many on this panel are involved in gene therapy. I think you have a very broad spectrum. So you'll get a good perspective.

There's an industry side to this that wants to see this technology go into the marketplace, and there's a lot of pressure that gets put on our government from that side, and some of that pressure is inappropriate.

We uncovered that in what happened to my son, and it was awful to know that.

So let the FDA make the right decision. Give them

the advice they need. Imagine these children as your own when you're reviewing this and you're discussing it. Put it in that perspective.

Would you want your child to be inserted in a gene therapy protocol when there are other viable alternatives to the treatment?

And maybe out of this should come some pressure on society to get a screening program for neonates so that we can detect this earlier, and maybe that's the most ethical thing that can come out of this.

Thank you.

CHAIRMAN SALOMON: Thank you very much for those excellent comments, and we will do our best to guide ourselves that way. I hope you're going to stay here for the discussions that follow and remind us if you think we're deviating from that. That's part of your role.

Again, what confused me a little bit is I have this list of people. So no disrespect intended, but Rachel. Yeah, come on up.

This is Rachel Salzman, and she's representing the Stop ALD Foundation.

DR. SALZMAN: Yeah. Can you hear me?

The disease we're actually focused on is adrenoleukodystrophy, but what it has in common with SCID is that it is X-linked. It is often fatal, and one of the current

therapies for adrenoleukodystrophy is a bone marrow transplant, but bone marrow transplant is also accompanied by significant mortality.

And as a result, one of the main focuses of our foundation is to work on novel therapies, including gene therapy, cellular therapies, et cetera. So we're very interested in this type of work.

I think I'm going to echo a little bit of what's been said, but maybe from my perspective and maybe pull together in just two or three minutes.

I think everybody in the room, the physicians, the patients, the families, the scientists, everybody is looking for a therapy that's going to be completely efficacious and completely safe. But by definition in reality that's impossible.

I mean, I think that there's a very well accepted train of thought in the medical community that in order for an intervention or a therapy to be truly efficacious, by definition it's going to bring with it some inherent side effects or some inherent risks that might be high, might be low, but the potential is there.

So I think we sort of have to go into that accepting that just as the reality of the way medicine works. We obviously want to increase the efficacy and decrease the risk or increase the safety.

So, therefore, speaking from the patient's perspective, they're going into this -- whether you're going in for a penicillin shot or you're going into gene therapy, you're assuming a certain level of risk automatically, and that's why I think that it's very important, and I also, along with Mr. Gelsinger, applaud what's going on here, this very open discourse about a risk that was encountered and came true.

I mean, I'm sure it was listed, and, you know, they've been talking about this theoretical risk for the last 15 years. So I think it's really good that it's being explored in such a timely fashion.

And I think it's important to continue to explore this adverse event, and what I've realized from the presentations is that ideally gaining this knowledge is going to lead to being able to decrease this risk not to zero, but just maybe to less than what it was before.

And ultimately that could involve doing pedigree analysis, microarray screening, modifying the vector, modifying the dose, working on defining what this second hit or epigenetic effect is, and you know, that's really important obviously, but if I just gave that assignment to everybody in the room, you could come back in 20 years and still not have an answer.

So it also might turn out that this was random. That's entirely possible. You can't rule that out either.

So I think that what happened was a theoretical

risk finally did happen, and it needs to be explored. At the same time, I think that should not hinder everyone that's involved in these types of efforts, their enthusiasm towards doing it, or their actual work of doing translational medicine and thinking about clinical trials.

I don't think that anyone, based on this meeting or this discussion should then be going home thinking, "I need to pause."

We definitely need to think about everything and understand it, but we would really like to see research continue with patients in mind of getting it into patients.

So ultimately, as for the 19th time, it is about benefit versus risk ratio, but risk means different things for a lethal disease. So you're dealing with the risk of intervention versus nonintervention, and that's a lot different when your nonintervention is a lethal prognosis that is very, very grave versus a disease that, you know, you're going to be bald forever. You know, there's a big, big difference.

So I just want to comment that I think it's very, very important that everyone continue to think about and work on clinical applications, and that when these clinical trials come up for review, that I know it's tedious and timely, but each one has to be considered on an individual basis because this disease has a selective advantage.

Adrenoleukodystrophy would not carry with it a

selective advantage, and that's an important difference.

You also need to consider the different transgene products. You need to consider what is the prognosis of this patient with this disease if it follows its natural course or what's the prognosis of this patient if he or she is able to take advantage of alternative treatment.

So that's kind of our perspective. Thank you.

CHAIRMAN SALOMON: Thank you.

(Applause.)

CHAIRMAN SALOMON: I have on my list Mr. Mike Susko of Citizens for Responsible Care in Research.

MR. SUSKO: Hello. Yes, I'm from the Citizens for Responsible Care in Research, and we're concerned in a sort of cautionary way about this field of research, and I guess there's a few initial reasons that come to mind.

Some of these are the official positions of CRCR, and some are my own philosophic views.

But we're in the field of gene research which has really revolutionized in the past 30 or 40 years. Our understanding has gone from like, you know, one protein to one gene effect to almost multiple genetic codes because the same gene sequences can be interpreted in different ways.

So the field is revolutionized, and our knowledge is incomplete, that being said. So we're really in a dynamic stage, and we're not quite sure, you know, how our knowledge is

going to be complete.

But part of the problem here is there is a fragility of the genome that we're recognizing. The genome has a lot of stability, but there's a certain fragility, and if you push it too far, you're going to get a real adverse effect, and this is why we're here today. We're getting the adverse effects of cancer that can possibly lead to death or have lead to death already.

So we really need to know how fragile is the genome under this specific stress of putting in an infection and to try to realign some of the DNA sequencing.

And so that's really unknown. In fact, this leads us to a point of, you know, looking at the negative data, can we get the accurate information in industry from all of the different animal studies. What's the percentage of mortality? What's the percentage of adverse effects?

That's a very important, knowledgeable thing to know. How fragile is the genome under these conditions?

And in terms of humans wanting to have the same thing done to them, what is their real risk? So that has important consequences.

All of this is very complex. I mean, to the layperson -- and we've talked mostly on the molecular level, and then we talk about some tissue response and inflammation. We go up to systemic responses of cancer. There's all of these

biological levels. They're all interwoven, and it's difficult in some ways to sort it all out.

But it points to a complexity that we don't fully yet understand. So how does this all translate then into informed consent? How do we translate this heavy science and in talking to an ordinary person, do you want this treatment?

It's difficult, but I suggest that our models, the model in an average person's mind is probably something like this. It's like there's a missing piece of my DNA, and I'm going to fix it like a patch on a tire.

But perhaps that's an inaccurate way to look at it. It's much more complex than that as you all know. It's almost like a series of clocks within clocks, like seven or eight different clocks, if you will.

And even for biological systems, you have to invent new words to describe how really the biology works. Like liquid crystal and holistic responses, tansigretti (phonetic), there's different words to describe the level of complexity.

So we need to accurately somehow convey that in informed consent that really things are very complex. We're not quit sure what's going to happen. Perhaps if we're surprised by cancer and adverse effects, our models have been somewhat flawed.

So let me then summarize as far as I would see some areas of concern. In informed consent, I think we have to

be careful to not give a false sense of security and let people think we know more than what we really do in terms of what would happen, given all of the complexity and all of the unknown fragility.

A second critical thing is to make available all of the negative data that's out there. That's good science. As you know, if something good happens or if something bad happens, and I think it's a struggle for the lay society to know just really what's happening. So I would emphasize that point.

And my final words would be I think good science and good ethics is compatible. It's like taking care of all the different biological levels. The highest is our ethical feelings, our intuitions, our thoughts on the matter, and then we have molecular interventions, but we need to integrate all of that.

And ultimately it would make for good business, too, because you would get better results, but maybe more in the long term.

So those are just some of my thoughts. Thank you.

CHAIRMAN SALOMON: Thank you very much.

I also have Dr. Phillipe Leboulch from Harvard.

DR. LEBOULCH: Thank you.

I'm also with Genetics Pharmaceuticals for the purpose of disclosure.

I just wanted to submit to the committee today our

own recommendation for the use of self-inactivating LTRs and chromatin insulators as a novel layer of safety. Both are retroviral vectors derived from Maloney and also from lentiviral vectors.

As it has been said many times today, the vast majority of untoward oncogenic events of insertional mutagenesis result, in fact, from enhanced inactivation by the provirus either from the LTI enhancers or from internal enhancer.

At the same time, the chromatin can negatively influence the expression of the transfer gene through silencing of position effect variegation. So what it would gain from shielding the provirus from the effect of the chromatic surrounding it and vice versa.

Richard Mulligan and Ellie Gabor in the late '80s pioneered the use of deletions of the LTR to inactive the transcriptional activity of the LTR, and you can do this by removing a portion of it, adding a protease signal and following with the transcription and chromosomal integration you inactivate both LTRs.

And we found, and we and colleagues, that even with very long, complex retroviral vectors and anti-viral vectors, this one for the gene therapy of therycenia (phonetic) and sickle cell disease is about eight kilobases long. We get perfect stability and no decrease in biotiters.

Now, at the same time, Gary Pheasantfeld

(phonetic) in the mid-'90s discovered a new class of elements in mammalian cells which are able to prevent the enhancement of a promoter by an enhancer blocking its activity, such as the CHS4 sequence from chicken, which works also in human cells.

As you see here, in the presence of that insulator, chromatin insulator, the expression after integration is dramatically reduced because the enhancer no longer works at a distance.

So we have been able to provide boundaries even with very complex retroviral and antiviral structures by adding at the same time, but we make the deletions in the LTR to self-inactivate the vector. We also place the chromatin insulator, and after reverse transcription and chromosomal integration, both LTRs contain the chromatin insulators and the boundary is established.

And in terms of transfer, what I just wanted to tell you today, it is feasible now to do this for most vectors, including lentiviral vectors even if the vecs. are very complex in structure.

This is a Southern Blot analysis with a 7.528 kb structure, and the decrease in titer from a very high, over ten to the ninth per mL titer, we have with the unmodified blood in vector, we are able to have less than fourfold drop in titer with perfect stability.

So just to close, I would like to say that perhaps

in the debate vis-a-vis the resuming of gene therapy trials for XSCID and extend this also to other gene transfer approaches for gene therapy, perhaps one would benefit from using internal promoters combined with self-inactivating deletion of the LTRs and chromatin insulators.

And of course, we have to do some work in parallel to demonstrate the power of those chromatin insulators, but since we know they can do some and they are mutual for the efficacy, why not incorporate them already in most vectors?

Thank you.

(Applause.)

DR. NOGUCHI: Excuse me. I just have one question. You mentioned that you were speaking of your results in sickle cell disease. Now, I believe that is not in humans; is that correct? That's in animal models?

DR. LEBOULCH: That's right. I don't know who is speaking, but that's --

DR. NOGUCHI: I'm sorry. Right here. Phil Noguchi.

DR. LEBOULCH: Oh, yeah. Hi. Exactly. That was preclinical models. It is not at all done in humans. We know that even with a very complex vector we can get high titers and maintenance of stability of transfer in the presence of those two modifications, which might be beneficial for safety purposes.

DR. NOGUCHI: I just caution everyone that when we talk about nature, when we say words like "it's perfectly," "we never see this," today is a good example of, well, you see it.

DR. LEBOULCH: Absolutely, absolutely right.

CHAIRMAN SALOMON: There another person on the list, Ms. Robb of the SCID Alliance. Can I welcome you up?

MS. ROBB: I didn't expect to speak today. So this is not a prepared presentation, but I was particularly concerned as I listened to everybody speak.

I'm a grandmother of an XSCID patient, one of the patients of Dr. Malech and Dr. Puck and formerly of Dr. Buckley. This is one who has had four failed transplants, and his condition is obviously deteriorating in a general sense. He's ten years old and he's the same size as another grandchild that I have who is six years old, but he looks like he's sick. He's little, but he looks like he's sick.

What I would like to make a point of is that this is an XSCID patient. It's not an autosomal patient, and therefore, there is no existing treatment for him at this point. He's failed the transplants four times, two from the mother, two from the father, and he is deteriorating.

I certainly want you to consider where you're going with this work, but we have been aware of his problem for nine and a half years. We have been aware of the plans at NIH for three years. These things have been put on hold over and

over, trying to be careful.

I'd like you to consider that when you consider the risk to the patient of a treatment that you also must consider the risk to the patient of no treatment because that's the other side of this coin.

I mean, there isn't, as with an ADA using PEG-ADA, there isn't the potential to put him on this $300,000 a year treatment, which really isn't feasible in a very practical way, but that doesn't even exist for this boy.

And I think that the work that they're doing at NIH has been very, very conservative, very considered. They have been moving at a very slow pace a far as pushing families into things. In fact, we're trying to push them into things at this point.

So that's something you must consider. I guess I've hit all the points here that I expected to cover, but I wanted to make sure that you were not being too intellectual about this, but you are also considering the quality of life.

Thank you.

(Applause.)

CHAIRMAN SALOMON: Thank you very much.

And again, as I said to Mr. Gelsinger, you, too, should get up if you think that we're not doing that at any point here.

Okay. So the last thing we'll do under the public

comment is a letter that will be read into the record by Gail Dapolito.

MR. BABLOCH: I'd like to make a comment, too, if I could, after.

MS. DAPOLITO: This is a written statement from the Council for Responsible Genetics in Cambridge, Massachusetts. They are unable to attend today, but asked for their comments to be relayed to the committee.

Who we are: the Council for Responsible Genetics, CRG, is the nation's oldest organization committed to educating the public on issues of biotechnology. Founded in 1983, CRG works to raise public awareness and promote debate on the social, ethical and environmental implications of new genetic technologies.

Our board of directors consists of scientists, physicians, lawyers, educators, and public advocates, each of whom have been involved in these issues at the community, national, or international levels.

Our central concern is that of public involvement and accountability. The public must have access to clear and understandable information on technological innovations, and it must be able to participate in governing the applications of technological developments.

Ever since proposals were first made to use gene modification techniques for potential human therapies, CRG has

fought to prevent the premature application of these treatments. Our board members and advisors with experience in their relevant areas were concerned that genetic modification could produce severe and unintended side effects.

This has been borne out in recent trials, and therefore, we are renewing our recommendation to adopt a different approach from the one currently in place.

As a first step, we propose a moratorium on all human gene modification trials.

The present risks. Overall the effects of both viral and nonviral gene therapy vectors continue to be poorly understood. After over a decade of human trial and more than two decades of animal research, gene therapy is still more of a theoretical concept than a sound medical course of treatment.

Techniques for making genetic changes in mammalian somatic tissues are still primitive. The death of 18 year old Jesse Gelsinger as a result of a University of Pennsylvania trial on in vivo virtual induction of genes was followed by reports of the resounding lack of success of hundreds of other such attempts.

Using ex vivo techniques, the Necker Hospital protocol to treat ADA deficiency hopes to circumvent the potentially fatal complications associated with in vivo methods. Nonetheless, there is insufficient ability to precisely direct gene transfer through the vectors chosen.

Retroviruses are difficult to target effectively, and as a result, they often may not reach the intended DNA location. This becomes a safety hazard in two primary ways.

First, these viruses integrated randomly into the genome of the host cell. After the transgene is introduced, inappropriate integration could disrupt important gene and cell functioning, precipitating cancer or other forms of biological damage.

Second, retroviruses may infect nontargeted cell types. Which of these is responsibility for the leukemia-like disease seen in one of the Necker patients is unclear.

Data from model organisms does not justify continuing human gene modification experiments at this time. Indeed, the NIH report on the Gelsinger incident acknowledged that there were no good animal models for the viral vectors used in that in vivo trial.

With regard to ex vivo protocols, animal studies provide a strong basis for precaution. For example, in the April 19 issue of Science this year, a team of researchers led by Jim Zong Li (phonetic) showed that the insertion of foreign genes into mouse bone marrow cells using a replication defective retroviral vector caused the animals to develop leukemia.

Why, in the face of potential cancer risks which could have been anticipated and were by us and other commenters, were retroviral gene therapy trials allowed to proceed?

This is a question that the committee will have to answer for any adequate review of the recent adverse event. At this stage, many of the scientists who are pushing the gene therapy agenda forward have financial stakes in moving the trials forward. Careful attention should be paid to the potential conflicts of interest that could lead to premature applications of these techniques and thereby compromise the safety of research subjects.

During the months that followed the Gelsinger incident, under improved reporting procedures, 691 reports of serious adverse events in gene therapy experiments were sent to the NIH. Over 98 percent of these incidents had not been previously disclosed.

It should be clear that trials cannot responsibly proceed under conditions of secrecy, commercial, or otherwise. Regulatory mechanisms should be put in place to insure the open distribution of data so that investigators can learn from each other's experience.

Furthermore, the case highlighted the lack of adherence by university researchers to principles of informed consent and to existing recombinant DNA Advisory Committee and FDA recommendations. In many cases, patients in gene therapy trials had not been made fully aware of the risk of severe immune system response, cancer and other adverse events.

As Abbey Meyers, President of the National

Association for Rare Disorders and a past member of the NIH Recombinant Advisory Committee aptly stated, "In the years that I sat on the RAC, I would see these documents time after time. Sometimes eight or ten of them would come in front of us at a meeting, and I saw lies; I saw omissions; I saw exaggerations. Patients were not being told the truth in the informed consent documents."

