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DEPARTMENT OF HEALTH AND HUMAN SERVICES

PUBLIC HEALTH SERVICE

FOOD AND DRUG ADMINISTRATION

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CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

AND

NATIONAL INSTITUTES OF HEALTH

NATIONAL HEART, LUNG AND BLOOD INSTITUTE

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WORKSHOP

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THURSDAY

SEPTEMBER 10, 1998

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The workshop took place in the Jack Masur Auditorium, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20892 at 8:00 a.m., Liana Harvath, Ph.D., Chair, presiding.

PRESENT:

KATHRYN C. ZOON, Ph.D., Director, CBER

LIANA HARVATH, Ph.D., Chair

DAVID STRONCEK, M.D., Moderator

GIOVANNA TOSATO, M.D., Moderator

PRESENT (Cont.):

JOHN WAGNER, M.D., Moderator

PAOLO ANDERLINI, M.D., Speaker

MITCHELL CAIRO, M.D., Speaker

RICHARD CHAMPLIN, M.D., Speaker

DENNIS L. CONFER, M.D., Speaker

JOHN F. DiPERSIO, M.D., Ph.D., Speaker

N. REBECCA HALEY, M.D., Speaker

MARY M. HOROWITZ, M.D., Speaker

JOANN E. KURTZBERG, M.D., Speaker

FRED LeMADER, M.D., Speaker

SCOTT D. ROWLEY, M.D., Speaker

PABLO RUBINSTEIN, M.D., Speaker

ELIZABETH J. SHPALL, M.D., Speaker

DONNA WALL, M.D., Speaker

C O N T E N T S

C O N T E N T S

P-R-O-C-E-E-D-I-N-G-S

(8:12 a.m.)

DR. HARVATH: Good morning. We're going to try and get started in the next minute or so, and I thought that I would give everyone a chance to find a seat. There's plenty of seats in the auditorium, and also take the opportunity to thank you on behalf of the Organizing Committee and the Center for Biologics and our colleagues at the NIH and the Heart, Lung and Blood Institute.

It's indeed a very great privilege and honor to be able to co-sponsor this workshop with the NIH, and Dr. Kathryn Zoon, the Director of the Center for Biologics Evaluation and Research will present the opening remarks and officially welcome you to this conference.

DR. ZOON: Good morning. Welcome to the stem cell workshop, and it's a pleasure to be here. This is the fourth in a series of workshops that have been co-sponsored by the Center for Biologics Evaluation and Research and the National Heart, Lung and Blood Institute since 1995 regarding the hematopoietic stem cells.

And I think this has been a very active area over the past two years. We've been engaged in many activities with various sectors of interested

parties both during FDA reform and discussions on scientific issues regarding stem cells, and I view these workshops as very important.

They're very important to the agency and to NHLBI one, I think, to identify important new areas of research that we need to find important answers to questions, and two, as we embark in effecting our tissue framework and the regulation of stem cells that we do it based on scientific knowledge and understanding to enable the technology without being overbearing.

And I think in looking at this, the importance of setting standards and understanding the scientific underpinnings and the necessary information to make appropriate decisions for helping patients using this technology becomes extremely important and requires the best minds and the best thinking to gather to deal with those issues.

And I really appreciate the attendance here today. It shows to me the interest in this area, and I'm sure during the course of the day, we will be joined by others if they can find their way into this building.

Our first workshop actually took place in 1995 when we held in December a cord blood workshop.

Subsequently, we had a workshop in February in '96 on peripheral blood stem cells. And then in '97, we had a workshop concerning the ethical issues in cord blood banking.

We and our colleagues from the NIH view this as very, very important. As I mentioned, the NIH is very interested in learning specific areas of research in this field that need to be pursued. We are continuing our public discussions on the current data available for the development of standards.

As you know, we put that notice out in the Federal Register, and we encourage people to continue to submit information to the docket. And I think that will be important from both looking at the peripheral blood stem cells as well as cord blood.

In January, we, as you know, we did put out the notice seeking comments on the issues related to proposed standards for unrelated allogeneic peripheral and placental cord blood and hematopoietic stem cell products. And we hope that by January of 2000 we will have adequate data to address the development of standards.

This public workshop, again, is a continuing dialogue, and we hope to learn as much as we can and share what we know with you. And we'll

continue to do so during the course of the next year and a half.

The Steering Committee for this workshop, I think, has done a marvelous job, and I want to thank them personally. This consisted of staff of the Center for Biologics and Heart, Lung and Blood. They included Liana Harvath from the Center for Biologics, and CBER members included Steven Litwin, Gerry Marty, Paula McKeever, Patricia Rohan, Giovanna Tosato, and Joe Wilczek. And NHLBI staff included Dr. George Nemo.

I want to thank you, first of all, for attending today. I think it's very important, and I wish you a very productive and successful workshop. I'd now like to introduce the Moderator of the first session, Dr. Giovanna Tosato, who's Director of the Division of Hematological Products in the Office of Therapeutics Research and Review. Giovanna?

DR. TOSATO: I'd like to welcome you all to the first session of the stem cell workshop. As you see from your program, there are three speakers. The first speaker is Dr. Liana Harvath, who is the Chief of the Laboratory of Cellular Immunology at the Center for Biologics.

She has been a point person for the development of the scientific and regulatory policy

for hematopoietic stem cell progenitor cells. She will describe to you the Federal Register notices and will discuss to you what the agency is seeking with these notices.

The second speaker is Dr. Mary Horowitz, who is the Scientific Director of the International Bone Marrow Transplant Registry, and she will talk to us about lessons learned from the Registry.

And then the third speaker is Dr. Paolo Anderlini from M.D. Anderson who will talk to us about some of his studies with cytokine mobilization of stem cells.

I'd like now to call on the first speaker, Dr. Liana Harvath. Thank you.

DR. HARVATH: Well again, on behalf of the workshop Organizing Committee, I would also like to thank you, and on behalf of that Committee, for your interest and your continued participation in this workshop and others that we've held.

And I'd also like to mention a special thank you to our colleague, Joseph Wilczek, who has taken care of many laborious details in order to facilitate the conference actually occurring and ask your indulgence that because of the numerous sites of construction on this campus that there will be

difficulty for a lot of people to actually find this auditorium or find their way into it.

I've been also asked to say that the telephone number that some of you were given, especially the speakers were given that I said if you had to be reached, you could use that phone number, we found out this morning that the construction has actually wiped out that telephone and that telephone number.

So I will get an emergency number for you so if your colleagues must contact you and that it's an absolute emergency, we'll have that telephone number available for you. I actually have it. I just didn't bring it up to the podium with me.

Well, as Dr. Zoon just stated, we have been actively engaged in hosting a series of workshops with our colleagues at the Heart, Lung and Blood Institute and also with other professional organizations. And if I could have the first slide please.

As Dr. Zoon just mentioned, in December of 1995, we co-sponsored with the Heart, Lung and Blood Institute our first workshop that dealt with cord blood banking, and particularly, we're focused on a scientific discussion regarding procedures for collection and storage of cord blood.

And there was, for those of you who attended, a very lively discussion on many very interesting aspects to this field.

The second workshop was just a few months later in February of 1996 on the topic of peripheral blood stem cells, again, focusing on a collection and a number of other parameters having to do with cell processing. And at that time, we had distributed, particularly at the cord blood meeting, our current thinking, at that time, on proposed regulation of this area, and received numerous comments to those proposals.

And in response to comments the FDA received on its proposed approach to regulation of stem cell products, FDA held a public meeting in March of 1997, and this was to discuss our proposed approach to the regulation of cellular and tissue based products, which is a very broad scope proposal for regulation of a variety of cells and tissues including hematopoietic stem and progenitor cells.

Then a year ago in September of '97, we co-sponsored with our colleagues at NHLBI and our colleagues at the American Association of Blood Banks and the American Red Cross, a two-day workshop focusing on the ethical issues of placental

umbilical cord blood banking, again, a very lively meeting.

What brings us here today then is to focus on, really focus on the science and take a pulse on the status of the science. Many people have expressed some concern to me, are you going to make decisions, is FDA going to make some sort of decision on what they hear here, and the answer is no.

We are conducting this workshop as a dialogue, and a dialogue based on scientific data. We know this is very much work in progress. We appreciate the excitement in this field. And so what I would like to do is to use this slide to talk about the specific goals of this workshop.

As Dr. Zoon just mentioned, January 20th of this year, FDA published a notice in the Federal Register, and this actually was a follow-up from the specific part of our proposed approach to cell and tissue based products really focusing on a call for data for unrelated allogeneic peripheral as well as placental umbilical cord blood cell products. And all of you should have a copy of this Federal Register notice in your folder.

In the presentation that I will give this morning, I'll just highlight some of the key

features of that where data are -- where we're actually asking the public to provide data for us in an effort to try and achieve the development of standards.

The workshop today is going to focus on several topics. We're going to have very experienced presenters in each of these fields, one dealing with the administration of cytokines to normal donors of peripheral blood products, peripheral blood stem cell products.

And as some of you may have noticed, we are also holding a companion workshop tomorrow on granulocytes for transfusion. And the reason being these two workshops being held as a pair of workshops is we appreciate the fact that many of you who collect peripheral blood hematopoietic stem progenitor cell products may also collect granulocytes from donors who are given this same, if not, the identical cytokines.

So what we wanted to do was have an opportunity for people engaged in both of these cell product fields to be able to attend the workshops without having to travel out here twice.

We will also hear about the current status of related and unrelated allogeneic peripheral blood stem and progenitor cell transplantation. And we've

asked our colleagues from the academic transplant centers who have been actively publishing in this area, we've asked our colleagues from IBMTR, ABMTR to speak to us about their experience with the registry data, and some of the statistical considerations that go into the evaluation of data in a large registry.

We've also asked our colleagues who are very active in the unrelated placental umbilical cord blood banking and transplantation field to present a snapshot of the current status of that field as well. And not shown on here, but in the last session of this meeting, we've invited our colleagues from the professional organizations, the American Association of Blood Banks, the organizations FAHCT and ISHAGE who have all been working to develop professional standards that are applicable to the collection, processing, storing of these products.

I would like to take just a couple of minutes for those of you who might not be familiar with our original proposed approach, or I should say, the proposed approach to regulation of cellular and tissue based products which is this broad-based proposed regulatory strategy for a variety of cells, and to hit a few of the salient features about this,

and then follow-up on the details that pertain to the stem and progenitor cell products.

This docket was released to the public February 28, 1997, and it outlines a risk-based system of regulation for a variety of cellular and tissue based products which include hematopoietic stem and progenitor cells. This proposal considers five overarching public health and regulatory concerns.

They include the prevention of the transmission of communicable diseases which is achieved by donor screening through histories as well as testing of the donors for infectious diseases.

Then the second area is necessary processing controls to prevent contamination of cells and tissues and that are intended to preserve their integrity and function for safe and effective use. These would be processes we've referred to as good tissue practices which are somewhat analogous to a good manufacturing practice in that they focus on how one conducts a series of procedures to collect their material, process it, store it, and distribute it.

The third issue is clinical safety and effectiveness, and we'll talk a little bit more

about that in the next slide, and the conditions under which the agency would ask for data to demonstrate clinical safety and effectiveness.

The fourth is necessary product labeling and permissible promotion for proper product use. And the fifth is monitoring and communicating with the cell and tissue industry. This would include basically registration with the agency as well as a listing of the products that are collected, processed, stored and distributed.

Regarding clinical safety and effectiveness, the proposed approach had stated that clinical safety and effectiveness data will be required for cells from an unrelated allogeneic donor or from products that are manipulated, and we have defined manipulation to include things such as genetic modification or ex vivo expansion.

Previous thoughts about or proposals about manipulation to include cell selection were not included in the revised approach because we recognize that this technology is moving very rapidly and will, perhaps, one day become fairly common practice. So manipulation in this proposed approach for hematopoietic stem and progenitor cells will be considered those two areas that involve a modification of perhaps the biologic function of the

cell or some either genetic parameter or perhaps cell cycle parameter which we may find an ex vivo expanded cells.

Another area where clinical data would be required would be when cells are used for other than their normal function, and the fourth would be products where cells may be combined with nontissue components. Now, we wrote this to apply to a broad spectrum of tissues, so some of these, you may say, do not apply or pertain to the hematopoietic stem and progenitor cell field. But when reading the document, please bear in mind that we had to write this for a broad spectrum of cell and tissues.

Now, this past year, there have been two publications that have appeared in the Federal Register, and this meeting is, and the talk that I will focus on in the remainder of my time, will really focus only on the first Federal Register notice, that was January 20, '98, which was calling for data for unrelated allo stem and progenitor cell products from peripheral as well as placental umbilical cord blood.

In May of '98, there was a proposed rule published, and that really will not be the topic of this meeting. Both of these have open comment periods, and depending upon the types of responses

we get to these various proposals, the agency may determine that workshops focused on those particular topics may be warranted in the future.

But this is just to let you know that we have been actively working in trying to disseminate this information, and we very much encourage your participation and thank those of you in the audience who have written comments to the docket, who've engaged in the dialogue because this is really going to be the best way for scientific based approach to the development of standards in this field to emerge.

Regarding the January 20th of this year Federal Register notice, as stated in that notice, we've kind of outlined our approach, and believe that for minimally manipulated unrelated allogeneic stem and progenitor cells that are intended for hematopoietic reconstitution that it may be possible to develop product standards, establishment controls, and processing controls.

This may be possible through the existing clinical data, and it may also be possible that there will be standards that emerge for subsets of patients, for example, pediatric population. There may be more data available for placental cord blood in that population than in the adult population.

So as we outlined in that Federal Register notice, we appreciate that the data may be substantiated or substantial in some of the product areas, and perhaps not as -- there may not be as much data in other areas. So we ask you to delineate that for us and provide that information to us.

If a processing establishment controls and standards can be developed through this process, then it will be possible for the agency to issue guidance for the product standards and these establishment and processing controls. And it would be the intention then that licensure could be granted for products certified as meeting those issued standards.

As stated in the original proposed approach of February of '97 and restated in the Federal Register notice, if sufficient data are not available to develop standards, then after a specified period of time, unrelated allogeneic stem cell products would be subject to IND and marketing application requirements.

We appreciate that many investigators have already voluntarily submitted INDs to the agency and are conducting their studies under IND. At this point in time, it is not a requirement. However, we

have given ample time and opportunity for dialogue in order to sort of forewarn people that if we do not have sufficient scientific data then we will require that these data be collected under an investigational approach.

Now, the request for proposed establishment controls include standards for personnel, facilities, quality management, standard operating procedures, staff training, competence, and process validation. They also include standards for record keeping, data regarding donors, processing, quarantines, storage, labeling, distribution, tracking, handling of errors and accidents, deviation from protocols, adverse reactions, and quality control processes.

Many of you have already developed through your professional organizations published standards for how to handle what are considered to be establishment controls. And this is just simply spelling out what the agency believes would be important to include in those controls.

The request for proposed processing controls would include standards for donor selection, informed consent, donor testing and screening, histocompatibility testing, collection procedures, product testing, volume reduction

methods, cryopreservation, storage conditions both in the liquid and frozen state, storage monitoring, transportation of the products, temperature limits, packaging and thawing.

These processing controls should also include standards for testing product contamination, product viability, and the manner in which you may select to test viability because we recognize that that could be something that could vary from one product to another, composition and functionality, and to include when and how you believe such testing is to be performed.

The proposed product standards should include the criteria for the acceptance of the unit including the volume, for example, the minimum volume the viable cell number which could be specified either as nucleated or mononuclear cells, storage temporal limits, microbial, or other contamination limits, and other characteristics, for example, CD34 positivity.

There may be other phenotypic markers that will be, perhaps, even more appropriate than CD34. But characteristics that you believe help you as the professionals that are collecting the products and the physicians who are administering these products

characterize what you feel will be the minimal acceptable criteria.

For the peripheral blood stem cell product area, the information we've asked for we've also asked you to consider including information regarding the treatment regimens of normal donors with mobilizing agents to include the type of mobilizing agent, the type of cytokine, for example, the duration of mobilization, how many days the normal donor was given the cytokine, and the number of apheresis collections.

We realize that there is a vast variability in this data, but we ask that you include the types of specifications that you consider to be important in this area.

The request for data for proposed product standards in this document provides a suggested format for the data submission. For example, for evidence of neutrophil and platelet engraftment and sustained platelet engraftment. And as you can read in the last, I think -- believe, the third page of this document, some of the final paragraphs, we talk about an absolute neutrophil count of 500 per microliter or greater the days to achieve that, and then the platelet engraftment would be the days to achieve a platelet account of 20,000 per microliter

or greater when the patient is transfusion independent.

And that sustained platelet engraftment would be to platelet counts to 50,000 per microliter or greater. These are very consistent with what you in the transplantation field have been using. And we in our advisory committees have been given as recommendations for evaluation of a variety of products in this area.

Product standards, also we include requests for data regarding the extent of HLA disparity, the nucleated cell dose per kilogram body weight of the recipient, and the extent and severity of graft versus host disease. We hope you will include your data in acute GVHD as well as chronic GVHD, the criteria you consider important for evidence of engraftment, and finally statistical methods for data evaluation.

Our biostatisticians insist that we put this in here, so they will be the people that will be looking over that kind of information, and perhaps in future workshops, if this turns out to be an area of concern, we can have some focus on that area.

So in conclusion, our intention is to continue the scientific dialogue, and we envision

that this may require some more workshops. And if we find that there are very specific areas where we need to focus on a particular scientific problem or some other type of issue, and we hear that from you, we will take the initiative to try and organize such a workshop in conjunction with our colleagues at the NIH.

We believe proposed standards should be supported by adequate data and other relevant information, that they be uniformed, and that perhaps we can achieve a uniform set of standards by consensus of interested parties working together. That's our goal and our hope.

And the FDA then would intend to issue through the agency's guidance document procedures then the set of standards that are derived through this public process. And you would be given, again, opportunity to comment on any of these procedures or policies that are put together through an open public comment period.

So I would like to, in the interest of staying on time and giving Dr. Horowitz time for her presentation, to thank you. Dr. Tosato didn't mention this, but I would like to just say that the way we're going to hold the discussion period is rather than ask speakers questions after each

speaker, if you would please hold your questions for the panel. We will then all step up to the front and do our best to answer your questions.

You have some blank pieces of paper in the back of your folders. If you do not wish to get up and ask a question at the microphone, you can write your question down, and we will have some of our colleagues coming down the aisles to collect them.

Otherwise, you're welcome to step up and introduce yourselves, and give your name and affiliation on the microphone because this entire meeting is being recorded and transcribed, and those transcripts from the meeting will be made publicly available. So we would like to know the names and affiliations of the individuals when they ask questions. Thank you very much.

DR. TOSATO: It's a great pleasure to introduce Dr. Mary Horowitz from IBMTR/ABMTR.

DR. HOROWITZ: Good morning. It's also a pleasure to be here, and I welcome the opportunity to share some information from the International Bone Marrow Transplant Registry. I know there are many people in the audience who are familiar with the IBMTR and the ABMTR. But for those of you who are not, just to put the studies I'm about to

present into some perspective, I'll just say a few words.

The IBMTR and ABMTR are voluntary research organizations that collect outcome data on allogeneic and autologous blood and marrow transplants from about 350 transplant centers in 40 countries. The IBMTR, which collects data on allogeneic transplants was actually established in 1972 and has been collecting this type of data for over 25 years.

This is just a map of locations of participating centers. We collect clinical data, and the data I'm going to present today is from multiple centers. And with this database, we are able to track trends in the use of transplants and techniques for how transplants are being done. And to start off the talk, I want to show you a very dramatic shift in autologous transplants that happened in the late 1980s through the early 1990s which was a shift from the use of bone marrow derived stem cells to peripheral blood stem cells.

In 1989 to '90, over 80 percent of autologous transplants were done using bone marrow, but as you can see, today, that is not at all the case, and almost all autologous transplants are done using peripheral blood stem cells. I might add that

there were no randomized trials comparing the two approaches during this period of time, and the change was extremely rapid.

There's been reluctance to use allogeneic peripheral blood stem cells or there was reluctance to use allogeneic peripheral blood stem cells because of the large number of lymphocytes in such grafts and the concern about graft versus host disease. But there is some appealing attractions of this approach also because of the large numbers of cells, and it's well-documented that in the autologous setting, hematopoietic recovery of both neutrophils and platelets is significantly more rapid when peripheral blood cells are used.

And in 1995, three small studies, three small single institution studies with a total of about 40 patients with all three studies combined were published in Blood suggesting that allogeneic peripheral blood stem cells could be used safely for hematopoietic reconstitution in the HLA identical sibling setting, and you can see what's happening.

Now, this 1995, one of those reports, appeared early 1995, and we see now that almost a quarter, and it's a little higher now for the 1997 figures, of allogeneic transplants are being done

using peripheral blood derived stem cells rather than bone marrow derived stem cells.

Most of those transplants are in the related donor setting. Right now, about 25 percent of allogeneic transplants use unrelated donor, and only fewer than five percent of those are done using peripheral blood stem cells, but in a related donor setting, about a quarter of the transplants are now using peripheral blood stem cells and that trend shows no evidence of plateauing. So I would expect that we're going to see the same kind of shift over the next few years that we saw in the autologous transplant setting.

The main focus of my talk this morning is really to present some data on the comparative outcomes of related donor bone marrow and peripheral blood stem cell transplants. This is a study that uses data that was reported to the IBMTR and to the European Blood and Marrow Transplant Group because much of the work in this field has been done in several European centers.

The co-chairs for this study are Dr. Richard Champlin, who will be presenting some data later today on the M.D. Anderson experience, and Dr. Norbert Schmitz of the EBMT and the University of

Kiel. I might add that those two centers were the centers that produced two of those reports in Blood.

The objectives of this study were to compare outcomes of HLA identical sibling bone marrow transplants with outcomes of HLA identical sibling peripheral blood progenitor self-transplants, and the outcomes we focused on were hematopoietic recovery or engraftment, acute graft versus host disease, chronic graft versus host disease, transplant related mortality defined in this study as a death in complete remission and leukemia free survival.

We wanted to choose a population of patients that represented the common indications for transplantation. About 75 percent of allogeneic bone marrow transplants are done for leukemia, so we included patients with AML, ALL and CML in first or second remission for acute leukemia, or chronic, or accelerated phase. Again, all of these transplants were done using an HLA identical sibling donor.

The grafts were non-manipulated, so non-selected peripheral blood or bone marrow transplants, no CD34 selection, no T-cell depletion. The years of transplant are 1995 to '96 because there really were very, very few peripheral blood stem cell transplants before 1995.

And the age we restricted to 20 or older, that's because the median age of recipients of related donor peripheral blood stem cell transplants is about 40 as opposed to the median age of bone marrow transplant recipients in general which is about 25 because very few children have received these transplants. So the data I'm going to show you is for adults.

With these inclusion criteria, we were able to identify 288 recipients of peripheral blood progenitor cell transplants and a relatively comparable group is, and I'll discuss that a bit in a few minutes, and 536 recipients of bone marrow transplants. These data were reported by 105 transplant centers. The distribution of transplant regions is shown here: 270 from North America, 378 from Europe, and the remainder from South America, Australia and Asia as shown.

The next few slides compare the characteristics of this patient population, their disease characteristics and their transplant strategies. As you can see, even though we restricted this to adults, there was a trend toward the peripheral blood stem cell recipients to be somewhat older.

The gender distribution was not significantly different, nor was the performance score pre-transplant. There was a trend toward a more acute leukemia in the peripheral blood progenitor cell group, and importantly, the peripheral blood progenitor sell group included a significantly higher proportion of patients with advanced disease.

And this is because in many centers this newer technology is being reserved for patients with high risk leukemia and lymphoma. An important consideration trying to look at the results of these transplants is the conditioning regimens in GVHD prophylaxis also differ in the recipients of peripheral blood and bone marrow transplants. Many more of the peripheral progenitor cell transplants are done after conditioning regimens that include total body irradiation.

There's been a significant trend away from the use of total body irradiation for allo grafting over the past few years in the bone marrow transplant setting, and the GVHD prophylaxis regimens were significantly different with a substantially lower proportion of patients receiving methotrexate which affect engraftments. That's an important consideration when we're looking at

engraftment as an outcome. So there were fewer of those in the PBPC group. And a higher percentage of these patients are also receiving G or GNC, a self-post transplant.

So we have two populations. We have large numbers. The populations are similar, but there are some important differences. So in doing our comparison, we use Cox proportional hazard, regression approach so we could adjust for other factors that might affect both outcome and the estimation of the relative outcomes between the two populations considering as potentially confounding factors the factors shown here, age, sex, performance score, disease, disease status, disease duration, FAB classification for the acute leukemias, white counted diagnosis, cytogenetic abnormalities and particularly in acute leukemias, conditioning regimen, graft versus host disease, prophylaxis and use of post-transplant cytokines to facilitate hematopoietic reconstitution.

The first thing we found in examining our regression models is that there was a significant interaction between disease and outcome, and the estimate of the relative risk of the various outcomes. And so all of the results that I'll show

you now are stratified by whether the recipients were transplanted for acute leukemia or for CML.

These show the results of analyses of hematopoietic recovery, acute graft versus host disease, chronic graft versus host disease, and treatment related mortality. The results are expressed as the odds ratios which approximate the relative risk of each outcome in patients who receive peripheral blood progenitor cell transplants versus those who receive bone marrow transplants.

This is the time to achieve an absolute neutrophil count of greater than 500. Virtually all patients in both groups did engraft, but the rate of engraftment was significantly higher, 2.6 times as fast and 1.7 times as fast for acute leukemia versus CML in the recipients of allogeneic PBPC transplants versus bone marrow transplants.

In contrast, the risk of grade two to four acute graft versus host disease was not significantly different with the two graft types relative risk of 1.09 and 1.28, nor was the risk of chronic GVHD significantly different between the two graft types with relative risk of 1.18 and 1.11.

There's one important thing that I neglected to say in describing the population. These patients were transplanted in 1995 and 1996.

The data set was established in late 1997. We elected to cut the study at one year post-transplant. So these data are really only on the first post-transplant year because we did not have enough follow up on a significant number of patients beyond that.

However, all patients had at least six months of follow-up and the median follow-up time was between seven and eight months. Treatment related mortality, again, defined as death in remission was significantly lower in patients who were transplanted for leukemia using peripheral blood stem cell versus bone marrow transplants. In fact, the risk was half as great, and this was significant at the .02 level.

In CML, the relative risk of treatment related mortality depended on whether the transplant was done in chronic phase versus accelerated phase. There was no significant difference in chronic phase. And one year treatment related mortality -- after HLA identical sibling transplants for chronic phase CML is pretty low after bone marrow transplants in general.

In accelerated phase, there was a significant reduction in the risk of transplant related mortality at one year. And I'll say that

over and over again as we go through the results because I think it's really important that, remember, this is only one year data.

I'm going to show graphically these results over the next few slides. All of these curves derive from the multi-variant model, so they adjust for the other factors that were shown on that slide that were significantly associated with outcome. So it's showing the independent affect of graft type independent of other co-variants.

As you can see, the time to recovery of an ANC greater than 500 was a median of four days faster with peripheral blood stem cell transplants. This is for acute leukemia. The difference was five days in CML. Other factors that affected ANC recovery were the use of growth factors post-transplant and the use of TBI regimens both of which facilitated hematopoietic recovery.

This is the adjusted probably of grade two to four acute GVHD after transplants for acute leukemia. As you can see, not only is there no significant difference, there is no difference. These overlap, and you will see the same pattern if we restrict the analysis to grade three to four acute GVHD although I don't have a slide showing that.

In CML, a slight difference again, not significant. The other factors that affected the risk of acute GVHD, were older age, advanced disease, and use of TBI, but again, there was no interaction with the affect of graft.

This shows the probability of chronic GVHD in this cohort. This is an older cohort, and older patients do have a substantial risk of chronic GVHD. And the risk at one year is at 50 and 60 percent with one year of follow-up. There is no statistically significant difference in the risk of chronic GVHD. This includes all grades both limited and extensive, but when we look at just extensive chronic GVHD, we again see no statistically significant difference.

When we look at a severity rating of mild, moderate, severe, we see no significant difference in the severity, but chronic GVHD can occur as late as two to three years post-transplant, and I think we have to really follow this cohort longer to be sure that there really is not a significantly different incidence. And this just shows the same results in CML.

This is the probability of transplant related mortality as evidenced by the relative risk of .5. There's a significantly lower probability of

transplant related mortality after transplants for acute leukemia, and that's regardless of whether these were done in first or second remission.

If one looked at CML as a group, we didn't see a difference, but there was a significant interaction and these are the chronic phase patients with no difference by graft type, but for those patients who were transplanted in accelerated phase, a very dramatic difference in the probability -- one year probability of transplant related mortality.

And now finally, adjusted probability of leukemia free survival derived from these models, and we see a significantly higher probability of one year leukemia free survival in those patients who received peripheral blood stem cell transplants versus bone marrow transplants for acute leukemia, an advantage only in the CML patients who are transplanted in accelerated phase.

So our conclusions are, we see a very convincing facilitation of hematopoietic recovery. I'm sorry. I didn't show you the platelet recovery. The curves look really the same as the ANC recovery with a significant shortening of the time to platelets greater than 20,000 with peripheral blood versus bone marrow transplants similar acute and

chronic GVHD rates in the first year, and again in the first year, we see lower transplant related mortality and improved leukemia survival in both groups.

Certainly, there's no evidence that the outcome in the first year is worse in any group by graft type. We continue to follow this cohort in the process of updating, particularly the chronic GVHD data so that we can have an additional year of follow-up. I had thought that that would be complete enough to be able to present some of that data, but we still don't have sufficient follow-up data on a sufficient number of patients, and it's better to present no data than potentially misleading data.