What is also not being clearly communicated to research subjects is that Phase I trials hold out no promise of efficacy. They are solely for safety and toxicity evaluation.

One more paragraph.

A main purpose of FDA oversight is to maintain controls over research to protect the safety and integrity of human experimental subjects. Genetic modification raises questions qualitatively different from previous drug treatment regimes. As a result, these studies should be reviewed with added care.

The implications of gene therapy go far beyond the immediate medical context, potentially changing the relationship of humans to their permanent biological make-up. Such issues make it imperative that the FDA take into account larger ethical questions before permitting gene therapy to move forward a proposal.

Based with the new evidence of risk, it is our position that the FDA should establish a moratorium in all

future gene therapy trials. Awaiting clear evidence of safety and efficacy, reevaluation should be undertaken of the Phase 1/Phase 2 framework for gene modification trials.

CHAIRMAN SALOMON: Thank you, Gail.

Can you introduce yourself and the group you represent?

MR. BABLOCH: Sure. My name is Jason Babloch, and I'm Vice President for Public Policy of the Immune Deficiency Foundation.

IDF is the national organization dedicated to improving the lives of primary immune deficient patients through research and education.

In an effort to be brief here, I would just like to read an E-mail from the mother of a severe combined immune deficient boy regarding today's topic who was unable to attend herself, and also provide the committee with our policy regarding gene therapy, and I quote.

"In regards to the gene therapy trials, I have very strong feelings about this issue. As a mother who has lost a child to this disease and has another son with the same illness, who by the way is alive today and doing very well because of another kind of experimental therapy, I believe the trial should not be stopped. When you sign a consent form for an experimental therapy, you are made well aware of the possible risk factors.

"Of course, you hope and pray that none of them happen to your child, and you go forward. What other choice do you have? To let your child die? I don't think so. So you take the risk.

"My son's in utero stem cell transplant was very successful. However, there have been subsequent transplants that have not been as successful. Does this mean stop performing in utero BMTs? Our physician would say no. Instead, he has learned something from each one that he and others have performed in hopes of perfecting the procedure.

"This is also true for gene therapy. We can only do so much research on animals before we have to finally experiment on human beings. Hopefully we have perfected the procedure enough at this point to provide a cure for an otherwise fatal disease.

"This setback has not changed my opinion of gene therapy, and I will still consider it as a treatment option for XSCID."

Signed Heather Daley.

I would just like to say the IDF strongly supports medical research directed at finding new therapies and potential cures for primary immune deficiency diseases. We agree that experimental treatments need to be well thought out and closely monitored, but in the end these types of trials need to move forward or we will never find treatments or cures for deadly

diseases.

While the information discussed today shows that gene therapy is not perfected, it holds much promise for the primary immune deficient community, especially for those patients who do not respond to other treatment options.

IDF supports continuation of gene therapy trials in the U.S. when adequate safeguards, including patient education on the risks associated with the procedures and follow-up are in place.

Thank you.

(Applause.)

CHAIRMAN SALOMON: Okay. If everybody would on the panel take out their tan folder, and you'll find in there a yellow sheet that's questions to the committee.

And the reason I do that is there were some important revisions in the questions from the time it was E-mailed to you. So I want to make sure everybody is on the same -- in this case, the same page literally.

Before I pose the question for the committee, I just have a couple of my own introductory comments. The first is we have a very specific charge from the FDA to this committee, and that is to comment specifically at least at the start, specifically on the safety and the feasibility and the appropriateness of going forward with gene therapy trials in patients with different forms of the disease SCID.

And we will do that because we are an FDA Advisory Committee, and the FDA gets to set the agenda.

It is clear to all of us, as well as to the people who have made public comments, that a number of the issues that we're dealing with then go beyond simply retroviral gene therapy in children with SCID, children or young adults, and there's no problem in us taking that up as a second thing, but we have to get done with the first agenda.

As for my own, sitting here saying, "Well, how can I take everything I've heard up till now and put a couple guiding concepts forward that, you know, we later can just shred them?" That's okay with me.

So these are just some thoughts I have. One line of reasoning came out that early transplantation, i.e., in the first couple of months of life, is, quote, successful and safe, unquote. And I'm not making this quote thing out to suggest I don't believe that. I'm just saying that that's a key point.

You know, we're talking about 90 percent successful rates. These are survival rates. I think one of the things Dr. Malech has made us more aware of is that those patients would survive. They'd be in the 90 percent, but, you know, the quality of life issues, et cetera, longer term follow-up, et cetera.

But if we just take this early allotransplantation is successful and safe, one response here would be to exclude

all such patients from these trials, and I think in the discussions we heard that's, indeed, being done in many instances, maybe not in Dr. Fischer's trial with this one month old infant, but maybe not.

And if we believe that, the idea of suggesting increased efforts to diagnose these early, you know, to me these are very solid, appropriate sorts of public health questions.

My feeling, however, though is regardless that still leaves a group that will either not be candidates for early diagnosis, won't get diagnosed early for whatever reason, won't get a perfect result even if it's much better, so there would still be a group of patients that will be potentially appropriate for the kinds of trials that have been outlined today and the kind of trial Dr. Fischer is doing in Europe.

So then I think the question -- so I don't think you can just say, "Oh, well, everyone will be done in the first month. So we ought to go home."

And so we still are going to have a group of patients that we can't forget, abandon, et cetera, that are potential candidates for the exact same trials that are being outlined by these people.

If you follow that reasoning, then I ask the question: what is it that we can do to make this safer? And that should be a major part of our talk. I mean, pieces of the puzzle I've heard today. Reduce the CD34 dose. We need to

discuss that.

We've heard a good counter argument to that, is you won't get engraftment if you reduce the CD34 dose much below a million CD34 cells per kilo. Maybe we need to discuss that some more.

Get better targeting of the progenitor cell. It's likely that probably ten or 20 really good progenitor cells would probably cure everybody. Can we really get down to gene transduction of that real primordial pluripotential stem cells?

Did the gene therapy in this case cause the leukemia? Well, I think we're going to have to butt heads on that. I mean, can we answer it or not? Maybe not, but I think the committee needs to get some sense of how far they believe that the gene therapy was involved in this.

Are XSCIDs or other forms of SCIDs more dangerous than other kinds of gene therapy? Now, here we get into this transition where we will go out of our agenda, but I think within the agenda we've given here one of the questions is should our attitude, should our advice to the FDA regarding gene therapy trials in XSCIDs or SCIDs due to all the other different kinds, ADA, JAK 3, et cetera; are there good, medical, biological reasons to give a different type of advice to these FDA on gene therapy trials in that group because of the potential that that group or those patients have different risks in the context of these gene therapy trials?

Vector design. Enhancer region. The LTR. The payload gene. The possibility of internal promoters. This one didn't have an internal promoter. So it's not an issue, but for those that might -- I don't believe any of them that were presented today had internal promoters, but that's something maybe we discuss near the end of the discussions.

What role is played by CD34 cell activation? All of the protocols I've heard require for these supposedly higher transduction efficiencies basically a soup of differentiation factors, flip three (phonetic) ligand, stem cell factor, GMCSF, IL6. I'm sure I'm leaving one out. TPO was another one I saw up there.

That may give you nice numbers. I mean we can be very proud of the fact now we've got 50 percent transduction, but if you get 50 percent transduction of the wrong cell types, then what's the point?

Other safety issues, I think, can come down the road. Suicide genes, for example, a lot of complicated issues are related to that.

So those are just my thoughts of pieces of the puzzle. I'm not trying to put them in any critical order, and I'm really not trying to buy us any one in one direction. There are probably five other things I'm leaving out, but that's just my best effort to take what I've heard since this morning and highlight it.

All right. So let's go to the question. Are there additional data or measures that clinical investigators need to provide before future and present clinical trials, i.e., the ones that are on hold, in SCID patients should proceed in the U.S.?

Please consider in your discussion each of the following as they pertain to the XSCID and, as well, other forms of SCID, such as ADA SCID, et cetera.

So then there are six subpoints here: risk-benefit of gene therapy; revisions to informed consent documents; alterations to the cell dose administered; alterations to the vector or the vector dose administered -- sorry -- mapping of the insertion sites on all clinical lots of cells prior to release for clinical use; and then alterations in vector design.

So I've held you guys back all day.

DR. MULLIGAN: I'd like to maybe shift the emphasis to the charge that we have versus the way that you posed it. You posed lots of very interesting things that, you know, those of us who know vectors and transplantation could talk about and talk about, and I'd like to suggest that we talk about what needs to be done as opposed to what is a guide if. That is, the vector types of things that we hear, they are very clever ideas and they make a lot of sense, and good vector people could differ on exactly what's the best feature, but I

would rate those as something that everyone is going to have their own chance to argue about what's the best kind of construct, but is that really on our radar screen at this point?

And I think the key thing that we maybe ought to initially talk about is, you know, quantitation of the risk. I think Stu made a very good point that I always harp on about the fact that it's obvious that if no gene transfer occurs, gene therapy is safe. That's clear.

But, in fact, even at this day when I hear in this session people saying, "Well, you know, I want to show you the safety statistics," I think it sends a very, very bad message.

In fact, I'm getting a message here.

(Laughter.)

DR. MULLIGAN: A very bad message from the point of view of how or, you know, what the consent forms really mean to people, what we're telling people. And I've always thought that that's a big issue, and I think that at the heart of this is that we haven't in the past been able to quantify the risk.

We knew there was one, and after all I've heard here, I don't think we're in any better shape to quantify the risk other than to treat some patients and see what happens.

And so I think that this is the crux of many of the issues that we'll face because I think from the point of view of asking what's the risk-benefit ratio, you need to know what the risk is. From the point of view of modifying the

consent form, you have to know how you're going to represent that risk.

So I would like us to focus on, you know, what we need to do and propose that the risk assessment might be a first topic.

CHAIRMAN SALOMON: Yeah, well, I certainly agree with that. That's the first thing we're going to do. Yes, let's do it. I agree with that, all of your comments.

Dr. Kurtzberg.

DR. KURTZBERG: I was just also going to comment on informed consent and say that I don't think that no matter how earnest you are or how detailed you are or how careful you are you can always transmit the information you are trying to transmit, and I think that when people are faced with a child with a fatal illness and they want hope, which you want to give them, that there are things that you hear and things that you filter out or things that you put more priority on because of hope.

And whether you have a vested interest in this because your child is sick or you have a company or you're a researcher or you're a scientist, we all have conflicts of interest when we make these decisions, and I'm not sure when you have a child with a fatal illness you can really have, you know, an uncomplicated, unbiased informed consent.

CHAIRMAN SALOMON: All right. Let's deal with

risk-benefit first.

Ken and then Dr. Harlan.

DR. CORNETTA: Yeah, I think there are two things that you can get sort of caught up on. One is trying to assess what is the risk for an individual who gets exposed to retroviral gene transfer, and I really don't think anybody here can even approach what that number is.

I think for things that are stated, that many times folks who have been treated never really got gene transfer, but I think relevant to some of the comments we had from folks in the audience who had patients affected with this disease is what is the risk to individuals who are facing alternate treatments, and it speaks to number A up there.

And what we have to try to od is look at what is the risk associated with gene therapy for this disease versus what are the risks for transplants. Now, I'm an adult bone marrow transplant, and I would love to see survival curves like we've seen for SCIDs. I mean, it really is very impressive.

We're also faced now looking at fairly limited numbers for SCID with gene therapy, but actually the numbers there are pretty good, too. It's a terrible thing that's happened to that one individual patient, but if this truly is even a ten percent risk for folks going with that disease, it's comparable or maybe even a little better with folks that are being treated with haploidentical transplantation.

So as a risk, we really now need more time to know is this really a ten percent risk or is this a one percent risk or is it a 50 percent risk, and we don't know that, but I think that's a critical question that relates to how we're trying to sort this out today.

CHAIRMAN SALOMON: Right. So just to make that point really clear, what we've seen today is for transplants not under three and a half months, and, Dr. Buckley, correct me if I get this wrong; we're looking at about 75 percent success rates, and in this case with the gene therapy done in the XSCIDs, and I didn't believe that it was different in the different disease types.

DR. BUCKLEY: Right. That's for the haploidenticals, and again, it depends on which center you're talking about. I mean, there are centers where the survival rate is 48 percent or 47 percent.

So I think, you know, it varies from center to center.

CHAIRMAN SALOMON: Okay. Ken?

DR. CORNETTA: Just one more point. Having been in transplant now for ten, 15 years, it's changed significantly, and I think when you're looking at a large number of patients, I think the standard of care -- we've seen transplant improve over time. So folks, yes, are getting better, but I also think we need to consider that in the context of gene therapy.

These are very early studies, and I think some of the questions that we'll address in the subsequent about cell dosing and about vector we'll also see potentially improvement in that over time, and I think that's just another thing to keep into consideration.

This is a bit of a moving target where we've seen improvement in transplantation. Hopefully we'll continue to see improvements.

CHAIRMAN SALOMON: I think that's a key point you're making. So just trying to stay with the numbers that were presented today, we're talking about 75 percent about -- as Dr. Buckley points out, in some places it might be worse, but in a good center like hers it's 75 percent -- and in this case it's one serious adverse event at three years in 11 patients.

DR. CORNETTA: But two patients did improve, too.

DR. MACKALL: But let me clarify. It's 75 percent survival. It isn't to say that the 75 percent, none of them had adverse events. The Alain Fischer trial had 100 percent survival.

This child hasn't died of this leukemia.

CHAIRMAN SALOMON: Great. Yeah, I mean I agree with that.

DR. MACKALL: So, I mean, I think we can't equate this adverse event with death. I hope he doesn't die, and he may not.

Now, since I have the floor, I'm going to just go ahead and make my point about risk-benefit. Ii come from a little bit of a different world. I'm a pediatric oncologist, and so for a living I give chemotherapy to patients with bad diseases.

And, for instance, Ewing's sarcoma, we have routinely a ten percent incidence of second malignancies after cure of patients with Ewing's sarcoma. The malignancies are treatment induced. They're induced by radiation therapy, and they're induced by the chemotherapy that we administer.

Etoposide is one of the most commonly used chemotherapeutic drugs out there, and it, depending on the schedule and regimen, can induce leukemia in up to five percent of patients.

So this isn't unique to gene therapy, and if we threw out every therapy in cancer that could cause cancer, we'd get rid of some of our most effective therapies.

CHAIRMAN SALOMON: Excellent.

Dr. Harlan and then --

DR. HARLAN: One general comment and then a specific one. The general one is that in clinical trials that we have entertained, we have advised patients about potential toxicities that then when they occur, they are shocked and say they never learned that that was a potential toxicity.

So there's a real -- informed consent is something

that's very easy to say and extremely difficult to really achieve. That's just a general comment.

The specific one is that it's a point that's been made several times. It's that I call it tortoise versus hare research. Tortoise research is stuff that's plodding along and is constantly improving. So informed consent there is also very difficult to achieve because the baselines in both are always changing.

The specific comment though, and it relates to what Crystal just said, is that we know at least what the toxicities are with haploidentical bone marrow transplant in this situation. That's something that we have some data to do.

And it would seem to me we heard from Dr. Kohn that an exclusion criteria is if there's not an HLA identical bone marrow donor.

The specific proposal I would put forth is that an exclusion criteria should be if there's not a haploidentical donor, in which case there's almost always going to be. At least we know what that profile is. Why shouldn't that be the baseline upon which gene therapy proceeds?

CHAIRMAN SALOMON: Okay. So to get that clear, you're taking a very different tact than the others took on risks-benefit. You're saying you're going to make the argument that in haploidentical transplants, we know the set of risks because we've done 300 in the United States and 500 in Europe,

and therefore, you don't buy the fact that the gene therapy has anything to add on top of that. So you'd put all of these on hold.

DR. HARLAN: I think that the approach that hearing -- I mean, the first law of medicine that I'm always going to fall back on is first do no harm. We don't really know the risks of gene therapy. We know the devil of haploidentical bone marrow transplant. So let's start with that, and patients that fail it, for whatever reason, it seems to me, to be the logical -- and we know it's efficacy, which isn't bad based upon what -- I mean, it's not perfect, but it's not bad based upon what Dr. Buckley told us.

And then upon that framework we test the less known therapy.

DR. SORRENTINO: Yeah, I just want to bring up one other point that came up today, too, that I think is very relevant to A, which is the 75 percent of the patients that survive haplo transplant, only half of them recover B cell function.

And while these patients can be treated with IVIG, it's an expensive inconvenience, at the least.

In contrast, the French study, nine out of 11 patients had fairly rapid return of B cell function, which is my understanding of the data. So, you know, one has to keep an open mind about this. I think this is potentially a very

important issue.

The possibility, and the numbers are small, is that gene therapy would be more effective for reconstituting B cell function in haploidentical transplant

DR. HIGH: Well, I just have a related point about analyzing risk-benefit, and I guess I can say along with Dr. Mackall that we're all influenced by our background, and I'm a hematologist who's not a bone marrow transplanter.

But one of the reasons, for example, that we don't do bond marrow transplantation for sickle cell disease generally is because of the up front mortality, and that's a little bit what we're talking about when we're looking at haploidentical transplants. If you say that something like 78 percent of those people survive, so there's a certain fraction of those people who will never have the opportunity to present to Dr. Malech to use gene therapy as a salvage therapy.

So I'm not sure that the right way to think about it is to use gene transfer as a salvage therapy. I mean, if the survival in the gene transfer procedure is 100 percent, then should you think of it the other way around?