I am going to present, though, some data that we have generated on a smaller cohort of patients on the costs involved in the early post-transplant care of patients who received peripheral blood versus bone marrow transplants. And this is the result of a collaborative study of the IBMTR and Charles Bennett, Theresa Waters at Northwestern University.

As you see, this is a smaller cohort of patients who received allogeneic transplants for acute leukemia, CML, or non-Hodgkin lymphoma at four

U.S. transplant centers. Thirty-three of these, again a small number, but some interesting data, 33 of these receive peripheral blood progenitor blood cell transplants, and the remainder bone marrow transplants all donors who were HLA identical siblings.

We have clinical data on these patients from the IBMTR. Cost data was derived from billing inpatient and outpatient billing data provided directly by these four institutions. We were able then to capture all of the resources for which charges were issued, and using ratio of costs to charges get an estimated cost. The cost covered from the graft procurement through the first 100 days post-transplant.

These are the characteristics of the patients. There, nothing is really surprising. It is -- these again were adults. Quite a significant proportion had advanced disease, and you can see in the three groups 20 percent received peripheral stem cell grafts.

This shows that the median total costs for allogeneic transplant by the disease and by the graft source. This is the difference between -- in the cost between bone marrow and peripheral blood

progenitor cell transplants. This shows differences by disease.

As you can see in the bone marrow transplant cohort, transplants for acute leukemia were significantly more expensive, had significantly higher costs than transplants for CML. We didn't see that difference in the peripheral blood progenitor cell transplants, but again, it's a small cohort for that kind of comparison.

We do see a significant at the 99 percent confidence level, significant difference in cost when we look by disease type with cost savings rangings from about $30,000 to almost $80,000 for peripheral blood progenitor cell versus bone marrow transplants.

When we analyze the drivers of costs in these transplants, most costs are driven by inpatient days, pharmacy, blood products, and the -- most of the savings observed with peripheral blood progenitor cell transplants derive from shorter hospitalizations, and fewer blood products, and some difference in pharmacy costs.

So now, again, as I emphasized in the previous study when we looked at clinical outcomes, there was only one year of follow-up. These are costs only through the first 100 days post-

transplant. It does include both inpatient and outpatient costs. But if there is difference in clinical complications later than that, that wouldn't be reflected here.

We are in the process of expanding this database to include more centers, and then of course more transplants, and trying to track costs out through the first year. But this is a labor intensive effort in terms of getting billing data from multiple institutions.

All right. You'll notice that I really didn't say much about donors in this presentation. That's because Dr. Anderlini will be presenting in his next presentation some of the IBMTR data on donor outcomes, at least in the short-term for peripheral blood versus bone marrow transplants.

But I have to say that in contrast to our plans for the recipients of these transplants where we do follow obtaining clinical data yearly on these patients for as long as possible where we do intend long-term follow-up, there is not really a coordinated effort at present for long-term follow-up of donors. Thank you.

DR. TOSATO: Again, we would hold the questions to the end of the session, and let me introduce Dr. Paolo Anderlini.

DR. ANDERLINI: Let me begin by thanking the organizers of the workshop for giving me the opportunity to speak here about normal donors and cytokines. My presentation will actually be largely focused on a specific cytokine, which is G-CSF and its safety and efficacy in blood stem cell donors for allogeneic transplantation. May I have the first slide please?

These initial slides were kindly provided by Dr. Champlin just to give you a general overview of the issues related to allogeneic PBPC donation in general, particularly with a donor evaluation and collection process. So the issues are donor eligibility criteria, the exclusion criteria, if any, or if they need to be defined, donor management, medical supervision issues, safety monitoring, and actually, the purpose of this slide was to come up with some kind of consensus statement which was at the previous workshop.

Obviously, in terms of eligibility exclusion, there are both donor and recipient considerations, the more possible risk of mobilization with G-CSF. The idea was to try to come up with some practice guidelines for donor management, monitoring short and long-term effects,

and the possibility of having a registry, an international registry for adverse effects.

And eligibility criteria are, in general, issues going from whether they should be different from marrow donors or platelet donors, issue of venous access, issue of age, issue of a possibility of accepting donors with hepatitis B or C in consideration of the specific scenario, and possibility of having exclusion, a potential issue related to donors safety which are more theoretical than actually established at this point.

The other thing I wanted to say, actually to include here is just a quick reminder. Most of you are probably very familiar with marrow harvesting which has a very well established track record, probably in excess of 30 years. Over a 30-year time period, there have been at least two documented fatalities which is a very good safety track record for any kind of surgical operation which bone marrow harvesting is in most cases still.

The life threatening complication rate, according to the biggest studies coming from the Fred Hutchinson on the IBMTR is probably about 25 percent. And according to NMPD data, specifically Dr. Stroncek's publication in Blood, the return to

baseline lifestyle in most donors for the NMPD takes about two weeks.

Many cases can be done as outpatients, but some cases require a brief hospitalization. And as far as the incidents of exposure to allogeneic blood products, there has been estimates as high as ten percent, particularly in older donors. But in general, if you look at the NMPD data, it's probably about one percent max.

And briefly, before we get actually to the normal donors which is a relatively recent development, I'm just going to go through some data on specific clinical scenario. G-CSF initially approved for use in severe congenital neutropenia, and a couple of years ago, there was an update on the experience of this long-term use of G-CSF in severe congenital neutropenia.

There was like a nine percent incidence of the developing of AML although many of you are probably familiar with the fact that this is considered by many a pre-leukemic state on its own. So it's hard to actually make a conclusion out of that. It's interesting that the risk appeared to be limited to severe congenital neutropenias. There was no apparently increased cyclic idiopathic

neutropenias with the use of neutrogen which is just our use of filgrastim.

And the risk appeared to be clearly linked to G-CSF receptor and RAS mutation including monosomy 7. With regard to aplastic anemia, particularly in the Far East, there have been several cases treated with G-CSF long-term, particularly in the pediatric age range.

There was a letter to Blood published a few years ago reporting six to seven pediatric cases treated for on the average of a few years with G-CSF, and there was a Kaplan Meier estimate of AML/MDS with 40 years of about nine percent. And interestingly, even here, in virtually all of the cases, there was an abnormality of Chromosome 7.

And very briefly on AML, we know that there are G-CSF receptors on normal myeloblasts and leukemic myeloblasts. If you do treat normal donors with G-CSF then you do a bone marrow, you usually do not see an increase in the percentage of myeloblasts. There may be some sensitivity in terms of G-CSF response in some AML/MDS patient although G-CSF has been used to treat post-bone marrow transplant relapse. So that may well be the exception more than the rule.

Now, I was also asked to review what has been our own experience at M.D. Anderson and actually what we have been doing, in general, for the past four years or so. The objective of the study was essentially to review what has been our experience at Anderson over the past four years with allogeneic blasts and cell collection in a large group of normal donors. And the two major end points of this analysis have been safety and efficacy of this, I guess, relatively new donation modality.

The study group actually includes 350 first time blood stem cell donors harvested over a four-year period with the analysis actually updated last June. These donors were actually distributed across a wide age spectrum with close to 20 percent of them 55 years of age or older. More than 90 percent of these donors had sufficient information on file for either apheresis yield or short-term adverse event assessment.

I would like to emphasize that donor evaluation and collection was performed within the framework that has been provided by the FAHCT guidelines. This slide is just to show in a graphical form the age distribution of these donors, once again, to emphasize that a sizable number of

them actually were either younger pediatric age range, I guess you could say, or older, in other words, in their 50s or 60, or even late 60s.

Our mobilization regimen calls for filgrastim to be given every 12 hours in a dose of six megs per kilogram until the collection is completed. Leukapheresis is usually started on day four of filgrastim administration although about 13 percent of the donors actually were started on other days, usually day five for scheduling issues and other reasons.

We apherese donors throughout venous access whenever possible. We process three times the blood volume which usually takes about three to five hours. Our target for collection is four million CD34 positive cells per kg. What we consider, however, as the minimal acceptable dose, cell dose for allografting is actually two million partly based on our and other similar experiences. You can successfully graft patients with this lower threshold dose.

The adverse events reported by the donors are the ones that you might expect, mainly bone pain, headaches, fatigue, and nausea. Much less commonly encountered were like non-cardiac chest pain, local reactions. About two-thirds, actually

more than two-thirds of the donors took analgesics which ordinarily is acetaminophen.

Grade two to three exists in this slide means the adverse events we just described were rated by the donors as moderate to severe. Grade four means that they dictated the discontinuation of the growth factor which happened in less than one percent of cases. Just for completeness, I did include here the case of a donor with a cerebrovascular event which occurred a few days after an uneventful stem cell collection which has already been published and reported in the literature. But once again, the relationship if this event, if any, with the collection is still unclear.

If you include what are the apheresis related problems, the overall dropout rate was still about one percent. In terms of follow-up, we are pleased to consider the infusion of a tolerable platelet rich plasma to minimize the apheresis induced platelet depletion in donors who complete their collection with low platelet counts.

I say consider because this is not done routinely depending on how low the platelet count is and whether the plan -- we plan to continue the collection or not. Otherwise, the adverse events

and the blood tests normalize adversive blood tests takes about a week, particularly the platelet count.

This slide summarizes the collection results in terms of pre-pheresis, leukocytes, number of pheresis, median CD34 dose. You can see about 40,000 is the median for the leukocyte count pre-pheresis. The number of pheresis is about 68 percent for one collection required to reach the target of four, the median CD34 dose about 6.6 times ten to the sixth per kg. This is the first collection, or if you want to express it in CD34 times ten to the sixth is 462.

This is just to show the same thing in graphical form. As you can see, the white cell count, the median is about 40,000. You do have outliers on both sides, people who barely move their counts, a lot of variability, in other words, and others who develop a very remarkable leukocytosis. Our current arbitrary rule is actually to do a dose reduction if the white cell count is in the 50,000 or 60,000 range, a 50 percent dose reduction.

Again, one versus more than one, but two-thirds, one-third, if you were to do the slide with only the older donors, 55 or older, you would have like a 54 or 56 percent requirement for one collection. In other words, that -- the one on your

left-hand side would drop, but it's still more than 50 percent collected with one pheresis.

Now, this is the expression in terms of total number of cells with a normal distribution, or just to get some idea. Obviously, if your cutoff is four million and the other standard is 70 kilogram recipient, then you should draw a line there around 280 just to separate the ones that actually are done with one collection, or if you use two, that would be like 140. So between 140 and 280. I think that if you had even more donors, that probably would approach a normal distribution.

Another way of presenting the data possibly is to show the number of cells per kilogram of recipient, again first collection. This is a box whisker plot assumed the standard 70 kilogram recipient which is a reasonable assumption you can make if your sample size is sufficient and large as this one. Even in this slide, the significant variability is evident. You can here just draw the line around four or three million as a threshold if you want to do that just to separate the one.

Additional information of the collection results, I guess, either we are lucky or have very, very good operators because our rate of inadequate peripheral access is only five percent, and in most

cases, the donor actually gets a catheter inserted which lately, in particular, has been mainly a femoral line to avoid the complication of central line placement. Actually, all these procedures were uncomplicated.

We usually don't pherese them more than three times, three consecutive times. In five donors, about two percent of the total who underwent three daily collections, the target dose was not reached, target as four. In four of them, however, we did get at least two million, and the fifth one, actually, had to undergo bone marrow harvesting.

We also looked at factors that can affect the yield of CD34 positive cells in normal donors. Basically, the idea was to see if you can identify up front people who don't mobilize very effectively looking at pre-donation parameters. And so we looked at approximately 120 donors age 40 years, the usual regimen.

The variable analyzed was the CD34 cell yield expressed as number per liter of blood processed. You really have to use this to adjust for differences among the donors in terms of blood volume and pheresis duration. So we looked at various factors, univariate analysis. The one that actually turned out to be more significant, even

though not strikingly significant, was age, sex with a little bit of a trend in terms of male donors mobilizing better, the baseline white count, the pre-pheresis white count, and day four versus day five to six meaning day five to six in general gives you a higher probability of achieving your target.

Obesity, interestingly enough, was also a factor.

This slide shows the correlation between age and yield. As you can see, the correlation coefficient is barely statistically significant, but it is statistically significant so there is a modest age related decline in the yield.

This is a correlation between the white cell count and apheresis yield. Once again, the -- modest correlation, but it's not particularly striking between the pre-apheresis white cell count and the apheresis yield as described previously.

When we did a stepwise logistic regression model, age remains statistically significant although not in a striking fashion. Day five, day six remains significant in everything else but pretty much fell off. So basically, I've come to the conclusion that at least you can look at the demographics and other factors. It is very

difficult to identify up front people who are not going to mobilize well.

Just briefly to acknowledge all of our collaborators which helped in this study, the BMTT members at Anderson as well as the clinic nurses which have been very helpful obviously in dealing with these donors.

As Dr. Horowitz just mentioned, with invaluable assistance of Melody Nugent and Mary Horowitz actually, I was kindly provided with some information about what they have in their database in terms of characteristics of blood stem donors for allogeneic transplants which have been reported by the IBMTR by more than 100 teams worldwide over roughly a four-year period.

As you can see, there were approximately 700 donors in their database, actually close to 800 I guess. Median age was about 38. The year of transplant, as you can see, there is an increasing number of them recently, particularly 1996 on. Most, actually, most of them were actually identical sibling. Some of them were twin or other related or unrelated.

Interestingly, there are some differences here between these results and ours, although they're not totally comparable anyway. Many more

donors in this series underwent more than one collection, two, three or even longer. There's no -- you really cannot say that they required this to achieve a target obviously because that type of information is not there. It just says how many phereses they actually underwent.

The donor complication rate, however, is pretty similar, about one percent. Thankfully there were no death from donation. The type of growth factors given was mainly G-CSF single agent. Another difference here is that a larger number of donors ended up getting some kind of central or catheter as opposed to getting a routine peripheral venous access.

In terms of donor complications, all we have to go by, I guess, is what was reported verbatim in the report form, and this is actually what they came up with. Roughly, you can say that here, about half of these complications vaguely appear at least capital related or venous access related. That's why it is very important to minimize, in our opinion, the need for invasive procedures. In some cases, it's not totally clear what actually is meant. I think that hypercalcemia is probably more likely hypocalcemia -- but anyway.

I should say, however, concluding the IBMTR component that these data have been obtained by the IBMTR statistical center and the analysis has not yet been reviewed or approved by the IBMTR Advisory Committee.

In terms of unexpected adverse events, what I mean by unexpected is something different from the usual bone pain, headache, and the thing that you expect, I guess, with G-CSF. There have been two reported cases of ocular complications, scleritis and episcleritis. One donor actually had a history of autoimmune disease.

There was a case report with splenic rupture and pathological evaluation showed extra midline hematopoiesis. And two events, one we briefly discussed it already. The second one was a myocardial infarction in a patient with known history of severe coronary artery disease shortly after the day -- the first day of his apheresis collection. And again, even in these two cases, it's unclear that there is any correlation between the procedure itself.

I should add a couple of extra case reports that are not in the slide. One was a case of acute gouty arthritis in a normal donor. The other one was what appeared to be an anaphylactic

reaction. But I will like to emphasize a few things. First of all, you don't have a denominator here so it's very difficult to actually put a percentage and have -- and say this is common, this is uncommon. These are just case reports, and in some of these cases, it's not totally clear that there is actually, indeed, a correlation like the one that I put at the bottom here.

There have been, however, no fatalities, and I would emphasize that, directly related to the procedure itself. Nevertheless, there are some scenarios which I guess should raise your attention. Obviously, if you have a donor that comes to you with a history of ocular problems, then that could be something you may want to take into consideration. Or if there is a strong family history of myelodysplasia or AML or a history of DVT or predisposition to thrombosis or others.

However, I would like to emphasize that these are not supposed to be contraindications. These are just things that you may want to take into consideration in your donor evaluation, and eventually, the decision should be based on the risk benefit ratio obviously for the donor and the patient.

A few things on what has come up the last couple of years what are called the post-donation cytopenias. We and other teams have found that about ten days, maybe ten to 14 days after donation, the neutrophil count of some of these donors drops sometimes leaving neutropenic levels.

A study from Dr. David Stroncek here has been instrumental. It was presented as an abstract. He essentially randomized donors to receive filgrastim and then to undergo pheresis or not. And this neutropenia apparently happened only in the ones who did undergo pheresis. So the idea is that maybe you do remove large numbers of mobilized progenitors.

It is something significant because in some cases you can have ANCs in the 500. However, it is self-limiting, asymptomatic and probably you're going to notice it only if you do a lot of blood counts. Just to give you a graphical, so you have the baseline, the before pheresis, and about seven days later, you have a statistically significant drop in the ANC.

The lymphopenia, this is true as well if you do lymphoid panels, lymphoid subsets. In many of these donors, you will see that in many cases, the lymphocyte count and many of the lymphoid

subsets actually drop, and this takes longer to normalize. This is a laboratory abnormality. There has been no clinical correlation for this.

And finally, the thrombocytopenia, now all of you are probably familiar with the fact that particularly with the continued slow pre-apheresis, you do decrease platelets to some degree. This happens mainly if you do two or more collections, or if you process more than two blood volumes. Roughly, it has been estimated there is like a ten percent drop for every blood volume you process.

There is also a contributory volume of G-CSF itself which probably causes a five to ten percent on the average drop in the platelet count. If you elect to do so, you can minimize this by doing autologous platelet rich plasm infusion. However, there has been no bleeding complications reported in any of these donors.

Now, to specifically look, I was interested in this part, how often this is going to be a problem. So I plotted what is the pre-apheresis platelet count in all of our donors. And you can see there is about a five percent of normal donors who will show up on the first day of collection with a platelet count of less than 150,000.

So it is not totally unseen. It's something that you do see probably about five percent of the time. Interestingly, these are donors who do well because your concern is well, okay then, I'm going to have to stop because, obviously, I don't want to push their platelet counts down. But none of these donors actually mobilize effectively. And many of these donors are actually donors who drop their platelet count substantially with the G-CSF. So it is there, but it may not necessarily be a major problem.

A few final issues. Is there such a thing as an optimal dose? There's clearly a dose dependent modelization of CD34 cells for doses up to ten micrograms per day. What happens beyond that there's not as -- has not been studied as well. Certainly what happens with higher doses you will have increase in the cost. You will probably, and not everybody agrees on that, an increase in adverse effects. So I think they should be studied, but I do not think they can be recommended routinely.

And on side effect those dependent there is not general agreement on this, but many investigators think they are, in particular, bone pain, body aches, and particularly if you go higher than ten. You may remember that we use a twice

daily regimen. Why do we do that? Well, because the elimination half life of filgrastim is actually three to four hours whereas the biologic half life is actually much longer.

This slide is actually kind of old, so there are actually now comparisons between the two. There are two studies, particularly a small study from Japan suggesting that twice daily if you split the dose in two administration, you will actually get superior or improved mobilization and collections.

And finally, I guess, the issue of the long-term safety. Now, if you do expect some kind of problem, acute myelocytic leukemia in general is a very uncommon event statistically speaking. So these events are going to be rare and probably delayed. And to detect increased risk of a rare event, you will need to follow probably thousands of donors for several years.

And also, do we have a control? Obviously, the idea of the correct control is marrow donors, and we don't necessarily have a lot of data. We really cannot compare with the general population because keep in mind, these are not just routine donors. These are HLA identical donors with, in most cases, at least with patients with leukemia.

Now, as far as the control, recently actually, there was a study from the Paris Group, a group of collaborators which followed up on about 800 marrow donors. Only half of them, actually, ended up being valuable with a questionnaire and a follow-up several years later, and they found one death from leukemia while the expected risk would be .5 percent in ten years.

I'm not saying this is statistically -- there are a lot of drawbacks in approaching this from a statistical standpoint, but I guess the conclusion is you cannot necessarily assume that marrow donors have the same risk to develop leukemia than the general population.

So the conclusion that we can at least draw is that the short-term safety profile, at least, is certainly acceptable, but just refers to the fact that we shouldn't, I guess, rest on our laurels. There is a need for a continued monitoring. The issue of dose reduction has been addressed in many settings. I guess what I just can say here that it's probably prudent to avoid excessively high leukocyte count or what actually constitutes the threshold is debatable.

And the more donors you're going to collect, the more you're going to run into special

circumstances, and peculiar donors which are supposedly hematologically normal but have some conditions like the ones we have actually described earlier. And so I mention just attention to these "special" donors.

For the cytopenias, I guess, the post-donation leukopenia is a little more than, I guess, a clinical abnormality. Whether you actually need to mind your blood counts afterwards routinely is uncertain. And as far as whether you should reconsider the reinfusion of platelet rich plasma, then I guess it should be left to the individual investigators, although keep in mind, that probably add costs and possibly risks because even autonomous blood products, you know, they have the problem of clerical errors and so on.

And as far as the long-term effects, I guess, the only way to address it would be to have a registry which is highly desirable, but logistics and cost are major problems. The accommodation was to try to have individual centers, at least in the interim to try to monitor to their own donors so that if and when a registry is established, they will have some data to enter.

Finally, we were asked to provide at least some opinion about what would be areas in need for

further research support. And I think in my opinion at least, two areas are in need of further research support. One is, as we just said, would be the creation of an international stem cell donor registry which is needed probably to monitor both short-term adverse events and possible long-term events, mainly myelodysplasia and leukemia.

Probably the best way to do that would be to have additional funding to the IBMTR and the national marrow donor programs so they can expand their data collection forms and get more information on the donors and the donation process because the information right now is relatively limited particularly for the blood stem cell donors.

The other area which should be considered is actually more study of the biological clinical effects of cytokine administration in normal donors. But partly I didn't put any slide on that, but there is some preliminary data using other cytokines in normal donors. So this is actually apparently going forward pretty quickly, and I think there is a need for information and study in that area as well.

Okay. So this concludes my presentation and thank you for your attention.

DR. TOSATO: I'll ask the speakers to join me here, and perhaps we can start a discussion based

on the three presentations we've heard. If anyone has questions written on the cards that we provided, perhaps they can be collected and brought here, or you can ask the questions in person. Steve, do you want to --

DR. NOGA: Yes. Steve Noga, Johns Hopkins Hospital, Baltimore. It's kind of a comment more than a question, but it's just something as we're getting into looking at allogeneic peripheral blood. A lot of us who have worked with bone marrow before this might take exception to a statement that you hear very commonly, and that's that there are no more toxicities and no more morbidity problems with allo peripheral blood than there is with bone marrow.

Now, of course, the data that Mary presented, and that is data of unmanipulated transplant, and that's true, there is no difference between allo peripheral blood and the -- and allo bone marrow in terms of unmanipulated products.

But as a transplanter, a lot of us might have exception with a 40 to 45 percent mortality rate related to the transplant, and over the years, a lot of us have worked very hard at trying to reduce that with manipulation, I've got to get this correct, Liana, minimally manipulated procedures for

trying to reduce this mortality, and it's just important to remember this as we get into this.

I mean we haven't even started this in allo peripheral blood yet, and that's important to remember because a lot of us in the manipulation field have dropped these mortality rates to around 20 percent. Yes, there's more relapse, but you know, mortality is kind of permanent. We haven't really figured out how to reverse that. We may be able to work on relapse. So as we go into this, we need to look at that.

And lastly, on the comment, when you showed the cost data, again, part of that's related to the fact that you're doing unmanipulated transplants probably, either allo or peripheral blood. When we turn around and manipulate products, we drop the cost by about 40 percent, and that even includes the cost of a selection column. So you know, it's just something to remember as we go into this.

DR. HOROWITZ: Well, I actually thought a consideration of T-cell depletion was somewhat beyond the scope of this conference, so I didn't address that. The reason that we chose in this study to look at unmanipulated or non-T-cell depleted both peripheral blood stem cell and bone

marrow transplants is because these are the most common.

Only about 15 percent of allogeneic transplants using bone marrow right now are T-cell depleted. The most common way of preventing graft versus host disease is with combining cyclosporin and methotrexate which is used in about two-thirds of all of the HLA identical sibling bone marrow transplants.

So the benefit of any specific approach that will -- that is designed to decrease transplant related mortality, of course, has to be examined. This is a moving target field. Obviously, you know, bone marrow transplants were used as the "gold standard" in this analysis, but they're not very golden. I mean, they still have a very high transplant related mortality rate.

Transplant related mortality rates in this particular cohort have to be considered in light of the fact that it was an older cohort, and most of the patients had advanced disease. And regardless of how you do a transplant, in that particular population, transplant related mortality still remains high.

DR. NOGA: And I agree. It's just saying we just need to remember that because, you know, I

hear over and over again how there's no difference in the rates, and you yourself show the slide that showed that we had this transition in the autologous setting from auto right into -- from auto marrow right into auto peripheral blood without many randomized studies or none really.

And here we go in the peripheral, and maybe this is a point to remember as we're looking at this and looking at possible grant applications in this line. These are opportunities.

DR. HOROWITZ: That's exactly why I show that slide.

DR. NOGA: Yes.

DR. TOSATO: Dr. McCurdy?

DR. MCCURDY: McCurdy, NHLBI. At a meeting where donors given growth factors were discussed extensively in Orlando at the time of an ASH meeting. I think it was probably about two years ago. Dr. Horowitz gave a very, I thought, excellent discussion of some of the statistical problems in following donors.

At that time, I indicated that the Institute would be happy to entertain discussions about follow-up on such donors to obtain long-term data on any complications that might occur. I can't promise funding anything, of course, and I'm less

directly involved now than I was then, but I think I can say that the Institute would still entertain discussions on donor follow-up, as was suggested a bit earlier.

DR. TOSATO: Would you like to introduce yourself?

DR. LEMADER: Fred LeMader of the San Antonio South Texas Cancer Institute. Given the context of the limitations of registry data and some of the data that was presented, since we are talking about promulgating new regulations for stem cells, I wonder if Dr. Horowitz and Dr. Harvath could maybe enlighten us a little bit.

As I see the data that was reviewed, we had some significant progress in autologous transplant. The technology was disseminated rapidly to the benefit of patients. That appears to be occurring as well in allogeneic transplant. And with the limitations of the data, it appears at least that accelerated phase patients and acute leukemia patients are benefitting.

How would regulations that might be promulgated improve upon the safety and the dissemination of the technology?

DR. HARVATH: Mary said I should go first.

DR. HOROWITZ: We don't collect that data, so we can't advise you.

DR. HARVATH: It's our hope, I think, any of us who have done scientific studies or clinical studies know that when you prospectively decide what kinds of data you're going to collect and what the parameters would be for the data sets you get in, when looking at those data then during the progress of the study, it's much easier, I think, to work with the data and sift through the information than it is to take retrospective data and analyze it.

Our goal with the regulatory process is to not impede the development, that is, not -- we want to stay out of the perception and also the reality of trying to impede the progress of the research, but rather to set what are minimal acceptable criteria based upon the knowledge at the time the groups get together to put the science together to look at the minimal acceptable criteria to try and prevent any kinds of problems that would pose a risk to normal donors as well as people who would be receiving a product.

And the whole premise of the regulatory proposal is really to contain the spread of any kind of communicable diseases. I mean that's the whole premise, which is why the focus has been on

allogeneic and unrelated allo. Now, what's learned from the related setting and what's learned from the autologous setting, those technologies and techniques certainly are going to be applied in the scientific and clinical arena to the degrees they're appropriate.

So I think what we want to do is try to make the best sort of scientific based sets of standards that are available, realizing full well that the rate the technology is moving, they're going to have to be revisited frequently.

Mary, did you have something to add?

DR. HOROWITZ: I don't think I really have anything to add to that. I mean what you're asking is really an unknowable. I think the concern that you express is that this is a field that's moving very, very rapidly, has been moving rapidly, has made a lot of advances.

I'd have to say the data that we collect, this is not retrospective data in the sense of the data collection. These fields are determined beforehand and are collected. We don't go back and do chart reviews. We collect the data in a prospective fashion.

The concern is that once regulations get established, they don't get revisited fast enough

for a field that changes very rapidly. There is no definite answer to your question. I just, you know, I think those are some of the concerns.

DR. LEMADER: And just quickly, I agree with your very last statement. I don't think the question was answered, and I think as we promulgate such rules, we do have to think about how we are going to improve safety, and help quality, and afford the knowledge in that area because I don't think you directly answered the question that I asked.

DR. HARVATH: I apologize if I didn't.

DR. TOSATO: Yes?

DR. STRONCEK: Dave Stroncek, Department of Transfusion Medicine, NIH. A couple of comments. One, I want to emphasize I think one of the biggest problems for donors is the variability and mobilization, and as a result of variability in the products collected, and research, if there is funding available, it should go into investigating better ways to mobilize stem cells.

And second, is that most, for sibling donor transplants, most people are using CD34 counts to quantitate the adequacy of collections. But as we're thinking about moving into the unrelated donor setting, that's not always possible or practical.

There's been some discussion on whether or not you can define a product as administering a certain dosage of G-CSF for certain duration of time and collecting one or two products with -- over a certain amount of time, and that could constitute a transplantable product.

Is there any comments if you think that might be a practical way to go, at least, for unrelated donors rather than using CD34 counts?

DR. TOSATO: Maybe I can add another question from the forum, again, on the same topic as how were your CD34 positive cells defined? You, in one of your slides, spoke about CD34 positive cells. This is an area --

DR. ANDERLINI: Okay. So one thing at a time. I certainly agree that part of the area of determining why people mobilize differently is an important area of study, and that should have been, I guess, more emphasized or specifically included in the second item in my two item list.

It would be important to know, obviously, why people mobilize differently. Now, it's not necessarily going to be cost effective to do that routinely because most donors will mobilize at least enough for a transplant, but certainly if your

target is higher or if you work in an unrelated donor setting, that may eventually be very helpful.

As far as the CD34 definition, these are, I don't want to get into all the details, but just as the standard flow cytometry measurement. And as far as the second point, Dr. Stroncek, as Dr. Stroncek knows very well, I mean, these are the topic of ongoing discussion as there is an attempt to come up with a protocol for first donation.

Now, as the field evolves, I think it's going to be easier to have real time CD34 measurements. Right now, particularly if you want to give many centers the opportunity to join this, I think that may not be possible. And I think that the possibility of just like two donations, in most cases, may actually be the simplest, and therefore, the most realistic way to go.

Now, in some cases, you're probably going to get too many. But then it may be up to the receiving center to dispose of those, but I think we should, at least right now, try to keep it as simple as possible.

DR. TOSATO: Dr. Champlin?

DR. CHAMPLIN: The -- Dr. Anderlini talked about the risks of G-CSF and leukemia. I just wanted to maybe emphasize the point that if the

disease is where leukemia has been seen, these have been states where leukemia develops anyway, for example, aplastic anemia, that people treated with immunosuppressive therapy have at least a ten percent, if not, higher risk of either myelodysplasia or leukemia developing.

And so my conclusion that leads to the data to date is that there is no evidence that G-CSF increases the risk of malignancy in a normal donor, and that all of the cases noted have been in diseased individuals with predisposition to leukemia to begin with.

DR. TOSATO: Time is getting short. Dr. Norcross?

DR. NORCROSS: I just had a question about the scientific basis on what Dr. Horowitz addressed about the stem cells did better in an accelerated phase, and whether you had any insight into whether that's a GVL or an NK mediated response that would be better with manipulated cells?

DR. HOROWITZ: I have no laboratory data to address the quality of the immunoconstitution after a bone marrow versus peripheral stem cell transplant. My read of the data, and this is speculation, you know, whenever we talk about why in a data set like this, is, first of all, if you look

at HLA identical sibling bone marrow transplants for CML, they have a low, a relatively low transplant rate of mortality.

These are patients that do well no matter how you do it. We've gotten pretty good with doing transplants for CML. Chronically, CML patients also come in without a lot of prior therapy. They tend to have a very good performance score, and they may be in a situation where -- recovery doesn't make a lot of difference.

I think the differences might lead to be the effect of decreasing the time to hematopoietic recovery in patients who are more ill when they start.

DR. FISCHER: Yes, Johannes Fischer from Duesseldorf, Germany. I want to get a comment on the peripheral blood stem cell collection on unrelated donors. We have done such collections for first stem cells capsules in now 93 donors, and still we are -- the mobilization of 12 micrograms G-CSF per kilogram body weight.

We have in those 92 donors collected more than four million CD34 positive cells in one collection in about 80 percent of the donors. And we are measuring this according to the ISHAGE criterion. So I think if you use such defined

protocol as the CD34 count could be on target, a value for deciding to do one or two collections.

DR. TOSATO: Thank you.

DR. SHAPIRO: R.I. Shapiro, Life Source. I have a question about the problem of weighing risk of communicable disease versus the benefit to the patient, and I think on Dr. Anderlini's slides, he showed one of the lines was, perhaps, you could allow donors with hepatitis B or hepatitis C, and I would be interested in Dr. Horowitz's read on this.

Is there a possibility of having extended eligibility beyond that of blood donors for stem cell donation?

DR. HARVATH: The proposed approach of February 28, 1997 clearly stated the criteria for which if there were infectious disease marker test positive when that would be permissible, and what I would like to do is just refer you to that because we don't have time to reiterate all of that.

But there has to be informed consent. There has to be documented knowledge of the transplant physician. But there are criteria spelled out in that proposed approach which would allow that.

DR. SHAPIRO: Okay. Thank you.

DR. TOSATO: I'm just going to take the last question.

DR. COLLINS: Nancy Collins, Sloan-Kettering, New York. This is more of a comment than a question. The previous answer as to how you look at your product as looking at a standard CD34 analysis, I'd like to find out that there really is no standard CD34 analysis, and anyone who has followed the literature over the past five years has seen the extreme controversy which has surrounded this issue.

And the number of studies which have taken place in this side of the Atlantic and the other side of the Atlantic are just not to say that this is not a very commendable and very important procedure which is being undertaken by a lot of investigators. But it's more to point up the difficulty which we have in looking at a product and trying to make standards or regulate things on the definition of what product is versus looking at more of a process-based approach. Thank you.

DR. HARVATH: Thank you.

DR. TOSATO: We will close on this note of caution, and we will reconvene in ten minutes.

DR. HARVATH: How about five after 10:00 we'll start the next session, and Dr. Stroncek will moderate.

(Whereupon, the workshop went off the record at 9:55 a.m. and went back on the record at 10:07 a.m.)

DR. STRONCEK: I'd like to begin the next session here. Could I ask everyone to sit down? We have -- I'm Dave Stroncek. I'm from the Department of Transfusion Medicine at the Clinical Center here at the NIH, and I will moderate this next session.

We have three speakers, and then we will have some time for discussion after that. The first speaker this morning will be Dr. Richard Champlin. Dr. Champlin is a Professor of Medicine, Associate Head of Hematology and Division of Medicine and Chair of the Department of Bone Marrow Transplant at the University of Texas, M.D. Anderson Medical Center.

He received his M.D. from the University of Chicago, Pritzker School of Medicine, and he did his internship/residency in hematology and his fellowship training at UCLA Medical Center. He's published numerous articles on bone marrow transplant and peripheral blood stem cell progenitors and self-transplantation. He serves on

numerous boards, and he's an officer of numerous professional organizations in hematology, oncology and transplantation.

Dr. Champlin will speak on Related Allogeneic Peripheral Blood Stem Cell Transplants, the M.D. Anderson Experience.

DR. CHAMPLIN: Thank you. It's a pleasure to be here and speak on behalf of the -- our group at M.D. Anderson. I should acknowledge from the outset that I'm going to present work done by a number of people including Paolo Anderlini, who you've heard already, Martin Kuerbling, and the most recent data I'm going to present is from analysis connected by Donna Przepiorka looking at the clinical outcomes of the transplants and trying to identify issues related to the composition of the graft and the outcome of the transplant.

The goal of allogeneic transplantation is to restore hematopoiesis after myeloblative therapy. At least, this was the way it was originally conceived as a way that one could just give much higher doses of chemotherapy and radiation than would otherwise be possible knowing it would ablate the recipient's bone marrow but then restore hematopoiesis with hematopoietic stem cells from an allogeneic individual.

Subsequently, we've learned, and I'll refer later in my discussion, in fact, the immunologic components of the graft are also very important in terms of the outcome of the transplant both in terms of graft versus host disease, graft rejection, but also the important immune graft versus leukemia effects.

The -- perhaps to summarize a lot of work by many people in the field, it's fair to conclude that blood stem cell transplant and bone marrow transplants are virtually the same. Anywhere you can do a bone marrow transplant, blood stem cell transplants work roughly the same way. There are some subtle differences, and we're going to get into that in a moment, describing different characteristics of each graft.

But the stem cells in the marrow and the stem cells in the blood appear to function in a very similar fashion. And so, again, from a regulatory standpoint, anywhere you do a bone marrow transplant, one could just as logically do a blood stem cell transplant.

Blood stem cells have the same major of properties and bone marrow stem cells in terms of self-renewal, ability to initiate long-term cultures, engrafted in SCID mouse, and now we know

from reconstituting hematopoiesis after myeloblative therapy in humans invariably restoring hematopoiesis.

One of the controversies which is still unclear is why does hematopoiesis recover more quickly after a blood stem cell transplant than a bone marrow transplant. Well, it may be just a matter of numbers, and I'll show you some data, again, from Dr. Przepiorka suggesting that that might be the case that there's more stem cells in the blood. And the other aspect there may be qualitative differences between at least the composition of blood and marrow stem cells.

Stem cells may well be heterogenous with some cells that have a set of kinetics that slow engraftment but sustained generation of hematopoiesis as opposed to others that have more rapid engraftment but a shorter life span. It may well be that blood stem cells are more enriched for these latter early acting cells, if you will, as well as the long-term cells which lead to variable reconstitution of hematopoiesis.

Again, the other argument that is held by many people is that it's just a matter of numbers, and there are just more of these progenitors in the blood.

It is clear though that the cells necessary for engraftment under reconstitution is in the CD34 positive subset of peripheral blood and bone marrow at least in man. There has been discussion that perhaps there is a pre-CD34 positive cell, a cell that is CD34 negative that may differentiate into one of these cells. But if one goes into a highly selected CD34 positive cells, one can achieve engraftment both in allogeneic, anatologic settings.

There is no simple gold standard in terms of what's the optimal composition of the graft or the number of stem cells, how to quantitate stem cells, but the best thing that we have at least on a day-to-day basis is the number of CD34 positive cells. This doesn't correlate well with the total white count, and it's not clear if looking at some of the CD34 positive subsets that may, in fact, biologically define stem cells better really operationally wouldn't allow us to define a better graft. So this is one of the sort of gray areas we think about regulation. How can you define a stem cell transplant when you can't easily define a stem cell itself.

The studies that we have done and Dr. Anderlini described were used G-CSF mobilized

peripheral blood stem cells where we collected the cells after four or five days. He showed you the data that after that mobilization period, we mobilized cells into the peripheral blood in a way that both total leukocytes as well as the CD34 positive cells and the CD34 positive Thy-1 are positive cells, again, reflecting the true stem cells. Components all mobilized in a roughly parallel fashion.

Martin Kuerbling published our initial work, I think, back in '95. This is one of his slides showing that when you see as much as a six-fold increase in your white count, but a 16 to 24 fold increase in CD34 positive cells or CD34 positive subsets encasing of the stem cell component.

So when, if anything mobilizes this stem cell component better than neutrophils alone, and allows, again, the effective collection of cells, usually with just a single paresis.

Lymphocytes are not mobilized in any great fashion, maybe two-fold, but most increase in the circulating numbers, but because when processes such a volume of peripheral blood, one ends up with at least a log order more lymphocytes in the final transplant than one has with a simple aspirated bone

marrow graft if platelets are not mobilized by G-CSF.

This just shows you the lymphocytes subpopulations, again, data that was published by Dr. Kuerbling. Again, roughly a one to two by one to one and a half log increase in the number of these cells compared to a bone marrow transplant.

The -- one of the questions is what is the minimal cell dose -- minimal dose of cells necessary for engraftment, and it's really unknown in the peripheral blood. In autologous transplants, a number of analyses have suggested as to you as one times ten to the sixth CD34 positive cells are enough for engraftment.

But with the allogeneic transplants, by and large, people have been giving great excesses in a number of CD34 positive cells. We ourselves have tried to target four to extend to the six CD34 positive cells per kilo just as an operational dose either a cell dose we try to meet for transplantation, but there have been several people have received lower doses, one with 2.5 times ten to the sixth per kilo, and that patient then grafted very promptly. So again, it's likely that we're above the threshold by a good margin.

The issue of time to engraftment Dr. Horowitz had discussed. In our initial studies, we saw that the neutrophils actually were not more rapid in recovery after a blood stem cell transplant than a bone marrow transplant, and a patient's not getting methotrexate. At larger numbers, again, there seems to be a small advantage with blood stem cell transplants in the medians here. But you can see that what really is different is not the median but the distribution, a much narrower distribution in recovery with blood stem cell transplants than you'll see with bone marrow.

You basically don't have these outliers, patients who are slow in reactors, and these are the ones that are then at risk for -- greater risk for infections and other complications related to prolonged neutropenia.

Dr. Przepiorka has just recently done this analysis trying to look at the impact of CD34 positive cells either from the bone marrow or peripheral blood on time to engraftment of neutrophils. And you can see that there is a clear correlation in that the source of cells, whether be it, the bone marrow or stem cells doesn't seem to be as important as the number of CD34 itself, again, suggesting that these cells are functionally

similar, and that CD34 cell dose itself is predictor.

Platelet recovery has been well documented autologous transplants to be more rapid with blood stem cells than with bone marrow, and this is certainly true with allogeneic transplants as well. At least to date, platelet recovery has not been effected by any of the available growth factors, although thrombopoietin is now being studied, and that one can see that when one see both rapid and again more uniform recovery of platelets after allogeneic blood stems transplants, and after bone marrow transplantion.

An analysis by Dr. Przepiorka looking at three parts of patients with advanced leukemia is treated at M.D. Anderson. We have two groups here that receive bone marrow transplants in our initial group getting blood stem cell transplantation. You can see the GVH prophylaxis in this group including methylprednisone and cyclosporin, and two different groups, one with methotrexate, one with methylprednisone in marrow transplants.

And you can see basically the same things that I just mentioned with more rapid recovery of granulocytes and platelets in the blood stem cell group compared to the bone marrow groups. Again,

without methotrexate, you can see granulocytes. The median was the same, although a tighter distribution on blood stem cells.

One of the things, at least that surprised me at the time was that we've seen an apparent reduction of regimen related toxicity, and again, this may be related to more profound and rapid reconstitution of granulocyte production, which again aids in wound healing and the reduction then in the appearance of toxicity at the preparative regimen.

Graft versus host disease, again, had been our major concern at the beginning of blood stem cell transplantation. Would the larger lymphocytes cell dose translate into more severe graft versus host disease both acute and chronic? And we and others have all found the same conclusion Dr. Horowitz, in fact, presented earlier, that acute graft versus host disease, at least overall, did not appear to be worse with blood stem cell transplants than with marrow transplants.

Again, the more rapid recovery of hematopoiesis led to more early discharge from the hospital, and encouragingly, the survival of patients within the first six months in high risk advanced leukemia patients was improved by the use

of blood stem cells then with marrow transplants, again, similar to what Dr. Horowitz had shown you earlier.

This is very recent analysis Dr. Przepiorka has conducted looking at the impact of cell dose on the outcomes in terms of GVH, and she found that CD34 cell doses were, in fact, more important or at least more significantly associated with GVH than CD3 T-cell numbers. And you can see that for people who have high CD34 cell doses, there's a higher rate of graft versus host disease.

And it doesn't matter whether you give them FK506 or cyclosporin as GVH prophylaxis. On the other hand, for people with lower CD34 numbers, less than eight times ten to the sixth per kilo, one sees that with FK506, there is a reduction of the rate of GVH compared to cyclosporin, and in fact, the rate of GVH is very low, in the 20 percent range.

So we have actually, arbitrarily, prior to the initiation of this study hypothesized that this may be the case, by giving a lower cell dose. In fact, we might reduce some of the GVH related complications. At least our own rule right now is to give no more than five million CD34 positive cells per kilo, again, with the hope that that might

reduce some of the immune complications of the transplant.

Chronic graft versus host disease have the same principle. This is for people with high cell numbers get more than eight times ten to the sixth for positive cells per kilo. You can see an extremely high rate of chronic GVH, again. In this group again, this has been reported by a number of groups now that blood stem cell transplants may be associated with a higher rate of chronic GVH compared to bone marrow transplants.

Interestingly, this was related to the CD34 cell dose. Again, when they lower CD34 cell dose and with FK506 prophylaxis, you can see that the rate of chronic GVH is now about 50 percent, similar to what we see with a bone marrow transplant. So again, it may be possible to optimize the composition of the graft than to improve these outcomes, and that more is not better, at least in terms of blood stem cell transplants. And so, again, there may be rationale to giving -- a given number of cells rather as many cells as one can collect from the donor.

And again, Dr. Przepiorka is here, and if there's questions regarding this data, she may be able to enlighten you further.

So the conclusions, again, in general, is that one can get a larger CD34 cell dose routinely from these people often exceeding 20 times ten to the sixth per kilo. Again, with a bone marrow transplant, one is lucky to get three million per kilo in terms of the CD34 cell dose. So much of the benefit is presumably related to the cell dose per se.

Again, one has the larger lymphocyte dose that may relate to both graft versus host disease and graft versus leukemia effects. More rapid recovery of hematopoiesis possibly less regimen-related toxicity, similar acute GVH overall, and again, the codicils I just told you about in terms of chronic GVH and with the encouraging findings, we may be able to control this by optimizing the cell number and GVH prophylaxis.

So our question comes back as to who should get a blood stem cell transplant versus a bone marrow transplant, and Dr. Horowitz presented some of the initial analysis of our joint efforts with the EBMT and the transplant registry to try to sort this out.

And so the first concern is who isn't really important to try to improve treatment here and the complications. You can see that people will

see that people with CML in chronic phase or acute leukemias in first remission have roughly half the rate of mortality as the more advanced patients. So the advanced patients, again, have roughly a 40 percent risk of dying from nonmalignant complications of their transplant, where it's generally a little 20 percent than the earlier patients.

So this is the group that is dying from complications that we hope that we can address, and at least in our initial analysis that the people in -- with CML in chronic phase of first -- acute leukemia in first remission didn't appear to be a major difference in survival in the early group. On the other hand, the people with advanced, particularly, CML once he's improved early survival related to treatment related complications. This isn't related to graft versus leukemia or relapse, this is just reduction of early mortality related to the transplant, graft versus host disease and infections.

And that you can see again, the bone marrow transplants doing much worse than the blood stem cell transplants. So at least in our own program right now, we're recommending blood stem

cell transplants for patients with advanced leukemias and CML in accelerated phase whereas we're continuing to do bone marrow transplants for patients with CML in chronic phase.

This is very updated data. You can see it's 1999, a productive year there. But she looked at the results of 1-Antigen mismatched transplants, again, would have an advantage with blood stem cells compared to bone marrow here. We all know that with any degree of HLA mismatching, the risk of graft versus host disease is increased.

And in fact, we were somewhat alarmed, at least in our own series, to have what appeared to be marked increase and the risk of GVH in these patients compared to bone marrow transplantation, and so that we have, in fact, stopped doing this at least within our own program and that we now would do bone marrow rather peripheral blood transplants for one minute managing mismatched donors.

And this is not necessarily been a uniform finding. I'm sure someone in the room will get up and present some data that are not this extreme, but it leads to something that we're very concerned about that there well may be more GVHD as we get into greater degrees of immune disparity.

I didn't bring the slide I actually intended, but there has been a lot of work using manipulated peripheral blood, and work by Martelli Reisner and others using so-called megadose T-cell depleted transplants. When one takes advantage of your ability with peripheral blood stem cells to get huge numbers of CD34 positive cells from the donors, then thoroughly deplete them of T-cells, one can then successfully achieve engraftment of those cells without graft versus host disease into haploid identical recipients. Everywhere it's been very difficult to make progress with bone marrow transplantation.

So clearly, the peripheral blood and its ability to generate large numbers of stem cells has opened the door to this group of patients that have not been effectively treated to date.

The other aspect is that we can use the immunologic aspects of the transplant in a therapeutic fashion, and what we have done and recently have published a number of articles related to this is to try instead of giving him a maxibly tolerated dose of high dose chemotherapy is to give a relatively mild dose of treatment, just enough to present rejection of the transplant by giving immunosuppressive drugs, again, preventing rejection

allowing engraftment of an allogeneic blood stem cell transplant that could then mediate a graft versus leukemia effect.

And in so doing, we use the transplant not so much as an hematologic supporting tool as an immunotherapy tool. In this situation, we can give additional lymphocytes as necessary to enhance that effect. We published this last month in the Journal of Clinical Oncology in chronic lymphocytic leukemia that this is a particularly encouraging approach where one can -- one does not see lysis of the tumor with low dose chemotherapy, but rather with engraftment of the cells.

You see the tumor melt away over a period of about a year, and we can help it along as it goes with donor lymphocyte infusions. And this just shows a tumor mass of CLL in the patient after going through the high dose chemotherapy. This was cells that hadn't responded to the chemotherapy, but with another infusion of lymphocytes from the donor, one, he sees complete resolution and complete remission in other individuals, and I'd refer you to that article about Esa Curry in the recent Journal of Clinical Oncology for a full description of this trial.

So in conclusion, one can use allogeneic blood stem cells both as a source of hematopoietic cells for reconstitution of the hematopoiesis but also as -- for immunocompetent cells, for immunologic based therapies, in our case, graft versus leukemia manipulations.

So I'd like to take my final moments just to maybe raise some questions as we think about regulation of stem cells. The question is, again, is allogeneic blood stem cell transplants an area that really needs regulation? After all, this has been an area of rapid development that has flourished, really, under the supervision of IRBs and without the involvement of the FDA.

We're talking about at least the cells -- the studies that I presented here, minimally manipulated cells. We all agree in the infectious disease considerations and good laboratory practices should be used should the FDA be involved in trying to clarify the indications for transplantation. This really is the practice of medicine, and this is an area that the FDA is not charged to be involved with.

This is, the FDA is charged to supervise the development and approve the development of drugs and devices, but is not specifically to be involved

with the practice of medicine. Clearly, this is an area that has been developed responsibly, one doesn't out of cavalier, people out there doing allogeneic blood stem cell transplants. This has been an area that really has been restricted to academic and research centers that are well supervised by their own IRBs.

Our concerns is that if one introduces regulations sort of prematurely, particularly, if one tries to incorrectly characterize the transplant and then impose rigid standards that would prevent us from really going forward with the rapid development in this field. It would actually retard rather than enrich the search, and it would inhibit rather than help patient care.

Clearly, the composition of the graft is important. It may vary, again, related to the application. For our graft, this is leukemia strategies. We want a very different graft than a T-cell depleted mismatched transplant using a double-aided regimen. So again, this is the practice of medicine where transplant professionals such as all of you in this room would use a fundamental understanding of bone marrow as well as blood stem cell transplantation to try to define

what is it, and the most active product for an individual patient.

And we need to very rapidly and flexibly move forward with research to try to define what is optimal in this regard. My own view is that this is an area where less rather than more regulation is actually required. Again, I have no problems with defining good laboratory practices, and the infectious disease testing that should be done to prevent infections from being disseminated.

But again, I have grave concerns about excessive regulation inhibiting research and interfering with the practice of medicine. Thank you.

DR. STRONCEK: Thank you, Dr. Champlin. We'll wait until all three presentations are done before we have discussion at the end. The next speaker will be Dr. John DiPersio. Dr. DePersio is a Professor of Medicine, Pathology and Pediatrics, Chief of the Division of Bone Marrow Transplantation and Cell Biology, and Acting Chief of Medical Oncology at Washington University, St. Louis.

He received his medical training and Ph.D. from the University of Rochester Medical School in New York, and he had internship and residency training in internal medicine at the University of

Texas Southwestern Medical School in Dallas. He completed a hematology and oncology fellowship at the University of California, Los Angeles. He also had post-doctoral fellowship training at UCLA.

His current research interests include growth stem cell factors, receptors and signaling, allogeneic stem cell transplantation, the generation of murine models for acute and chronic graft versus host disease, and murine models for the treatment of graft versus host disease.

Dr. DiPersio will speak on Related Allogeneic Peripheral Blood Stem Cell Transplants: The Washington University Experience. Thank you.

DR. DIPERSIO: Thank you very much, and what I'd like to do in the next few minutes is review our experience. I'd like to thank the organizers for inviting me here, and allowing me to share with you our experience.

Well, as you know, the problems related to allogeneic transplantation, historically, have resulted in major decreases in survival related to initial cytopenias in toxicities related to the transplant. Depending upon the state of the patient at the time of the transplant, this has resulted in ten to 40 percent treatment of related mortality.

Second major obstacle has been acute and chronic graft versus host disease. I'll talk a little bit about this if I have time at the end, and then of course, the major problem as we resolve number one and we start to make some in roads in number two, is that we're faced, unfortunately, with incredibly aggressive diseases and the biologic resistance that we're facing now, especially in patients with acute leukemia who have received high dose ARA-C in the past is a very, very major problem.

Well, the advantages of peripheral blood stem cell transplant are obvious, and I won't bore you with them. You've heard a lot already by Dr. Champlin. But basically there are a number of clear-cut advantages listed on this slide. There are also some disadvantages in that some donors require central lines, et cetera, might there be increased risk of graft versus host disease and increased risk of CMV. You've heard a little bit about that already.

When we started this in mid-1994, over 200 peripheral blood transplant procedures in the past, we had no idea about what the rates a central line placement would be, how donors would tolerate G-CSF,

et cetera. So all these were unknown when we started.

I'll give you primarily the data on the first 100 patients that we transplanted because that data, as far as both acute and graft versus host disease are a little bit more mature.

This is the first cohort of patients that we transplanted using mobilized peripheral blood. This is G-CSF, 10 micrograms per kilogram given for the standard period of four days, and then pheresis on day number five. And you can see that the interesting this is that the day after infusion, there reproducibly is an increase in the white count.

We're still trying to figure out using chimerism studies what this is due to. But then the white count drops. When you actually look at the period of neutropenia in these patients, it's extremely short. It's only five or six days at the very most, and then counts came back very quickly. These patients received cytosporin and methylprednisone for graft versus host disease prophylaxis.

So this was a very impressive and brief duration of neutropenia, and more importantly, these products have approximately two times ten to the --

approximately six times ten to the 11th HLA compatible platelets in each product. So that represents two single donor platelet transfusions each time you give a mobilized peripheral blood product. I should also mention that we use 20 liter exchanges for all of our normal donors.

And this is the median, excuse me, the mean platelet count for all the patients in this initial 21 patient cohort showing you that the vast majority of these patients had a nice increment in their platelet counts at the time of infusion, and most of these patients never drop below 20,000 never mind below 10,000. So the vast majority of these patients require very minimal platelet transfusions.

Now, based on the initial blip and the impact of these platelets that contaminate these products, we asked if -- would a second infusion of mobilized peripheral blood or the infusion of HLA compatible granulocytes further reduced the period of neutropenia, and this work was done by Randy Brown in our group and by Doug Adkins who is in the audience, who will speak tomorrow.

And so we did another cohort of about 15 patients, and these are -- this is the median ANC of the second cohort in which you see this little blip again on day two which is not as pronounced this

time. And then the second infusion of mobilized peripheral blood occurred on day plus three, and you can see that there is a major increment in the white count, and that these patients had only approximately one to two days of neutropenia.

So now, we've gone from a typical, you know, 12 to 15 days of neutropenia down to five, now down to one. And this is work from Doug Adkins' study, in which he'll present some of this tomorrow, in which granulocytes were given on days three and day six from the same H like compatible donors. And these are the ANCs of the control group receiving mobilized peripheral blood alone on day zero, and the neutrophil receiving mobilized peripheral blood on day zero and granulocytes on day three and day six.

And if you look at the difference between the ANC count and the control group in blue, and the neutrophil infusion group in red, you can see that there's a significant difference in the peak neutrophil counts on days four, five, seven, and eight suggesting that, again, using this approach, these are radiated neutrophil products. Using this approach, we've reduced the absolute period of neutropenia down to one to two days. So this is essentially an outpatient procedure.

Now, this is an example of a patient receiving two peripheral blood stem cell products. The white count is in the solid circles, and the absolute neutrophil count is in the open circles. And you can see that after each infusion, there's an increase in the neutrophil count. The platelet count never drops below 10,000. The neutrophil count never drops below 100, and the patient grafted promptly both platelets and neutrophils.

This is sort of a typical, a little bit faster than the usual because he's a little younger than many of the patients that we transplant, but he received, actually, no packed red blood cells, no platelets. He was not febrile. He received no antibiotics. He was in the hospital for a total of 17 days. And his hospital based charges were about $57,000 which was essentially all pharmacy charges.

So this is the initial 50 donors looking at -- this is very much similar to what was presented already by the group from M.D. Anderson, so I won't belabor this. But about 90 percent of our donors could mobilize greater than two times ten to the sixth with a single 20 liter exchange. Approximately 63 percent could mobilize more than five times ten to the sixth, and about ten percent of our normal donors require central venous access.

We, fortunately thus far, have had no significant complications with central venous access, although we have had one donor who developed unstable angina during his mobilization phase. He was a young man actually with no history of heart disease.

This is data published several years ago by Randy Brown showing that as far as I know, the first clear-cut association between the number of CD34 cells infused in the allogeneic setting where the rate of engraftment, these are Kaplan Meier's probability of neutrophil recovery and platelet recovery.

And you can see that both -- in both situations, if you have more than five times ten to the fifth CD34 cells per kilogram, then you're going to have rapid platelet and neutrophil recovery, very similar to the data published so far in the autologous setting. And this is the data; I'm looking at higher numbers of CD34 cells, and you can see there's not a big advantage of infusing higher numbers of CD34 cells.

Well, the important issues in peripheral blood stem cell mobilization relate to the quality, not only the quantity of stem cells mobilized. The impact of mobilization on other types of cells such

as T-cells, T-cell subsets, NK cells, dendritic cells and the effects of all these things on graft versus host disease, and identification of the occasional poor mobilizer which in our center it ranges between four and five percent of the normal donors could not mobilize adequately.

This is data on the first 50 patients looking at the mobilization of white cells, lymphocytes, both T and B cells, NK cells after five days of G-CSF. So you can see that there's a significant mobilization two to three -- two to four fold, actually, of not only white cells, but also lymphocytes, lymphocyte subsets, B and T cells. The etiology of this is unclear because as far as I know, these cells do not express G-CSF receptors at least at the RNA level that we looked at by PCR.

Randy actually made a very important observation and noticed that in the few patients, in the few normal donors that mobilized poorly, they had very low numbers of resting CD34 cells in the peripheral blood. In fact, they had less than 1,000 in the peripheral blood, and those were the patients, those were the normal donors who could not be mobilized with G-CSF adequately to reach the target of two times ten to the sixth.

And we had several that had normal blood counts but had extremely low amounts of mobilization similar to the few patients showed by the previous speakers. And this is the relationship between the probability of achieving our threshold with a single pheresis and the resting CD34 number. So the resting CD34 number in our hands correlated with the ability to mobilize in a single collection and also identified a particularly high-risk patient normal donor for a poor mobilizer.

We then looked at the normal population, and this is 400 normal platelet donors, and we measured resting CD34 levels in these 400 normal platelet donors. And as you can see, there's a wide array of resting CD34 numbers, but most of us in the room here have about 2,000 to 3,000 CD34s circulating in our peripheral blood. But as you can see, there's about three to four percent that have less than 1,000, and those we think are the -- at least at high-risk for being very poor mobilizers with G-CSF.