MS. LORI KNOWLES: I just have sort of two preliminary questions that I need some information on first in order to look at the risk-benefit ratio analysis. The first was the causal question that you brought up and said that, you know, was going to be necessary for us to but heads on.

I don't know how we can do a risk-benefit analysis until we actually address whether there is -- how we look at that risk in relation to the causal factors with respect to the introduction of the virus. So my in thinking sort of logically through it, I would need to know really what everybody thinks about that first, then to be able to look at the risk-benefit analysis. That's just the first comment.

The second is I would like to know sort of what the quality of life is for the neonate group, the benefit in that case.

DR. BUCKLEY: Let me respond to that. The quality of life for the patients who have been transplanted in the newborn period has been excellent. We don't have any that would fall into Dr. Malech's category over here.

But I would like to make a comment about the either/or type thing. I think that if you're going to say, well, you have to first have an HLA identical transplant or a haploidentical before you're allowed to do gene therapy, then I think you're not going to have the same situation that Dr. Fischer had in Paris where you had such great success because he had de novo gene therapy, and he's not done any gene therapy in patients who have been previously transplanted.

Maybe that's why he's successful, is because he did it in the de novo situation, and so I don't think you can eliminate that as a choice.

I would think that, again, when you're weighing the risk-benefit you'd have to say, well, suppose you do a haploidentical and it doesn't work out and the child has chronic disease. Then you do the gene therapy, and maybe the gene therapy won't work either because the child has a damaged thymus or some other problem.

But I just think that we should not ignore the possibility of putting in this array of choices here de novo gene therapy.

CHAIRMAN SALOMON: The question I have back to you is you're clearly one of the world's experts on doing bone marrow transplants in this group of patients. So if you had unlimited resources to create a center, the World Center for SCID Care, to the extent that you haven't already created it, by the way, at Duke with your own work --

(Laughter.)

CHAIRMAN SALOMON: -- but if you had the World Center, what would you do? Would you just continue doing your bone marrow or would you bring Dr. Fischer because now you've got all the money in the world and everyone will come to you?

So would Dr. Fischer dome and will all of these guys come and we'll all be doing stem cell gene therapy there right next to your bone marrow transplants?

DR. BUCKLEY: If Dr. Fischer wants to move to Duke, that would be wonderful.

No, I just think that the reason that he was able to go forward so well is the fact that he was not confined by having to do this in a previously transplanted patient, and I think that we should not ignore that fact.

And I think that, you know, he's had this adverse event, but he's also had 100 percent survival rate, and he has patients who don't take IVIG, and so I think it's very impressive what he's done.

CHAIRMAN SALOMON: Ms. Ballard.

DR. NOGUCHI: Excuse me.

CHAIRMAN SALOMON: Oh, I'm sorry.

DR. NOGUCHI: At this point what I'd like to do is just remind people of -- I'll paraphrase some of the FDA regulations concerning clinical trials because it is at the heart of what we're discussing here.

As a physician, Dr. Harlan is absolutely right. First do no harm. But the clinical trial involves human subjects in which the outcome is not predicted and it cannot be known, and in fact, it is the mechanism by which we as a society and worldwide have determined that where things cannot be known a priori, there is a set of conditions under which experiments are taking place with human subjects who may also happen to be patients.

This is one of the critical internal conflicts that we as physicians and as scientists have in that as

physicians we want to do the best and not do any harm, but doing a clinical experiment is to find out what are the risks, what are the potential risks, what are the potential benefits.

In that context of a clinical trial in which the patients are patients, but in the context of their trial, they are human subjects participating in an experiment to answer the question: does this therapy work or does it not work? If it does, what are the risks involved with that?

And it is really an unfortunate, but a very central issue here. Are we talking about medical treatment? Are we talking about clinical trials?

CHAIRMAN SALOMON: Ms. Mallard.

MS. BALLARD: When you talk about risk versus benefit, there is no risk free therapy at this time, no matter whether you're talking about a bone marrow transplant, gene therapy, the haploidentical transplant, a chemotherapy transplant. There's risk in all of them.

Yes, we need to know the measure of risk. If there's any way possible to give the information to the families at the time they decide on a therapy, if there's a way to give some reasonable measure, but the family needs to decide which risk they wish to take on versus the success rate of the therapies also.

CHAIRMAN SALOMON: Dr. Kurtzberg and then Butch and then Dr. Harlan.

DR. KURTZBERG: I have two comments. One is that when you talk where the risk of the disease was already one in 100,000, when you quote a risk of ten percent, one percent or 99 percent, it has a very different meaning to them than the disease that they already acquired, which had a much less likely risk, but here they are with it. So that's the first.

Two, I would like to put forward to the FDA that do not harm can also -- you can be doing harm when you withhold promising treatment, and if you don't test in a responsible way promising treatment, you don't make progress, and if you have a disease that is already doing considerable harm, that should weigh into the equation, and I don't think do no harm by itself carries a compelling argument.

DR. TSIATIS: As a statistician, I've been trying to think about some of the comparisons that are being made, and I'm trying to decide if we really have evidence that the risk for gene therapy right now is worse than the risk of some of the other methods of treatments, like, you know, the bone transplantation.

And I'm not clear. I don't feel like I have the correct numbers in order to make that assessment. For one thing, when one is assessing risk, you don't just look at the number of events over the number of people. Really what's important is how many person-years of exposure has there been on a particular treatment and how many events were seen during

those person-years.

And certainly there's a lot less of that with the gene therapy than there is with some of the other modalities of treatment, but unless I could actually see those numbers in comparison to each other to make the judgment that it is much worse right now, at least from a statistical point of view I don't feel I see necessarily that it is much worse risk right now than other methods.

DR. HARLAN: Do my comment is I don't disagree with anything that's been said, and I think the ideal solution would be to get truly informed consent, but what plagues me is what I described earlier, that patients don't necessarily hear it, but also the statistic that everyone should know is that if a town has one surgeon, there will be 1X gall bladders taken out, and if it gets two surgeons, now there's going to be 2X gall bladders taken out, and the population as a whole does just as well.

So the gist of what I'm trying to say is I'll bet you that the patients in France sort of know that bone marrow transplant is an option, but what they hear is this gene therapy is probably better, and so that informed consent process gets very murky.

And I agree with what Dr. Kurtzberg said about that we need to take risk to advance therapy, but when we know a therapy and we know something about the risks of a therapy, I

think that we should always start with that first.

That's the only point I wanted to make.

DR. COFFIN: I wanted to get back to the denominator issues. Actually my friends sometimes call me Dr. Denominator because I always ask questions like this.

But it was mentioned that we can't know the number or easily know the number of person-years in gene therapy, but I think that's -- what I would like to know if we can know is the number of person years that are engaged in this particular trial just to begin to get a handle on at least the local risks right here.

Because I don't think gene therapy overall is at all relevant to the particular case we're discussing for the reasons that have been -- you know, when you don't have genes inserted, they're very safe. We can all agree with that probably.

So what is the number of patients and number of years of total experience in this particular trial that we're discussing? Do we have that information?

CHAIRMAN SALOMON: Well, just roughly from the data we got that we were presented, there were 11 patients, and probably the mean follow-up on that 11 is a year and a half because the longest is about three-some years.

DR. COFFIN: Fifteen years or so.

CHAIRMAN SALOMON: Yeah.

DR. COFFIN: So we have one adverse event of this sort in basically 15 patient-years.

CHAIRMAN SALOMON: Right, and then that would be --

DR. COFFIN: So far.

CHAIRMAN SALOMON: Yeah, and there I think we already pointed out one of the things that makes it really hard is that I was showing mortality data that Dr. Mackall pointed out was an error. I was actually not making an error. I was saying it would even be more dramatic if you didn't take it that far, but unfortunately that's kind of where we're at.

In terms of years? I have no idea how to do that. Dr. Buckley, you'd have to help me with that. I mean, probably looking at 20 years with maybe a mean follow-up of five to ten years in 300 patients?

DR. BUCKLEY: It was first done in 1968, but it really didn't take off until the early 1980s when T cell depletion techniques came out because before 1981, unless you had an HLA identical, they all died.

So I think probably the comparison would be with everything from 1981 on.

CHAIRMAN SALOMON: And of course, the weakness there, John, is those are mortality data obviously, and the point we've already made is that it's 100 percent survival in the gene therapy trial, but we're talking about a serious

adverse event, and we really don't know what the quality of life is for the 300 kids at five years, let's say, that were taking as the mean follow-up. So it's a pretty tough comparison.

DR. COFFIN: The other concern here is it's not like a chronic toxicity in an ongoing drug treatment. I mean, this is potentially a very heterogeneous disease. The question is: are there more of these? Are there more sort of smoking guns waiting among these other kids to show up in future years?

And so it's a very different thing to come to grips with in many other sort of risk-benefit analyses. We don't know that this is the final story on this particular set of patients, and we won't know that for a great many years really.

DR. BUCKLEY: Can I just comment relative to that?

You know, I think your point is very well taken because even though we followed patients at my institution for 20 years, you saw the graph that the first speaker had showing the decline in Trks. We don't know whether these people are going to have a normal life expectancy or not. I think only time will tell that.

And if there's a more effective therapy that will last their lifetime, then certainly we should try to find that.

CHAIRMAN SALOMON: Dr. Allan.

DR. ALLAN: I think as Dan knows, I'm not a transplanter. So I'll just make that clear in the beginning.

I'm not a gene transfer person either.

I would sort of reiterate what John said, which is, you know, we were brought in here to sort of like give you some advice, and the data is just getting out there and has just happened, and so it's very difficult to make some sense out of what to do and how to proceed because there's not a lot of data.

I mean, there's a tremendous amount of data that was presented this morning. I was overwhelmed by how much data was presented, but still, I mean, you've got one in 11 kids that has what looks like leukemia, and you don't know -- I don't know how effective chemotherapy is on bone marrow transplant patients, and I'm sure other people do, but so you don't know if it's going to be 11 out of 11 down the road. You don't know if it's age related, and that's one of the things we sort of touched on this morning.

So you don't know if it's like the young they are, the greater at risk they are for getting some sort of leukemia. You also don't know if doing gene transfer into bone marrow has, let's say, a greater risk than if you're using peripheral T cells because I don't know if there are any peripheral T cell lymphomas or leukemias in the early studies that were done with gene transfer.

So, I mean, you've got a bunch of unknowns. I mean, these could potentially be risk factors, but I don't know that they are.

I mean, the age thing is certainly important, but you also have to consider, you know, as other people mentioned in X-linked SCID is that that there aren't other treatments if the haploidentical bone marrow transplant fails.

And so, I mean, I think it's a very difficult process, but it's not one that you can simply say, "Well, gee, we should just go forward because, you know, it's one out of ten and it looks like that's the way it is."

And, on the other hand, it's like, well, are you going to shut down all of the clinical trials because of one leukemic patient.

DR. NOGUCHI: Welcome to my world.

(Laughter.)

DR. ALLAN: I'm basically saying, I mean, we're still in the same boat we were before we showed up today, except we have a lot more data.

DR. NOGUCHI: I don't mean to be facetious, but you will notice that we did not talk about any risk-benefit ratio. In fact, for biologics and for rare diseases, each human subject who happens to also be a patient that's entered increases the knowledge base. It is very small. It is very incremental, but it is constantly changing.

You have potential benefits. You have potential risk. What we know today is not what we'll know tomorrow, but, yes, that is exactly what we're seeking advice on and to air

this in public so that people understand that this is not a trivial issue.

CHAIRMAN SALOMON: Okay. Rich, Bruce, and Stuart, were you wanting to? If you can turn yours off, I'll get to you.

DR. MULLIGAN: Well, I was going to talk on what Phil just said, and it's interesting that we're very happy that we have some better statistics about bone marrow transplantation as a form of therapy. And I wonder 15 years ago or so when Rebecca was, you know, going through developing those methods why that would be different than it is at this point. That is, you know, we do clinical trials in the way that Phil talks about them to get statistics about whether things are safe or not safe, and I don't know how -- well, I would say I don't think we will be able to quantify the risk in any other way than doing proper clinical trials to get those.

And if we all agree that having the marrow transplantation statistics are very valuable because we have something to compare to, then why wouldn't this be a good way to get the statistics to do the comparison?

DR. TORBETT: I guess, I feel somewhat the same way. The question here is how high the hurdle will be. How high do we want to set this hurdle before we go on?

And hearing all of the data and listening to Dr. Denominator, it's hard to figure out where to set that bar, and

I guess that's what's being asked. Looking back over Dr. Buckley's past history, again, if the bar would have been set exceptionally high, we probably wouldn't be having this conversation now and thee patients would be dead.

So I'm waffling back and forth, and I guess I'm trying to get a sense of where to set that bar, and it's just not altogether clear.

DR. BLAZAR: As a bone marrow transplanter, the way I would look at this is if I had an HLA identical sibling donor, I would probably move to that because, just to be concrete about this, the statistics there are quite good, and we can deal with many, but not all, of the problems.

For the HLA nonidentical, despite Dr. Buckley's impressive data, most of us proceed with caution under those circumstances.

The gene therapy approach is also being pursued with caution, and I think the concern I have is really the long term propensity and likelihood that some of the other clones in these patients may become leukemogenic. We don't know that, but I don't see, just you know looking at this as a parent and as someone who does bone marrow transplant, a very large difference in the risk between gene therapy and haploidentical bone marrow transplant worldwide.

I do see a difference with HLA identical transplant, just to put this out there on the floor, and I would

feel less enamored with going ahead with that if I had an HLA identical sibling. But I would feel perfectly similar in the risk-benefit ratio of gene therapy at this point versus a haploidentical transplant.

And if I had the option and it was a newborn, I would probably try the gene therapy approach with some trepidation, as I would with haploidentical transplant, having some trepidation.

DR. ORKIN: I think I've given some thought to what the risk is, and I think I'll be exactly where John is. We don't know the risk. I don't think we're going to be able to figure out the risk no matter how long we sit here today.

My gut feeling is the risk is probably higher than we thought it was, but what we thought was is probably different for everybody in the room.

But actually as I just scan back to what was said in the report seven years ago, we basically said -- and I think it still holds, and I won't take any credit for writing this because the report was written by a whole slew of people -- because the clinical experience is so limited it's impossible to exclude long term adverse effects and only longitudinal clinical studies will actually show that.

And so I think the comparison also with chemotherapy is very accurate. Any chemotherapy trial is fraught with many adverse events, probably a higher frequency,

and we don't know what the cumulative frequency events will be here, but probably the only prudent thing to do is watch carefully and do what can be done to extract the most information from the single case and any cases that come in the future.

DR. KURTZBERG: I agree with that and would go further to say that there shouldn't be any reason that you couldn't come away from this defining stopping rules like you would for any other clinical trial, which would, you know, if so many cases and so much time develop this complication, that's too much and then we stop and we go back to the lab.

And I think that's where this should go right now.

DR. ORKIN: On to the next case.

MR. BLAZAR: I want to put Joanne on the spot and ask you. Joanne, I wanted to ask you what you would do with those different scenarios if you had an HLA identical sibling versus a haploidentical versus gene therapy, just to get another.

DR. KURTZBERG: Well, I would go with the HLA identical sibling first. I think that one is the easiest call to make.

Between gene therapy and a haplo, I would also throw cord blood into the equation, and I think all of them are unknown and equally either potentially beneficial or risky and should be studied.

And I think as long as the rules are well designed and the studies are well designed, that they should all be pursued at this point. I think personally I'd probably go with the cord blood, but that's what I do.

I'd probably do gene therapy before I'd do a haplo.

DR. COFFIN: I think clearly, despite all of my misgivings about what the future will hold for the other treated patients, there's going to be some class of patients for whom the present vector strategy will probably offer the best balance between these, and I think it's up to the people who have the real clinical experience who probably can make the best judgments on this.

But going forward, I think it's going to be very important to start very seriously developing preclinical models rather than relying on the clinical outcomes to tell us that. There's a good possibility for developing preclinical models to tel whether, you know, these self-inactivating vectors which sound so great on paper but really require a test before we can, you know, say this comes through.

I can visualize mouse models that will actually not be that difficult probably to develop that would give us a very good handle on what the potential for certain vector strategies is for activating adjacent genes, and I think should be really started to work on posthaste for all gene therapy, but

particularly for this particular case, and perhaps for any other successful gene therapy, is going to run into the same kind of issue as least when retroviral vectors are involved.

So I think we should start very quickly on getting that kind of thing going. But I think also there will be some class of patients that will be obviously suitable for continuation of gene therapy with the current status.

CHAIRMAN SALOMON: We're getting close. There's a group of people who want to talk. Let's do it: Barbara, Butch, Ken, and David.

Okay. We can go in that order.

MS. BALLARD: First, I'd like to ask him to ask that question to me about choosing what therapy to use because I'm the parent here. And one of the questions, I know Dr. Kurtzberg is about to leave. Actually I'd like to ask her if she'd listen a minute.

In your cord blood comment, ADA deficiency SCID, chemotherapy is not a very good option in most cases of ADA deficiency SCID. The statistics have been very poor of them surviving chemotherapy because it tends to do too much damage to the liver.

Do you still consider cord blood an option for an ADA deficient SCID?

DR. KURTZBERG: Yeah. I think in this ere of transplantation, if you measure B sulfan (phonetic) levels and

you do the right supportive care and you get a child early, before they have five infections and are colonized with aspergillus and viruses, that, yes, those children can do well.