And we also were wondering if this was just an individual observation made on one day, or whether this would be a consistent observation that we can make over time. So we took a number of platelet donors, and we followed them for six

months. And we measured their resting CD34 numbers. And interestingly enough, they stayed relatively constant over 6 months. So the ones that were high stayed sort of high. The ones that were low stayed sort of low. And I'm not sure what the significance of this is except that this was kind of a fingerprint for each normal donor.

Now, this is -- consistent with that notion, this is the distribution in the normal allogeneic population of CD34 cells in the peripheral blood before mobilization, and this is the distribution in our autologous transplant patients showing a marked reduction in the circulating CD34 numbers. And this is consistent with the notion that patients undergoing autologous transplant for breast cancer and for non-Hodgkins lymphoma have a great deal more difficult mobilizing with cytokine alone. And this suggests the fact and is consistent with the notion that a significant portion of these patients and a very small proportion of these patients cannot be mobilized with G-CSF alone.

Well, the identification of poor mobilizers in the auto setting is well known, and some of the other things that we've looked at is pre-mobilization platelet counts and pre-

mobilization flt-3 levels. As you know, flt-3 is the only hormone that I'm aware of that varies inversely with the marrow cellularity and probably the stem cell mass. So we were interested in looking at flt-3 levels as an indicator.

And before I actually go through that, I just thought I'd show you what happens to stem cells in an allogeneic transplant recipient who's receiving mobilized peripheral blood, what happens to these stem cells over time. These are a series of patients, I think, a total of 21 all together in which we did tracking studies in which we followed the appearance and disappearance of CD34 cells in the peripheral blood after infusion of a single large product.

And as you can see, there's a nice spike in the CD34 numbers within minutes after infusion as you would expect. And these levels were drawn from a separate site, not from the central catheter, so there was no chance of contamination. And then they drop rapidly so that within six hours, they return to baseline. So the actual cells circulate very briefly and then disappear. Where they're going is unclear.

Now, the other interesting thing we didn't expect to see was that during the transplant period

as you would expect, the levels of CD34 in the peripheral blood of these transplant recipients is extremely low. But then at the time of engraftment, there is a huge surge of CD34 cells which mobilize into the peripheral blood at the time of engraftment. What the survival advantage of this would be is unclear to me.

But the interesting thing is that we have taken the CD34 cells and purified them on a number of occasions and shown unequivocally that they're 100 percent donor in origin. So these cells that mobilized and circulate in the peripheral blood at the time of engraftment are the donor cells that were infused at the time of the transplant.

So, obviously, the bone marrow microenvironment or the stromo microenvironment is being remodeled very dramatically at the time of engraftment. And I suspect that the mechanisms relating to what causes this is also underlying the mechanism of basic mobilization in general.

Now, getting back to the flt-3 level business, since we were interested in looking at the correlation between flt-3 and CD34, this is actually the flt-3 level's measured by ELISA at the time of the transplant and the time of engraftment. And as

expected because the marrow is ablated, the flt-3 levels are extremely high at the time of transplant.

And then at the time of engraftment, even though the marrow cellularity here is zero, at the time of engraftment when these CD34 cells from the donor starts circulating, the serum flt-3 levels drop precipitously.

We also thought well if there's an inverse relationship between flt-3 and CD34 numbers, maybe we'll see that at the time of mobilization because when you mobilize patients, you see increasing numbers of CD34 cells circulating. So we looked at 70 normal platelet donors, and the flt-3 levels were about 53 picograms per mil. And then we looked at auto transplant patients, and there were 52. And then when we mobilized these auto patients, the levels dropped precipitously to 11. So it was consistent with a notion of an inverse correlation.

When we looked at the allo, their resting levels were a little bit lower suggesting that patients that undergo repetitive platelet donation actually have perturbed hematopoiesis. But when we mobilize these normal allo donors, their flt-3 levels dropped. And in the allo recipients, of course, you've seen this already, that the flt-3 levels at the time of transplant, before transplant

are 58, at the time of transplant 336, and then six hours after transplant when there's this spike of CD34s, the levels don't change appreciably.

So there's not an absolute correlation. In other words, the clearance of flt-3 is not clearly related to the circulating numbers of CD34 because if that were the case, this level should have dropped a little bit.

This is the relationship between the post -- this is the relationship in red, the post-mobilization CD34 numbers here, and then the baseline CD34 numbers in the blue, and you can see the flt-3 serum levels. So as -- before mobilization in the blue, you can see that the flt-3 levels are high, and at the time of mobilization, the CD34 numbers go up, and the flt-3 numbers go down.

Now, we went back and said, okay, maybe this is important, and I must admit, I'm not completely clear yet how -- what the relationship is here yet. I think it's going to take a little bit more work, and we also have to work on our flt-3 assay a little bit more. But this is the relationship in our auto transplant patients between pre-mobilization flt-3 levels and CD34 levels per kilogram per liter pherese at the first pheresis.

So we thought this would be an accurate way to portray the data, and you can see that all the red dots represent the patients that we couldn't reach one times ten to the sixth CD34 cells per kilogram. And it turns out that those are the patients that have serum flt-3 levels before mobilization in excess of 100, 150.

And we certainly know for sure that if your serum flt-3 levels are in excess of 200, the chance of being able to mobilize as an auto patient with flt-3 alone is almost negligible. So I think this is an important -- this may become an important predictor of how we can pull out the people that it's just senseless to try to mobilize.

Now, we've looked at all of these normal donors too, and we haven't found any normal donors with very, very, very high flt-3 levels. So this is the -- someone asked previously about the eniological component of these grafts, and these are T-cell mitogenesis assays before and after mobilization with G. And you can see that the T-cell mitogenesis responses are a little bit decreased after G mobilization.

Again, I'm not really sure why this is. It could be that there are just more contaminating monocytes although the number of lymphocytes in

these in vitro studies are identical from lane to lane. And also in the post-transplant period, although I have no comparison to bone marrow here whatsoever, I'm just showing you one glimpse of what happens to mobilized peripheral blood allo recipients.

The PHA and the OKT-3 mitogenesis assays remain very depressed around to one year. And when we look at NK activity using K562 targets, they remain very depressed out to one year as well. So in spite of infusing all of these T-cells, we still are left with patients, it's probably not surprising because they're on immunosuppressants that have suppressed T-cell function.

And also consistent with this, the rates of CMV reactivation appeared to be increased. Again, this is not a randomized study. This is just using our historic allogeneic transplant controls in the first 50 alloperipheral blood stem cells. And you can see that the percent at risk for CMV viremia is about the same.

The incidents of first viremic episodes in our CMV patients was 25 percent versus 62 percent, second, viremic episodes, 11 percent versus 25 percent, third, 2.8 versus 8.3. And the incidents of CMV disease is extremely low, but a little bit

higher, but not statistically significant in the peripheral blood group.

So this is the rates, and this has been reviewed already ad nauseam, so I won't bother you. This slide shows you that using IBM -- these are not matched controls like you saw from Dick and Mary. These are just IBMTR patients that Mary was kind enough to give us that had been treated with methylprednisone and cyclosporin only. They weren't matched in any other way. So this is not a good comparative group.

But the rates of acute graft versus host disease stay at grade two to four and three to four are approximately the same in a BMT in the peripheral blood groups whether we used our own historic controls or the IBMTR controls. However, that rate is a chronic graft versus host disease initially appeared to be greater, and the actuarial risk at two years is over 90 percent. That's very, very high.

This is data with a median follow-up of almost 2.8 years. So I think this is getting out there to some of the longest follow-up for rates of chronic graft versus host disease. And the actuarial risk of developing chronic graft versus host disease at two years is a little over 90

percent. And most of these patients have extensive graft versus host disease. It's mild to moderate, and it does affect the performance status of over 50 percent of the patients.

So this is also an interesting prod looking at the proportion surviving. I should say that all of these 200 patients that were transplanted, none of them were transplanted with low-risk disease. They all had relapsed or resistent AML. None of them had CML in chronic phase. None of them were AML in first remission. So these were all very, very high risk patients. So this is a respectable, I think, at two or three years, a respectable long-term survivorship rate.

And what Randy did is Randy then did a sequential studies looking at since we had a number of patients that couldn't be mobilized optimally, Randy then pulled a G-CSF data together, some of the old patients, and then did a trial which he looked at G plus GM in which he used ten of G and ten of GM, and then GM alone, ten.

We stopped this trial at the end of ten patients. You'll see why. This is the number of CD34 cells mobilized in these normal allo donors. So 8.9 in 11.0, this is actually statistically different, and this is, of course, statistically

different resulting in us prematurely terminating the trial. So that the GM-CSF alone in these allo donors was a very inferior mobilizing agent.

The number of CD3 cells mobilized was dramatically lower when G-CSF was -- when GM-CSF was added to G-CSF. This was a very big surprise, and the more of these patients we looked at, the difference between these groups has increased, actually. And also, when you use GM-CSF alone, the number of T-cells in these grafts is lower probably because the total TNC is lower as well.

And the number of dendritic cells that was mobilized is -- these are the number of dendritic cells in these grafts from the G-CSF group and the G plus GM, so that you're getting about twice as many immature dendritic cells in the G plus GM. And with the GM alone, you're getting a lot of dendritic cells. And the interesting thing is that if you look at the activation marker on dendritic cells, CD80, we tried CD86, but the antibodies for CD86 are not very good. So we looked at CD80 as an activation marker for mature dendritic cells in the peripheral blood.

The patients mobilized with G-CSF alone had almost no expression of CD80, while at least 60 percent of the DCs in patients receiving either GM-

CSF alone or G plus GM-CSF had very, very bright expression of CD80. So number one, that we think GM-CSF is actually mobilizing dendritic cells, it's not only mobilizing them, it's activating them as well. This is consistent with some of the in vitro data, but it was sort of surprising.

So the kinds of things that we can use peripheral blood with now -- we can actually add this as Dick Champlin had mentioned to minimal conditioning regimens. These are some of the regimens that we've used. We've used only high dose aroseda condition patients, and we've gotten almost complete engraftment in seven patients. I think one patient failed to engraft.

But the problems with these patients are that they all relapsed. These were patients with resistent leukemia. Doug Adkins in our audience, along with Gary Spitzer, while they were at St. Louis U., thought up this scheme, and they started it sort of simultaneously, now at Georgetown and at Wash. U., and they use single dose TBI which is a cytoxan which is an incredibly well-tolerated regimen with almost no toxicity and morbidity. When you add this to mobilized peripheral blood, the results are pretty remarkable and how easily patients go through transplant. And of course, we

might be able to improve our stem cell and stem dendritic cell mobilization by using other combinations of cytokines which we're looking at now.

So the -- this is the data from Doug's study looking at single dose TBI and mobilized allogeneic peripheral blood, and what I'd like to say is the number of days in the hospital is 21 days, the length of stay, and the number of average days in the hospital through day one hundred is only 26 days.

This trial which had about the initial number of patients, 30 patients has a 95 percent survival at 100 days. And that's pretty remarkable for allogeneic peripheral blood stem cell recipients, and the number of days they're receiving blood products and antibiotics is very minimal. So this is really -- we have much more trouble now with our auto patients than our allo patients.

So the future directions related to control of chronic graft versus host disease which is a huge problem in this, unfortunately, in this unmanipulated peripheral blood population of recipients, and to assess the stability in grafting using peripheral blood. And I think there's a lot to be done with this.

We have seen and observed decreasing blood counts post-transplant. A number of patients develop thrombocytopenia post-transplant. We haven't really looked at it quantitatively, but it needs to be done. There's an important need. Things need to be done with functional and physical T-cell depletion, and we're working on genetic manipulation of T-cells and one or two more slides just to show you that this is another approach.

We're using various suicide genes which you're all familiar with, and we're using epitope tags to mark these suicide genes, and we're using mouse models. So I think one of the nice things that could be -- if I could put in a plug for a little less pure clinical kinds of support, and a little more translational support. The kinds of things that we could do to sort of modify or mitigate chronic graft versus host disease using these translational approaches in my view would be very much needed and would be very beneficial in the long run.

So we actually generated fusion suicide genes that are expressed in the surface of cells. These are a single CDNA that functions as suicide genes and epitope tags. We just happen to choose the CD34 as an epitope tag because it's FDA

approved, and we love the FDA, and we wish the FDA would give us more money.

So these are what these genetically modified T-cells look like right now. They have CD34 in the surface, and they have suicide genes fused and framed through a linker region, and we've proven that this is expressed. We proved that this functions very well, and we also mutated the TK gene so it's very, very active now, ten to 20 fold more active than the native TK. And we've shown in a mouse model system using a transgenic mouse in which all the T-cells are expressing this fusion suicide gene in the periphery.

Here's an example, I think. This is the mouse actually. It's a transgenic mouse. It's blue. It's H-2 of b/k, and for the transplant models, we're using H-2 disparate recipients, the BALB/C which is H-2/D and the FVB which is H-2/Q. And we've gotten two founders which are high expressors for this fusion suicide gene.

This is a wild type litter mate control which is genotype negative, and you can see there's no CD34 expression in any of these cells. Here's the transgenic, next slide. I have like one more slide, I think. This is the transgenic animal. You can see that the peripheral blood. There's very

good expression in the CD2, CD8, and CD4 compartment for these genetically manipulated T-cells expressing this fusion suicide gene.

And we've shown that these cells die rapidly in response to gancyclovir compared to litter mate control T-cells, and that we've done transplants. And this mouse model would be a very ideal system to test the optimal way of T-cell suicide, when to T-cell suicide, where these T-cells go, optimal conditions for mitigating graft versus host disease, and also for testing the immunogenicity of genetically marked T-cells.

And so this is the first experiment. We just got this back last week. This is very encouraging in which we've done a transplant. It's kind of hard to see. This is day 21 after a transplant from H-2 disparate bone marrow donor which is T-cell depleted, and we add the transgenic or nontransgenic T-cells to these animals. These animals die of overwhelming graft versus host disease.

And so what we've got is that this particular, I'm having trouble reading it. This is the non-transgenic, I believe. So this is a transplant recipient at day 21 in which he's

received just nontransgenic T-cells. So you can see there's no CD34 expression here.

Here is the transgenic -- here's the nontransgenic with gancyclovir. You can see that gancyclovir has a little bit of a nonspecific affect on reducing the number of CD3s. Here's the transgenic recipient. This animal always dies of overwhelming graft versus host disease right about now, and you can see that all of the T-cells or most of the T-cells in the peripheral blood of this recipient our CD34 suicide gene expressing.

And then when we treated these animals, these guys all die, and when we treated these animals with gancyclovir, these T-cells disappeared. And these animals live. So we have a nice mouse model, so I think that's the kind of things that may also benefit us in the long term.

Allogeneic peripheral blood stem cell transplants results in comparative outcomes and rates of acute graft versus host disease when compared to allogeneic bone marrow, addition of second allogeneic peripheral blood on day three, or allogeneic granulocyte infusions on day five and seven, reduce the neutropenia period to only one to two days, rates of graft, chronic graft versus host disease appear increased, although this will -- we

need to see the results of the randomized trials to really know for sure, resting CD34 platelet count of flt-3 levels predict autologous and probably allogeneic donors who will be poor mobilizers.

CD34 numbers circulating, very high numbers at the time of allogeneic peripheral blood stem cell and graft, and the reason for that is unclear. Circulating levels are inversely correlated with flt-3 levels. Allogeneic donors mobilized with both G and GM-CSF yield higher, numbers of CD34 cells and fewer T-cells, and those receiving GM-CSF have more dendritic cells and more activated dendritic cells. And whether that will have an impact on graft versus host disease is unclear.

Future efforts to selectively deplete or genetically modify T-cells in the allogeneic peripheral blood stem cell setting may reduce rates of chronic graft versus host disease. I think you for your attention.

I'd also like to thank all my collaborators, Randy Brown, who's a PI of all of the peripheral blood allotrans, Doug Adkins, who is in the audience, who is the PI for the granulocyte studies, and my lab colleague, Tim Lay who has

helped me with the laboratory-based studies with the mouse model. Thank you.

DR. STRONCEK: The next speaker is Dennis Confer. Dr. Confer is a Medical Director of the National Marrow Donor Program, a position he's held since 1993. He's also a Clinical Professor of Medicine at the University of Minnesota who received his medical degree from the University of Nebraska Medical Center, and he has fellowship in hematology and oncology training at the University of Minnesota.

He has been a faculty member at the University of Minnesota, and the University of Omaha. And was the Director of the Bone Marrow Transplant program at the University of Omaha. When it comes to unrelated donors, Dennis knows everything and does everything.

DR. CONFER: Thank you, David. I haven't donated. I am listed. I'd like to thank the organizers for inviting me to present some information from the National Marrow Donor Program. The first slide. There we go.

I'm going to present data on unrelated donors who have donated peripheral blood stem cells and facilitated through the NMDP programs. Basically, all these data derived from second

donation requests. You can see here that as of the end of July 1998, the NMDP had facilitated over 7,300 transplants of bone marrow using volunteer unrelated donors.

We have also received over 500 requests or about seven percent of these transplants, 500 requests for additional marrow or peripheral blood stem cells. So this donor's donated bone marrow once, and then the transplant center comes back and says that the recipient needs additional marrow or peripheral blood stem cells from the same donor.

You can see these requests are almost evenly divided between requests for additional marrow and requests for additional peripheral blood stem cells. About 70 percent of these requests are because the initial graft is either functioning poorly or has failed to engraft. About 30 percent are related to recurrence of the recipient's original disease.

Initially when we began collecting peripheral blood stem cells, we tried to do these according to a standardized protocol. This was formalized, however, in 1996. We submitted an investigational new drug application in late 1996, and the protocol under this IND opened February 1, 1997. So I'm going to talk to you about data

collected under this protocol that opened in February of '97.

Under this protocol, all of the donors received filgrastim at a dose of approximately ten micrograms per kilogram subcutaneously days one through five. Leukapheresis is then performed on day five and optionally on day six at the discretion of the collecting site and the donor center.

The dose of filgrastim is rounded to an integer number of vials in order to make it easier to administer the drug. You just give a set number of fixed vials determined on the recipient's weight. The actual doses range from about nine micrograms per kilo up to about 11 and a half micrograms per kilo.

Donor evaluation and follow-up is collected on a series of forms that the NMDP terms the 400 series. These collect data pre-mobilization and then during mobilization -- during the collection of the peripheral blood stem cells and then follow-up data on the donors.

Because we are collecting donors from multiple sites, we have over 100 donor centers, over 100 collection centers, and now over 40 apheresis centers -- collecting this data is oftentimes a challenge. We've tried to introduce a number of

processes to improve on data collection. These include a Forms Due reporting that goes to the centers. These reports are generated monthly. They display all the forms that are currently due for a given donor. They show forms that are past due, and they also list forms that have been submitted but with errors identified.

In addition, at the time data are entered into the STAR computer system at National Marrow Donor Program, we do some on-line validation checks. This includes range validations. We cross-validate for consistency within a form and also cross-validate for consistency between forms so that one form can't have data that is inconsistent with data previously submitted on another form. And mandatory forms are also identified and data for those fields is required.

Additionally, everyday the transplant or the collection facilities, the donor centers will receive an error report. This is transmitted electronically on the day that the form is keyed into the computer, and this will list the particular form and the specific error that was identified during those data validation entry on the previous slide.

And once a month, they get a summary error report that lists all their errors that have not been resolved at month end, and also displays an error message. For the data I'm going to present today, we have about 85 percent of the required forms have been submitted. We're working to improve this form submission rate.

I should tell you that a similar effort to improve form submission from transplant centers has been very successful. Among recipients, we now have more than 99 percent of the required forms have been submitted and successfully entered into the computer system.

So this is looking at donors between February 1, as I indicated, and August 8 of this year. During that time, we received 119 requests for peripheral blood stem cells in the second donation setting. Out of these 119, there have been 34 donors who received filgrastim. Now, this is much lower than the number of requests submitted. About 40 percent of these requests are cancelled by the transplant center.

These recipients are extremely high risk because they have graft failure or relapse of the original disease. And frequently, they will either get worse or they will get better after the request

is submitted, and that leads to cancellation of the requests. In some cases, the collection site, the donor center was unable to perform the collection, and that resulted in these requests being changed to requests for a marrow donation.

So among these, 32 donors have provided products that were infused into the recipients. In 17 of the donors, there was a single collection involved. In 15 of those donors, there were two collections involved. The bulk of the data I'm going to discuss today involved 31 of these 32 donors. One of these donations was so recent that no forms are yet due.

In one case, filgratim was administered, but a collection did not occur. This happened because the recipient died during the administration of filgrastim. The recipient expired so the donor's filgrastim injections were stopped. And similarly, in one case filgrastim was administered a product that was collected, but after the product was collected and before it could be infused, the recipient, who obviously was critically ill, expired.

So on those 31 cases that we're interested in, the median time from the marrow collection to PBSC collection was about two and a half months.

You can see the minimum is about four weeks or one month. The maximum time, one and one half years.

The donors, the 31 donors, ten were female, 21 were male. This is a little bit different than our distribution of marrow donors where about 60 percent are males and 40 percent are female. The median age of these donors was 38 years, the maximum was up to 60 years, minimum 24 years.

You can see that the, not surprisingly, the majority of these donors are caucasian, but in seven instances, the donors were non-Caucasians requested to make the peripheral blood stem cell donation. This comprises about 20 percent of the total, which is actually higher than the representation of non-Caucasians in the marrow donation population, and we have to -- we'll look into this factor.

Relevant to the data collection issue, you can see that in 23 of the cases, there was only a single donor at 23 centers, and this isn't surprising since we have over 100 centers. Three donors were requested from a single large center, and five donors were requested from a single very large center. I do not believe this is an indication that marrow from these centers is, in any

way, inferior. It's the size of the donor pool that's available at those centers.

So turning now to data on the donors, this slide shows donor white blood count, and it starts with the baseline at the time of their pre-mobilization evaluation, and then we collected white blood count data on day one, on day three, day four, pre-collection on day five, and pre-collection for those donors donating a second product on day six.

These values were drawn prior to the administration of G-CSF. So this is actually another baseline value. This is following two doses of G-CSF, and prior to the third, et cetera. Each of these shows the median value in the diamond, the minimum value among the donors in the green triangle, and the maximum in the red.

And what you can see is that after two doses of G-CSF, there's the expected dramatic rise in the white blood counts. This continues during the administration of G-CSF. You can see that our maximum white counts were over 60,000 in these populations. Our protocol contains a provision that if the white count reaches 65,000, there is to be a 50 percent dose reduction.

This shows absolute lymphocyte count. Dick Champlin showed similar data which shows that

there is some mobilization of lymphocytes which is not nearly as dramatic as the total white count or the neutrophils. In some cases, however, there's quite a dramatic increase in peripheral blood lymphocytes.

This slide shows the platelet count during filgrastim administration. It's beginning to show that after four doses of G-CSF, there probably is beginning to be some decline in the platelet count that is irrespective of the collection day apheresis collection itself, and obviously, among those donors who've already had one collection and are scheduled for a second collection, there's a further decline in their platelet counts across the board. We'll look at that in a little more detail later.

This now looks at donor symptoms by day. You can see that the bone pain starts before the first dose of filgrastim. A couple of these donors seem to be symptomatic when they started. In that regard, it's important to note that this is population of extremely stressed people because they've already donated bone marrow.

They thought that they were setting out to save someone's life, and they found out that, in fact, isn't the case. And so they're being asked to make another donation, and they are really under

stress, and I think that it shows in some of these symptom profiles if you'll notice that during the rest of the talk.

But clearly, after they receive G-CSF, the bone pain obviously goes up. Sixty to 70 percent of the donors are reporting bone pain on days three, four, and five. I don't know whether this decline is real. Maybe it's anxiety after that first collection is over. This shows severity of the bone pain, and again, the bone pain appears to be most severe on days three, four, and five with one donor in each case saying that it is very severe on day three and four.

The maximum severity of bone pain looks to be on day five when among those people reporting bone pain, 47 percent said it was mild, and actually 53 percent now were saying that it was moderate bone pain.

This slide shows the sites of bone pain by day during filgrastim administration. You can see that back pain in red and hip pain in yellow seem to be the most common sites. Thigh pain, knee pain, and rib pain are reported at about equal frequency during the administration of filgrastim.

This then looks at other symptoms assessed by CALGB toxicity scores, and very common, as you

all know is headache, grade one, grade two headaches which are severe but relenting, and grade three headaches which are severe and unrelenting. This resulted in a dose reduction for this particular donor of 50 percent. This was the only dose reduction that occurred in these 31 donors.

Other prominent symptoms, myalgia and arthralgia. This number here refers to the day of maximal reporting of these symptoms for each of these groups which is the data that's displayed here. Myalgia, arthralgia, very common. Malaise and fatigue is also very common. Insomnia is a common complaint among people receiving G-CSF occurring in this experience in about 30 percent.

And then we look at a bunch of less severe but not necessarily minor symptoms, less severe in terms of their reported frequency, being reported in one to three donors each. Again, the days of maximum reporting are shown here, tend to peak around day four or three of the G-CSF administration.

I point out that fevers surprisingly seemed to be reported in about a fifth of the donors during the administration of G-CSF. You can see that nausea is not infrequent and anorexia vomiting occurred in one donor and was mild. And flu-like

symptoms also are reported in about 20 percent of the donors.

This then looks at ECOG performance score during filgrastim administration. Zero is normal, one is physically active but with some minor physical impairment, two is less physically active, and you can see that day three, four and five donors are starting to report ECOG performance status one, and at day five, one of our donors felt physically impaired.

Turning our attention now to the collections, first collection and second collection. First collections tend to be larger in terms of the blood volume processed. Median volume processed was 15 liters in these donors, minimum was ten liters, maximum is limited by NMDP standards to 20 liters.

The second collections are smaller. The median was 12, minimum was seven, but again, one donor had a 20 liter collection on day two. This shows the time of collection, duration of collection, first collection, second collection. Again, first collections tend to be longer than the second collections.

The median time here is 210 minutes, three and a half hours, for the collection; the maximum, six hours for a collection. On the second

collection, the median time is dropped down to just a little over three hours; maximum is still out there nearing four hours.

This now looks at platelet count, pre- and post-apheresis, on day five. So this is the platelet count on the donor prior to getting connected up to the machine. This is the platelet count on the donor following the apheresis procedure. The donors are just ordered here according to their pre-count. These numbers don't necessarily mean anything.

Basically, the average fall in platelet count with the apheresis procedure is about 33 percent. You can see in some cases there's a very minimal fall in the platelet count. Importantly, you can see here that in three cases, the donors started either at or below 150,000 platelets per microliter. As Dr. Anderlini suggested, perhaps some of these donors were handled very gingerly during the collection process to prevent further declines in their platelet counts.

One, two, three, four, five donors finished at or near 100,000 platelet count after the first collection. Fewer people had a second collection, but this shows data on platelet counts on those who had a second collection. This is

interesting. You can see that more than half of these donors actually started with a platelet count on the second day that was below 150,000. In significant numbers, more than half of them finished with platelet counts at or below 100,000. And in two cases, the donors finished with platelet counts below 50,000 after their second leukapheresis.

And I believe that's a cause for concern. There were no bleeding episodes reported in these donors. In fact, there were no serious adverse events reported in any of these donors or adverse events reported except for one case of intractable insomnia in a donor. And the one donor out of these 31 required central venous access. So in 30 cases, we were able to collect with peripheral venous access alone. Only one required central venous access.

We turn our attention now to follow-up of these donors following collection. This shows the baseline values, so this was the value that was recorded prior to any administration of filgrastim or any apheresis collection. And this shows laboratory values obtained two weeks post-donation, one month post-donation, six months post-donation, and one year post-donation.

It's important to point out that we have five donors eligible for one year follow-up. Two of those just became eligible, and so we don't have data on them. So this represents data on the other three donors who were eligible for one year follow-up. You can see that there does appear to be a decline in the white blood count at two weeks following donation in the median and the minimum and maximum, and with the apparent recovery by one month.

Absolute lymphocyte counts, this has already been reported for related donors. Lymphocytes also fall in the unrelated donor setting. Here at two weeks, the maximum count is closer to the baseline median, and minimum count lymphocytes below 1,000 at two weeks and again at one month with recovery of lymphocyte numbers over subsequent follow-up.

Absolute neutrophil count, as has already been discussed by Dr. Anderlini, in these donors also drops at two weeks post. Absolute neutrophil count also drops at two weeks post-donation. It's not -- it's variable, but in some donors, it's approaching neutropenia, neutropenic levels, and then the neutrophils recover to baseline levels.

And also as has been alluded to, there is a rebound elevation of the platelet count following cessation of the G-CSF and the apheresis procedures at two weeks, and then they return to normal at one month and subsequently, as near as our data show.

We then also asked the donors about their experiences with symptoms post-collection. We interviewed them two days post-collection, one week post-collection, and then weekly thereafter until they were completely recovered. And you can see that when asked about bone pain two days post-collection, some 30 percent of the donors are still experiencing bone pain. By one week post, only one donor was complaining of bone pain, and this again, is similar to the baseline data.

Malaise and fatigue are also present two days post-donation, being reported in more than 35 percent of the donors. This malaise and fatigue quickly declines to baseline levels at one week and beyond with these donors.

Myalgia, arthralgia perhaps is persistent in some donors two days post-donation, but then quickly returns to baseline levels. And ECOG performance scores, same song next verse. You can see that at two days, one week and two weeks, we still have some donors who are saying that they feel

somewhat physically impaired. This represents one donor in both the one week and two week follow-up, before that donor felt completely recovered. And again, this may be due to multiple factors that go beyond the administration of G-CSF and the leukapheresis collection.

So important summary observations, I think it's clear that the experiences of the NMDP donors mirror the published literature and what's been presented already today for related donors and for those volunteers who've gotten G-CSF and given peripheral blood stem cells under research protocols.

In our experience, serious adverse events have not been encountered, and that's encouraging that the numbers are small. Evaluation and long-term follow-up of these donors requires really a comprehensive system for data collection and monitoring. In that regard, I think that donor outcome is paramount in this activity. I think that the National Marrow Donor Program has the world's largest comprehensive database of marrow donor outcomes, but we recognize that that database is limited in terms of its long-term follow-up data.

We're taking steps now to rectify that, and to collect long-term follow-up data on bone

marrow donors, which, as Dr. Anderlini has already reported, is really preciously scant in its nature, and it really needs to be clarified that, in the long-term, marrow donation, in fact, is also safe. Similarly, it will be important moving forward to collect long-term follow-up data on peripheral blood stem cell donors.

We are prepared to expand this database to begin to include donors who are providing peripheral blood stem cells as in the first donation setting as an alternative to bone marrow for that first donation. One of my concerns is related donors. I think that we've seen that centers can collect excellent data on related donors, but I think that data on related donors is not being collected at all centers, and I think that should be rectified.

In that regard, I think it's sometimes unstated but implied that related donors can, should and will do almost anything for their family member. But I think that no drug should be given to a related donor and no procedure should be administered to a related donor that's not also appropriate for an unrelated donor. And I think that's an important concept to bear in mind.

Both of these donor groups are normal volunteer donors, and related donors deserve the

same protections that I think we've tried to put in place for the unrelated donors. And I think that it's important to keep that in mind as we move forward. So that's the end of my comments, and I guess we'll move to the panel. Thank you.

DR. STRONCEK: If all speakers could come up for a panel discussion. You can -- people can either fill out the papers with your handout, that was with your material when you came in, for questions or ask at the microphone. Please identify yourself and where you're from when you ask the question. Dr. Leitman?

DR. LEITMAN: A question for Dr. Confer. In the 31 donors that you've analyzed so beautifully, 17 underwent one donation and 15, two. Retrospectively, there should be, as required by that protocol, a CD34 analysis of the product, which is not generally, in fact, in the vast majority of circumstances, it's not available prospectively.

In the retrospective analysis of those products, could you determine in how many percent of cases the second donation was not required because a target CD34 had been reached on the first donation?

DR. CONFER: Well, we haven't -- I don't have the specific numbers. We're still looking at the CD34 data. They're collected at multiple

laboratories, and as we've already talked about, there are problems with standardization and utilization of the same methods, and interlaboratory variation is prominent.

So even once we've looked at those CD34 data, I'm not sure that they're going to be particularly enlightening. We're also noticing that there can be fairly significant differences between CD34 counts obtained at the collection site, and then once the product is transported to the transplant center, the value that the transplant center obtains on that very same product. And so, it bears careful observation.

I think that as we move forward, it will be important to establish some kind of a central laboratory for quantifying CD34s to make some sort of an effort to create a gold standard for the entire program.

DR. STRONCEK: Dr. Snyder?

DR. SNYDER: Yes. Ed Snyder from Yale University. I just wanted to make a comment about what Drs. Champlin and LeMader had commented on as far as regulation. I'd like to support their comments. I think their points are very well taken and need to be borne in mind.

To provide some perspective though, the blood industry over the years has worked closely with the FDA, and in retrospect, I think we can look back and say that the FDA's efforts have had a positive impact on the public health, and have improved the safety of the blood supply.

And it took a while for us to learn how to work together, and I think that is the key to this entire concept. I think the FDA's approach, and we're at a point in stem cells now where we were several years ago with the blood supply. I think the FDA's approach is one of cooperation and working together, not by fiat which I think would be wrong.

The Committee that is going to be writing the next set of standards with FAHCT and NMDP or the members ADB has FDA representation on that Committee. And I think the agency, from my perspective, I'm speaking personally, is sensitive to the industry's concerns, and has worked this into their approach, and I'm hopeful that we will be able to work together, keeping in mind Dr. Champlin's and Dr. LeMader's very important concept so that we can improve the safety of the blood supply, yet make sure that the research required to move the envelope to further patient care is not impeded. And I'm

hopeful we can achieve both of these goals by working together.

DR. HARTZMAN: Bob Hartzman from Navy. I'm sorry. Did you want to?

DR. CHAMPLIN: I just wanted to maybe respond a little bit to that. I think the -- everybody, again, wants the same ultimate goal, to have safe and effective transplants being performed. The issue is how to get there.

What the FAHCT organization and NMDP both have been doing in terms of trying to develop voluntary standards and accreditation systems to be sure that centers that are performing transplants have quality assurance programs in place, are monitoring their own patients and the engraftment of the cells, and the cells are, in fact, meeting both infectious disease and good laboratory practices standards.

That this is, in my view, the way to go to look at the process of the system. Again, right now, trying to define a product which again has been a point of contention, as we have discussed regulatory aspects, is an area of great controversy. How many CD34 do you need? What is, you know, what is important there? What is the important aspects

of the -- aspects of the transplant? This is a moving target that is evolving quickly.

Again, different rules may apply to cord blood as opposed to bone marrow and peripheral blood. So again, I would be very concerned about prematurely putting in place some arbitrary and perhaps incorrect definition of a product when we need to sort of support research and further development in a quality assurance monitored fashion.

And again, my own view is that this could just as well be done as it has been done under IRB academic and research institution monitored circumstances and not necessarily with a central Big Brother-watching-you approach of having the FDA applying a broad national standard.

DR. STRONCEK: Okay. Dr. Hartzman?

DR. HARTZMAN: Thanks. I'm going to state the obvious first. Obviously, this is not a trivial procedure for the donor. And I think that it's been brought up also before that it's the paramount concern is that we don't put donors at excessive risks.

I'm aware of at least two cases where donors have died in some period a few days post-donation. It's not really clear that it was the

donation that caused their death, but even these rare kinds of events, I think there has to be some kind of reporting system somewhere in the system that they can track these kinds of things, and so that there is an awareness at least so that donors are aware that these are possibilities. That's one issue.

The second issue is the amount of pheresis product requested. From my donor center, we fairly often see requests that I consider outrageous. There are huge amounts of cells that are being requested. And we work it out and kind of negotiate a level that seems to make some sense. But I actually think there's some -- I believe that there's some limit to the numbers of the cells that can be collected from donors from a safety standpoint. You know, can you collect -- is it okay to collect ten percent? Is it okay to collect 50 percent of their theoretical CD34 cells? I think there is some limit in -- I think there may be some need to regulate that. Thanks.

DR. CONFER: Yes, Bob, I think one of the things you're pointing out is some of the issues in trying to establish a protocol that meets the needs of transplant recipients and also meets the needs of

the donors and is acceptable to their advocates at the donor centers, and it is a big issue.

We have to make compromises in trying to put together the protocol for primary donations, and we've been -- that's one of the things that's really impeded our ability to start offering peripheral blood stem cells in the primary donation setting is to figure out how to prevent conflicts like you've experienced in the past, and how to streamline the processes and yet, insure that donors who donate can do so safely, and at the same time, that adequate products are provided in at least greater than 95 percent of the cases for the recipients. And so it's a tricky process.

DR. CHAMPLIN: I would just comment that in the related donor setting, as Dr. Anderlini had presented, 99 percent of the time, we can get enough cells from the patient to use two times ten to the sixth CD34 positive cells per kilo as a minimum dose.

And so it's really only in the situations that you're doing complex cell processing where you're looking at extensive T-cell depletion, for example, in the mismatched transplants, where you want the megadose collections that are necessary for that. And then in some situations, it's difficult

to get that large number of starting cells ideally you'd like to have.

DR. HARTZMAN: Yes. I agree. I think there's a level, just what you're saying, where it's safe, and you can virtually always expect it to work. But there are those circumstances that are more in the research area in terms of depletion, just as you said, that I -- that there's a limit to which you can ask somebody to be pheresed.

DR. CONFER: But I also think that within a single center, it is possible to more reliably collect these stem cells and have adequate collections the vast majority of the time, and I think Dr. Fischer alluded to that in describing the Duesseldorf experience with unrelated donors collected at a single center.

But when you start collecting donors at multiple centers, it's very hard to standardize the procedures and processes to provide adequate products in the vast majority of cases.

DR. STRONCEK: One of our panel, I guess, Dr. Hartzman's kind of asking, if one of our panel members wants to comment on it, is there any data, either animal or human data, that might suggest there's a limit to the number of stem cells we can take as far as the donor is concerned?

I'm not aware of it. And even though apheresis is very efficient and may collect 30 or 40 percent with the stem cell circulating, I have no idea on what percent of a person's total stem cells that represents, or I don't think there's data that we deplete people of stem cells.

DR. DIPERSIO: Yes, I'm not sure, but it's interesting that when you collect stem cells, you actually collect more than you predict based on circulating numbers prior to pheresis. So the procedure itself mobilizes in some normal donors phenomenal numbers and in others not many. It's kind of an odd thing. I'm not really sure what this is due to, but there's no evidence that there's a limit as yet.

DR. CHAMPLIN: And even in donors undergoing these megadose collections, there's no short or long-term deficits of hematopoiesis that certainly have any clinical relevance, and Dr. Anderlini showed, again, there may be a physiologic sort of neutropenic sort of phase as you're getting back to your baseline, but these people, by and large, are normal no matter how many cells we take.

DR. HARTZMAN: That's true in the short-term, but we really don't know in terms of long-term

that when you really start taking significant fractions of somebody's total cell mass --

DR. CHAMPLIN: It's fair to say we don't have a twenty year follow-up on the donors, but of the follow-up, that we do have, we have not seen any problems.

DR. WEBB: Ian Webb, Dana Farber, Boston. My question relates to those handful of severe side effects to the donor being splenic rupture, and I'm wondering if the panelists would like to comment on whether that's, in fact, a real phenomenon in terms of the relation to the G, and if so, what mechanism they propose for that?

DR. CONFER: My comment would be that it's not a handful yet. It's a finger. It's one case. And it was -- it's an interesting case report to read because it's kind of -- when you read it, you end up feeling like you're not sure what exactly happened.

It wasn't clear in this donor whether the donor was normal pre-mobilization, whether the donor, in fact, might have had splenic enlargement pre-mobilization. Now, the investigators did go to look and see whether there was any evidence of a viral infection that could be causing splenomegaly and didn't find such evidence.

Although there was extramedullar hematopoiesis, it was described in the report as scattered. It's really, I think, unclear what happened in this case, just as it's unclear what happened in the case that Dr. Anderlini described where the donor post-donation had normal blood counts, returned to her home, and then suffered fatal cerebral vascular accident.

It's not clear what's happening in some of these cases. NMDP has had the experience of donors dying before donation. So it's possible that a donor could be fully evaluated, determined to be in good health for a donation, and die actually prior to the donation which is, I think, you'd almost have to say it's probably not related to the collection. It would have to be some kind of anticipation.

So these cases are really tough to sort out. There was a death of a related donor just two weeks ago in the United States. A young woman in her 30s who donated bone marrow, and then post-donation within a few hours, suffered a massive myocardial infarction and died. So the number of deaths, they're not zero, and it bears monitoring and registering, I echo Bob Hartzman's comment there.

DR. STRONCEK: Anybody else?

DR. KURTZBERG: Joanne Kurtzberg from Duke. Can anybody on the panel comment as to the suitability of mobilization of peripheral blood stem cells on minor donors in the related setting?

DR. CHAMPLIN: We've done it. Dr. Anderlini might want to comment directly, but we've gone down to at least age four without major problems. Obviously, vascular access becomes a limiting feature there, but the young donors mobilize well. And it's by and large worked out.

DR. KURTZBERG: I guess I'm not asking can it be done. I'm asking which do you think is less risky for the minor donor, a bone marrow harvest or one or multiple phereses with mobilization?

DR. CHAMPLIN: I think they're both safe. I mean each has its own aspects as you well understand. It's the trade off of general anesthesia to the vascular access issues in the small patient, and again, issues of informed consent of small children in these type of collection procedures, but I think you can do it either way. And clearly, the young donors mobilize great, and we've had success with the transplants.

DR. ANDERLINI: Just to comment briefly on what has been discussed briefly by the splenic problem and this donor. Splenomegaly is a well-

documented fact of long-term G-CSF administration in severe congenital neutropenias. And I can actually remember at least two cases of normal donors who did have bona fide splenic pain after G-CSF.

So my impression is that there is a small minority of people who are, for whatever reason, more sensitive, and they may develop splenic congestion or maybe even some degree of foci or extramedullar hematopoiesis in the spleen which causes the splenic pain.

I agree that the details of that case report, and all we have to go by is obviously that case report, is not extremely clear what happened to the donor who had several other circumstances happening including a chest tube and so on.

But I think there is probably something true in an effect on the spleen which is probably minor in the vast majority of donors, but could be apparently more prominent in some of them.

And as far as the donors, the pediatric donors, as Dr. Champlin pointed out, the real issue is the vascular access, that these are policies that if they don't have vascular access and they need a central line, since they have to go to the O.R. anyway, they might as well get a bone marrow harvest done there. But that we have collected from I would

say a sizable number of donors as young as four years old pretty uneventfully.

DR. STRONCEK: There have also been -- there's also some machine considerations on pediatric patients. The blood volumes outside, extravascular blood volume outside with apheresis is quite high, but the machine -- there just needs to be some modification of procedures, and a number of centers do collect stem cells on kids.

DR. KURTZBERG: You know, we collect auto stem cells on kids all the time as small as eight kilos, but you can't do a 20 liter exchange, and I would wonder if you could get a yield that would be sufficient for an adult donor.

And if you have to do multiple phereses and put in a line, I personally think a harvest is less morbid. But I think there are issues of risk that the sibling is going to be put under that should be considered by people other than the parents. That's all I'm trying to say.

DR. STRONCEK: I guess Dr. Przepiorka was next.

DR. PRZEPIORKA: Yes. Przepiorka, Baylor, Houston. The panel, both the current one and the previous one, addressed speed of engraftment, but I think one other area has to be the incidence of

graft failure and durability of engraftment. And Dick alluded in one of his slides to the fact that the RFLPs appear to be donor once the patient doesn't graft.

In the Anderson series of about 150 patients who have received a standard myeloblative regimen, there's actually only been one person who's had secondary graft failure. That's very similar to the incidents in the bone marrow patients, and actually, that underscores Dick's first assumption that wherever you can use bone marrow, you could probably use stem cells safely as well.

DR. CONFER: That's a good comment, and it also raises another issue that we've been grappling with in NMDP, and that is if there is an incidence of graft failure following peripheral blood stem cell infusion, what's the backup? Are we going to take the same donors and mobilize them again and collect more peripheral blood stem cells?

There are some data to suggest that you can mobilize people again, and you will collect similar numbers of stem cells with the second mobilization. But if we have concerns about long-term safety, et cetera, then one might question the wisdom about multiple mobilizations for a single donor.

But then the other question is are we going to take them to the operating room and collect bone marrow if the peripheral blood stem cells fail? And that becomes an issue in deciding who can donate peripheral blood stem cells because if we select people who really aren't candidates for bone marrow donation which has been a fairly common suggestion to me, is that oh, this is great because now all these people who can't qualify to donate bone marrow, can donate peripheral blood stem cells.

I don't like that because if those grafts have a failure rate, then we may indeed come back to those donors and start saying, well, now we really should collect bone marrow, recognizing that this donor isn't really a very good candidate to provide bone marrow, and that may cause us further headache and distress.

Our plan is that at the outset of this protocol for primary donation, nobody will be able to donate peripheral blood stem cells who can't also qualify to donate bone marrow. And we're going to set the bar high, and we're only going to lower it when we're confident that it's possible to lower it.

DR. CHAMPLIN: I would certainly agree with you that in our view, that if you're not medically stable to give bone marrow, you probably

are not medically stable to give blood stem cells either. And again, there's been a few cases that Dr. Anderlini indicated where people with pre-existing cardiovascular disease got into trouble during the collection procedures.

Also these people, just on a practical basis, when they're getting their bone pains, they have pains in their chest. And if they've got cardiac disease, you're not clear, is it just the G-CSF effects or is it something more serious. So again, one shouldn't compromise on the safety of the donors, and blood stem cells probably are just as risky as a bone marrow collection.

DR. CAIRO: Mitch Cairo, Georgetown. This is somewhat a follow-up to Joanne's comment. And I'm addressing it mostly, I think, to Dick. In pediatrics, we obviously do a lot of allogeneic transplantation from nonmalignant diseases, and there's some data to suggest that human life -- reconstitutions probably similar using mobilized bone marrow as using mobilized mass related for blood stem cells.

So in a nonmalignant setting, with the suggestion that there's more chronic GVHD using mobilized peripheral blood stem cells, do you think there's any scientific reason to think that

utilizing mobilized bone marrow instead might achieve the same results without less chronic GVHD in the nonmalignant patients?

DR. CHAMPLIN: The -- there's a lot of interest now in mobilized bone marrow, and -- but there's only been a small experience. There was an abstract at the ASCOG meeting by Rodi and coworkers from North Carolina who suggested you got the same benefit with rapid engraftment without and increase in chronic GVH, but it was, again, a very small series.

DR. CAIRO: Right.

DR. CHAMPLIN: So, we're actually interested ourselves in exploring that prospectively. I know many places are, but it has not yet been confirmed that there is this benefit, but hopefully there will be.

DR. DIPERSIO: I'd just like to add one word of caution there, and that is that the concept of using mobilized bone marrow is counterintuitive to what's been observed in mouse models for mobilization. In all the mouse models for mobilization, I think with the exception of mice mobilized with flt-3, there is actually, during the mobilization phase, there is a decrease in the

number of progenitors in the marrow during mobilization.

So there actually is a movement of marrow progenitors out into the periphery or egress into the periphery. So the actual quantitative numbers decrease during mobilization in mouse models. I think it's not been shown at all in humans what the deal is, but I think that's just one word of caution.

DR. CHAMPLIN: You may want to, I mean, it may be appropriate to collect them after perhaps three days of simulation rather than at the time that the cells are peaking in the peripheral blood. So again, the scenario, that's caused a lot of interest in the medical community and needs to undergo definitive evaluation.

DR. STRONCEK: A couple of questions, written questions. One is for Dr. DiPersio, and the question is how did you measure the very low CD34 levels on normal donors? Did you have to use any special techniques to measure, to get accurate counts of like 1,000 per mil?

DR. DIPERSIO: This is an adaptation of the method by Rosco and all, so it's very sensitive. It uses lineage panel, CD34 marker, two different

fluorochromes. It's a two-color analysis. A hundred thousand events are used.

So one way to get around it is to develop a nice assay which is reproducible using a nice flow cytometer which I'm sure everybody has and uses in the audience. The other is to sort instead of 40,000 events, 100,000 events. So we can detect reproducible numbers above 1,000 per milliliter of blood or ten per microliter of blood.

DR. STRONCEK: Another question is in regards to normal donors and patients who mobilize poorly. Do you or anyone else have any data on giving such donor stem cell factors plus or minus thrombopoietin? I guess other mobilizing agents. Would you recommend G plus GM in patients that don't mobilize poorly -- mobilize poorly?

DR. DIPERSIO: I think hopefully this stuff that I preliminarily presented here will be presented by Randy Brown at ASH this year, and so the reality is that there's a dramatic effect of GM, meaning that it mobilizes much less well than either G or the combination.

And the other dramatic impact was not so much on more CD34 cells being mobilized with G plus GM, but many fewer T-cells, and also this dendritic

cell issue. So I think that there are, there's lots of room to explore other possibilities.

And hopefully, in normal allo donors, once the, you know, phase one, two, and certainly phase two studies are completed with some of these other growth factors that are sort of more interesting, not more interesting, but more novel, I should say -- we'll be able to test these in normal allo donors using a single or just a few donor exposures.

DR. STRONCEK: I think that everyone would agree though that, especially for an allogeneic sibling donor transplant or an unrelated donor transplant setting that considerable experience should be obtained in other groups before we go.

DR. DIPERSIO: I think the other thing is that one has to realize that the vast majority of normal donors are mobilized with what we're doing now. So one could make a very strong argument to not rock the boat until we really have explored all the long-term and short-term effects of these other cytokines first. I'm sure these words will not be heeded.

DR. STRONCEK: Question for Dr. Confer. Were the collections performed by the marrow donor program under an IND.

DR. CONFER: Yes. The collections I described were under an IND. The one that was submitted in late '96, protocol opened February 1, '97.

DR. STRONCEK: We are scheduled not to lunch for a little bit yet. Does anyone else have any questions or comments?

PARTICIPANT: One question. Could the panel comment on any concerns about the possibility of the growth factors producing any kind of an immune response? Has anyone looked for the development of any antibodies to G or GM, or is there any concern that this is not something we should be concerned about?

DR. DIPERSIO: I think that, well, I think that anytime you inject a recombinant protein, especially subcutaneously, one should have serious concerns about antibody production. I don't think that there's been any evidence of severe neutralizing antibodies that I'm aware of with G yet, but certainly with other cytokines, there have been.

And it's very interesting that in the primate models, primarily because we're using human recombinant proteins in these pre-clinical primate models, almost every cytokine will induce

neutralizing antibodies in that setting. So I think the risk is always there, and I think the risk is most significantly there for patients and normal donors getting subcutaneous injections with multiple donor exposures, repetitive donor exposures. That's the biggest risk.

I think if the single donor exposure is the only thing that happens, the risk is probably extremely small.

DR. CHAMPLIN: But I think it's fair to say that there has not been any problems, to date, with a cytopenia syndrome related to G or GM, to my knowledge. If somebody knows something, he should speak up. Because I think these factors seem safe from that perspective whereas antibodies have been more of an issue with interferons, for example, in other products.

DR. DIPERSIO: I guess I'm specifically, I agree with Dick 100 percent. There's nothing that I know of that's happened either, but there are, as you know, there's G-CSF and there's G-CSF. There are other G-CSFs that are going to be available in the future when this becomes a generic, number one.

Number two, there are other modified forms of G that are now being tested in clinical trials, and there are other companies who are looking at G-

CSF molecules that are fused or chimeric with other molecules. So I think those are some of the things we have to watch out for in the future.

DR. STRONCEK: My understanding, though, of knock-out models for G-CSF, is that animals aren't severely neutropenic so I haven't heard about any neutralizing antibodies, but if they're not looked for, it might be difficult to pick up.

DR. DIPERSIO: They're going to be very difficult to pick up. In the knock-out model, the knock-out mouse is a completely normal mouse, but it has about 20 percent, ten to 20 percent of the normal neutrophils but is completely normal in every other way, very much like the MPL knock-out.

PARTICIPANT: On a more mundane level, I want to ask Dr. Confer if you had evaluated from the donors that were both bone marrow donors and peripheral stem cell donors, their evaluation of -- if they had to do it again, how -- which one did they prefer? Would they prefer another stem cell collection versus bone marrow? What kind of subjective information do you have on that?

DR. CONFER: That's a good question. We actually have an ongoing companion study that surveys these donors, and surveys a variety of psychosocial factors, and also, their perceived

inconveniences and side effects with the two procedures.

It's very difficult to ask them which one they would prefer because again, I think the answer overwhelmingly is that they would have preferred that the bone marrow that they donated first worked. And so their second experience is extremely clouded by that first experience having in some way failed.

So you can't ask them that question directly because you don't know what to do with the answer. What we do ask them is how -- is what they perceive as inconveniences and problems with the procedures. I'd say that the magnitude of inconveniences and side effects are similar with the two procedures, but they're quite different, and that's obvious.

With bone marrow donation, donors experience their symptoms after the donation. They miss work after the donation. They have problems lifting, carrying, sitting, et cetera, after the donation. With the peripheral blood stem cell donation, they clearly have the bulk of their symptoms prior to the donation.

They miss work prior to the donation because they're trying to find their way to the site where they can get their G-CSF injection because

we're trying to collect data during the injection. And so we have to do it in some kind of a controlled fashion.

We can't just send these donors home with a bunch of vials of G-CSF and say give yourself a shot. It doesn't work. So they're inconvenienced by that, and they tend to feel like the time convenience was greater with the G-CSF donation than with the marrow donation.

The other thing is that, after they've donated bone marrow, they are roundly applauded by almost everyone they meet. They went to the operating room. They went through this seemingly big procedure. They got an anesthetic, and the peripheral blood stem cell donation by comparison is an emotional letdown.

You go and you have to lie still for four or five hours. And so there's a different set of experiences, and it's important to continue evaluating these.

DR. STRONCEK: Concerning donors, myself who has dealt with donors for a number of years, I'd like to think the situation is where the science and the clinical medicine dictates the best possible component to collect for the transplant recipient.

Then we as people that deal with donors would assess how can that product be collected and provided in the safest manner possible both for the donor and the recipient, and assess if -- well, there may be some situations where it isn't safe to ask someone to donate.

But whether or not one procedure might be a little more safe or a little more inconvenient than another, I don't think is always that critical in whether or not we move forward with other things.

PARTICIPANT: No. I agree with that. I just was wondering whether or not the donors had any strong feelings about it. Again, if you get to the situation where -- it may be that scientific data shows that there's advantages and disadvantages that it may be -- do you think it would ever get to that it could be a donor choice on which procedure?

DR. CONFER: I don't think we're at the point of donor choice yet. In fact, in the unrelated donor setting, we really want to avoid that. At this point, I think it's really critical for the physicians caring for the recipient whose life is on the line to determine what stem cell product they feel is desirable for that recipient.

As we've already heard today, I think that in the unrelated donor setting, the vast majority of

these peripheral blood stem cell transplants are going to occur in recipients who are judged to have very high risk disease, where it's known that unrelated donor bone marrow has a high failure rate, where transplant related mortality is its highest, and where the potential benefits of peripheral blood stem cells will be most obvious.

And I think it's important that the transplant physicians indicate which stem cell source they would like during this developmental period. And then I think that we have to present the transplant center's choices to the proposed donor in some kind of a balanced and fair way.

But it's way too early, both in terms of the recipient outcomes and in terms of the donor outcomes to tell the donor, hey look, this is a toss up. You choose. And so we're trying to really avoid that, and I think it's essential at this time.

PARTICIPANT: And also as part, have you followed up the data on, it was 50/50 split on the second donation, it looked like. What were the outcomes there? Can you summarize the outcomes? Is that possible?

DR. CONFER: Yes. We've done an analysis of the outcomes. It's still in process, but if you look at survival, again, these are high risk

recipients. If you look at survival of those recipients who've been infused with stem cells, it's approximately 22 percent at two years, which is actually a little bit surprising. You might think it would be a ten percent or a five percent.

So significant numbers of these patients do survive at two years following infusion of stem cells. At this point, there is no difference in the survival of the peripheral blood stem cell recipients and the bone marrow recipients. And I think it's, in large part, the numbers are totally inadequate. But if you look at the Kaplan Meier curves, they're indistinguishable.

It's interesting that even among patients who have a second donation request submitted and then they don't get a stem cell infusion, their survival is also about 20 percent. So it's apparent that some of these people get better on their own. If stem cells aren't available, the doctors try other things that are sometimes successful. So overall in this population of people, about 20 percent of them turn out to be long-term survivors.

DR. STRONCEK: One last question, Dennis. The question -- can you talk a little bit about the rationale, why it was elected to use an IND for collecting blood stem cells by the NMDP?

DR. CONFER: If I can remember. In the second donation setting which we were collecting peripheral blood stem cells, trying to use sort of a standardized approach, but we weren't using a standard protocol, some of the donor centers, many of our donor centers are blood centers.

The blood centers were used to regulation and oversight by the FDA. Many of these blood centers were concerned about the idea of using their machines to collect peripheral blood stem cells, and then ship these stem cell products across state lines without an IND, and that was probably one of the major factors.

We really wanted to standardize the process for donors and collect data on donors. So we wanted to have a unified protocol, and it made sense in the process to also address this concern about oversight regulations by applying for an IND. So we elected to do it under an IND application.

And our plan at this point is to -- is that we will absolutely continue the first donation protocol also under the IND mechanism.

DR. STRONCEK: Well, if there's no more questions or comments, that concludes the second session. And the third session this afternoon will start at 1:30 in this room.

(Whereupon, the workshop went off the record for lunch at 12:06 p.m.)

A-F-T-E-R-N-O-O-N S-E-S-S-I-O-N

(1:30 p.m.)

DR. WAGNER: Could people have a seat please? We'd like to get on with this afternoon's discussion. Could people have a seat please so we could begin with this afternoon's session?

We're going to be changing the topic this afternoon. As you heard this morning, we've been talking about peripheral blood stem cells and some potential issues in terms of how these cells might be evaluated in terms of defining a product, and what kind of results have been observed with allogeneic peripheral blood stem cell transplantation.

There are a number of issues that are a little bit different when talking about umbilical cord blood, and could I have the first slide please, or do I control it? Go back.

Basically, this is just a cartoon of some of the issues that we need to discuss this afternoon. And certainly, this is only a cartoon that just helps us, serves as a construct, but definitely to define what the issue is.

First off, as a transplant physician, what makes it difficult for me and for other people in this room is how do we know what kind of umbilical

cord blood product we're actually getting? There are numerous banks, particularly in Europe, where there are many banks, almost one or two in every country in Europe and in a variety of places elsewhere around the world, have created banks for unrelated transplant purposes using umbilical cord blood.

As a transplanter, I have no idea how those banks have actually been developed, what kind of standards that they have, what kind of quality assurance assays they do in terms of enumeration of colony forming cells, nucleated cells, CD34 positive cells, but more importantly, infectious disease markers, or issues in terms of genetic, potential genetic diseases.

And I bet what we would find if we pulled every center that collects cord blood something different, and what I need to know as a transplanter is what is good and what is not good. So, as you can see here, there's a variety of choices. One is just accessing the cord blood, which is a major hurdle.

But I can tell you what in practice what happens frequently is only a small number of these banks are actually being accessed because of a trust between the transplant physician and the banking --

the bank itself. And as you know, the New York Blood Center currently represents the world's largest repository of umbilical cord blood and certainly serves as a standard by what we, at least in practice, use today.