I think the trick is to do due diligence with supportive care and also to get these kids early, before they have a lot of organ damage from infection.

MS. BALLARD: I'm not talking organ damage. I'm talking the ADA deficient SCID specifically without the ADA enzyme leaves the liver unprotected. I only know of one ADA deficient SCID ever to have survived chemotherapy.

DR. KURTZBERG: Well, I don't think your statistics are accurate. There are more than one, and it can be done, and there are choices for chemotherapy now, and there are levels that can be followed, and with supportive care, it is definitely possible, and we've done it and so have other centers.

MS. BALLARD: Okay. But then back to your question of how to choose. Obviously if there's an HLA identical sibling, that's the optimal situation, but unfortunately in many of these cases that's just not an option.

Between the gene therapy and haploidentical, right now the statistics are looking better for gene therapy. That still has to be a very personal decision. You have to decide do you wish to be part of a clinical trial, and as such, as a parent, you become part of that clinical trial yourself, and not

everyone is going to opt to do that just because there are ten kids with B cell function who are doing wonderfully.

It's a commitment to a long term trial, and the parents do have to understand that, but they do understand that, and that's the thing that you've got to understand and give the parents some credit. They do understand when they go into these trials, and many will choose not to. Many will choose to go with what is the standard therapy, but the option needs to be there.

DR. BLAZAR: I think we're actually saying the same thing. I don't disagree with anything you said. I was just trying to put myself in this place, which of course is impossible, but I don't see a major difference in right now what we know about the risk between haploidentical and gene therapy, and then it becomes a very personal decision.

MS. BALLARD: Right. I agree. I think there's risk to every therapy available right now.

CHAIRMAN SALOMON: I think we're getting close to where we need to be.

Butch, Ken, and David.

DR. TSIATIS: As I listen to the clinicians, there seems to be real uncertainty between the use of haploidentical transplants and gene therapy, and I wonder if anybody has given any consideration to doing a randomized study, and then we could put up front the risk and benefits.

CHAIRMAN SALOMON: Well, I mean, there's always good things to be said about randomized studies, provided that you can coordinate them properly and get large enough numbers, but you'd be the one to tell us that.

I mean, I don't know that that's necessarily -- we're not in that position at the moment.

DR. COFFIN: When we present a study, whether to do a randomized study, I think the issue -- and I'd like to hear what other people say -- is that the number of patients we're talking about per year in the United States would mean that a randomized study would take many, many years before we'd be able to actually make a conclusion.

CHAIRMAN SALOMON: Yeah, I think we know the answer to that. I mean, that's what will happen.

David and then Ken.

DR. HARLAN: Yeah, I just would like -- I made the modest proposal earlier about the haploidentical, and Bruce's question to Joanne, I think, made me change my position.

I understood from the presentation that the haploidentical prognosis was better. If the Duke team itself, with the best statistics, feels that it's a fair tradeoff, then I get back to your question. The ideal situation is to bring Dr. Fischer to Duke and let patients decide.

Short of that, it sounds fair to me.

CHAIRMAN SALOMON: Ken.

DR. CORNETTA: Maybe just to talk a little bit as we talked before, Ms. Ballard, I think the hardest thing for a patient perspective and from a family perspective is being faced with a potentially lethal disease and not having any choice, and when you have two modalities that there is not clear what may be better or the other, empowering the families to be able to make that choice is also an important thing, too.

So as we deliberate that, we need to keep that consideration.

CHAIRMAN SALOMON: So if I -- I mean, I tried to just let everyone go on this because that's very appropriate, and I think just the natural flow of this brings me to what I think we could consider now as a consensus, and here I could get myself into big trouble, but that's why I guess I get paid the big bucks for up here.

(Laughter.)

CHAIRMAN SALOMON: So the -- just kidding, just kidding. I don't get paid.

So under the thing of consideration of risk-benefit of gene therapy versus alternative therapies, I think that really it's obvious to everyone here that what the FDA wanted expert advice on this first question was should they put all of these skid trials on hold and keep them on hold until, you know, such-and-such information that we would uncover in our brilliance today would therefore allow them to come off whole.

And I haven't heard that. What I've heard from this group is that this was an absolutely clear serious adverse event, and all of us are scared about it and all of us are aware of the fact that it has implications for the safety of this whole thing.

However, one adverse event, serious as it is, in the context of the whole field as we've refined it together in our presentations and our discussions -- I won't try and articulate that again -- is not enough to advise the FDA to put all of these programs on hold.

It is enough perhaps, and we'll go there now, to talk about the consents. It's definitely enough to talk about, you know, specifics about whether or not we could refine the safety profiles for these program to go forward.

But just for Point A, can we say that there's a consensus here that these trials as proposed and as we modify them in the next hour, it's okay to take them off the hold and go forward?

DR. WOLFF: I'd like to make a comment.

CHAIRMAN SALOMON: Please.

DR. WOLFF: You could say to have them go on, but as someone else had mentioned earlier, how many events have to occur before one feels that it should stop and should we establish something like that now?

CHAIRMAN SALOMON: Yeah, I think we should discuss

that. I'm just saying I think the first key point is that this serious adverse event doesn't pull a trigger on all of these gene trials. I mean, I don't think it does. I haven't heard anyone, when the final discussions rolled around, that I heard anyone at this table saying they thought it did, and that's the first key thing we're here to do, is to give advice on that point.

Alison.

MS. LAWTON: I think I agree with you, but I think that in saying that it should be allowed to go forward you have to consider all of the other things that you will do at the same time. For example, the inclusion and exclusion criteria, the informed consent.

So with that caveat, then I agree with your statement.

CHAIRMAN SALOMON: Then let's agree that that's the advice we're giving right this second and go on to talk about the informed consent and some of the details. And then I think to be fair and actually address Alison's point here, when we're doing, we'll go back and I'll ask the same question again and see whether someone is so unsatisfied with the resolution of that that they would change that.

Is that fair enough? Okay.

DR. NOGUCHI: Excuse me, Dan. Thank you for getting us to this point. It's remarkable.

I would like to follow up. I think it was Dr. Denominator, our good colleague, John Coffin. In doing this I think the statement has been made or implied that part of the reason to focus on these trials is, in fact, this is a first reproducible, demonstrable evidence of gene transfer that has an effect.

And comparing that to the other trials is not really even mixing apples and oranges. It's almost like shoes and apples. And if we could get a little bit of a consensus on how people feel about other trials vis-a-vis safety profiles for this particular trial, that is, does the fact that we have 150-odd trials that have had retroviral vectors not in SCID trials -- how much bearing does that have if, in fact, the level of gene transfer is not very high compared to what we see in SCID?

CHAIRMAN SALOMON: It's funny because we've been arguing for two weeks about how to put that into this agenda.

I mean, personally I don't think that this should -- again, this serious adverse event needs to get integrated into -- this is my opinion now -- needs to get integrated into the informed consents of all retroviral gene therapy trials. Is there anybody at this table after this discussion that would disagree with that?

The reality, as we've been told, and certainly I'm personally aware of this, is that we generally do just that. I mean, any retroviral gene therapy trial consent form that I've

ever seen or written basically acknowledges the possibility of insertional mutagenesis.

So that I think the next step really is probably this is not going to involve any significant rewriting of extant consent forms or ones in the future. I think the real issue is one posed in a more real world, and that is how do we get this out to all investigators to highlight that they need to go back to these people and say, "Hey, there was an adverse event. It was due to insertional mutagenesis. You need to be extra specially aware of that, not just that it's, you know, a line buried in acres of text in a typical consent."

Does anybody disagree with that?

Richard?

DR. BLAZAR: I think that Phil may be trying to weasel out of us whether we want to have an opinion of what all the other clinical trials have to offer us in terms of safety, and I'm happy to, you know, harp on this and say that my opinion is it has not really given us virtually any information other than the other parts, the least important parts of gene therapy, like can you use sterilized bags for your transplantation.

That is, you know, obviously those are the things that you have to do in a proper fashion, but I would just say that I think the answer is we might want to come up with some opinion about whether we think now in retrospect how the data that we've had in the past really has helped us and whether

we've, in fact, gotten that across even in the consent document.

I mean, I've never seen a consent document that actually has said that, well, there's been no reported serious adverse toxic or deaths from gene therapy, but, in fact, many in the field think that there's been so little gene transfer that we would have detected it. That's usually the missing piece.

CHAIRMAN SALOMON: That's a good point. Yeah?

DR. SORRENTINO: I just want to make two comments. One is about the efficiency of gene transfer. You know, I think it's important to note that in the case of SCIDs what we're really seeing is very high levels of gene transfer due to this tremendous selective advantage that's in the T cell compartment.

In fact, the level of stem cell transduction as reflected in the myeloid series is actually very low. So it's not really that gene transfer has gotten so much better and so much more efficient. It's the biological nature of this disease.

And to address, you know, the point that Phil brought up, which is what about the other gene therapy applications, you know, we've discussed this extensively at St. Jude, and in many ways you can really think of two major categories: clinical gene transfer trials where the transgene is intended or proven to be therapeutic, and then there's the second class that, you know, we have talked about quite a bit,

and it is a very tough issue, and that's marking trials where retroviral vectors are used to follow the biology of a cell population in a patient with the intent to derive a better therapy for that group of patients in which the individual patient cannot derive any benefit.

And I would argue that this is a very important issue, nd I'd be interested in what others here think about now with this new information from France. How does that impact marking studies?

CHAIRMAN SALOMON: Okay. But I think we need to finish with this particular agenda, and I totally agree that some discussion of this is very appropriate and, I think, necessary before we leave here this evening.

Kathy.

MS. KATHY KNOWLES: Is it appropriate to start talking about informed consents now?

CHAIRMAN SALOMON: Yes.

MS. KATHY KNOWLES: Okay. Several items. Gail has got an article from, I think, one of our Seattle papers about I believe it was the National Academy of Sciences is now moving forward in terms -- you have a series of articles. I think Rosanna gave them to you. If you could please pass those out.

A very large institution, unnamed but many of you know who it is, has been cited by a number of national agencies

for a lack of informed consent problems extending over an 18 to 20 year period of time.

This was a real problem, and in fact, the institution is being sued by its patients, actually the families whose patients actually died.

Secondly, in terms of informed consents relevant to this particular issue, these need to be complete. They need to be accurate. They need to be written in an understandable language and give full disclosure to both the potential positive and the potential negative outcomes.

And I also agree that other informed consent for other retroviral clinical trials should contain language relevant to cancer as well.

And that's it for the moment.

CHAIRMAN SALOMON: So I think that what we need to do is come to some sort of a consensus about what we want to recommend to the FDA to do about consent forms in the existing SCID trials that are on hold and also on all -- I think it's fair to talk about this in the context of all retroviral gene therapies trials.

I think one of the things that I want to see incorporated in an advice to you is something that on "Front Line" Mr. Gelsinger had a -- one of the key things that they came out with on really a superb "Front Line" show on the whole incident with his son was a point where he went to an NIH

conference in the Natcher, where everyone at this table has been at one time or the other, and having gone in there thinking that he was told that there was like a 50 percent response rate, I believe was the line, when the scientists got up and started talking, it suddenly became, well, it's statistically not significant and all of that.

And he's going, "Where in the hell is this 50 percent response rate?"

So I think that to the extent that there's anything in these consent forms, coming back to what Rich Mulligan has been talking about, if there's anything in these consent forms talking about how safe retroviral gene therapy is, that needs to come out. I mean that's my personal opinion, and others on the committee should engage me on that.

And that there should be a very specific reporting to the FDA that we have shared this thing, this particular case. There is a cancer that's possibly, probably, however the wording wants to be, and then all of these consent forms be looked at until and everything put on hold until you can at least show that these things have been changed in the consent form, informed consent process.

Jon.

DR. ALLAN: As a clarification, when you're talking about informed consent and whether to add, you know, the possibility of cancer risk, I mean, to me I don't know how

many gene therapy trials are involving bone marrow, gene transfer into bone marrow. Maybe you can tell me, but because I mean, I'm sure it's not just for SCIDs.

DR. NOGUCHI: We think at the present time it's roughly 50.

DR. ALLAN: So I wouldn't be averse to putting in the very defined wording that says, you know, there's an adverse clinical event or whatever. In the informed consent it says, you know, you have the risk of getting cancer from this.

You know, I don't have a problem with that.

DR. NOGUCHI: Just to clarify, part of this we already anticipated and part of the reason for waiting until today's discussion is to get some better understanding of exactly what we're talking about here.

I think it's very clear that gene transfer in XSCID is associated with an adverse event that is related to the treatment.

Now, that's a very powerful statement about what the reality is, and we do feel that's information that all individuals who are participating in retroviral vector trials should know. Again, we focused on first the trials most closely related to Dr. Fischer's trial, and again, with the idea that those who are most closely affected here at first is what Dr. Fischer had requested.

The general population, yes, we were planning to

do that, but we would certainly appreciate help in how you think we might phrase this to the broader category of retroviral vector clinical trials.

DR. ALLAN: Well, that's why I was getting more to the point, which was like how many bone marrow clinical trials are going on that involve gene transfer? Because, you know, we don't know whether it's only in this one SCID patient or whether it has something to do with bone marrow transduction or what.

CHAIRMAN SALOMON: Bruce, Rich, and Ken.

DR. TORBETT: Having not gone through bone marrow transplantation and perhaps some of my colleagues can share with me, in the consent form for the more serious cases, are all of these kinds of problems, such as graph versus host, all explicitly stated such that an individual knows that there's a tremendous risk for the treatment?

And perhaps with gene therapy there should be a similar kind of -- now that there is an adverse event -- a very similar kind of informed consent.

DR. BLAZAR: We do a long process of education. There are referring physicians. There are tapes sent. There are nurse coordinators that meet independently of the physicians to make sure that there is as much ability to transmit the information without having the direct care involved.

And after all of those are said and done, then we have two sets of conferences, a conference on the out patient, a

conference on the in patient, and the forms are typically ten to 12 pages long, and we go through each of them in extraordinary detail to the point where people really don't want us to have gone into those kind of statistics, but we do anyway.

So it's a very long process of education that begins all the way from the referring physician, and it's not done emergently. It's done several weeks before the patient might be transplanted.

DR. MACKALL: I mean, just to add to that, in a form such as this, and it's appropriate, we focus very much on the document. But the document is really just a document, and it amounts to a laundry list of things that can happen.

A good physician sits down and looks a patient in the eye and say, "This is what's likely. This is what really concerns us."

And so sort of the dictum is informed consent is a process. It's not a piece of paper, but just by necessity we end up focusing on the document in the official realm.

DR. BLAZAR: I think earlier maybe Lori asked the question or made the point that in the risk assessment you really do have to have a sense of what was the relationship between the insertion, and I think for the consent document the most powerful thing is to have an opinion from us about that answer.

And I would propose that it's something not like

may have been associated with, but probably contributed to. And I think that would go a long way in getting the point across.

DR. CORNETTA: I guess maybe just to get a consensus here about whether we feel that what's been presented today is definitive evidence that this has caused this T cell outgrowth, or do we feel that the data, while I think it's fairly compelling, is still at the point where it is probably related or some other terminology? Have we really come to some consensus whether this is really cause and effect at this point?

CHAIRMAN SALOMON: Well, as I said in my introductory comments, I presumed that we would have to butt heads on this one. So I believe that you can always come up with another explanation. There's a lot of IQ points sitting around in here in this room today, but I believe that until I see some other data, that this was caused by the insertion of the vector. That's my sense. It is causal. That's my sense of it.

DR. RAO: Would you at least say it's one hit in a two-hit process?

CHAIRMAN SALOMON: Oh, yeah, absolutely, and I think it was very clear from the biology of T cell leukemias and LMO2 in hematopoietic malignancies that it's not a one hit process. So absolutely. We discussed that.

DR. COFFIN: As I said before though, don't take any comfort from the fact that it's not a one-hit process. Even

if it's not genetically a one-hit process, it can be in practice a one-hit process where that one hit is enough to initiate the process even if more hits have to happen along the way. They inevitably will in many cases. So don't hide behind that.

DR. RAO: It's how you write the consent form. You have to be accurate, and that's all I'm trying to say.

DR. COFFIN: I would write it in a way that left that issue out. Not lying about it. I just would not bring it up in a consent form.

DR. MACKALL: I don't think a patient really understands the two-hit hypothesis. I mean, the point is if they didn't have gene therapy, they wouldn't have gotten the leukemia.

PARTICIPANT: I agree.

DR. COFFIN: My concern also would be that the physician would misunderstand that and use that to lessen the impact.

CHAIRMAN SALOMON: Dr. Kalle, Dr. Baum, do you guys want to opine on this? Did this do it or not? How would you craft your sentence?

DR. KALLE: Why, I think the scientific discussion and the discussion what the patient consent form should contain may be erring on two different sides where I would feel for myself it would be perfectly okay to err in the patient consent form to what's saying, yes, the gene transfer caused the

leukemia in this case for all we know today.

Whereas the scientific accuracy would, of course, demand us to be much more strict. So I think in the patient consent it should be aired on the side that this is very probably one of the contributing causes, but that would be my personal opinion.

CHAIRMAN SALOMON: Dr. Baum, do you want to comment on this, please?