But what we're going to have this afternoon is a couple of discussions on a variety of issues that might help formulate or focus some of the issues that need to be discussed in terms of how the stem cell source might be better managed.

We're going to have, actually, Dr. Mitchell Cairo from Georgetown University, Pablo Rubinstein from the New York Blood Center, and Joanne Kurtzberg from Duke University relate to use some of the experiences in terms of creating banks, in terms of transplant outcomes, and, hopefully, we'll be able to have a better idea of how we might be able to standardize this collection and testing of this umbilical cord blood stem cell source.

So because of time issues and a number of us have to leave because of catching flights, I want to begin by introducing Dr. Mitchell Cairo, who is currently at Georgetown University. He's going to talk to us about the NHLBI Multicenter Cord Blood Banking and Transplantation Study. Dr. Cairo.

DR. CAIRO: Thank you, John. First of all, I'd like to thank Liana and the organizers for kindly inviting me. It was a long plane ride to get here. Also, I feel a bit privileged to be leading off this session to have Pablo and Joanne following.

I think most of you know that they both represent the Mark McGwire and Sammy Sosa of cord blood banking and transplant. And after they talk, I'll let you determine which one's Mark McGwire and which one's Sammy Sosa.

What I'd like to accomplish is to just give you a little bit of background of why many of us got interested in cord blood collections and their uses and alternative for allogeneic stem cell transplants, and then spend the rest of the time talking about the National Heart, Lung, and Blood Institute project which involves the creation of several cord blood collection centers, several unrelated cord blood transplant centers, and a medical coordinating center and where we are to date.

As John mentioned, we talked earlier this morning about the use of bone marrow, and for the most part, peripheral blood in obtaining hematopoietic stem cells. And although there are other sources hematopoietic stem cells such as in

fetal liver and other sources, the topic of the discussion today will be cord blood.

Many years ago, it was identified that very early primitive hematopoietic stem cells is identified by LTC-IC and HPP-CFC was significantly fold increased and circulated unrelated cord blood, umbilical cord blood compared to that of unmobilized adult bone marrow.

Similarly, it was identified that looking at committed progenitor cells, CFU-GEMM and CFU-GM, again, there were a several fold increase in term cord blood and actually even higher in preterm cord blood compared to that and adult peripheral blood.

And when you looked at proliferative rates as assayed by famine in suicide studies, again, they were increased.

What was also noted that although the CFU neg content was approximately twofold higher compared to that of adult peripheral blood, it wasn't as high as some of the more committed progenitor cells, and the earlier hematopoietic progenitor cells as I've mentioned on the previous slide. And that may have some reason for the outcome, I think, you're going to hear from Dr. Kurtzberg later on regarding platelet reconstitution.

Now, our group and many others have identified that core blood is also very receptive, if you will, to ex vivo expansion, and this is just looking at a variety of cytokines, and this particular was looking at IL11-G-CSF or IL11-GM, a stem cell factor, and you can see in two to three weeks you get a 75 to 100 fold increase in the white count and similar increases in CFU-GM.

And there have already been some phase one pilot studies looking at the possibility of ex vivo expanding cord blood cells for a variety of reasons including enhancing hematopoietic reconstitution.

Now, along with the fact that there are increased members of early and some committed progenitor cells, there's also a differential regulation of not only hematopoietic but also immunoregulatory cytokines, and this is just work from our group looking at either increased expression or decreased expression in the number of growth factors which is important in terms of the neonate and may be less important in terms of utilizing umbilical cord blood, except as I think it relates to immunoregulatory cytokines.

Now, the other important features besides it having an increased number of progenitor cells is that it appears that the immunoeffector cell

differential and cord blood is significantly different than that from peripheral blood, and this is an example is looking at CD34 RO expression in adult peripheral blood compared to that in cord. And the reciprocal changes in CD45 RA such that there are many more naive cells that circulate in cord blood, and very few memory cells compared to that in adult.

And when one looks at immune functional responses and whether this plays an important role in being able to give more disparate allogeneic grafts using unrelated cord blood is not clear yet, but it's certainly suggestive in that secondary T-cell alloantigen proliferation and cytotoxicity is decreased in cord blood compared to that of adult blood.

And likewise, there are a number of mediators, and most importantly, gamma interferon, TNF alpha and CD40 ligand that are decreased in immunoregulatory cells from cord blood compared to adult. And that may also play an important role of why there is a potential of having less toxicity using similar disparate grafts between cord blood and peripheral blood.

So back in 1995, the National Heart, Lung and Blood Institute put out an RFP to establish

several blood cord collection centers, and the objectives were to develop some standard operating procedures which I'll discuss in a few minutes, and to collect approximately 15,000 to 20,000 umbilical cord blood units over two to four years with a mixed ethnic balance roughly in this proportion that would be utilized for a transplant study that would be done by a group of institutions that were also selected on the following RFP.

And this RFP, then, was designed to establish several unrelated cord blood transplant centers that would treat children and adults with malignant and nonmalignant diseases and accrue approximately 350, 400 patients a year. And during this time to establish a uniform political protocol that would have unified approach to all these particular items listed on the slide.

So after review, several cord blood collection centers were approved, and the current group right now is at Duke University with Dr. Kurtzberg and UCLA with Dr. Fraser and myself here at Georgetown. In addition, there was another RFP that was put out to establish a medical coordinating center, and the one that was chosen after review, competitive review was the EMMES corporation that John referred to in his earlier slide, and I'll tell

you a little bit more about that later. They act as the medical coordinating center.

And originally seven transplant centers were approved. Currently, there are now six in the program. They're all listed here along with the PIs associated with each of those. And as you can see, Dr. Kurtzberg is also PI of the transplant center, and Dr. Wagner represents University of Minnesota and also the following investigator.

Now, in addition to that, three HLA laboratories were chosen to serve as the reference laboratories for all HLA typing and for the confirmatory typing, and the three are at UCLA under Dr. Checka, Dr. Terasaki, University of South Carolina with Leanne Baxter-Lowe, and also the Navy Medical Research Institute in combination with Georgetown University with Doctors Hurley and Hartzman, who you've heard from earlier today.

So this is how the structure works. NHLBI, obviously, is in contact with everybody and serves as a center for coordinating an entire project. There are the three banks that collect and store cord blood. There are the transplant centers. The Medical Coordinating Center then serves to collect all the data from the cord blood banks regarding the units that are collected and their

compatibility, availability, the infectious disease screening, et cetera.

The cord blood transplant centers then access the Medical Coordinating Center when a potential recipient comes available, and the HLA reference labs then serve as doing the HLA typing and the confirmatory typing for both the donor and the recipient.

In addition, there's a data and safety monitoring board led by Dr. Beatty who serves as an external review for the project, and they're constantly reviewing the standard operating procedures for both the banks as well as the transplant study, and you heard from Dr. Horowitz earlier today who serves on this board.

So now I'm going to talk a little bit about what we've developed. For the banking issues, we have set standards for educating maternal donors, a variety of ways of informing donors that this project is available through various means. Here at Georgetown, we have a phone number called 4-LIFE which is easy to remember, education of health care professionals, in services to staff and patients, and we also have brochures that are now in seven languages.

Briefly, in terms of obtaining consent from maternal donors, we don't begin that until approximately 36 week, in the third trimester, and up until that time, the beginning of active labor, it's done on in a confidential way. The interview is usually done by a research nurse. We usually reaffirm consent if they've given consent prior to coming in the hospital, and they can obviously withdraw from the study at any time. Up until the point, if the cord blood is collected and it has been reserved for a patient recipient undergoing a transplant, it cannot be retrieved after that time.

Similar questions that are done with many blood donor related issues, blood transfusion, history, genetic, immunological disorders, sexual history, issues of confidentiality and linkage are very important. It's important that we protect the donor from being identified, but linkage is important for a very brief period of time because of the need to go back to the donor if we identify an infectious disease, potential problem, or some genetic abnormality.

We use blinded bar code labels for confidentiality. There's only one form that's kept for the linkage, and that's kept in a locked secured

place. We have a multi-level security system throughout each of the centers.

Briefly, I'm going to walk you through the collection process. We use a specific collection kit provided to us through the project from NHLBI from Medsept Corporation that contains CPD-A and several collection stands. This is a collection stand that was designed at Duke University. And you can see the placenta hangs in this direction, and collection is done by venipuncture under sterile technique.

This is what a collection bag looks like. There are several opportunities for venipuncture of the cord. The CPD-A is already contained within the collection bag has various other places along the way if we need to inject her with other samples.

In terms of the separation and sample preparation, although out standard operating procedures have been well worked out, we're always trying to refine those, and all these procedures are going to be published fairly soon in the Journal of Hematotherapy, and very soon thereafter put up on the website by EMMES Corporation, and that will be available to the general public.

Briefly, red cells are depleted by HES separation and then leukocyte separation by

centrifugation, and then various samples are obtained for certain studies. For the crowd preservation, it's done by controlled rate freezing with DMSO, I'll show you what the freezing bag looks like, and then it's transferred to a quarantine stores location.

This is the processing kit also manufactured by Medsept. This is the main processing bag in which the collection is transferred into. This is a transfer plasma bag, and this is the freezing bag, and I'll show you another look at that with some cord blood in it.

This is just showing prior to centrifugation, and this is the leukocyte rich plasma extraction that's done. And then this is a picture going into the cassette of the final product. Right now, although we're in discussions of possibly changing the format of this and having actually two bags for possible use of ex vivo manipulation in the future, but right now, we have a 5 cc aliquot and a 20 cc aliquot that's put in a little canister, and then it's frozen.

Several of us are using the BioArchive by Thermogenesis, but there are other freezers that can be used. This one happens to have a robotic arm in which the cassette, then, is then put in a location

and recorded in the computer, and then is retrieved up again without having to go into the freezer to take one unit out from another unit.

We do the fairly standard type of infectious disease screening. We do it on maternal samples to avoid taking any cord blood, and we are doing a great degree of graft characterization on all the units, CD34 and a variety of subsets of CD34 listed here, and also CD34, CD38 negative. In addition, a number of lymphocytes, subsets are also analyzed at the time of crowd preservation.

Colony forming assays are also being done under standard procedures using the MethoCult stem cell technology system, and we're looking at BFUE, CFU-GEMM, and CFU-GM. HLA typing initially is done by -- in a serological level for A and B and high resolution for DRB1. There's hope that at the end of the project that we'll do a retrospect of high resolution analysis of the units that got transplanted for both A and B.

And the units remain in quarantine until there is a negative medical history that's obtained, verification of the consent, a normal delivery history, and nothing abnormal with the neonate. All the infectious disease markers come back, and there's no evidence of microbial contamination.

Once all that is identified, the unit moves out of quarantine. It moves into permanent storage, and that information then becomes available to EMMES for the transplant centers to access. And thawing procedure is, for the most part, a slight variation of what Dr. Rubinstein published using dextran 40 and albumin.

And then we have a quality assessment program that in compliance with other regulatory agencies. And additionally, there's an external oversight that's done by NHLBI organized through the Medical Coordinating Center.

Now, moving onto the transplant study, the primary end point of the transplant study is to demonstrate durable engraftment as defined as an ANC greater than 500 for three days by day 42, and the important secondary end points are platelet engraftment that is, platelet count greater than 50,000 untransfused for seven days, and red cell engraftment is defined by reticulocyte count greater than 30,000 for two consecutive measurements.

Other secondary end points as you would imagine include disease free and overall survival, incidence and severity of acute and chronic GVHD, and important transplant related complications. The patients that are eligible for this multi-center

study are patients with AML or ALL as defined with high risk in first CR induction failure, second and third CR, and first and second relapse, other malignant diseases, CML, those in chronic phase who have failed to identify an unrelated bone marrow donor over a year's period, JMML with certain criteria, MDS, and then lymphomas that are either primary induction failures or have demonstrated chemosensitivity after first CR.

A number of nonmalignant diseases, it's an important part of the project, include marrow failure syndromes, a number of metabolic disorders, a variety of immunodeficiency diseases, and then a hodgepodge of other diseases.

Now, the HLA compatibility requirement, as I said, DRB1 is done by high resolution, and A and B are done by serological level of DNA typing. The patients that are eligible for study can be a four of six, a five of six, or a six of six of the blood type matching, and there are various cell compartments that has to be a minimum of one times ten to the seventh nucleated cells per kilo per the recipient, and we have a couple of different cell categories. One cell is between one and three, and the other is greater than three for both malignant and nonmalignant diseases.

The preparative regimens have also been formalized for the patients with a malignant disease or severe aplasia, TBI and cytoxin, ATG and certain nonmalignant diseases are busulfan and cytoxan and ATG other than the ones listed. For patients who can't have TBI because of previous other toxicities or infants, there's a bumelphalan regimen that will be used with ATG.

And then there's a preparative regimen for Fanconi anemia which, I think, you've seen before which is cytoxan and ATG, and fractionated TBI which we're doing in combination with Dr. Wagner at the University of Minnesota which has an ongoing study, and then a BuCy regimen for other inborn areas of metabolism.

GVHD prophylaxis has been finalized to be cyclosporin and Solu-Medrol beginning on day five and tapering according to certain criteria on day 19. Supportive care, everybody is to receive G-CSF and PCP, HSV fungal, IVIG and CMV prophylaxis are for the most part standardized but with some institutional protocol variation.

We're also planning an immune reconstitution study that will be headed up by Doctors Parkman and Kapor, the Children's Hospital in Los Angeles, looking at subset reconstitution,

antigenic T-cell functionality, phage stimulation, and also looking at CD40 ligand expression that we've done periodically over the first 36 months post-transplant.

Lastly, our accrual objectives are to accrue approximately 350 patients over a four-year period. Hopefully, they'll be 50 percent adult and 50 percent children. We are anticipating 75 patients in each of the four malignant cohorts, that is, between one in three and greater than three cell category. Approximately 30 patients will probably fall in the nonmalignant category and 30 patients who can't have TBI.

There have been early stopping rules that have been built into the study having to do with primary graft failure, severe acute GVHD, day 100 survival, and each of those will be evaluated in each of the cohorts I mentioned separately.

So I want to close with, Liana asked us each to think about what the future might hold in terms of future research, and I've decided just to pick on ex vivo cellular engineering. I think there is potential in this area. As you'll hear from Dr. Kurtzberg, there's certainly room for enhancing hemological reconstitution, specifically platelet reconstitution but also myeloid reconstitution.

There's also room to enhance immunological reconstitution. Our group and others are interested in cancer vaccine development ex vivo, and also a great deal of interest, of course, using core blood either for gene replacement or more importantly potentially for gene therapy.

So in summary, our plans are that we'd like to collect about 15,000 to 20,000 units over two years. We just got going over the last three months or so, and have approximately 500 units that have been banked. So any day now, the first patient will be coming up for transplantation.

We hope to complete the clinical studies and then analyze the graft characteristics and other variables to correlate it with engraftment and GVHD. I think most of all we hope, at the end of this study, to be able to finalize standard operating procedures for the most cost effective way that we can collect cord blood so it can be used most readily and easily.

And then I think it's also important we pursue, investigate, and initiate pilot studies for ex vivo cord blood engineering.

I'd like to thank all the members of the Steering Committee of the NHLBI Cord Blood Project. There are many people, and I particularly want to

mention Nancy Kernan, who is actually the study Chair who is not actually part of the banking or the transplant study, but serves as a steady guiding hand for the rest of the Committee.

I've mentioned several other people earlier. Also in particular, I'd like to single out Paul McCurdy. I think without Paul's vision and his inspiration, this project certainly wouldn't taken place, and he's continuing to be an important consultant to the project. And I think from EMMES Corporation, the Medical Coordinating Center, Shelly Carter and Liz Wagner have contributed significantly to the development, and I think ultimately, the success of this project. Thank you.

DR. WAGNER: Because of the issues of flight schedules, if there's any really burning questions, I think, for Dr. Cairo, you should ask them now. However, there will be people here like Joanne Kurtzberg and other members of this cobalt study who will be able to discuss some of the issues of the study if you should have them later on.

Is there anything in particular before we go onto the next presentation that you'd like to talk to Dr. Cairo about? Okay.

The one thing that has actually been shown is the work that Pablo Rubinstein is going to

present to us now has certainly been critical to the development of umbilical cord blood transplant. He's learned much about the banking aspect of umbilical cord blood and some of the difficulties of this. And assuming he has a goldmine of transplant outcome data and probably has all the data, the only one that has almost all the data on outcome as well as the banking, what product you're actually getting.

But I think that, assuming this is going to give us clues, and I hope that the NHLBI project will extend that because then what we're going to be able to do is to control for what goes in as well as what comes out, and I think that hopefully, that will be able to extend what Pablo will be teaching us now.

I think that without any further comments, let me introduce Dr. Pablo Rubinstein, the Director of the New York Blood Center, Cord Blood Banking Project.

DR. RUBINSTEIN: Good afternoon. Thank you very much John, Liana, the organizers for the invitation to our group to be represented here. I also have to express our recognition to the NHLBI who initially supported research application from us group which was initially approved in 1992.

May I have the slides please? The placental blood program at the New York Blood Center started collecting placental blood for transplantation in February of 1993. The program consists of several laboratories, IV blood center, immunology, neurogenetics and stem cell growth factors, and the number of colleagues outside, Joanne Kurtzberg, other transplanters, people infectious disease, et cetera.

What I will show you this afternoon is a -- something about the most salient aspects of the work that has been done by our group in developing systems for the collection of placental blood. But before I start on the more practical and mechanical aspects, I'd like to show you these as a reminder that the hero in placental blood transplantation is the mother.

Whatever risks are incurred in placental blood collection and donation all reflect on the mother. It is the mother who will be asked all kinds of indiscrete questions, the mother who has to agree to receive back the results of testing and donate blood for those testings, and it is mother without whom nothing like this could be done.

Now, what are the tasks for a bank? There are really several groups of tasks, but the first

one refers to the unit of cord blood or placental blood, as we prefer to call it, and these have the number of steps which are understandable, I think. Procurement, that is the collection and all attendant aspects including informed consent, processing, testing, typing, and perhaps most importantly the management of all that information, because practical potential for using this blood depends exactly on managing the information collected at all of those tapes in an effective and very efficient way.

So how can we do a collection? There are only two major ways in which one can approach these. When these two has the obstetrician or some member of the obstetrical team collect the blood directory in the delivery room or the birthing room during the third stage of pregnancy, I'm sorry, of labor.

That is when the cord has been severed, the baby's out of the picture, and now the few minutes until the uterus will eventually throw the placenta out. During this period there are uterine contractions and people can insert a needle into the umbilical vein or simply open the clamp at the end of the cord and let the blood run out into some recipient.

This is history -- the earliest method for collection, and it's certainly one that requires very little else than the cooperation of the obstetrician. It is, however, the one method that involves a temporary minimal probably very innocuous distraction of the attention of the obstetrical team. It is our opinion that if you're going to do this, you should let the mother know that there might be some increase in risk.

All of our obstetrician friends say these risks are minimal, and we agree, but whatever risks there are, the mother should be aware when she consents. Now, the alternative to this is the way we do is very similar to what Dr. Cairo has just shown you, and there are good reasons for that similarity, and I will also show you a picture about it. And if then -- when the placenta itself is born, it's taken immediately to an adjacent small laboratory where trained personnel will prepare the cord to achieve very good aseptic condition, and will preform the phlebotomy from it.

It's taken into blood bags with ACD or CPD anticoagulant. There are differences between these two, and it's an issue. There are some advantages with CPD particularly because the volumes are not predictable. And then there is informed consent.

There is no consensus exactly about the informed consent issues. For practical reasons, and also to take into account the number of practical results that have been observed, we have chosen to perform the informed consent after the collection and in the immediate post partum after the mother has recovered.

There is ample opportunity, however, to provide information to expectant mothers during the pregnancy. The consent itself is rather complex. It includes specific parts in which the mother is asked specifically to allow us to keep the placenta blood unit for unrelated transplantation, allows us to probably -- to submit to a very intensive interview which focuses mostly on the existence of risk factors for infectious and genetic disease, allows us to perform a medical chart view both for the mother and the child, allows us to take a blood specimen for her, hopefully at the time of routine collection of specimens for after partum, allows us to take a saliva specimen from the infant to look for CMV by culture in our case, our methods, and then to allow us to perform infectious disease testing in both her and the baby's blood including HIV and report back the results to her through her

physicians. So this consent is an involved procedure and takes substantial amount of time.

There is even a pre-consent form, but I will not go into that. The procedure we use are very similar stand. As you can see, the placenta is placed upstairs in the trucks, and is wrapped in it, you can see here the cord. The first part of cleaning involves throwing some alcohol on the cord to remove all clots and attached material, and then they use iodine swabs. It's a procedure where the time for each step of the cleaning is regulated, and it has achieved very remarkable reduction in the placental bacterial contamination. In our group, it has been well under one percent for, now, several years.

After the collection, the blood is brought to the processing laboratory at the New York Blood Center. And in the Blood Center, then we obtain adequate blood for the performance of a number of tests, as you can imagine, bacterial culture, before and after processing, infectious disease serology, complete blood cell count. We rather prefer to do hematopoietic progenitor quality count than alternative ways of identifying progenitor cells because it not only tells us about the existence of

the progenitor cells, but it tells us about their function.

We know that for two weeks they are growing. They're multiplying, and in my opinion, this is valuable knowledge. ABO and Rh typing are performed, but in practice, we have seen that they are no value to the procedure because grossly mismatched, the worst mismatches possible are perfectly well tolerated. And finally HLA typing, about which I will say a little more later.

Infectious disease testing is exactly testing for bone marrow donors or, indeed, for transfusion donors except that it's done in the mother and the baby, and it is also accompanied by CMV culture from the baby.

By now, you can imagine in making all this adequate there has been an ample opportunity to make mistakes in identifying the vessels, the tubes and the articles that are prepared. From the beginning, we have designed a method based on bar coding, which was already described by Mitch, and which removes some of these risks, but that risk always exists because even if you use bar code, somebody may stick the wrong bar code in the right tube, and that way it would be potentially problematic. So the system

is designed so that we can catch those errors if they occur.

Now, the processing of this blood in our laboratory includes two parts. The first one is the reduction of volume, and the reason for this is purely practical. When we process large unfractionated blood, then the volume is high. The bags in which this blood are frozen, also large, and they occupy a lot of room. So a large freezer will contain only relatively few bags.

As Mitch also indicated there has been considerable research in performing this type of volume reduction. This is a little complicated because, it was initially a little complicated because there are reports in the literature that say that any volume of cord blood will carry with it a heavy penalty in terms of the number of things that are lost.

This procedure, for various reasons, does not have that problem, and in fact, it is not extremely difficult to recover practically all of the mononuclear cells. There is loss of granule size and, of course, platelets, but the final suspension contains essentially all of the mononuclear cells present in the collected volume. It involves an enhanced sedimentation with one

percent ATS followed by a five minute centrifugation of 50 g.

There is no need to wait after the addition of the ATS because we are not looking for lyophilation. What we are trying to achieve here is balancing the electrostatic charges, the zeta potential of the red cells so that we can separate them more easily.

Cord blood has a very low sed. rate, usually under one milliliter per hour, so this addition allows us to be extremely efficient and five minute centrifugation at 50 g. is enough to obtain a supernatant into which most of the white cells and practically 100 percent of the mononuclear cells go.

And after separating these, we spin -- we give it a brief but hard spin, and we remove the excess plasma, leave 20 mil. here in this bag, and then we do the cryoprotection to go into the freezing of this unit. Cryoprotection is done by obtaining a final concentration of ten percent DMSO and one percent dextran 40. The hydroxy ethyl starch is the same one that comes from here.

The addition of these extracellular cryoprotectant is very usual for theoretical reasons that have been empirically shown to help. And then

the unit is frozen at the control rate most recently using these thermogenesis BioArchive freezer.

It's always a question -- it's obvious that any procedure in which you fractionate something involves losses even if we cannot see them as systematic and measurable, but we have tried to see whether the step of processing modifies the engraftment ability of this blood. And as you can see, when we have issued whole blood transplants, the first 3,600 units in our inventory were whole blood, the attention of an ANC 0500 occurs 80 percent of the time. And when the unit has been reduced, it's 82 percent of the time by day 52. So there's no significant difference here, and that minor improvement is just a numerical feature.

But this requires some care. I don't know if you noticed that Mitch Cairo showed that the bags have to be wrapped to maintain the shapes of those bags during centrifugation, and that's critical because if you don't hold the bags so that they maintain their shape, since they are half empty or more than half empty, upon centrifugation, they will collapse, a lot of cells will get into the creases, and then you will not be able to recover those.

So we have designed holders that can be used in standard centrifugal cups and allow us to

maintain that shape, and that is critical to the recovery. And after the units are frozen, they are stored, and this is a convention of a liquid nitrogen freezer, if not, BioArchive system.

And these standard freezers come with the capacity of 1,200 liters of nitrogen. We can store up to 400 units if they are not volume reduced. And with volume reduction, we can go up to a little over 1,000 units in the same container. And with the BioArchive, we have 3,660 units.

We also need to plan for the future. There will be new tests. There will be need to repeat old tests, and so we set up repositories. These repositories are of two kinds. Sample aliquots -- we store viable lymphocytes that can be used for in vitro assays and proliferation and so on. We have genomic DNA that is recovered mostly from the granule sites and will create the red cells in the pellet. All red cells are just separated at the time of volume reduction, and we keep plasma also from the tip.

But in addition, we insist that our freezing bags have a tubing, a piece of tubing that is liquid nitrogen grade so that we can store segments that are integral to the unit, and these are invaluable for the demonstration of identity of

the blood with the testing articles. It's, again, a check on our ability to maintain the integrity of the laboring throughout all the procedures.

Naturally, the storage and the thawing of these units have to be connected. Techniques and the methods used are not independent, and they should be tied one to another. Storage in all cases then under liquid nitrogen level. Every unit is kept under the liquid nitrogen so there are no changes in the temperature of storage.

The shipping, when we ship to transplant centers, is done in dry-shippers. These are devices that are cooled by liquid nitrogen and maintain temperatures below -145 for time at least five days, and after nine days, depending on the manufacturer and the capacity of these devices. Thawing is important because it is at the time of thawing that you can rescue many cells that would be otherwise lost.

The loss at the time of thawing occurred because you are bringing out from freezing self-suspensions equilibrated with DMSO, a very high concentration which achieve high osmolarity. And so it is important, in our view, to avoid the destruction of cells that occur when this material

is injected directly into blood where the osmolarity is point three.

And to achieve these in a way that there's nothing incurred major hassle of the transplant center, all that is really necessary is to thaw quickly, dilute with a mixture of dextran and albumin, probably dextran alone is sufficient dexranina isotonic fluid, and then centrifuge for 15 minutes so that you can remove the supernatant.

This step of dilution has to be done more or less slow, but mostly has to be done with movement. And then after centrifugation and the elimination of the supernatant which is useful to us because it allows you to do bacteriological studies in large volume without having to relinquish any of the material for the transplant. And so the last step is to resuspend according to the instructions of the physician.

One important aspect is a delay in typing and matching. We have very similar procedures also of the NHLBI group. Let's forget about the traditional explanation of the -- where we recognize just three loci. Today we know that there are many more loci in haplotype(ph), and probably several loci other than A, B and DR are important. And DR,

by the way, a hydrosolution, we deal with four genes, but they are beta as opposed to just one.

So for preparation for transportation, we perform a compilation of the typing, both of the unit and the donor, and we supply DNA for the transplant centers to perform confirmation at their HLA laboratory. And the high resolution is completed at that time to bring the unit up to the current level if it has been tested several years ago. Surely now we have better ways.

Matching is done using serologic definition for A and B, and hydrosolution, the highest available at the time short of sequencing, naturally, for DR beta. Increasingly, however, we are resorting to sequencing for DR beta. And in this slide, there are several variables, the age, the cell dose, and the HLA mismatching. There is controversy, and we don't know all the answers yet, and the numbers are relatively small.

But from the first 550 patients in our study, the results show that compared to a single -- to zero mismatch, there is a higher risk for transplant related events in transplants where there's one mismatch or two mismatches. So these precautions about HLA are important, and there is no definition yet of the issue, and there is more

research needed here which will be forthcoming I'm sure.

So we record and utilize data from the units, and these are all of the rather obvious things. Incidentally, we repeat that genetic testing is necessary, but the criteria for deciding what diseases to test for are not completely defined yet. In our group, we have arbitrarily decided that we will test for two kinds of diseases, for diseases, rather, that fulfill two conditions.

One, that can be transmissible by bone marrow. There are many genetic diseases that have nothing to do with the blood or immune systems, and they should pose no problem. And the second is that they should occur with the frequency about one in 10,000. Why one in 10,000? Well, it's an arbitrary decision. We don't know any better. But these really should be considered and should continue to be considered.

The other aspect is the mother's questionnaire. Any suspicion of a disease that can be gleamed from the mother's history about the family history of the mother or the father should be followed up. The typing for HLA includes the mother, and this I think is an important precaution for several reasons. One is that in many cases it

helps in the definition of difficult alleles, and perhaps even more important, it provides you with another check on the identity of and the relationships of the neonatal blood that you're going to transfuse in that unit.

All the information that you have, the questionnaire of the mother, all the aspects that you know about delivery, and so on, depend on that link. So it is important that be -- it should be possible to demonstrate without question that the baby that you are going to transplant, I mean, the donor whose blood you're going to transplant relieves the child of the mother who has provided all the information.

Now, I'd like to say something about the other aspect of the cord blood bank which is the search. All of these procedures up to now is to have tissue available for transplantation. But now it has to be used. So we, like NMDP and all of the other agencies doing this require such requests. And these provide us with identifiers and some information about the patient and the ethnicity of the patient as well as histocompatibility testing.

We require a copy of the lab report. We don't -- we begin the process just with the typing as transcribed into the search request form by the

transplant center. But we require HLA lab report, a copy of the regional one, and the reason for that is, again, that there are mistakes incurred when people transcribe things. And people in HLA ourselves are not immune to those. So it's very important to have this lab report. I should tell you that we have detected overall almost ten percent errors in the patient's typing as it is reported to us.

And now I'd like to say a little bit about the ethnicity. Everywhere we talk about ethnicity, we know that the distribution of HLA antigens is different in different ethnic groups, and that it is important to have HLA compatibility. So we have looked for ways to optimize the proportions of donors of the different ethnic groups to take into account the polymorphism of the different ethnic groups.

And that is the reason why we have now for over a year worked at Brooklyn Hospital, which as you can see here, has a distribution of ethnicities very different from our original hospital, Mt. Sinai. The ethnicities are listed here. Yellow is Asian, black is Black, Hispanics are green, and Whites are gray, and combinations are blue.

And the major difference here is, of course, the decrease of caucasoid component and the increase of the Black and Hispanic. This has changed the configuration and the percentage of units in our inventory. In this slide, you see three bars for each ethnic group. The last two describe the frequency among all patients and the frequency among all transplanted patients.

For example, here, for our Black patients, about ten percent, a little over ten percent of all the requests come from patients that are Black, and a little over ten percent of all transplanted patients are Black. But among the unit, it's 25 percent. And a similarity exists for the Hispanic patients. Among caucasoid patients, the inventory, therefore, contains less than the proportion among patients requesting transplants. It is very interesting that the probability of getting a transplant really is more or less identical to the requests.

In this slide, we see the probability, the percent of donors, I mean the percent of patients in this -- who receive transplants of donors from the same ethnic group and all the other ethnic groups. If we begin here, these are caucasian patients, and the donors for the caucasian patients have been

almost all or the great majority caucasian even though the probability of being caucasian among all donors is about 50 percent.

The Hispanic donors contribute a little to this group, but the Blacks or Asians are neutral in this respect. Now, for all the other groups, the situation is different. For the blacks, there is an important contribution from the caucasoid.

The contribution from the Hispanic ethnic group is more or less as expected from the frequency in the overall population. And the Hispanics are somewhere in between. Asians, there are very few patients and few donors, but still there have been transplants, and these are, again, mostly from Asian donors or caucasoid, but it's impressive that the majority have been Asian despite only five percent of the donors in that group.

Here is a combination of ethnicity and mismatching. And as you would expect for caucasoid, the probability of receiving transplants with zero mismatches is more or less the same between caucasoid and non-Caucasoid donors, and the same is true of one or two mismatches. But for the other ethnic groups, the expected increase of the frequency of ethnically matched donors for zero mismatches is very clear both for Hispanics and

Blacks. And if you increase the number of mismatches, then you can see that ethnic mismatched donors were chosen.

To choose a donor, we don't look at the ethnicity. We only look at the DR. So the conclusion of this is that the ethnicity is a terribly important concern for the bank at the time of setting up the bank. It's not our concern at the time of transplantation necessarily. At the time of transplantation, the concern is the HLA matching.

Now, there's another way to look at this. There are still mysteries in the SEOEC. There's clearly a different frequency of transplant related events depending on the patient's identity, but this is not dependent on whether the patient is ethnically matched or not matched to the donor.

There are a number of other aspects that are important to the banking effort. I have been shown and evaluated, and we know whether or not they are significant. For transplant related events only, the patient's age is not significant, but the cell dose, the disease, the mismatched HLA types, the performance in the United States or foreign, and the distinction between ethnicities are all significant.

After engraftment, that is when the patients, all patients, some engrafted, the majority, but for those that engraft and after engraftment, the important things are patient's age. The white cell dose is no longer important in our statistic. The disease also, and this refers to specific conditions, is also not important although it was important before. It was important for engraftment, therefore. And the matching, the U.S./foreign are significant, that the white/nonwhite are not significant.

It is complicated to evaluate some of these things. I will not go into detail here, and these are the criteria for the selection of a unit. They include consideration of HLA matching, cell dose, and the risk factors for the patient that are dependent on the clinicians evaluation only.

From our point of view, also the status of the units in the inventory is terribly important because at any time we are starting units for transplantation. Say there are 100 units at any one time in the process of study. So they are reserved to some extent, and these introduces a complication in the selection process.

But finally, the decision is made by the clinician with interaction with the bank. These

are, again, a critical step because it's the only way we can evaluate all of these things and go back to re-evaluate a unit condition. Just so that you remember, we have been transplanting with several gross mismatches, and these are the proportions of mismatches. The majority of patients have received two out of six mismatches. So these are four out of six matches. That is a major most frequent group in our collection.

The rest of what I had to say refers to the procedures that I used in our bank once a unit is identified and reserved. The confirmation by both laboratories takes place, hydrosolution is obtained and are dated for DRB1, and finally the transplant center reserves a unit. From that time on, it only rests for them to give us a date when the need the transplant at their place. We insist that that should happen before cytoreduction, and these are the numbers of transplants that have been done until last year.

I'm happy to tell you that now that we are going to be five years since the first transplant performed by Joanne Kurtzberg, we have issued tissue for 700 transplants. And these transplants have been throughout the world. Thank you very much for your attention.

DR. HARVATH: John Wagner apologizes that he had to run off because of the airline strike in Northwest that's affected many of the flights in and out of Minneapolis. So he had to catch a flight, and I decided to pinch hit for him. And it's a great honor and privilege to introduce the next speaker, Dr. Joanne Kurtzberg who is a Professor of Pediatrics at Duke University and one of the leading experts in the world in transplantation, especially of cord blood. Joanne?

DR. KURTZBERG: Thanks, Liana. And it's really a pleasure to be here and have a chance to show you come of our work. Just to review very quickly a little bit of history about the clinical transplantation of cord blood.

The first person to put cord blood into a living organism was Ted Boyse, who performed urine experiments back in the 1980s showing that he could rescue ablated litter mates with cord blood from other litter mates, but I don't think at that time anybody thought that would have much practical application to human transplantation.

His studies were followed by work Hal Broxmeyer did comparing bone marrow derived and cord blood derived progenitor cells showing you work that Mitch already really portrayed, but that cord blood

was enriched on a frequency basis for progenitor cells, and that those progenitor cells were proliferating at a higher rate. And that makes them a better target for retroviral gene transfer which may have importance in the future.

Arlene Gluckman performed the first human cord blood transplant in 1988 in this boy who has Fanconi anemia and was six years old at the time. His parents had conceived a child who was healthy and HLA matched, and through a multi-disciplinary, multi-institutional, academic and industry, a collaboration, the cord blood was saved, frozen, transported to France where the transplant was performed when the baby was six months of age in case she would need to get a backup donor.

This child did very well and engrafted, as one would have expected with HLA matched sibling bone marrow. He's ten years out from transplant now at the medical center and has done well, has not had any abnormalities or any unexpected complications. Of course, in Fanconi, there is a unique problem of fixing the hematopoietic system but not necessarily fixing the patient, and this patient has not developed any secondary malignancies, but we know now as we follow more recipients of transplants with

Fanconi that they are at risk for other cancers later in life.

Now, that work led to other transplants in the related setting when the setup could occur, and Nancy Kernan and John Wagner reported in the Lancet in 1995 and updated in Blood in 1997 a collected experience from many centers with related transplants which demonstrated that engraftment was feasible in children, and of course, this was only done in children because adults really didn't have parents having offspring who could serve as donors.

And in the setting comparing this to sibling bone marrow white count and platelet count recovery was delayed, and surprisingly, there appeared to be less acute graft versus host disease. This is a slide John Wagner prepared comparing incidents of grade three and four GVHD, I'm sorry, it's overall grade two to four, and then the lower line three and four GVHD and recipients of HLA matched cord blood compared to young recipients transplanted at the University of Minnesota with HLA matched sibling bone marrow.

And you can see and see the axis here is only 20 percent. But there was a difference in the incidence of GVHD in those two populations, although they are not randomized. This is just a

retrospective look. Also interestingly, there were a few haploidentical transplants done in a related setting, and when there was disparity at the noninherited maternal antigen, there was very little severe GVHD, but when there was disparity at the noninherited paternal antigen, there was a higher incidence of severe graft versus host disease, suggesting that in this setting there's some tolerance conferred by the graft.

I think that that's important to note, and one area of future research may be, particularly in kids with genetic diseases, like this little boy with Val Major, that haploidentical sibling cord blood could serve as the donor source of reconstituting cells in early transplantation and essentially correct gene therapy.

We performed six transplants like this over the past six years, four in kids with genetic diseases, and two in kids with leukemias, and five of the six engrafted, the one who didn't was a child with Fanconi anemia, and the other kids did not have any severe GVHD and really acted like a matched sibling bone marrow would have been expected to act. And several of these kids are now out almost three years without any chronic problems with correction of the genetic disease.

Now, you've heard many times today that banks have been supported by the National Heart, Lung and Blood Institute for unrelated transplantation to really address the problem of donor identification in an alternative fashion. Their first bank was funded at the New York Blood Center in 1992, and all the transplants that I will tell you about today were supplied from that bank. And more recently, as Mitch told you, that three additional banks have been added to the pool.

Now, the frequency of cord blood transplants have increased dramatically, I think, over the past couple of years, and so maturing data on long-term follow-up is just beginning to come. In our own institution, we've performed 165 transplants to date, and we now have 90 patients who've been followed for periods of time that are greater than six months, the longest being followed for five years.

I'm going to spend time, though, summarizing data which is combination of work performed at Duke and the University of Minnesota over the past four and a half to five years. I'm looking at transplants and outcomes. And in the group that I will show you, 24 of the patients are adults, and the remaining are children, and the

total patient number is 143. You can see by frequency that the majority of the transplants have been performed over the past couple of years.

The criteria to be included in this analysis was having greater than 42 day follow-up, although all the patients now have greater than 100 day follow-up. Having this be the first allogeneic transplants, some of the patients had already failed an autologous transplant to be able to have conditioning for the transplant. So there are no children with immune deficiencies who are not ablated included in this analysis, and to have that HLA match zero to three antigen mismatched or matching cord blood graft available from the New York Blood Center.

Of the 143 patients, about two-thirds had malignant conditions. These are not surprising diagnoses. They would be what you would expect in any pediatric transplant program. I will mention, though, that all the patients had high risk disease. Many of the leukemic patients were either in late remission or relapse. The CML patients with the exception of a couple were either in blast crisis or accelerating phase. The JCML patients were also accelerating.

And then one-third of the patients had nonmalignant conditions more likely to be found in a pediatric program, but Fanconi anemia, a few with acquired severe aplastic anemia, Blackman Diamond syndrome, and then some in the metabolic conditions including osteopetrosis, crybais disease, Lechnyhan syndrome, Hurler syndrome, and all DNALD.

A few had immune deficiencies that were partial, so they did require ablation, and a few had secondary AML or MDS related to treatment of a primary -- different malignant condition.

The median age of the group was 7.2 years. The oldest patient was 58 years. That was actually a stock broker who lied about his age to get into our program. We didn't figure it out until he was already there. Median weight was 21.6 kilos with the largest patient in our series 92 kilos, a little bit more males than females, and about a 50/50 split on the patient being CMV positive, of course, all the units were CMV negative.

This just shows you some demographics of the units. The median volume was 84 mils with a range of 40 to 214, and that is not how the units were selected. Median cell dose was 3.6 times ten to the seventh cells per kilogram with a wide range, as you can see, which really related markedly to the

patient's weight. Median CD34 dose was 7.6 times ten to the fifth per kilo, CFU-GM dose 1.3 times ten to the fourth per kilo, and CD3 dose nine times ten to the sixth per kilo. And these numbers were all measured, these three on the post-thawed unit for consistency because we didn't have all the data on the pre-cryo unit, but this count refers to the pre-cryo count.

Now, there were some differences between the Duke and Minnesota practices, and we didn't agree to a common protocol before deciding to do this analyses. So let me point those out to you. At Duke, we gave all patients empiric support with G-CSF from day zero, and that was at a dose of 10 mcgs per kilo per day, and that was kept going pretty much for the first two to three months post-transplant. Minnesota initially did not give patient G-CSF but later did switch over.

At Duke, patients under the age of two did not get TBI regardless of diagnosis, and older patients who had a metabolic disease did not get TBI, or patients who had been treated for a prior malignancy and had a contraindication for TBI, and those patients received a chemotherapy based preparatory regimen which is busulfan and melphalan for malignancies and busulfan cytoxan for

nonmalignant conditions. At Minnesota, all patients were given TBI.

At Duke, we started our GVH prophylaxis with high dose steroids in combination with cyclosporin, and at Minnesota, they used an intermediate dose steroid. So here we started with a pulse of 10 milligrams per kilogram which rapidly tapered over about ten days to 2 milligrams per kilogram. Here, the highest dose a patient received was 2 milligrams per kilogram.

At Duke, we did do a greater number of patients with more disparate grafts and adults. Also for the malignancy patients, the chemotherapy agent combined with TBI at Duke was melphalan where at Minnesota it was cyclophosphamide.

Definitions you've seen. Mitch presented these same definitions as he was explaining the design of the NHLBI study which will be done, but we define engraftment as the first of three days to reach an ANC of 500 and graft failure as failure to reach that ANC by day 42. So even if a patient engrafted after day 42, they were scored as a graft failure.

HLA was typed, as Pablo just showed you, at a serologic level for class 1A and B, and a molecular level for DR beta 1. And I think I should

say that we had different strategies for selecting units as we proceeded through this work, and I don't know that we know the best one yet, but I can at least share with you how we changed.

Initially, we looked for the closest match thinking that that would probably be the best thing, but over time, we said that we would look for the closest match at DR and sacrifice class one if we needed to do that, and then currently, what we're doing is looking for sort of the closest match at DR combined with the highest cell dose. So if I have to choose between a large four of six that matches a DR beta 1 and small five of six, I will pick the large four of six, and I'll show you the data that has led me to make that selection.

In terms of the group that we're going to look at today, you can see the majority of the patients received grafts that were either mismatched at one or two antigens by the criteria that I mentioned. There were a few who had three antigen mismatches and about ten percent who at A, B, and DR beta 1 were six of six matches.

Okay. As far as engraftment is concerned, 87 percent of the patients reached an ANC of 500 by day 42; 93 percent reached that point overall. The median day of reaching an ANC of 500 was 25 days

with a wide range out to 59 days. HLA disparity did not impact on engraftment of neutrophils, so you can see the three antigens, two antigens, one antigen and zero antigen mismatch grafts and no difference in these curves.

We had a question early on as to whether we would get as good engraftment with TBI -- without TBI as we did with TBI. So we looked at that, although this was not a randomized comparison and saw that the kids getting the chemotherapy based prep regimen, if anything, had better engraftment than those getting TBI.

Now, this is one of the pitfalls of univariate analysis. If you remember, I said kids with metabolic conditions and nonmalignant conditions did not get TBI. So this curve is weighted towards smaller and younger children who, just by nature of size, got a higher cell dose.

We also looked at the effects of G-CSF and saw a difference in time to engraftment in the patients getting G with about a nine day window of earlier time to reach ANC of 500 in the group getting G. And again, these were not randomized. These were Minnesota patients. These were Duke patients. But later, Minnesota did add G to their regimen so that some of the patients in this curve

also came from Minnesota. But we thought that we would continue to use G as support for this kind of transplant.

There also is a relationship of cell dose to time of neutrophil engraftment, and the curve looks nice, but I think we lost some patients who are very large and graft early and patients who are very small and graft late. So it doesn't necessarily hold true patient to patient. Open circles here are patients who did not engraft, and you can see that they received cell doses that are in the range of patients who didn't engraft.

And it really doesn't matter what parameter of cell dosing you use, whether it's nucleated cell count, mononuclear cell count, CD34 cell count, or CFU-GM both. They all correlate with each other, and they all correlate with time to engraftment. In multivariate analysis, the only thing that came out as an important factor in predicting myeloid engraftment was cell dose here shown as CD34 cell dose.

And you can see there's a distinct difference between patients getting less than three times ten to the fifth CD34 cells per kilo, and again, this is measured on the post-op sample, and those getting higher doses. And the group here has

less engraftment and certainly a longer time to get to engraftment than the group getting the higher dose.

Graft failures seem to have clustered in a few diseases. We have a number of patients now with CML who were either in accelerated phase or blast crisis who had persistent disease or just outright graft failure. We've only done one patient with severe aplastic anemia, but that patient got a high cell dose, and did not engraft, and then two patients with Fanconi anemia who also did not engraft after receiving high cell doses.

And I think these really may be red flags, and may be diseases where the cell dose threshold may really impact engraftment, and I think we need to proceed with greater caution in these diseases using blood transplantation.

Platelet engraftment followed neutrophil engraftment. It took a median of 2.7 months for patients to reach a platelet count of 50,000 without transfusion support with a range that went out to eight months in some patients. All the patients who engrafted to 50,000 engrafted completely and ultimately reached a count over 100,000, but it definitely could take many months to get to that point.

HLA did not impact platelet engraftment in this group. But again, CD34 cell dose did, and there's a more dramatic curve here where about half the patients getting less than three times ten to the fifth CD34 cells per kilo would be predicted not to engraft platelets, again, suggesting that's an important number.

Graft versus host disease occurred in the moderate severe category grades two to four in 37 percent of patients. Grade three and four occurred in 14 percent of patients. We had broken this down looking at the 24 adults defined as patients over 18 compared to the children, and don't see a difference in the curves.

The incidents of GVHD or severity did not appear to correlate with the HLA disparity of the graft. And again, most of these are either one or two antigen mismatched grafts, but we didn't have any difference in incidents of severity based on that mismatch. And when we looked at grade three to four, the same analysis held up, did not predict more severe or less severe GVHD.

In a multivariate analysis, the only variable that came out as significant was CD3 dose per kilo, and what that had -- when that exceeded 1.6 times ten to the seventh CD3 cells per kilo,

there was a higher chance of developing grade two to four GVHD. We did not plan this, correct for this, or adjust this in any way. This is just a retrospective look at the data. But the HLA disparity, at least in these patients, did not come out as significant in terms of predicting GVHD.

Now, chronic GVHD has occurred in 11 percent of patients, the majority of whom have limited disease, and all of whom have been treated successfully. None of the patients have gone on to develop the serious sequelae of chronic GVHD like scleroderma or any persistent immune cytopenias. And this has been relatively mild. Again, we don't have as much adult data, but it does not look, so far, like there's a higher incidence of chronic GVHD in the adults.

Immunity constitution is an interesting topic and another one I think really deserves better study. This is looking at PHA counts in Duke patients between day 60 and 90 post-transplant. These analyses were performed in Rebecca Buckley's lab at Duke, and in this assay, a count of 100,000 or above is considered normal. And you can see that between these days when everyone is still on immunosuppression, about half the patients are approaching this 100,000 count.

This is a little bit deceiving because these patients are all markedly lymphopenic at this point. So even though their cells may proliferate, they may have an absolute lymphocyte count in their blood of 100. So that they may work, but there's not enough of them to do a lot of jobs.

If you look over time at PHA counts, again, and these are months post-transplant now, you can see that patients are truly corrected at a year post-transplant, and we stop immunosuppression at nine months and seem to maintain that as they go out back to normal life. Patients in this stage in our program have been immunized with the usual vaccines and have responded.

We've had one of 90 patients we followed have pneumococcal sepsis 18 months post-transplant, and that patient was successfully treated, but we're not prophylaxing anyone except the few patients who have chronic GVHD.

But again, even though the counts are starting to come up and here these patients are still profoundly lymphopenic, and they don't get to a lymphocyte count over 800 until about 12 months, and that also correlates with getting to a CD4 count over 200. There's also an interesting phenomenon of B-cell proliferation in this phase which -- B-cell,

not proliferation, but B cell growth before T-cell growth.

In the patients with malignant conditions, there's been an overall probability of 25 percent relapse, and we interpret this as a good finding because most of the patients were very high risk and either in relapse or in late remission. The majority of those relapses have occurred in the first year.

For reasons that I can't explain but which we duplicated at Minnesota as well as observed at Duke, patients not getting G have a higher chance of relapsing than those getting G, and I would love help in explaining this, but it has led us to not be in a rush to stop G-CSF. These patients received G-CSF for the first 60 to 90 days and then did stop, in contrast to these, where no G was given.

We have learned a number of things about managing these patients and decreasing morbidity and mortality associated with the procedure. We started at Duke with a high dose methylpred or triple drug per Nelson Challett's Stanford regimen to prevent GVHD because we expected to see severe acute GVHD given the number of mismatched grafts we were transplanting.

When we didn't, and when we did see a very high incidence of infection in this group, approaching 50 or more percent and when Minnesota had the same incidence of GVHD that we did, but with a lower dose of steroids, we cut back on steroids. And this shows you that when we went back and looked at the higher group -- both groups together that the group getting intermediate dose methylpred with cyclosporin had half the non-release mortality as those getting the higher dose methylpred with cyclosporin. And so now, all patients are back on this regimen.

The incidence of GVHD was not different between those two groups which helped us make that decision with relative ease.

Now, the overall survival of the entire group, and this is event-free survival is 44 percent at two years. I'm going to show you the things that did and did not impact on survival. Age related disparity did not impact on survival, and, of course, this is univariate analysis, and there may be biases inherent to the selection of the units, but our two antigen mismatched units were doing at least as well, if not better than, our one antigen or our zero antigen mismatched units.

It didn't matter in our series whether if you had two antigen mismatches, they were both in class one or one was class one and one was class two. And if you only had one antigen mismatch, it didn't seem to matter whether one was class one or one was class two. These are small numbers and may not reach significance because they're small rather than because this is real.

Things that did impact on survival were, first, age. You can see that the under two year old group has roughly an 80 percent event free survival. Again, this is rated toward kids with non-malignant conditions but does include some babies with infant AOL and AML and JCML. Between the older children and the adults, there was not a difference in overall of entry survival. And again, in these two groups, the majority of the patients had malignant conditions. But there are -- there were two patients in this group with other inherited diseases, and about a third in this group with non-malignant conditions.

Overall, those with non-malignant conditions have a better event free survival than those with malignancy. But in multivariate analysis, again, the only thing that comes up as significant is cell dose, with those getting, again,

shown as CD34 here less than three times ten to the fifth per kilo having an 80 percent transplant related nonrelapse mortality, and the other patients segregating out to 55 or better percent.

Age did not fall out. HOA did not fall out, but cell dose did. And for that reason, we focus many of our efforts in collaboration with Anstrom on methods to enhance cell dosing by ex vivo expansion. I'm going to show you a little bit of that work. Anstrom has made a closed sterile profusion system which was originally designed for bone marrow cell expansion, but has now been applied to cord blood and peripheral blood stem cells.

And media goes here, and it's cooled in an incubator, I'll show you in the next picture, and then the cells are inoculated here. Now, in the original system when bone marrow goes in, it lays down stroma, and then the hematopoietic cells proliferate, I'm sure in part, by interacting with the stroma. But in cord blood cells, there really is none to very little stroma laid down. So the proliferation is happening through a different mechanism, and we may not be stimulating the same cells.

These cells are profused with media that does contain horse and fetal calf serum and also

pixi, epo, and flip-3 ligand. The profusion is 12 days in this incubator which is computer run, and can detect any changes in temperature or leaks. Cultures are done two days before the end of the infusion to make sure that no contamination with bacteria or fungi have occurred, and if not, the cells are harvested at day 12 and given to the patient.

Now, because the units that we've been using are all frozen in single bags, we have not had the luxury of being able to time this the optimal way because we can only thaw these bags once. With the new bags that Mitch showed you, it will be easier to go back and take an aliquot of the cells and do something with them, and later come back for the rest of the cells.

But what we did in the now 27 patients that we've transplanted is we took on day zero of the transplant, we thawed the unit, and we took somewhere between one and three times ten to the seventh cells per kilo and gave them to the patient, unmanipulated, just like we always would, and we took the remaining cells and put them into the expansion device and expanded them for 12 days, harvested them on day 12, and infused them intravenously without any other preparation, and

then we looked at the usual things, engraftment and GVHD in overall event free survival.

I'm just going to show you a little bit of data, but if you look at cell doses, this was the median cell dose in the group, and this was the unexpanded cells plus the expanded cells for the total cell dose. Median CFU-GM was rather low in the unexpanded component, but markedly augmented with the expanded cells, and the thing that we keep -- the subparameter that we saw the greatest expansion in was the CFU-GM where the fold expansion ranged from 50 to 250 fold.

The overall cell count expansion ranged from about one and a half to five fold. We did not expand 34s, and in fact, because of the way we did this, in some cases, we diminished the CD34 dose because we took that portion of the graft out for expansion.

I forgot to put the recovery slide in, but if we look at recovery, we had absolutely no difference between data ANC of 500, or a day two platelet, or a red cell transfusion independence in the group receiving the augmented cells versus historical controls that would have received the same unexpanded dose or the same total dose with expanded cells.

But what we did see was if we looked at overall event free survival and this is at 100 days, the ex vivo group has a superior 100 day event free survival compared to, again, historical controls. Now, I don't -- I hope that this is real, but it also may be that we're just getting better at doing this somehow. And that because we did have improvement in our survival by year anyway.

But this does stand out to us particularly when we compare a group getting two times ten to the seventh cells of the total cell dose with those getting two times ten to the seventh cells as an unmanipulated though supplemented with ex vivo cells. And there, event free survival of the first group is about 15 percent, and in the latter group, it's about 80 percent. So there may be something that we're adding to that we can't really identify right now in an easy number, that is getting helped.

But I think the two things that have to happen are, one, that we can expand pre-transplants so that we can give the expanded cells on the same day that we give the unexpanded cells; and two, that we optimize the cocktail or the conditions that we expand under. For instance, we're in our lab looking at supplementing with a placental fetal layer that's irradiated but priming the expansion

device, and that does give us better expansion.

We've looked at addition of SCF, and MDGF and G-CSF to the cocktail, and that also greatly enhances expansion.

So in conclusion, we feel that banked cord blood can substitute for bone marrow as a source of reconstituting stem cells in a transplant, that TBI is not necessary for engraftment, that full HLA compatibility between donor and graft is not required, that chronic GVH is uncommon, and in the long run, this may turn out to be one of the most important benefits of this source of stem cells particularly in young kids without cancer where we really don't want the morbidity associated with chronic GVH to be a problem.

And we do believe that graft resistent leukemia effects are preserved despite the fact that there is less graft versus host disease. Our biggest obstacle right now is infections. Depending on cell dose, we see infections in the first 100 days in anywhere from 20 to 80 percent of patients. And these infections span the range of bacterial sepsis, fungal disease, and a lower incidence, about eight percent of either CMV or adenovirus viral disease.

And we don't know if these infections are really due to some defect or problem with neutrophil recovery. We have patients who are running ANCs at 15,000 but who still come in with bacterial sepsis. And one theory is that we're having recapitulation of the neonatal neutrophil development. And maybe we're having a lower neutrophil total body load even though we are using G-CSF to push all the neutros we have into the blood.

So there may be a maturity of neutrophil function, a lower load of total body neutrophils overall, or some delay in neutrophil recovery. And then we know that we have delayed immunoreconstitution as compared to an HLA matched sibling. I'm not sure it's delayed as compared to an unrelated bone marrow, but certainly in the whole first year, the parameters that one would use to measure immune function are low and the lymphocyte count is low.

But later on, we're not seeing any selective defects like absent B-cell development or anything like that. We do get full reconstitution eventually. So future directions that I think are necessary are, one, to optimize and really explore better ways to supplement ex vivo expanded cells; to also look at supplementation in the patient of

cytokines that would accelerate in vivo expansion; to think about adoptive cellular therapies that could be used or created from small numbers of dendritic cells held back from the graft so that we could immunize against the adenovirus or maybe even bacterial antigens; and then, again, to really suggest that to have identical related cord blood may be a great source of cells to correct certain genetic diseases.

I'll just show you a few pictures and some acknowledgments at the end. These are twins with severe hyperplasia. They were both born prematurely, but this child had RDS and BPD and was on a ventilator for many months. This child did not have that complication, and you can see how that impacted on their growth. He was transplanted first. He was transplanted six months later. In this picture, he's 18 months out, and he's 12 months out, but they both got BuCy ATG and four of six unrelated cords, and both have full immune reconstitution now.

Twins with Karbés disease. She is the healthy twin. She happened to be an HLA match to her brother who was the affected twin, and she was not a carrier. And her marrow, I wish we had had cord blood, but her marrow was used to correct his

disease when they were five weeks old, and this is their picture at a year. And although his development is not quite up to hers, their sibling who was the index case had died at 13 months of age. So he's clearly had much less insult from the disease than he would have.

And if we could look at situations like this between siblings, we might have cord blood in the freezer and not have to subject a young baby to a harvest.

This is an interesting Fanconi clan. These are all cousins from Alabama. He received sibling bone marrow. He received mother's bone marrow. She received a three out of six unrelated cord blood, and she's three years out in this picture. And she received an aunt who was HLA identical about four months from this picture which is why she still looks cushionoid. She had some acute GVHD. But this child had no other donor source and really has done as well as the more traditional transplants.

So let me stop there and acknowledge some of the people who've contributed to this work: the physician and nursing and laboratory groups at Duke. From Minnesota, John Wagner, Stella Davies, and Todd DeFor who did all the statistical analyses.

Obviously, Doctors Rubinstein, Stevens, and Carrier from the New York Blood Center, from Anstrom, Alan Smith, Doug Anstrom, and Christian Goltry, from Thermogenesis, Phil Coelho, and from Medsept, Sandy Mulligan, and NHLBI for really their funding of all this work. And I'll stop there.