DR. BAUM: So yesterday I was writing such a comment in a patient informed consent, and it said there is a risk and we cannot say the frequency right now. We cannot predict the frequency, and we have to balance this against the other issues.

So it has to be fairly open, and for this specific disease under consideration here, I would say like the other people in the panel that we should go for gene therapy only if there is no HLA matched donor available and if the patient agrees or finds that the risk of gene therapy may be lower than that of a haploidentical transplantation.

CHAIRMAN SALOMON: Okay. So, yes, Dr. Malech.

DR. MALECH: I would emphasize also the important point between what you tell a patient and what you believe scientifically, and I really feel that what you really can say today is that we can find no other reason than the gene therapy for what caused this.

Therefore, what you have to tell the patient is that X number of patients in the world have been treated with this, and one got a leukemia, and right now it could be higher than -- what is it? -- eight percent, but right now we know that at least eight percent of people, meaning one out of X number, got leukemia and that we cannot rule out the gene therapy. And it's got to be as straightforward as that.

The other comment that I would make is that one of the things that has been driving me crazy at NIH, but is actually a good thing is that various IRBs -- and we have multiple IRBs at NIH -- are starting to come. It used to be that every institute's IRB would have sort of different ways that it would ask people to say things.

And now what's happening is that at least some parts of the document are becoming dictated. Now, they may not be the best language, but at least everybody is saying exactly the same words. It isn't the whole consent, but little portions, paragraphs, things.

And I would say that this issue today is so fraught that I would almost like to see some consensus of what's said and that everyone says exactly the same thing, at least, you know, what is said around it.

And I think that's a key point here.

CHAIRMAN SALOMON: Yeah, I have no problem with what you said except one major issue, and you're welcome to come

back at me with it. I liked the way you put it, but I don't like this numbers game. I mean, you know, if it's eight percent. I don't think that that belongs in here.

It's a serious adverse event. It happened. That's it, because we have no idea what the numbers are. Because then someone else is going to say, okay, sure, in the 11 SCIDs.

Then I'm going to say, well, but it wasn't going to happen to my T cell gene therapy patients, or someone else will say, "But you know, we did 300 T cell gene therapy marking studies and never happened."

Do you know what I'm saying? I just don't like the numbers part.

DR. MALECH: I think there's two issues here about that. Number one is that my experience with patients is that they want to be given two things. They want to be given some kind of real numbers because they don't understand statistics.

And then they want to know what would you do, and obviously in the "what would you do" is always a fraught thing, but at least we can take care of the numbers issue.

And I'm not dictating a specific way to say it.

CHAIRMAN SALOMON: Can I make my personal comment about numbers issues that I do in my own practice of organ transplant patients now is it's 100 percent if it's you and it's zero percent if it isn't? So it's one patient.

DR. MALECH: I don't think we're saying different things. I feel that whatever we do, whatever the consensus is, it would be a good idea if we all had exactly the same thing, whatever that consensus.

DR. CORNETTA: I guess from the numbers, it is when you tell a patient eight percent, they're trying to figure out that, but describing a study where you say there were 11 patients treated and one of them developed a leukemia, that is something that's a fact that they can deal with, and that's probably the most informative thing for them, and that may be something that could be a suggestion to be included.

DR. NOGUCHI: I'd just point out that we don't want to be in a position where we as professionals somehow don't see the patients and the patient's representatives. We have them here.

What would you like to hear?

MS. BALLARD: I agree with what he was saying, mentioning that out of 11 cases there has been the one case that developed leukemia. I think that's the most -- it's the easiest way for a parent to take a grasp of what the reality is.

DR. ALLAN: The thing that sort of bothers me a little bit because, I mean, when I hear consent form, you know, my worry is that if you're a clinician, you're trying to recruit patients or human subjects for this clinical trial, and it is a clinical trial. You know, if you say, you know, there's a

chance this is going to give you cancer, you know, it's documented. There's an association that's directly related to the use of this vector in leukemia in one out of 11 children.

At this point we don't know if it's going to be higher. It may be a lot lower, but we don't know if it's going to be higher, and if you can present it that way to a patient, then they'll have the informed consent, but if you go, "Well, you know, there was one out of 11 that got leukemia. We don't know," you start to minimize the serious nature of the fact that one of these kids has leukemia from this retroviral vector.

DR. BLAZAR: I mean, I would echo that, and when Harry -- the way he put it, he said in a way, again, out of all these patients one had it, and I think is what Jon was talking about, about the couching this in terms of the number of hits or whatever. I think it should be as potent and direct a pitch as possible.

CHAIRMAN SALOMON: Yeah. I mean, I'm pushing for that as well.

I mean, I think the message you're getting from everybody, Phil, is that if -- I know certainly I'm thinking personally now -- if someone is going to come back later and say, "You guys had this meeting. You gave the advice to the FDA it's okay to continue with the SCID trials. It's okay to continue with the other retroviral trials," and then whatever happens down the road -- and I'm in a position where someone

from the public, someone's father, mother, brother, whatever, gets up and says, "Well, you know, you didn't do informed consent. You know, you said this. You said" -- I mean there has got to be a way, if anything just to make it really clear, that if we're going to go forward, the deal is that the FDA has to really step up with it and make sure that there is, and I like Dr. Malech's point; you know, just that there's wording in all of these consent forms that is the same, and that we all agree on it, and we're not going to get bitten by it later in a public forum.

DR. NOGUCHI: I think that we appreciate that advice, and I think that that's exactly the kind of information we will take back.

I still would like to make sure that we understand what the actual patients and patient surrogates would like to hear.

What I think we can say, just in a general concept, is if -- and based on the current data -- if you didn't have a gene therapy trial for XSCID it's very unlikely you'd develop this leukemia, or the other way is there is a treatment called gene therapy. There is a leukemia that's developed in one out of 11 patients, and it seems to be related to the treatment or it is related to the treatment.

I think it is a fact that it is related to the treatment. Whether it's one, two, or ten hits, if you didn't

have the gene therapy, would you get a leukemia? And the available limited worldwide data would suggest really not very likely.

So I --

CHAIRMAN SALOMON: I mean, I think the way the statement ought to go is what I think was framed by others here, and that is all of the scientific information that we have available at this point says that this did it.

DR. NOGUCHI: Kathy, what do you want to hear?

MS. KATHY KNOWLES: I'd like to propose that this Institute of Medicine and National Academy of Sciences report be looked at in terms of the integrating that into your work. I know you're working on part of that already. But I think I mean in terms of all research centers should have boards to evaluate scientific merits of an experiment and potential financial conflicts of interest. All experiments should be previewed by an ethics board. Consent documents should be designed to inform participants in research, not protect research centers from liability, et cetera, et cetera.

CHAIRMAN SALOMON: Can I ask Mr. Susko and Mr. Gelsinger if they would care to comment also on this discussion of exactly what should be in an informed consent? And certainly anyone else in the audience that would care to comment.

I mean just give us a reality check. Have we done what we said we'd do or haven't we?

MR. GELSINGER: One of the comments on the IOM report was that they changed the name of the document to a disclosure document, not informed consent.

From a patient perspective, you know, I'm not an uneducated man, and when we got involved in gene therapy I had a basic understanding of what was going on, what was going to happen, not at the level you guys understand it. So there's no possibility of me understanding that without going through the whole education process that you have undergone.

So the patient can't see that. He's not ever going to see that. What you have to do is disclose to him all the pertinent information that he can absorb, and now you're going to have to deal with their education level. So you're going to have to make it very plain and stated very simply.

And you should err on the side of caution, absolutely. You should warn these people that there is a very great possibility that this gene transfer caused this condition in this child.

Informed consent is a misnomer. We really need to get away -- we need to get away from that. It doesn't work. It gives people the illusion that what they're signing -- now they know what they need to know, and it's not there. It's not true.

CHAIRMAN SALOMON: I mean, to be fair, this form can't do that.

MR. GELSINGER: No, I understand.

CHAIRMAN SALOMON: But we can advise the FDA what to do with the flawed instrument that we have, and certainly your point doesn't change the value of the points you made. It does need to be made to a different forum. That's all, that limited piece of it.

MR. GELSINGER: Right. I understand.

The compassionate use aspect of this should be seriously considered. We have a grandmother here. I don't know if she's still here or not, but I'm totally supportive of that aspect of it. When you analyze the risk-benefit of this, I think those people will be willing to take that risk.

So you know, when you go into that consideration in these clinical trials where the alternative therapies haven't worked, you know, then the use of this gene therapy is totally appropriate, and I don't have any objection to that, you know.

And you know, that should all be in the informed consent process, too, you know, that this information is transferred to them. You know, you've failed on these other therapies. Now you have this as an option.

But I'm getting off the subject of the reason I'm standing up here.

MR. SUSKO: I think I would emphasize in the informed consent also the unknown quality. We have one out of 11 adverse events and a child might die or might not, but we don't really know the real frequency.

So you might say, "Well, yes, we have this risk, but you know, it's unknown whether there will be more or less even." So the unknown quality should be fairly admitted, the truly experimental nature that's going on, I think.

And also just to reiterate, common language is very important. You don't want to use like insertional mutation. It can cause cancer, you know, something that ordinary folks would understand, and certainly one hit or two hit.

I just want to make another comment. Maybe cancer is caused by a series of repeated stresses that add up, you know. So you can't just say one thing is causing it, but the net effect is the same. You're going to be left with cancer.

So just a plea for common language that might let ordinary people understand. Admit to what you really don't know because it is truly experimental.

Thanks.

CHAIRMAN SALOMON: Any other comments about informed consent from the panel or from the audience?

MS. LORI KNOWLES: This is just really brief. I actually just really want to second that point about the nature of the scientific uncertainty. I mean, there are a lot of people nodding their heads, but this is a comment that Mr. Gelsinger brought up. This is a comment that our last speaker brought up, and we've been talking about the nature of

scientific uncertainty in this area.

I do think it needs to be explicitly and clearly articulated in these informed consent documents as well.

CHAIRMAN SALOMON: Are you okay with that, Phil?

DR. NOGUCHI: Absolutely. Thank you especially for that guidance. We will be working on this.

CHAIRMAN SALOMON: Okay. The next issue here, I think Alison introduce it again, but it has come in and out, and we've had a lot of discussion on it, and that is, I guess, should I call it an exclusion criteria? Anyway, the consensus I've heard up until now, again, take this apart if it's not what you think, but the consensus I've heard up till now is that if you have an HLA identical donor, you shouldn't be -- these patients should be excluded from these gene therapy trials as of right now.

I mean, if you have an HLA identical, the first thing is that you would be excluded. However, if the choice is that you have a haploidentical donor, or cord blood, trying to pick up a theme that was brought up by Dr. Kurtzberg, that you then could make an informed decision to do either the gene therapy, to do haploidentical transplant or to do cord blood.

Are we okay with that or not?

MS. LORI KNOWLES: I thought also that it would be important to exclude the neonatal group that responded very well and that had the very high quality of life, which is why I asked

Dr. Buckley when she was here.

CHAIRMAN SALOMON: Okay, yes. That's right, and in fact, that was my first introductory comment, was the early LOs did well. So okay. We need to discuss that.

MS. BALLARD: Even Dr. Buckley brought up the fact that Dr. Fischer's success may be pertinent to the fact that he worked with kids that had not already had bone marrow transplants.

And let's face it. Mostly the younger kids are the ones you're going to be transplanting or going to be trying to do therapy on, one of the two.

CHAIRMAN SALOMON: Okay. So that's an unknown. Let's not say -- I mean that's what Dr. Buckley said.

MS. BALLARD: Right.

CHAIRMAN SALOMON: But we don't really know that, but that's okay.

MS. BALLARD: But even she brought up that point.

CHAIRMAN SALOMON: Yes, she did. No, you're right.

Rich and then Bruce.

DR. MULLIGAN: You know, I agree technically with this, but I think that as a policy issue, it may haunt the FDA at some point because if we get to it after we finish with this, what about all of the other diseases?

In a sense does that mean that this body is going

to, you know, do the same sort of thing for each of the many diseases? That risk, is it an appropriate thing to walk through these, have us walk through these as opposed to state basically this is the best we think.

But I'm just wondering whether we should be legislating that here.

CHAIRMAN SALOMON: That's fair enough. Certainly we're not going to take on all retroviral gene therapies right now in terms of that sort of detail, but I thought that it -- and the answer here could be that we don't have a consensus. I'm okay with that. Just to me my job is to reflect the opinions of this group fairly.

So if you don't believe that we can say that this or that group should be an exclusion, that's okay. Put that on the record.

DR. MULLIGAN: I think that we do have a consensus, but I think I'm just saying technically we have the consensus. I'm questioning whether the policy is the right policy.

I mean, we've clearly gotten the message across, and they can certainly take it home as a consensus, but it does raise, I think, the important question of how the diligence is going to be put to each of the many other cases that will surface when we finish with this one here.

CHAIRMAN SALOMON: Well, the reason that I think

it's important to deal with is in the context of what Alison was saying in that, okay, she's tentatively all right with agreeing that there's a consensus that we should go forward depending on what sort of restrictions we do to mold the existing study.

So I don't think we can hide from that and then come back to it.

Dr. Puck.

DR. PUCK: Even in Dr. Buckley's successful studies in the first three months, those were not successful in terms of B cell reconstitution, only survival. So when you consider that they didn't do any better in their B cell reconstitution than the patients transplanted older. I think we really don't have enough information to exclude those necessarily at this point.

DR. CORNETTA: Also, having been in these situations, there are always exceptions. So I think for us to be arguing is it three months or is it four months or three and a half months that you put this line, and then the first patient that will come along there may only have one potential haplo donor and that has hepatitis, you know, and you get into these things.

So I think you have to give some credit to the clinicians that are treating them to be able to sort these issues out because it ends up being more exception than rules in these situations.

DR. MULLIGAN: I think the safest thing to do rather than trying to define what the alternatives are, say, is that if you have a suitable HLA identical sibling donor, that's the preferred treatment. If you don't, then this is an option rather than trying to make all of the other definitions.

CHAIRMAN SALOMON: I actually agree with that. I'm very comfortable. That's what I was saying earlier.

Is that okay then with that discussion?

DR. KALLE: That was already the case in the French trial also.

CHAIRMAN SALOMON: Okay. Alterations -- I'm trying to follow this through. So C is alterations to the cell dose administered.

The only thing that hasn't been discussed enough just because Dr. Kurtzberg had to leave, so let me share with you. If you notice, when she was leaving I was kind of talking to her a little bit on the side.

So her comment was that with cord blood, matched or unmatched transplants, no T cell depletion, most of you here know, but just for those who don't, the neonatal or fetal T cells contained in cord blood are typically so unreactive immunologically and immature that you don't have to do the T cell depletion typically to avoid the GVH.

And in those patients she said she can get success with about one times ten to the fifth CD34 cells per kilo, which

would address an issue that, John, you brought up and others picked up on, you know, that one would significantly reduce the number, the theoretical number, of hits to get an insertional mutagenic event.

I don't know. I mean, that's cord blood. I don't think then that the point here is that there's any -- I don't think Dr. Kurtzberg implied that there was enough data now to put everything on hold except cord blood, but I mean --

DR. CUNNINGHAM: You know, I respect Dr. Kurtzberg's opinions about cord blood, but most of us who transplant have been brought up with the idea that using -- and I'd like to hear what Ken has to say about this -- using at least two times ten to the sixth is important for engraftment.

And it may be that cord blood is actually a very special type of stem cell that may have a different potential from the type of stem cell that we're using in a post natal situation.

So I think we have to be very careful, but it's like the previous discussion about numbers, limiting us where that's concerned.

Sorry. I should also point out that if you remember our patient, we actually used two times ten to the five cells per kilo in the first transplant, and we got no marking. So I don't want to draw too much from one patient, but I think that's an important point.

CHAIRMAN SALOMON: I actually was interested in that and wrote it down, except then you gave them 3.2 times ten to the sixth and didn't get any markings. So I kind of --

DR. CUNNINGHAM: With the 3.2 times ten to the sixth we do see marking. It's just we haven't seen T cell reconstitution yet.

CHAIRMAN SALOMON: Okay. John and then Ken.

DR. COFFIN: These arguments are sort of all from precedent, which is all fine, but I'm not convinced that it's absolutely written into stone that these numbers will be necessary. My question is just is it possible to design a study to actually investigate the issue. Can one take patients and start them with a small number and watch what happens for a few weeks, a month, and then step them up? Is that -- I know nothing about this subject. So is that a possible thing to do?

DR. CUNNINGHAM: Maybe Dr. Puck would like to talk about this as well, but you know, the issue is with these children we have to wait 90 to 120 days anyway to see if there's a response. So we're putting those children at risk for those 120 days by, say, starting at, say, one times ten to the five, which we don't know is an optimal dose.

Thirty years of transplants suggests at least in myeloablative situation that you need approximately two times ten to the six CD34s per kilo. Now, that's a very rough number. So, you know, I don't think there's any data there to design

that kind of trial, and you may put the children -- you know, we go back to the risk issue. You may put the child at risk for a question that is not possible to be answered in a small group of patients.

DR. COFFIN: Well, if there are more cases of malignancy among these children who have been treated so far, one might revisit that risk issue, of course.

DR. CORNETTA: I think there are a couple of points. One, we've been talking about CD34 numbers, and generally those have been derived from folks that are getting ablative transplants, and I don't know in regards to the numbers that Dr. Kurtzberg was generating whether those were folks that got ablated, but I suspect they probably were.

So it's hard to know how that relates to a disease like X-linked SCID where you're generally not ablating these patients, and the more I've been thinking about this, the more my head is spinning about a variety of issues.

One, I think the discussion sort of assumed that CD34 is the stem cell, and it's clearly not. And so if you want to decrease the cell dose, again, should you be actually looking at a more purified population so that you're starting with less ones, especially in this case, because we aren't trying to get myeloid engraftment. We specifically want to get T cell engraftment, which is slow to begin with.

The big issue which I think is unsettled for gene

therapy is the issue of competition, which is probably related somewhat here, but certainly for other types of bone marrow transplantation, what is the normal pool that you're competing with?

And we see a potential here for an outgrowth of T cells, but what is the population we really need to hit? It's a really complicated issue. This may be something where we need to challenge the people that are doing these trials to really think through these issues and be asking questions to try to do that.

But I'm not sure we're going to define the science here of these complicated issues.

CHAIRMAN SALOMON: But I think the one thing, as I said in my introductory comments is we all realize now that the CD34 population contains a whole lot of cells, and the true stem cell there is somewhere between .5 percent and maybe two percent in a bone marrow, right? Depending on where you get it, mobilized or cord blood.

So, I mean, the real question would be are there ways to target the true stem cell that's within the CD34 population, and if you could do that, then one would significantly reduce the risk of insertional mutagenesis just by doing the mathematics.

DR. CORNETTA: Now, would you also change their engraftment potential? These are really difficult issues.

DR. NOGUCHI: Before we go on, I'd like to take off cord blood from the discussion. That's a separate set of investigations that should not be considered any standard of therapy at all. It is under advisement as to how we are going -- we called for data to see if we could create standards for cord blood bank allotransplantation, but we are not finished with that. So if we could take that off the table.

CHAIRMAN SALOMON: It's off.

(Laughter.)

DR. CORNETTA: But, Dan, I think the comments that we've talked about CD34 in these populations relate to any type of transplantation. It's not just cord blood.

DR. MULLIGAN: I mean, I think this is definitely a -- we should sensitize people to be thinking about this, but it's definitely a research issue. It's nothing that could be -- we could advise to only use such-and-such.

And just to kind of move us ahead, I would think the vector dose would be exactly the same sort of thing, you know.

CHAIRMAN SALOMON: I mean, right. I think the one thing I've heard is that these vector doses are getting around one copy per cell, you know. So I think there's no reason to talk about vector dose change unless somebody disagrees with that.

Mapping. We've got to deal with this, guys,

because this could be a real problem for investigators in the field, and that is mapping of vector insertion sites on all clinical lots of cells prior to release for clinical use.

I mean, that's a classic FDA move.

(Laughter.)

CHAIRMAN SALOMON: I'm sorry, but you can tell from the way I put that that I do not -- I haven't heard anything that would scientifically support doing that, and again, that's up for discussion.

Bruce.

DR. TORBETT: I think it would be like Stu said. Basically you'd use all of your samples and then there is no risk of getting leukemia.

DR. MULLIGAN: I think also Stu's point, which is very important is that there is an increasing sense and there's many more targets that would be kind of dangerous targets, and so it's probably a fruitless approach to take.

I think Christof talked about whether you might use that approach in the course of looking at the patients to in addition to looking at counts, see if there's any specific clonal outgrowth or something like that.

I even wouldn't say we've advised people that you had to do that, but that would be something that would be probably an important part of a good clinical analysis.

CHAIRMAN SALOMON: That's actually a good segue to

where I was going next. Before we go any further just because this is such a critical thing, I think is there any dissention by anyone? I mean, does anyone think that we ought to be recommending insertional mapping on the clinical lot before release?

Okay. Is that clear? Good. Thank God.

So the next question though is in the sense of safety, what should be recommended to the FDA with respect to modifications of these existing SCIDs protocols with respect to, you know, should they be looking for fill in the blank, you know, an expansion of a specific T cell alpha-beta clone, expansion of a gamma-delta clone, a specific insertion site that emerges as a single clone.

Anyone have any comment on that? I mean, my personal opinion is that we probably should request each of these protocols to come up with some sort of a safety monitoring procedure that includes this new data, but that's my opinion.

DR. COFFIN: Yeah, I think close monitoring for outgrowth of single clone, proviral integration clone is the most telling thing to look for, and I think that's actually something that should be incorporated into all retroviral vector trials.

Wherever one can access the -- like as with blood, where one can access the population of cells that have been treated, I think one of the things you can really do is look for

the clonal outgrowth of specific proviral insertions, which may or may not actually be an oncogenic event. It may reflect other kinds of selection or just a chance event, but I think that's the one thing that can really be looked for with certainty.

DR. SORRENTINO: As I think about that point, the problem I have with it is what would you do with the information if you see the emergence of a clone in a patient that has a normal peripheral blood profile?

And you know, secondly I'd like to point out that in the early studies of gene transfer in mice, it's actually typical to see a polyclonal hematopoiesis become oligoclonal, and then with time become monoclonal in the absence of any functional abnormalities.

So, you know, while the information would be scientifically interesting, I'm not convinced that it would increase the safety margin in any way or provide useful information to the clinician.

CHAIRMAN SALOMON: I actually would take an opposite point of view. I mean, from my thinking here now, we've said there's definitely been an event that to the best of our knowledge has produced a cancer, you know, in the context of all the refined language we use.

I don't think you can really go forward with these trials and not incorporate some sort of monitoring, you know.

If you're showing it has occurred in this kid, at

13 months this clone was diagnosable, and we really didn't know what was coming, you know, at 20 months.

Yeah, sure. I'm not saying that you would suddenly now treat the kid with chemotherapy at 13 months, but I sure as hell a few years from now want to know how often this is happening. You know, if it turns out that in the first 100 patients that really get successful retroviral mediated gene therapy, that 25 percent of them show clones and ten percent of them get leukemias, I want to know that, and the only way I'm going to know that is if you're monitoring it.

So that's my point of view.

DR. MULLIGAN: I mean, also we did some of the work that Brian is talking about in the early days doing gene transfer infections, and I thought it was very important to give the field a sense of how many cells you're infecting and also looking at therapeutic indices.

You know, in our case, as you know, often there is only one or two contributing stem cell clones, and over time a new one would all of a sudden contribute, and it wasn't necessarily a leukemic clone, but it often had different characteristics in terms of transcription and so forth.

And so adding that in would give you something very important if like you were seeing some therapeutic effect and then all of a sudden completely disappeared. That may be because another clone is making a contribution. I think it's

quite helpful.

DR. COFFIN: I think a lot of the times if this is a bad event, it will be at an identifiable integration site. I mean, we saw that today, and we can see it again. As soon as you see a clonal provirus coming up, the very fact that you can see it means you can also sequence the integration site.

If that happens to be in the first entrant of c-myc, for example, I would start to get very concerned, and I would consider starting that patient on therapy right away.

I mean, we have a lot of knowledge of what are really bad spots in the genome to have a provirus in, and that could give a head start on therapy even before.

The other thing is that if you see that, then you could also at least in principle get those cells and ask whether they were becoming monoclonal for T cell receptor rearrangements and other bad signs of overgrowth of clones that shouldn't be there.

CHAIRMAN SALOMON: Alison, Linda, Dr. Puck, and then Mahendra.

MS. LAWTON: I just wanted to ask a question because I'm not familiar with the methods used about this recommended screening, and so I have a question of if you're going to start doing this how frequently would you need to do it. How easy are the methods to do that?

Because that obviously has a big impact on whether

this would be an appropriate way forward.

DR. KALLE: Of course, we only have the experience from this one case. We had thought about a strategy. Of course, this is more at the scientific level right now because it is not a GLP procedure type of study, at least not at the current point in time. It is more complicated than just a PCR reaction. It's probably something that takes about two days to accomplish for a whole set of samples.

What we do say though is with the dynamics of the clone growth that we have seen, probably screening it every three to six months would be sufficient if we would argue from the base of this case, as well as from the mouse leukemia that we have looked at, both of which have taken a significant amount of time of clonal proliferation to develop.

DR. WOLFF: I agree with John about trying to find where the clonal integration sites might be because you could end up knowing that it's a specific oncogene that was previously involved in human cancer and that some treatments are already becoming known for that specific type of alteration.

For example, if it was in a retinoic acid receptor alteration or something, you might want to give retinoic acid to the patient, but that's just a general idea.

DR. PUCK: I just want to make a point that I think this is a research question that is extremely worthwhile, and it's certainly reasonable to ask every clinical trial to

include it, but recognize the difference between a research test and the kind of test that would be a GLP or CLIA approvable test, which you know, this has definitely not reached that level, and therefore, the idea of using it to design therapy has to be really questioned at this moment.

DR. COFFIN: I'm talking only in the context of research trials right here, but all approved GLP tests started out as research tests at some point or another and then worked up that way when it was seen they were necessary.

DR. NOGUCHI: You should not be afraid of doing a test that you can do. I think the point is if it can be done, the next question is of standardization and reproducibility, but if you don't do it and five years from now suddenly some of the previous bone marrow transplants with the retroviral vector in a non-SCID patient developed something, you are not going to be really in a good position to say, well, I just didn't have a GLP test for it.

We don't have a GLP test for West Nile virus. We're going to be pushing on that, and we will get it done, but you have to try.

CHAIRMAN SALOMON: Mahendra and then Rich.

DR. RAO: I guess the point has already been made, but there also seems to be some advantage in doing the test because you saw them become monoclonal or oligoclonal much more rapidly in that one case than you saw in the others, and then

you looked at the time course.

So just a temporary profile might give you some clue, not just necessarily on research, but may also give you some warning in an early case.

DR. MULLIGAN: And I just think we could just recommend that we think this is important and not say you have to or anything, but just that this is something we think is an important test.

CHAIRMAN SALOMON: Ken.

DR. CORNETTA: Maybe I'll over a different and that is to require archiving of samples because I think you're getting -- having gone through these trials, you're adding a tremendous amount of work onto a variety of trials, and I'm still not clear. I think people are talking whether this should be an X-linked SCID or this should just be all retroviral trials.

If you have one percent of the sales that have the vector in it, you still have to go and look. Is that clonal? What does it mean? Is it going to -- you can do all of this work, and six months later that clone may not even be there.

We probably need to think through these things, and you may need to set some boundaries of if it's 50 percent of your sales are marked, looking then to see whether that's a clonal population.

But right now it's a very loose discussion of, you

know, we're looking at what potentially may be a really rare event and not be clinical and require people to do a lot of work.

And I think even the LAM PCR, I think, is proprietary still at this point. No?

So but I think these are certainly going to complicate especially as trials move into Phase 2 trials or even Phase 3 where you have lots of patients put on, and we may be getting a lot of data that may not be useful.

CHAIRMAN SALOMON: I don't disagree with that at all, as long as we are square about the concept that the response to this new data that there was an insertion is something that we need to be very clear can't be forgotten in any retroviral gene trial going on right now.

Now, I don't think just sticking stuff in minus 80 freezers and forgetting about it is enough for me. At the same time, I absolutely acknowledge the reason of your point, that if it's one percent and a month later it's gone, I'm not interested in, you know, creating this overarching, you know, craziness or running around doing a test like that.

So I think you're right. It should be refined. I think that was Rich's point as well, but I also don't agree that, you know, just solve this by archiving.

DR. BAUM: I just wanted to make a kind of unpopular comment here. This also has an economic impact, such

a recommendation. Already now its monitoring is highly expensive of the stem cell gene therapy and other gene therapy approaches, and I don't know what the costs would be, but I consider these extremely high, especially when it comes to GLP type testing.

And this definitely, at least from the European perspective, would draw any attention of the pharmaceutical industry out of this business, out of this area.

And many people say -- we hear it in the comments of the ASGT -- we need the support of the industry to develop the field. We cannot go ever beyond Phase 1 trials when we don't have it.

And such a recommendation has an enormous economical impact, and we really have to consider whether this applies to specific what I would call intermediate risk or high risk applications and whether it has to be applied to all kinds of retrovirus or other virus manipulations.

CHAIRMAN SALOMON: I think that's fair, too. And that comes under the idea that it would be appropriate, as Ken and Richard said and so you as well, is that we refine it and make sure it was applied in appropriate settings.

DR. MULLIGAN: It could be a time dependent thing, too. I mean, I think what's important is that we give the sense that we're very concerned based on this patient, and so there's great apprehension that, in fact, it could happen to every

patient.

And until we maybe have less of that concern, I don't think it's unreasonable to look at these existing patients in a very careful way.

CHAIRMAN SALOMON: I would take the position also, and I think the FDA is -- I'm most comfortable in terms of coaching advice in this sense, and that is it becomes something that each sponsor for each trial gets to address.

I mean, we don't have to come across as saying this is absolutely a blanket, but I think we should say to the FDA that in any case in which the sponsor creates or, you know, is proposing a trial, whether that sponsor be in an academic institution or in a company or in some hybrid, that they have to account for this issue and how they're going to deal with it.

If they can convince the FDA that for this or that reason it's low risk and, you know, they can only do it every six months, which by the way I think would probably be fine, you know, that's fine. That would all be appropriate, and I think we still can stay with a very clear message to the FDA that to the extent that we've learned today that an insertional event caused something that was diagnosed -- well, at 13, 14 months it first showed up and the kid had a leukemia at 30 months. That's going to reverberate in my mind every single time a retroviral gene therapy trial is stuck in front of me from now on.

DR. BAUM: Maybe one last short question here. Would you ask a pharmaceutical company that develops an alkylating agent for chemotherapy to develop a method that allows clonal tracking of mutogenized (phonetic) clones? Because the incidence of secondary leukemias could be ten percent?

That is basically what you're asking now for retrovirus methods. So it just needs to be kept in mind.

DR. NOGUCHI: Yeah. Can we get back to the specifics here? And then you could perhaps amplify it. I think we take the larger issue of retroviral vector integration both the therapy that has an adverse event in general as a concern, but what about the SCID trials? Are you saying or what is the consensus saying here? That this is a nice research thing and in one case it predicted possibly that X number, 12 months before that leukemic event could be detected clinically? That was a nice thing, but think about in the other XSCID trials, or is it something more than that?

DR. ALLAN: Yeah, I'd like to sort of comment on that. One is that the technology that you're talking about is really for mapping. You're actually mapping the integration sites, and we really don't need to know at least initially, and if you're doing clinical patients, you don't need to know what the integration site is. You only need to know whether it's monoclonal, oligoclonal, polyclonal, and you can do that fairly

cheaply by PCR and --

PARTICIPANT: Restriction.

DR. ALLAN: Yeah, you can do a Southern Blot, restriction through Southern Blot if you have to, but, I mean, probably that's not going to be sensitive enough.

So I don't know that it's prohibitively expensive or that difficult even, and I think that, you know, if you're going to od it every six months, especially in the case if you've got cancer in one out of 11 kids, I don't think it's unreasonable to expect that.

DR. KALLE: Yeah, I just wanted to comment that it is a PCR assay, that of course, we have made it available to a number of research laboratories by also teaching other laboratory members from other labs how to do it.

Of course, to do it in many different labs would cause other problems of standardization, but we would, of course, be cooperative either way.= to help out with this.

DR. TORBETT: I'm speaking toward the SCID trial. I'm trying to get back to what Phil was bringing up because I think that we already have noticed that there could very well be other or at least there has been one leukemic event, and I think following at least these groups of patients or others that are transplanted similarly, right now it's imperative until we get a little bit more information to make other decisions.

We've had an event. There's a way of tracking it,

and I think we can go on. Whether or not it's general for all retroviral therapies I think is a bigger question, but from my own, you know, perspective, I think at least for these kinds of trials, it needs to be done.

CHAIRMAN SALOMON: I can't leave here without being really clear. It needs to be done. That's my opinion.

Again, we've got to be a little careful here in that -- and I'm perfectly happy that the diversity here is a group of people in our second row here who have got, you know, reasons to want or not want to do this, but I think from my point of view and from the point of view of the panel, I think in SCID trials you've got to do this.

Now, exactly how you've got to do it I'm willing to allow each of the sponsors to discuss it and come to an agreement with FDA staff, but I don't think that anyone is saying that it shouldn't be done.

Am I misreading anything? Ken and Kathy?

DR. CORNETTA: I think requiring a monitoring plan be developed in each protocol is key, and again, there may be differences which each protocol in the populations that may change those. So having a blanket statement here today would be inappropriate.

But I think requiring that there's a monitoring plan for this and all protocols would be the recommendation.

CHAIRMAN SALOMON: Kathy?

MS. KATHY KNOWLES: I think monitoring is very important, but also, too, the point that Abbey made, and I'm going to kind of reinforce it, about establishing the gene therapy clinical registry system is really, really important as well.

DR. NOGUCHI: Yes, we heard that very clearly.

MS. KATHY KNOWLES: I know you did.

CHAIRMAN SALOMON: Okay. Yes, Dr. Malech.

DR. MALECH: I actually would strongly agree with you, but I think that one of the words that Dr. Cornetta brought up was boundaries and trigger points, and so I think obviously each of us investigators are going to have to go to the FDA and say, "Here's what we propose."

And it's almost certainly going to be more than just storage of samples. It's got to be something like assessment of clonality. But then once you get to that point, it's an infinite number of things you can do.

You can sequence each of the clones. You can go on, and so there also need to be trigger points. That is, something has happened to the patient in addition to clonality. For example, in Dr. Kohn's studies, he had oligoclonality, but it wasn't a problem. So we have to have other trigger points.

I don't want to define them here, but we'll obviously be proposing them to the FDA that will say: okay. We have monoclonality and such-and-such happens to the patient.

That triggers a bigger assessment.

But I think that's how it has to be thought about.

CHAIRMAN SALOMON: That's excellent.

So I'm thinking at this point it's a few minutes after five. We didn't do the very last thing, which was alterations in vector design. That's -- Carolyn, you're giving me a significant look.

I mean, I'm willing to take it on if the group is willing to take it on. I'm just thinking that I'm not sure what it is we're going to tell you in terms of vector design at this point.

MS. WILSON: I think that some of the comments we heard from various scientists earlier made it clear that it's an important research question, but probably not something that would influence the clinical trials that are ongoing at this stage, unless others feel differently.

CHAIRMAN SALOMON: Well, let's just make sure we agree with that.

I mean, the only thing I'd throw out if somebody -- I mean, if we would say that -- this is getting pretty far out onto a limb -- that it's the enhancer element in the LTR of this particular vector that was the problem.

I just can't believe that I'm going to get anyone to agree that, you know, now we should mutate that enhancer element or something like that, right? I mean, no one is going

to go with that.

DR. MULLIGAN: I would agree that, you know, for someone who likes to meddle with these vectors, there's so many things that appear that they're really fancy work, and then when you really look at them carefully, you have to be very careful. You would never at this point say you have to do this.

I do think that the suicide vector feature, which is another one that has really never worked as well as --

CHAIRMAN SALOMON: I didn't pay him to do this, Phil.

DR. MULLIGAN: But I actually predict that at some point in the future we may sit here and advise you that that may be a critical feature, but not at this point.

DR. NOGUCHI: I think in general we like to say we are dealing with an issue right now that has risks and it has benefits, and our preference is let's deal with that before we start changing things, which will ultimately multiply all of the concerns and all of the ambiguities enormously.

Here we have an opportunity to try to understand this particular trial, this particular disease, this particular therapy.

DR. TORBETT: I'll have to agree with Rich though. I think that some type of suicide system in the long term is going to be really effective in a situation like this, and I think that's something that should be considered.

CHAIRMAN SALOMON: I think the other comment that I would have is if we think about everything we've heard today, it isn't anything that we couldn't have figured out a few years ago, but the idea of using powerful internal promoters in these genes is something that I think we just can't resist, but kick to a little higher level of concern as we go forward in other retroviral gene therapies.

Rachel and then Linda.

DR. SALZMAN: I want to make one comment back on characterizing the patient subject population. Since Dr. Fischer had mentioned that this one patient number four had a family history of medulloblastoma, that there might be some predisposition to cancer. So I just wanted to add the comment that I think it is important to do family pedigrees, maybe to do some -- I don't know about this, but I know there are certain genetic mapping can target certain genes that are related to certain cancers just to further characterize, not to include or exclude them; just to characterize the patient subject population.

DR. WOLFF: Well, I believe it's a consensus that there's a decision to continue these trials, but is there any limit to the percentage of patients who would have leukemia that would stop the trials in any way? Should this be discussed?

DR. NOGUCHI: I would like to say that we would like to think about that. However, because of the nature of

this particular trial, I would say that no matter what happens, the next one may get leukemia or it may never happen again. That still does not negate the necessity, I think to examine what we're dealing with.

This is a rare disease. We are not going to really ultimately have the luxury of trying 14 different approaches to it. So I think the notion of stopping rules is a very good one, but I don't think we know enough to be able to define that two is too many. We simply don't know.

If two is too many, what if they're different leukemias? What if one is a carcinoma and one's a leukemia, as an example?

So I think you've given us all much more than we had even hoped for, and for any future adverse events, don't worry. We'll be right back here talking about it.

(Laughter.)

CHAIRMAN SALOMON: Okay. So it's always a problem around five, somewhere around five o'clock. People are on different coasts, hit planes around four because that's just the reality of plane travel these days, and the rest of us who are staying don't care how long we stay, but many of you have homes to get to, et cetera.

So I think before we get to the last part of the meeting, which is to go on to discussing the recent site visits and things of FDA research, is there anything else, Phil,

Cynthia, Carolyn?

I mean, have we covered the waterfront here in that?

DR. NOGUCHI: Let me just say on behalf of all my colleagues in the government this is extraordinary. We got advice from the community, especially the affected community, and we're very grateful for your help.

CHAIRMAN SALOMON: Then to all of the audience who's very much invited to stay for what will be some very interesting presentations of FDA research, but will not any longer deal specifically with issues of gene transfer and in SCIDs, for all of you thank you very much for being here and participating in a very critical process, I think, to all of the experts and everyone on the panel.

But everyone on the panel can't leave. Okay? So should we take a five minute break?

Let's take a five minute break, but maybe not leave the room.

(Whereupon, the foregoing matter went off the record at 5:20 p.m. and went back on the record at 5:30 p.m.)

CHAIRMAN SALOMON: Okay. One of the important functions of the FDA staff particularly in CBER is, in addition to doing all of the many different kinds of regulatory work, is that they do, many of them at least, take on the additional

responsibilities of doing basis research. And one of the responsibilities of the BRMAC, the Biological Response Modifiers Advisory Committee, is to site visit on a periodic basis these laboratory efforts.

And as a scientist myself who writes NIH grants and competes with his colleagues to do the best job possible, I have to say that these are a really, really big deal, to have your who laboratory efforts, directions, focus and productivity reviewed periodically.

And to be honest, whenever I do these site visits, and I've done my share over the last five years, I, number one, always get an incredible amount out of them because these are very intelligent people, and they're doing very interesting work.

Number two, I'm always amazed and reassured that the work that they're doing directly complements the regulatory activities, and that's something that I've never seen any exception to that and continues, therefore, to fuel my enthusiasm for support of research within the FDA.

I am always impressed with how much they do with little. Whenever I go to the NIH I am always saying, "Oh, those guys have got it easy," Mahendra, but whenever I go to the FDA I think, "My God, how do they do this?" And yet they maintain their productivity and the quality of their research.

And finally, after this last one, I must make a

personal comment. After this particular group which you're going to hear about now, I was so impressed that I essentially had an epiphany where I just said, you know -- I came back. I cut two of the five research projects in my lab. I just cut them. I said, "You know, if one of these guys had come and audited my lab, I know when they walked out they'd go, 'Wow, I don't know about this guy's focus.'"

You know, I was thinking in terms of the way I was thinking about them, and my epiphany was if I can go and do that with them and that's the process that's doable, then I shouldn't shy away from doing it on my own, given that no one is doing it to me except through my NIH grants, and I just really had this remarkable response to this this time.

So anyway, I don't know whether all of my post docs are happy about my trimming some of the projects, but I think it was definitely a good thing for me as well.

So with that introduction, what we're going to hear initially, Dr. Amy Rosenberg for the Division of Therapeutic Proteins is going to introduce her people, which in this case is Dr. Michael Norcross and the group under him, and then we'll hear a little bit about the research.

DR. ROSENBERG: My name is Amy Rosenberg, and my division is the Division of Therapeutic Proteins located in the Office of Therapeutics, and our division regulates protein therapeutics with the exception of monoclonal antibodies and

some recombinant blood products. We have a very wide variety of products. We have nearly 40 licensed products, as well as numerous products under IND under development.

Among the products we have under development are chemokines, and to expertly regulate chemokines is no small feat. Particularly difficult are potency assays, which are critically important to assessment of biological activity.

Dr. Michael Norcross, who is our principal expert in chemokines, is crucial to the regulation of these products and is working on development. His research directly impacts on our ability to assess potency assays for these chemokines.

He was located in the laboratory of gene regulation. Our division has been reorganized for consistency and efficiency, and he is now in the laboratory of immunology.

The other part of his talents, that of an immunologist, are utilized in regulating the numerous tumor vaccines and vaccines for autoimmunity that we deal with.

So I will let Mike explain his work to you, but please be aware that his research is key to our ability to properly regulate the products I've just told you about.

DR. NORCROSS: Okay. Thank you.

Can you hear me okay? I guess.

All right. As she said, my name is Mike Norcross. I'm in the Division of Therapeutic Proteins.

I suggested we change the name to Division of Non-

Drug Proteins, but nobody would go for that. That's sort of an inside joke.

Anyway, the studies in my laboratory are mainly focusing on chemokine and chemokine receptor, structure, and function, and with a main emphasis on HIV infection and immune function.

The current lab staff includes Jin Hai Wang, a staff fellow who presented at the site visit on his work on host factors regulating chemokine receptors.

Ennan Guan, a biochemist who did much of the chemokine biochemistry, and Greg Rodriguez and Mike Phalen (phonetic), who was here years ago and now has come back to the FDA.

I wanted to just briefly mention that there are a number of biological function of chemokines that now we in the FDA are involved in in regulation. Chemokines are involved in cell migration and a number of systems, for example, leukocyte trafficking, homing, hematopoietic stem cell mobilization, inflammation and immune responses. This is to recruit cells into an inflammatory site.

Recently it's been found that cancer metastasis, that cancer cells or tumor cells can have chemokine receptors on their surface, and they may be involved in metastatic seeding of sites, maybe going towards a chemokine gradient.

Of course, allograft rejections, atherosclerosis,

and angiogenesis and arteriogenesis, and we've had a number of applications actually addressing different biological processes here as a therapeutic target.

Chemokines can mobilize hematopoietic stem cells. Chemokines can actually suppress the replication of stem cells and protect the marrow from chemoablation. Angiogenesis, chemokines can both stimulate blood vessels and inhibit blood vessels, and we've seen other applications of trying to stimulate arterial perfusion.

And as Amy mentioned, one of the issues is potency assays, and there's been really a problem trying to get reproducible assays. It was mentioned earlier that, you know, usually things start as a research method, and then to try to apply that for using it for product characterization is really the major obstacle that we're seeing now with chemokines. We need basically a Viagra for potency assays, but anyway, we're working on that.

So why are we interested in -- I was going to mention the number of biological activities, but the chemokines have revolutionized the HIV research field with the discovery that chemokines or at least a cocktail of chemokines, RANTES, MIP-1 alpha, MIP-1 beta had potent anti-HIV activity, and it was followed up to find out what the mechanism was that receptors for those chemokines were also receptors for HIV.

So chemokine receptors are now termed co-receptors

with CD4 for HIV binding, entry, and fusion into the cell.

CCR5, the chemokine receptor for these RANTES, alpha and beta, mediates macrophage-tropic virus infection. Another chemokine receptor, CXCR4, mediates T cell tropic virus infection, or X4 virus. These are viruses found in late stage HIV, and those ligands for those receptors, STF alpha and beta.

So you can see from this simple model that the concentrations of chemokines in a site where virus is replicating and the expression of receptors in those cells that are the target cells are critical to regulating how much virus will grow and actually probably are important for the disease progression.

So the areas that I've been interested in mainly are in the regulation of chemokines, both their expression and their structure, and factors and host factors that regulate expression of chemokine receptors.

And so I'll just briefly go over the research projects in that we're basically broken up into two areas. One is chemokine protein structure and function, and those started with studies where we discovered that proteinglycans were binding sites for chemokines on cell surfaces, and that they were required or played a role in potentiating the anti-HIV activity of RANTES, one of the antiviral chemokines.

We have been interested in a dipeptidase called CD26. It's on cell surfaces, and we found before the chemokine

revolution that this enzyme was important for HIV replication in cells, and we discovered that this same enzyme, the dipeptidyl peptidase, cleaves to amino acids off of chemokines. And we think that chemokines are -- believe and have shown -- that chemokines are probably one of the main substrates for this enzyme at least in the immunological system.

And just to briefly tell you that the CD26 is on the cell surface of T cells and macrophages, and it's also secreted into peripheral blood, and its unique property is that it cleaves to amino acids off the N terminus of a chemokine. It has either an alanine or a proline, and missing just two amino acids out of 70 totally changes the activity of the chemokine. Both either it loses activity or it changes its receptor specificity, and we believe that this enzyme system regulates the activity of chemokines in a way that I think it's a feedback mechanism in some ways to shut off inflammation, but it's also another mechanism to regulate the kinds of T cell helper cells that you'll get in an immune response.

I'm not going to go into the details of the data that I presented at the site visit or that we're working on to support these models.

So with our interest in the natural enzymes that process chemokines, we became interested and have studied the structure and function of naturally secreted chemokines, and we have made several important observations, and one was that when

we started to look at the chemokines secreted by T cells and macrophages, we found that at least one set, MIP-1 alpha and beta, which have potent anti-HIV activity, were found as a heterodimer.

And the field, even as of this year, generally believed that chemokines worked as monomers, and we found the first example of a dimer, and it really raises a lot of interesting questions about how a dimer of a chemokine may work to cross-link receptors or to induce signaling pathways that really weren't considered previously in this field.

We've recently published a paper on the MIP-1 data, the natural form of MIP-1 data secreted by T cells, and found that this chemokine not only binds to a receptor that mediates HIV entry, but now gains the ability to bind to other receptors.

And we think that this chemokine may be important in suppressing immune responses late in T cell response through activation of a cell called a regulatory T cell.

The other have of the projects that we're working on that Dr. Wang and his colleagues have been working and defined and we have published on is the regulation of chemokines and receptors by host factors, and host factors, particularly chemokines.

And we look at this in two different ways. These are the ligand dependent regulatory pathways, and we've looked

at T cells with CCR5 expression, and primarily we've been interested in the role of Interleukin 2 and Interleukin 12 in stimulating production of MIP-1 alpha and beta in T cells. And we think that this might be an interesting combination of chemokines actually in therapy for HIV.

The other half of these types of projects or what we call ligand independent regulation, that is, ligand chemokine independent regulation of the receptor expression, and we looked in monocytes and macrophages, and what we found is that chemokines IL-4 and IL-13 and GMCSF had a very potent activity in suppressing the expression of chemokines on these cells.

And that correlated with a resistance to virus infection. And we went on to and Dr. Wang did some elegant experiments to look at the mechanism of how this was regulated by looking at tyrosine phosphorylation, phosphorylation of receptors, the recruitment of focal adhesion kinases, and a very eloquent mechanism of how cytokines can actually feed back and turn off chemokine receptor expression, and we think that this mechanism, I think, is important in vivo actually for regulating the sensitivity of virus infection in vivo.

And I won't mention really too much about interaction of chemokine receptors.

And also with host factors and HIV entry and pathogenesis, we published recently or within the last year on TGF beta and mediating depletion of T cells by inducing

apoptosis in normal activated T cells, and we have some ongoing work on really another aspect of pathogenesis in virus entry where we found that an amino acid transporter was involved in the translocation or the signaling through that transporter could modify transcription and nuclear translocation of HIV by disrupting an intermediate filament.

And I'll just finally finish up on what we're currently doing in our future directions in these areas, and we are continuing to look at chemokines secreted by normal T cells, particularly interested in antigen presenting cells, dendritic cells, B cells, and CTLs.

We've been looking at other chemokine processing mechanisms. We've discovered another enzyme that I think cleaves chemokines that aren't sensitive to the CD26 enzyme, and I think these two pairs of enzymes are the main enzymes that are regulating or metabolizing chemokines.

We're following up on our observation about the chemokine dimer. We believe that this is a potent and novel anti-HIV agent, and we are looking at the receptor signaling and the cross-linking kind of potential that you'll get from a dimer of chemokines in contrast to a monomeric form of an agent.

And I mentioned about the MIP-1 beta. We think that this is involved in recruitment of a regulatory T cell that will turn off an immune response once it's started.

We had proposed to look at, to go into the mouse

model. We mainly work on human systems, but we're interested in addressing the CD26 in these new knockout mice to actually address the question about processing and what effect in vivo it will have, and also with T cell helper cell polarization.

And then finally, we're continuing studies on the mechanism of this ligand independent pathway, this cytokine pathway, by looking at roles of endocytosis, clathrins, the G coupled receptor kinases, arrestins, and the STAT proteins in the signaling pathways.

So briefly, that's a very quick overview of what we've been doing and what we plan on doing in the future.

So thank you.

(Applause.)

CHAIRMAN SALOMON: In the past, we've, when we had more time and it was earlier in the day, I encouraged discussions of these things. I would hope you'll forgive me if we just go on to the next one and then maybe if we have questions.

Anyone want to throw anything at me on that one? No? Okay.

Michael, no disrespect.

DR. NORCROSS: No.

CHAIRMAN SALOMON: Absolutely.

Okay. Phil, would you like to introduce for the Office of Cellular Tissue and Gene Therapies the programs of Dr.

Raj Puri and Dr. David Essayan?

DR. NOGUCHI: Yes. Thank you.

And I apologize. I wanted to use the restroom in between here.

Recently the FDA and CBER has announced a new and launched a new office that will cover cellular therapies, tissue therapies, and gene therapies and other assorted things related to that. As part of that reorganization and as we go along, I think that the programs of the Division of Cell and Gene Therapy continue in their scientific approaches that you have reviewed previously.

One new part of the reorganization that has just occurred is Dr. Raj Puri has been named as Acting Director of the Division of Cell and Gene Therapies, and so that I think some of the concerns that were expressed in the draft report are probably very pertinent as you look at what he's doing to date.

Dr. Raj Puri, in addition to leading a program for molecular tumor biology, is also co-Director of the NCI-FDA microarray facility and has done a very good job at moving some of the standardization forward on that.

I don't believe he'll be talking too much about that. I just have to put in one very nice thing here. He recently went over to NCI to show them some of the chips that FDA has made versus what NCI has made, and it was, I think, a fairly uniform agreement that, well, they'd like to learn how we

make it better than they do.

And in addition to Dr. Puri's review of his laboratory program, we had another review program of Dr. David Essayan, who is in the transition period moving his research program from the Johns Hopkins University to the Center for Biologics and really is just beginning to establish his own program separate from Hopkins, but within our own research facilities.

I'll leave it at that.

Raj.

DR. PURI: First I'd like to thank Dr. Salomon, Dan Salomon, and the subcommittee, site visit subcommittee, and the Biological Response Modifiers Committee for your time in reviewing the scientific program in the Laboratory of Molecular Tumor Biology.

As Dr. Noguchi mentioned, my lab is composed of two principal investigators: one, myself; another is Dr. Essayan. And you are going to hear from him his research program, which we all presented at the site visit.

Next slide, please.

I think my presentation is in that computer. There is some technical glitch.

I will indicate some of the names of my staff who has done all of the work which I will present here today.

Our research stems from a serendipitous discovery

of the expression of receptor of immune regulatory cytokine in studying the muting tumor cells to study the mechanism of action of tumor biology and cytokine in the context of tumor biology.

We found that Interleukin-4, which is a PH-2 derived cytokine, the receptors are overexpressed on muting tumor cell, and since then we studies a variety of human solid tumors. We found, as listed here, a large number of human solid tumors expressed receptors for this cytokine.

Not only the receptors are overexpressed. We have shown over the number of years, almost more than a decade of this research, that these receptors are also different from that of immune cells and the receptor structure is different in addition to signaling construction to these receptors is different on tumor cells.

I have presented some of these data in my site visit was held in 1997, and I will not talk about a whole lot. Ever since then we have taken the advantage of this knowledge of overexpression of IL04 receptors on tumors in producing a fusion protein where the IL-4 is fused to a bacterial toxin made by a bacteria called Pseudomonas aeruginosa, and this molecule called IL-4PE or IL-4 toxin now is in clinical trial for the treatment of recurrent brain tumor, the deadliest form of all cancer.

About seven years ago, a new cytokine was cloned called Interleukin 13, which is a cousin of Interleukin 4, and it's also made by the Th2 cells, and we were obviously very

interested to see if its receptors are also expressed on tumor cells.

And I will show you in this slide ahead that pleasantly, a pleasant surprise that these receptors are also overexpressed on certain number of cancer cells.

Next slide, please. The slide before.

So we have arbitrarily divided our research program in three major areas. One is to study the biology of these tumors and the discovery of novel surface molecule on solid human tumors.

And second, which I will not talk here today a whole lot, looking at the mechanism of action of signal transduction through these two receptors and in the tumor cells and in the immune cells.

And thirdly, the third aspect we are studying is the targeting of these receptors for a possible treatment of human cancer.

Next slide, please.

As I indicated that Interleukin 13 receptors were found to be overexpressed in a variety of different tumors as shown here, some of the cells lines and tumors, glioblastoma multiform cell lines, primary cell culture, tumor specimen, AIDS associated Kaposis tumor, certain percentage of squamous cell carcinoma of head and neck, and some ovarian carcinoma.

But in contrast to the tumor cells, normal immune

cells on which IL-13 has prominent biological activity express very few or non IL-13 receptors. T cells do not have IL-13 receptors, and IL-13 does not mediate a biological response on T cells.

But in B cells and monocyte, the receptor numbers are only few, but the tumor cells express a lot, many on the cell surface.

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In the numerous studies we have reported vigorously in various journals, we have proposed that IL-13 receptor is three different type, which is similar to Type 1 cytokine receptor superfamily. In Type 1 IL-13 receptor, the IL-13 binds to two IL-13 binding proteins called alpha 1 and alpha 2 chain.

And alpha 1 chain forms a productive complex with the IL-4 receptor alpha chain, and this receptor configuration is present on a variety of solid tumor cells, such as malignant brain tumor, kidney cancer, and squamous cell carcinoma of head and neck.

In Type 2 IL-13 receptor, alpha 2 chain is absent. Instead, these two chains are present, and both chains bind Interleukin 4, and this type of receptor not only comprises and forms a Type 2 IL-13 receptor, but it also forms Type 2 IL-4 receptor, and these two chains may be a signal transduction introduced by Interleukin-4 and Interleukin-13 in

nonhematopoietic cells.

The Type 3 IL-13 receptor was another player, which you heard today a lot about the gamma chain. Gamma chain, as you heard, that is a component shared between IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 receptor system.

And because Interleukin-4 has similarity in biological activity to that of Interleukin-13, it was believed that gamma chain is also a component of IL-13 receptor system.

However, our studies have demonstrated that gamma chain does not bind Interleukin-13 by itself. It does not participate in the formation of IL-13 as separate complex. But if you do express gamma chain in nonhematopoietic cells, such as renal carcinoma cells, this chain can interfere, binding up Interleukin-13 and signaling transduction through Interleukin-13 in those tumor cells.

Next slide, please.

To confirm this model, we performed reconstitution studies, and we published two papers in Blood in over two or three years, and those studies are summarized here, the two manuscripts I'm rising on this slide, which supports our proposed model that IL-13 receptor is composed of three different types.

And to summarize that IL-13 binds to IL-13 receptor alpha-2 chain with high affinity and alpha-1 chain with low affinity. One chain is co-transfected in Chinese hermister

(phonetic) ovarian cell with IL-4 receptor alpha chain. It forms a high affinity IL-13 receptor, and these studies were performed by Dr. Kosi Kawakami in the lab.

In addition, Dr. Kawakami found that IL-13 structural institution (phonetic) is internalized after it binds to IL-13 ligand, but it does not mediate signal transduction through JAK or STAT kinase pathway.

And as I indicated, IL-13 receptor alpha 1 chain forms a high affinity receptor to alpha 4 receptor chain and does also mediate signal transduction through JAK/STAT pathway.

Next slide, please.

Dr. Kawakami discovered in the lab and in paper to be reported, published last December in General Experimental Medicine. He found that IL-13 receptor alpha 2 chain has a unique property which we did not expect. The receptor chain gene transferred into the human breast and pancreatic cancer inhibited the tumor growth in the immunodeficient animals.

As you can see here, two different clones of breast cancer and pancreatic cancer continue to form large tumor. Needed to sacrifice due to ethical reasons, but in transfector cells, you can see there is a delay or no occurrence of the tumor at all, and this reoccurrence of the tumor later on was associated with the loss of alpha 2 chain expression.

Next slide, please.

We have found numerous studies. We reported that

in that paper, but to tell you that there's an in interesting phenomenon that infiltrates in our inflammatory cells and neutrophils that seem to produce IL-13. As you see here, the core localization studies performed by Dr. Kawakami.

And in addition to, we looked at a chemokine production such as Interleukin 8 is made by those cells.

Next slide, please.

So this program is currently ongoing, and our future plans include to study the incidence of receptor expression in vivo in tumor samples by different techniques. We will continue to study the structure and significance of this receptor expression in tumor cells, particularly with regards to the expression of alpha 2 chain.

And Dr. Noguchi mentioned that we are using a microanalysis to see what type of pathways are associated with IL-13 receptor alpha 2 chain, why nature provided so many of these receptors for the immune regulatory cytokine, which is supposed to have biological activity on the immune cells, but not on the cancer cells.

In addition to that, we are looking at the regulation of IL-13 receptor alpha 2 chain because it is only overexpressed on the tumor cell, but not on the immune cells. Therefore, if we can figure it out how to regulate this receptor which can be helpful in developing therapeutic modalities for the treatment of cancer.

We want to further expand our studies which we reported in JX Med. that what is the mechanism of inhibitional tumor growth with alpha 2 chain, and of course, because this receptor chain is only expressed on tumor cells, we ask the question: is this tumor, this receptor chain, is it novel tumor associated antigen?

Next slide, please.

But in the meantime, we have taken the advantage of knowledge of this expression of IL-13 receptors on solid tumor cells, and we created a fusion protein. As I indicated in case of Interleukin-4, here in Interleukin-4 we connected IL-13 to Pseumonas exotoxin made by a bacteria, Pseudomonas aeruginosa, and it has three domains.

The binding domain is replaced here by Interleukin-13 and gives a new characteristic to the molecule. It will only bind to the cell if they express IL-13 receptor, and it will only kill the cell if enough molecules go inside the cell.

So Dr. Bharat Joshi in the lab -- next slide, please -- made this molecule in large quantity in the lab by recombinant DNA technology and expressed that in E. coli, and he perfected the technique to produce large quantity of this protein in a very short period of time.

And we asked the question of various fellows in the lab: Dr. Rapat Hussein, Dr. Mariko Kawakami, Dr. Bharat

Joshi, and Dr. Kogi Kawakami, and Pam Dover studied that, and they found that the tumor cells which express high number of IL-13 receptors were highly effectively killed by IL-13 toxin.

Consistent with the lack of expression of IL-13 receptor on immune cells, the IL-13 toxin was not active or did not kill those cells in the tissue culture, and the Il-13 toxin made a remarkable anti-tumor effect in three tumor model which we have reported in malignant glioma model, ascaposis model, and head and neck cancer model.

Next slide, please.

This is just a cartoon which I just said that IL-13 toxin bind to the IL-13 receptor or expressed on the tumor cells, and this toxin internalized process intracellularly and caused the protein synthesis inhibition leading to the apoptotic and necrotic cell death, and the cells dies where the normal cells do not have IL-13 receptor have low number. This molecule does not go inside the cell, does not get processed, and the cell don't die, and providing a good therapeutic window where you can target IL-13 receptor with this molecule.

Next slide, please.

So based on our preclinical studies, our data has resulted into the initiation of three Phase 1/Phase 2 clinical trials, and by the way, this technology has been licensed by the NIH technology transfer to the biotechnology companies who are undertaking these clinical trials.

The first clinical trial, the drug is infused directly into the brain tumor. The study being undertaken by new approaches to brain tumor therapy, a study headed by Skip Grossman at Johns Hopkins University with nine other clinical centers, where they infuse the drug and monitor the safety, tolerability, and clinical responses.

In the second trial, which is being taken at the Memorial Sloan Kettering, M.D. Anderson USCF, and Yale Cancer Center, where the drug is infused first. The tumor is resected, followed by infiltration of this drug surrounding the tumor cavity.

The third trial just initiated in Israel, as well in Germany and the Cleveland Clinic, where the drug is infuted preoperatively and the tumor resected, where the tumor response and the safety is being monitored.

Next slide, please.

So as I indicated, that IL-13 receptor alpha 2 chain binds IL-13 with the high affinity, and Dr. Kawakami has found in the lab that the tumors cells which do not express alpha 2 chain, if you put alpha 2 chain in those cells, you can sensitize them to the cytotoxic effect of IL-13 toxin.

And similarly, he has shown that in vivo if you put this alpha 2 chain, sterilely transfected tumor cell, when you form the tumor in the mouse and retrieve with the drug with IL-13 toxin systemically, you can mediate systemic tumor

response.

Now he is undertaking third generation experiment which is similar to the clinical situation. Form the tumor first, and it will model of human disease, and come back and do a plasmic mediated gene transfer in vivo in the tumor, followed by IL-13 toxin systemic therapy.

In addition to DR. Sitteram in the lab is generating antibody to IL-13 receptor IL-2 chain by phage display technology because alpha 2 chain is overexpressed on the tumor cells, and one can use an antibody connected with a toxin molecule and make a recombinant fusion protein for the targeted therapy of cancer.

And she's also expressing excessive domain of alpha 2 chain to generate a unique region for some of our studies as I indicated.

Next slide, please.

So this is my last slide, and I wanted to bring to your attention how this research impacts on our regulatory process. Cancer is a most difficult public health problem, and our research supports informed decision making for accelerated development of novel therapeutics.

Our research anticipates public health needs and supports informed decision making in the protection and prevention of public health. It provides insight into the mechanism of action of safety, chemistry manufacturing, and

control of growth factors, cytokines, cells, and gene therapy that we regulate.

It provides insight into development of standards and new technologies such as indicated, that microarray technology that we have developed.

It helps development of identity and potency of tumor therapeutics and vaccine that we regulate in Office of Cell Tissue and Gene Therapy, in the health development and retention of expertise in the cutting edge areas of medical research which we need to evaluate those cutting edge research as we see at CBER.

And I'd like to thank you for your kind attention.

(Applause.)

CHAIRMAN SALOMON: Thank you, Raj.

DR. ESSAYAN: David Essayan.

Which mic is working here? Can you guys hear me?

Okay. I want to thank the committee and the audience for their endurance.

My laboratory has two main projects. The first one is regulation of T lymphocyte responses by specific phosphodiesterase, or PDE enzyme isoforms. Our second project is the pharmacology and regulation of a novel IL-17 homologue, which we and our colleagues at Hopkins recently cloned. We call it ML-1, but it has also been dubbed Interleukin-26.

Just to remind everyone the steady state

intracellular levels of cyclic nucleotide second messengers are regulated at the point of degradation by the cyclic nucleotide phospodiesterases.

Our laboratory was the first to demonstrate that inhibition of PDE-4 with the resultant increase in intracellular cyclic AMP specifically down regulated proliferation and cytokine generation from peripheral blood mononuclear cells and allergin specific T cell clones.

We went on to demonstrate that the effects of PDE-4 inhibition were mediated through concurrent activation of Type 1 and Type 2 cyclic AK, otherwise known as protein kinase A.

These were all memory responses. We have since become interested in looking at the effects of PDE-4 and a more recently described PDE-7 and their inhibition on neoantigen responses and induction of T cell tolerance.

Along that line we've used a model whereby mice are sensitized to ovalbumin in the presence or absence of Rolipram, our model Type 4 PDE inhibitor, and then rechallenged at a later date either once or several times with ovalbumin.

Using systemic rechallenge we've demonstrated a significant dampening of the proliferative responses to ovalbumin from cultured splenocytes. Our control PDE inhibitor, which is a PDE-3 inhibitor called Milrinone, had no such effect.

Looking at bronchial challenge, we've demonstrated that the Rolipram treated mice show a marked decrease in their

bronchoalveolar lavage eosinophil counts, as well as abrogation of their bronchial hyper responsiveness. We feel that these findings are consistent with induction of antigen specific tolerance.

Future aims for this line of research include comparison of PDE-4 inhibitor effects between Th1 and Th2 type stimuli. Work has been done with Th2 stimuli.

We would also like to go on to identify the potential synergy with concomitant signal 2 blockade and would also like to look at the potential synergy or antagonism with concomitant PDE-7 inhibitor administration.

Our laboratory, which is actually just a grandiose term for my technician and I, have also been working since 1999 on our second project at which point we identified with my long term collaborator, Dr. Shao Ku Wang, an open reading frame on 6P12, which showed significant homology to Interleukin 17. We named this ML-1 and went on to demonstrate that ML-1 was constitutively expressed in basophils and Th1 cells and up regulated on activation in T cells and in clinical bronchoalveolar lavage samples.

The following year we defined the tissue distribution of ML-1 compared to Interleukin 17, expressed ML-1 in a variety of systems, and went on to also generate a murine polyclonal for Western Blotting.

Recently we've demonstrated that ML-1 induces

secretion of Interleukin 6 and Interleukin 8 from endothelial cells in a manner similar to Interleukin 17, but also up regulates expression of ICAM-1 on bronchial epithelial cells in contrast to Interleukin 17.

Even though the ML-1 receptor has not been identified to date, we've gone on to show that ML-1 activates ERK1 and ERK2 kinases, but not p38 or the JNK pathway.

And most recently, sine the site visit, we've also gone to show that pulmonary administration of an expression construct for ML-1 induces neutrophil influx in the pulmonary bronchi, and here we just show the dose dependent up regulation of IL-6 and IL-7 and, in contrast, Interleukin 17, increasing the mean channel fluorescence for ICAM-1.

And here we show the transient up regulation to phospho-ERK1 and ERK2, but no changes in phospho-p38 or JNK.

Future directions for this project include characterization of the expression of ML-1 in specific disease tissues; characterization of the biology of ML-1 on additional immune cell types; investigation of the pharmacologic regulation of ML-1 by immunomodulatory agents.

We're in the process of generating ML-1 transgenic mice at the core facility at Hopkins and ultimately would like to generate ML-1 knockout mice and then would also like to look into the cloning sequencing and functional analysis of the ML-1 five prime UTR.

The mission relevance of this data I will close with. We feel that these projects increase our expertise in T cell biology and immunopharmacology, which enhances our ability to review a variety of products for a variety of indications.

We also feel that our research findings are relevant to the pathophysiology of treatment and treatment of allergic and immunologic diseases.

I would note that PDE-4 inhibitors are currently in late phase development at our sister center, Center for Drugs.

I will close with that. Thank you very, very much.

(Applause.)

CHAIRMAN SALOMON: Okay. So if I remember the drill, then what we need is to clear the room and go into a closed session where we'll vote on approval of the site visit report.

Certainly before everybody leaves, let me thank Michael and David and Raj and everyone else in their groups for a really excellent set of presentations when we visited.

(Whereupon, at 6:17 p.m., the meeting in the above-entitled matter was concluded, to reconvene immediately in closed session.)