DR. HARVATH: We have one abstract presentation by Dr. Donna Wall, and Donna is the Director of the St. Louis Cord Blood Bank program, and what we'll do is we'll let Donna give her presentation, and then Dr. Kurtzberg, Dr. Rubinstein, and Dr. Wall will form a panel at that time. And then I'd like to invite some of our colleagues from NHLBI, if Dr. Jensen or Dr. McCurdy are here, and they would also like to sit on the panel in case there are any questions regarding the NHLBI study. Dr. Wall.

DR. WALL: Thank you very much for the few minutes here. What I would like to do is just focus on one little bit of area of contention in the way we run our cord blood bank in St. Louis, and to provide information to justify that approach. And I think it's important to do this at this time that regulatory guidelines are being developed.

The bank in St. Louis got started in 1996 with a lot of help from Dr. Rubinstein, and

actually, a lot of help from the NHLBI granting operation that helped us get our act together. The fundamental basis of our operation is that we are a community-based cord blood banking system where community obstetricians and midwives perform the collections during third stage of labor.

We have, and I'll walk you through our thinking, and our approach is to make sure that we have this as a very safe and practical alternative for cord blood banking. The collections are performed only on documented singleton deliveries. The only major catastrophic approach to collections during third stage labor, which is that time period Dr. Rubinstein described, where the placenta is still in utero, infant is delivered and over in the isolette, and the obstetrician is waiting for the placenta to deliver to finish up the delivery process.

The only major risk, serious risk that we have been able to come up with is that there'd be an undiagnosed twin that has not yet been delivered with a shared placental blood source, and the potential of tragedy if a collection was performed prior to the delivery of the twin. For that reason, we only perform collections on documented singleton deliveries.

Very similar to the NHLBI project, we have consent and medical questionnaire reviewed by the parents prior to delivery with discussion with our cord blood staff, and we perform the usual patter of viral and bacterial testing as well as hematopoietic and HLA testing.

The scope of the program is that we have over 300 obstetricians and midwives in the area. This is now the majority of delivering physicians in our region practicing at 40 obstetrical units within 150 mile radius of St. Louis. We have collected over 10,000 cord blood units over -- since we've been in operation and have banked 3,200 of these units.

We have, during this program, we have done no active solicitation for donations and basically have operated pretty much on good will of the community, public interest in the program, word of mouth from expected parents, and a few brochures in delivery room offices. This is really a no brainer concept to sell to expectant families. In this last month, we received over 600 donations to the unit.

The important points in maintaining a quality cord blood collection program that utilizes this front end approach which is different than the

NHLBI and Dr. Rubinstein's program is that we maintain a very close communication from the cord blood bank directly with expectant families. So there is a phone conversation as well as written material with the expectant families.

Secondly, we have an active in service program for collection teams, and importantly, we continually monitor physicians and midwives for the products that they send into us for bacterial contamination, clotted products, inadequate labeling. If there are problems, there is direct communication back and reinservicing.

With this approach, we have roughly three major pathways that cord bloods that are donated to us undergo. Initially, we're banking about half of our cord blood units, but since our supply has outstripped the resources at a laboratory, and wearing my clinical hat, since large cell doses is critically important in core blood transplant, we have repeatedly upped the minimal cell dose that we are banking.

We are now banking only units over eight times ten to the eighth cells, total manipulated cells. So this is a little bit of an artificial pie, and so a few more go over to research, et cetera. By doing that, the major reason for not

banking cord bloods that are sent to the unit is small volume and small nucleated cell count. There is absolutely no attempt made to change this factor because we did not want obstetricians changing delivery practice. There is absolutely no pressure exerted on delivering families.

As we started the program, it became apparent that the most important thing we needed to do is to calm down the parents to make sure that they understood that the collection would not occur if there is any risk at all or any difficulty at time of delivery. The other areas that have needed control and development of procedures have been in setting up appropriate transportation and the usual blood banking issues with labeling and processing controls.

Since the start of the unit, we have had a progressive fall in the infection rate with community obstetricians collecting. There is a trend toward slightly increased percentage of units having bacterial contamination timing in with new residents in July and et cetera.

Interestingly, we have been able to, for me it's been interesting being able to show the benefit of pre-screening families with medical histories and not collecting cord bloods on families

that have had viral histories in the mothers. And so we actually have a very low viral seropositivity in the mother. This is the result of 3,000 of our banked cords. Our CMV rate has been stable throughout at 38 percent.

Of the cords that we have collected, we have used -- 28 have been used in transplantation, half at Cardinal Glennon Children's Hospital, half at other institutions, and more to justify our approach to bothering to use all the different centers, there's been a split in where these units have come, many from small community hospitals as well as the larger birthing centers.

A spinoff of having this type of a third-stage collection program is that you now have a procedure, policy and hardware available to perform collections in centers in more remote locations. And this is -- we have been solely expanding this component of our program, which we call our directed donor program, where for families of a larger geographic region where there is a potential that a child who -- an already existing person in the family could be needing an allogeneic transplant that we will bank the cord blood unit of the next offspring.

To date, we have had 69 units collected. We've used one for transplantation. We are up to four of the units we've been able to identify the familial condition in the newborn, and we now have three SCID babies that we've identified and have actually been able to facilitate early treatment for the babies. And we have been able to bank all but three of the other units with using basically the same education procedures that we have for our cord bank program.

So in summary, it is very feasible to have obstetricians doing third-stage collections of cord blood units. We have very acceptable bacterial contamination rates where the quality of the products is excellent, especially given that one is now allowed to direct your usage of the products, choosing units that are of certain ethnic mix of higher cell counts for banking. So that's my two cents. Thank you.

DR. HARVATH: I'd like to invite Dr. Wall, Dr. Kurtzberg, Dr. Rubinstein, if you would join one another at the table, and we'll give the audience an opportunity to ask some questions.

Also, you probably noticed it's a lot warmer here. They've turned off the air conditioning because a lot of people were freezing

the first part of the session. So I think what we will do in order to give people a break before the final session, we will plan that we will take a break at 3:50 and come back here 4:05, and start the last session 15 minutes late, and use that to cut into the time just so all of you have a chance to stretch and take a break.

I see Dr. Rowley is at the microphone, so I'll let him start.

DR. ROWLEY: Actually, I have, I think, probably two difficult provocative questions for Dr. Rubinstein. One question is what criteria do you have for who do you release cord blood units to? We heard Dr. Champlin this morning talk about the difference between regulating a product and regulating the practice of medicine. And you have set standards for the cord bloods that you bank, but I'm asking you do you have standards for who you will release them to, and can any transplanter come and purchase a cord blood unit from you?

And the other question I'd like to hear you talk about is your use of post-collection consenting. Has that been validated in the sense that you know that the answers to the health questionnaire are going to be answered truthfully? Because it's the health questionnaire that protects

us from the window period before a person with high risk behavior becomes positive in the virology testing.

DR. RUBINSTEIN: You were right. These are not easy questions, but they are relatively simple to answer. The first one relates to how do we decide who can get a transplant. In the United States, it is relatively easy because most of the transplant centers are affiliated with NMDP, and there we have no qualms.

When they are not affiliated with NMDP, we have occasionally given units to transplant centers when we are reasonably certain of who it is that is asking those units. The criteria are a little arbitrary, admittedly, but we ask the person who has done the training in the principles of that transplant center, and I have to say that in the experience within the United States, the outcomes of those transplant centers are not significantly different from those of the NMDP approved centers.

For other countries, the situation is a lot more difficult. In many places, we have had to resort to the opinions of colleagues who are well-known in this area, and we have tried to document in each case the reason why a given transplant center was approved for receipt of one of our units.

In retrospect, sometimes we have been overly optimistic perhaps, but it has not happened more than for those who are approved officially. So I don't think that we have been wrong very many times.

The other question, I really don't know very well how to approach it. Would you mind repeating it, Scott?

DR. ROWLEY: Well, the other question was how you validate the truthfulness of your health screening when you come to the donor after the delivery as opposed to having the donor come to you? I mean, in the blood industry nowadays, the donor walks in the door. They have, you know, they want to donate whereas in your situation, you're going to the mother afterwards and saying, well, we collected it, and now we want to ask you these, I think you used the word, intrusive questions. And do you know that they will answer those questions truthfully?

DR. RUBINSTEIN: No, but it would seem to me far more likely that if these people have no interest whatsoever in the process, they are less likely to hide from us information that might be important than those who have a personal reason for wishing to donate.

There is no way that I know that we can evaluate the truthfulness other than having the controls in the laboratory and the checks that we can make from the clinical chart of those patients.

DR. PRICE: Tom Price from the Puget Sound Blood Center in Seattle. This is kind of a curiosity question for Dr. Wall. One of the huge barriers to setting up cord banks has to do with the expense of doing it. Ten thousand cords is a reasonable piece of change. Can I ask you how you funded this?

DR. WALL: Come on, now. I'm not going to give away my secrets. No, philanthropy from the St. Louis region, a number of small startup grants, and a lot of fast talking.

DR. HARVATH: Dr. Stevens?

DR. STEVENS: Just to make a follow-up comment on the question about the validation of the risk factor data. I'm not sure that it would be related necessarily to the timing of when the consent was obtained, but I just wanted to point out that there hasn't been a whole lot of validation of risk factor data in almost any donation setting including the ordinary blood transfusion.

We ask the same kinds of questions of volunteer blood donors, and we don't know very much

about the validity, in fact, of the answers. And often enough, when donors come back and we find out that they were positive despite the fact that they denied risk factors, in retrospect, they do admit to risk factors.

The whole issue of the validity of this information, I think, is an important one, and is a very complex science, I think, all by itself which probably deserves some special investigation, but I'm not sure there were be a correlation with when the consent is obtained.

DR. KURTZBERG: You know, even in the matched donor, and I put that quotes, we find about ten percent of the time that dad is not dad, and so, those kinds of things happen in a living donor setting as well.

DR. DIPERSIO: I have a couple of questions for Dr. Kurtzberg. First, the 11 percent rate of chronic graft versus host disease, that's taking into account all the censored patients?

DR. KURTZBERG: Yes.

DR. DIPERSIO: And this is out of two years. So this represents, I just want to get this straight, this represents 11 percent of the patients that are living out to two years, is that right?

DR. KURTZBERG: Yes. It takes into consideration -- it's a Kaplan Meier plot. So the data onset of chronic GVH is in there and patients --

DR. DIPERSIO: So it's an actual probability?

DR. KURTZBERG: Right.

DR. DIPERSIO: Okay.

DR. KURTZBERG: And patients are censored if they die.

DR. DIPERSIO: So that's in your population, probably in the order of four out of 40 patients that are living out at two year?

DR. KURTZBERG: It's not that many. Actually, in our population, we only have one patient with active chronic GVH beyond a year post-transplant.

DR. DIPERSIO: The other thing was the issue of G and relapses. That's, of course, in two different centers with two different conditioning regimens, is that right?

DR. KURTZBERG: That's right.

DR. DIPERSIO: So there's --

DR. KURTZBERG: We did -- our first thought was it's melphalan versus cytoxan, and we did go back and look at that, and there is a

nonrelapse advantage to using melphalan, but when it was put into multivariate analysis, it still came out.

DR. DIPERSIO: The other thing is where is the data to suggest that any level of mismatching is bad in this kind of procedure?

DR. KURTZBERG: Pablo has it.

DR. RUBINSTEIN: Well, I had to talk about the banking issues. But any level of mismatch can be seen to affect the rate of the acute graft versus host disease. It goes up from about six percent for those that have no mismatch to somewhere in the range of 25 to 30 percent for transplants across one, two or three mismatches. It doesn't go up, and I'm talking now about only the severe GVHD, grade three of four.

DR. DIPERSIO: I guess what I mean is that if you look at overall survival and outcome, there doesn't seem to be much of a difference between a two and a three antigen mismatch and a one antigen mismatch. So really the major, I mean the way I looked at the data was the major factor was CD34 dose or cell dose is by far the more important predictor of outcome.

DR. KURTZBERG: In our data at just these two centers, that's the way it looks, and I don't

know how much of that is affected by biases or the types of support of care that we deliver or biases in unit selection, but Pablo can speak to a larger group of people.

DR. DIPERSIO: Of course, the three antigen mismatched unrelated cord blood has got to be fully mismatched. It's very highly likely that if you go ahead with high resolution class one typing, you're going to find various sequence differences. So they're completely mismatched. My question really has to do with why are you limiting yourself to a three antigen mismatch, and why aren't you just transplanting cord blood samples with high cell counts?

DR. KURTZBERG: You know, I guess we can always find -- I mean, we really have not found ourselves in a situation where we couldn't find at least a three antigen mismatch, that we haven't not transplanted someone because of not finding a unit that matched at least that well.

DR. RUBINSTEIN: I have to point out that in the overall data, it is very clear that the survival decreases with the number of mismatches, and this is significant. The number of mismatches is associated with the probability of engraftment as

well as that of survival and the probability of transplant related events.

So there is a discrepancy here between the overall data and the data that John has reviewed for us. But I think that when you put together two centers, and these are the largest centers using our blood, when you put together two centers, the opportunity for stratification for factors is maximum.

For example, University of Minnesota for a very long time restricted themselves to either perfect matches or five out of six matches and only recently started adding two mismatches to their range of possibilities. Whereas John, from the beginning, was willing to explore the more mismatched patients.

So if they're aware, for example, of a different overall probability of survival in the two centers, then you could either maximize the effect of HLA or minimize it depending on which of the two centers has a better overall probability of survival.

DR. DIPERSIO: I have one last question. Sorry to hog the microphone here, but you know, the engraftment data that Dr. Kurtzberg presented was very remarkable, I thought, because it's the only

time I've ever seen any data supporting the fact that G causes a more rapid ANC recovery to 50 and 100.

I mean in every auto study ever done and every allo study ever done, the ANC recovery time for patients given growth factor or not given growth factor is exactly the same. It's just the steepness of the curve is different. In other words, the time at which the counts start to come back is the same, but the steepness of the curve is much different.

But in your curves, we were looking at no ANC recovery until day 20 in the no G-CSF group, and then ten days earlier in the G-CSF group, up to an ANC of 50 or 100 which is pretty unusual. I wonder what are your thoughts about that?

DR. KURTZBERG: I'm not sure. We added G at the beginning just to standardize our approach. I was afraid that the different practitioners in our program would not be able to resist starting it if we didn't have it in the protocol. And so we put it in for everybody.

I don't know. I think that we're mobilizing very early. I think that as soon as there's a neutrophil, it's coming out in the blood. And I don't know if that's different from bone marrow. I can't really explain it except that there

may be an overall slower engraftment so you can see a bigger difference at a lower count than you would with bone marrow. The total loading dose is lower. You're seeing a bigger effect at low counts. That's the only thing I can think of.

DR. LANE: Tom Lane, San Diego. I just realized after hearing the last couple of discussions you probably can't answer the question that I'm going to ask which is sort of related, which is based on some of the data particularly from Duke regarding the efficacy of two antigen mismatches. How many cord blood units are needed?

DR. KURTZBERG: I can say two thoughts about that. Just because you can do it, doesn't mean it's the best thing. Okay. And I don't think we know the answer to that part. The other thing is when I'm picking a four out of six, I don't pick it the same way that maybe, you know better than me, John Wagner, when we're picking it, it's Pablo and me, and Malito who is in his lab making a decision about a unit, and there's a lot of factors taken under consideration.

There are often linkages, and often preferential beta one matches. I don't think necessarily everybody will do it that way. So I think there's really still a lot of questions to

answer before we know the answer to your question, but my guess is 50,000 to 100,000.

DR. LANE: That figure originally came from Ellie Gluckman, was it, and maybe you could clarify this. At least I've seen 100,000 from Gluckman, and I thought that was the figure she used to explain the number that were needed to answer the question about the effect of HLA matching. Does anyone have a comment on that?

DR. RUBINSTEIN: I cannot remember exactly how she arrived at that figure.

DR. LANE: I don't know either.

DR. RUBINSTEIN: Probably not in a very systematic way.

DR. LANE: Okay. One additional question, if I may. There are two things about the NHLBI protocol, if I understand correctly, and really I'm asking for clarification, one is that I understand that no cord bloods from mothers who test positive for CMV by serologic means will be used? That's not true?

DR. KURTZBERG: No, that's wrong. Mothers who are IgG positive are allowed, but if the moms are IgM positive, then the units are not.

DR. LANE: I see, it's IgM. Good. And what is the status of Look Forward, and how does that play into this?

DR. KURTZBERG: Each bank has devised their own proposal for Look Forward, and I don't know the details of the other two, but I can tell you that at Duke, because of the demographics of our population, we have a large number of our donors followed by what's called the Duke Health Service, and that relates to people at the University as well as people out at seven different public health clinics.

And there's already a network established to follow the babies through that system. And so we're taking advantage of that and doing chart reviews two months, six months, and two years after the baby is born to see if the baby has developed any significant illnesses. In addition, both the baby's pediatrician and the mother are given self-addressed stamped postcards that say all kinds of things that range from "I want out and I don't have to tell you why," to, "my baby's been sick, and blank," you fill it in, et cetera.

It's explained in the consent form, and they have to be willing to consent to participate in that part of the program.

DR. MCCURDY: McCurdy, NHLBI. I was intrigued very much by Dr. Wall's presentation because she, particularly the part about the directed donations. It appears to me that if this, if cord blood transplants work really well, and you can, indeed, do two antigen mismatches or even one antigen mismatches, data put together, I think, by Dr. Beatty, Dr. Pat Beatty from the NMDP files, would suggest that you could cover the country with a reasonably small number.

I have no idea exactly where 100,000 came from, and I don't want to claim any priority for it, but I've been using that number to cover the country for several years now, and it came straight off of Mt. Sinai. Actually, Dr. Beatty's data would suggest that you could do a pretty good job with matching for most ethnic groups with, I think, somewhere in the neighborhood of 15,000 to 30,000.

But to get back to the question of directed donations, if you do, indeed, need only 100,000, then you do not need a bank in every city. Which means that to serve the directed donation market, and I have no idea how large it is, but to serve that, you have to collect it at a distance and send it in for processing.

So I would encourage you to do as thorough a job as possible to determine the value of these units, both following up on transplantation and carefully demonstrating how many are infected, what the experience of the obstetrician collecting them has to do with the amount infected, maternal contamination, CFU counts, and so forth because that's the only way we're going to know and learn whether you can indeed do what you suggested, collect at a distance and send it in.

DR. WALL: And the important piece of this is, this is the difference between a matched sibling allogeneic cord blood transplant versus an unrelated donor cord blood. So it's worth going through the effort to do it, and the units go through the whole quality control that our other units are handled with.

PARTICIPANT: Yes. This question is directed to Dr. Wall. Of the 28 transplants which have been, have occurred as a result of your being drawn from your inventory, do you have any information on patient outcomes?

DR. WALL: It's just starting to come in. The information on thaw characteristics of the unit as best controlled with the units we thawed from Dr. Rubinstein's bank in our laboratory with the units

we thawed from our bank in our laboratory, and their thaw characteristics are identical.

We're still very young in the timeframe for any of the mature data such as Dr. Kurtzberg and Rubinstein have presented. We are getting engraftment, and we're just way too early yet. The bank's much younger.

PARTICIPANT: Over what period of time have the 28 transplants taken place?

DR. WALL: They've all been in the last year and a half.

PARTICIPANT: Thank you.

DR. HARVATH: Mary?

DR. CLAY: Mary Clay, University of Minnesota. Just a quick technical question. One of the issues that we've struggled with has been genetic testing from both a cost analysis basis and also the effect on the donor, something that's not talked about very often. Could any of the panel members comment about the current consensus or thinking about genetic testing?

DR. RUBINSTEIN: I assume this is testing for genetic diseases. Depending on the ethnicity of the donor, of course, the situation is different in populations with a high frequency of a certain

dominant gene or dominant elite expression sufficiently to detect it.

It's comparatively simple, but many of these are very infrequent. So that is the first step that you have to determine. So far, there are no guidelines, so you must establish a criterion.

We have sought one in 10,000 and higher. We should actually test for them even if there is no specific anticipant. And we have used hemoglobin abnormalities as an example. In populations of African-American descent and out of the Mediterranean populations, it is important to perform hemoglobin HBLC, also perhaps in people from Southeast Asia and other regions of the world.

Other testing is strictly conditioned on the histories. And so you are dependent on the history taken of the family. The -- overall, the yields of these testings are not very good in the sense that we don't detect very many, but for hemoglobin, of course, it is a must.

DR. KURTZBERG: You know, another control that I think all the banks are using is that if the CFUs don't grow, not to know the reason, but that unit would be excluded because it could be a signal of some marrow failure syndrome that's coming.

And also, it's not economical to do genetic testing prospectively and in, say, metabolic diseases, but for any recipient of a unit where the recipient has a metabolic disease, the unit is screened.

And that's why the cataloging and banking of all the test samples is so important, so you have something to go back to if you have a unique patient where you wouldn't want to transmit a carrier gene or whatever. And that's one principle I think all the banks are following.

I also think it's important to stress that this is only affecting blood -- diseases expressed in the blood for the most part. So you know, if it carries the CF gene, it shouldn't matter, et cetera and so forth. I think the question on future genetic testing, things we can't predict, some screen that may come up for cancer or Alzheimer's or who knows what, that's harder. And we've all sort of skirted the question in a large degree.

I mean, our consent form says future tests may be developed that may be applied, but it doesn't go into any specifics.

DR. RUBINSTEIN: I would like a quick stab at the question that Tom Lane made about the numbers in the inventory. The answer to that question

requires clarification, first of all, of the issue of HLA influencing the clinical outcome.

If HLA does influence the clinical outcome, we need to go further and decide at what level of resolution you can still see the effect of HLA. Once you answer those two questions, it will become easy to calculate because we have data for haplotype and antigen frequencies in the major populations.

The figure of one in 100,000 could be extremely optimistic under one set of conditions or rather pessimistic under others. But we need, at the moment, a sort of useful figure to work toward, something that is reasonable as we now have. And whether systematic or not, the figure of one in 100,000 is a nice round number, and it looks feasible. And so I think for the moment, that's a good target.

DR. HARVATH: Because of the time, I have to ask you, is your question relatively short, and --

PARTICIPANT: It is short, very short.

DR. HARVATH: Okay.

PARTICIPANT: It will heat up the room. In view of the discussion about the validation of infectious disease screening, you know, history in

the mom before or after delivery along with the informed consent, has there been any consideration of doing follow-up testing on the mothers that would be equivalent to the follow-up testings that are done on living donors of semen and surgical bone as well as the donor retested plasma which is 112 days? I knew I'd be popular.

DR. KURTZBERG: That's not done for living bone marrow donors. Just a point to make. It's not done on living bone marrow donors routinely.

But there's testing of this on the day it's harvested. I mean I think we all, especially with some of the more sophisticated testing techniques, I think you could probably have a two or three week window, and I joke about this, but most people in terminal pregnancy are not going to practice high risk behaviors that month.

PARTICIPANT: I mean, that's absolutely, I mean most people that we usually associate with, many people that will donate, that's not true. The reason it becomes a moot point with living with transplant donors, with bone marrow transplant donors is that you've already transplanted so there might be a case for not doing it.

But here you have some lag time before it's utilized. So you could hold it in quarantine

unless it was needed. So I think it's something to consider.

DR. HARVATH: I'd just like to make one more point, and Dr. Wernet was not able to make it here. He was going to speak about his organization on NETCORD, and Dr. Visser had asked that Dr. Rubinstein may make a comment about NETCORD. I would like to refer all of you to his abstract, and wondered if Dr. Rubinstein could make a very short comment on this since you've been involved with it, and then we will take a small break so that we can all sit through the remainder of this meeting.

DR. RUBINSTEIN: The NETCORD organization is a grouping of the existing cord blood banks in Europe and some of the United States banks. The purpose of it is to establish procedures in which screening and matching and so on can be done with higher efficiency than up to now, and perhaps to develop standards and better methods for communication with the transplant centers.

The initial work for NETCORD is to agree on a common set of standards and certification protocol such that, in fact, we can exchange unit with certain reasonable assurance that they are equivalent. That has not been done yet, but there

is a series of meetings proposed in which this process will be hopefully terminated.

And then we will have an organization that collectively at the moment has about 25,000 units. So it could be an important part. It could also be a very difficult problem because from what we have seen, the criteria that have been used up to now are somewhat different. So that it will be necessary to validate not only the current procedures and standards but those 25,000 units.

So there is a lot of work to be done, but it is a beginning of an international grouping of these banks that will facilitate the task of the transplant centers.

DR. HARVATH: Well, on behalf of the Organizing Committee, I would like to thank all of you very much for participating in this session, and making these contributions. Okay. I don't know what time is the official time. I guess we go with the clock on the back of the wall. From here, it looks like it's about five or six minutes after, I don't know. Is that what you see?

How about if we convene back here at 15 after, give everyone an opportunity to stretch, and then we'll promptly start at 4:15.

(Whereupon, the workshop went off the record at 4:04 p.m. and went back on the record at 4:17 p.m.)

DR. HARVATH: If we could begin the last session so that a number of people have to catch flights, and we'd like to give the last speakers an opportunity. Again, on behalf of the Organization Committee I would like to acknowledge and thank our colleagues in the various professional organizations who have so diligently worked to come together to develop professional standards.

And what I would like to do is to introduce all of them at the outset, and then just simply allow them to present on behalf of each of the groups. The first presentation will be by Dr. Rebecca Haley regarding the American Association of Blood Banks' approach to professional standards.

Then we will have three individuals speaking as a collective group on behalf of FAHCT and their standards, a representative of ISHAGE, the President of ISHAGE, Dr. Scott Rowley, Dr. Elizabeth Schpall, President of FAHCT will speak, and Dr. LeMader representing the American Society of ASBMT. Is that Blood and Marrow Transplantation? Yes. I always want to say Bone Marrow Transplantation.

So all of them will present in order, and present to you the progress they have made and their professional standards. And if time permits, we will have a short panel discussion. Dr. Haley?

DR. HALEY: Thank you, Dr. Harvath. Good afternoon. Thank you for the opportunity for allowing me to speak today. My name is Rebecca Haley. I'm a Senior Medical Officer of the American Red Cross BioMedical Services. And today I'm talking to you in my capacity as the Chair of the Hematopoietic Progenitor Cell Program Unit of the Standards Committee of the American Association of Blood Banks.

The AABB is the professional association representing 8,500 individuals involved in blood banking and in transfusion medicine, and, in addition, we represent 2,200 institutional members, including community and American Red Cross blood collection centers and hospital based blood banks and transfusion services that collect, process, and distribute, and transfuse blood and blood components as well as hematopoietic progenitor cells.

AABB members are responsible for virtually all of the blood collected and more than 80 percent of the blood transfused in the United States. Throughout its 50 year history, the AABB's highest

priority has been to maintain and enhance safety of the nation's blood supply. The AABB is dedicated to ensuring safe available blood supply and blood components and is committed to helping ensure the safety of HPC therapy in large part through the development of standards for the collection and processing of these cells.

The AABB has had a long history in standards since 1957. The AABB has issued standards for voluntary compliance in blood and blood component collection, processing, and transfusion. Our standards are refined every 18 months through a deliberative process that combines the elements of scientific peer review, clinical experience, expert advice, and regulatory analysis.

The AABB has published HCP standards since 1991, and is very appreciative of the FDA's efforts to provide liaisons to the Standards Committee and to other AABB committees. Last year, the Food and Drug Administration proposed a new regulatory scheme for HPCs and other tissues. The AABB applauds the FDA for the creative approach that it has taken recognizing that these new technologies do not necessarily fit into existing regulatory framework for drugs and biologics, I might also add for blood.

The AABB is supportive of the FDAs recent proposal that would require establishments to register with the FDA and provide a listing of standard products. We are particularly pleased that as part of its proposal, the FDA has expressed a desire to work with private organizations in establishing national standards for the collection and use of hematopoietic progenitor cells.

Recognizing that voluntary organizations such as the AABB have considerable experience in standard setting, the agency has proposed a system under which it will review and adopt industry specific standards developed in professional societies. We welcome the opportunity to participate in this public/private effort to establish standards for HPCs.

Professional organizations have played an important role for professionals and institutions engaged in the emerging field of hematopoietic progenitor cell collection, processing and transplantation. Cooperation among these organizations has been instrumental in developing standards and accreditation programs for HPC activities and keeping professionals abreast of challenging developments and technologies in this fast changing field.

The existence of these professional organizations, which collectively represent every expertise and discipline engaged in the field of HPC therapy, offers a unique opportunity for cooperation and collaboration among the government and private professionals in the regulation of the field. In the response to FDA's request for standards, a work group has been convened to develop standards.

The following organizations have been invited to participate in this standard setting process: the AABB; the American Society for Apheresis; the FDA; the Foundation for the Accreditation of Hematopoietic Cell Therapy; FAHCT, which represents ISHAGE, and ASBMT, and the NMDP. In addition, two public members will participate. One of them is an ethicist, and the other will be a woman who has been transfused with HPCs as a part of her breast cancer treatment.

The goal of this work group is to create one set of comprehensive standards, and we're confident that we can work together to accomplish this goal. The standards writing effort will be a departure from traditional approaches. In the past, standards have been a collection of specific technical requirements and somebody would have a

problem, and so then somebody would sit down and write a standard to answer it.

They would be arranged by how the cells traveled through the collection process or through the laboratory, and it was a mixture often of standards, work instructions, and then a lot of times there would be a little treatment advice thrown in so that it would be kind of a grab bag of things that were not very intelligible if you're trying to look at it from a system point of view.

We are attracted to a different model. We would like to do a standards document proposed on the ISO 9000 model. The reason that we think this would be nice is that it's a general quality plan as an instrument for accomplishing a mission. You start from the top with some preset categories. It's systematic, complete, conducive to continuous improvement, and so we think that as standards change -- writing has changed tremendously in the last even four or five years, that this may be a good model to head for.

Our group has chosen a model that is similar to the one above. It won't be exactly like the ISO model that you're going to see when you visit a biomedical equipment facility, for instance, because that's not exactly what we do. We're

incorporating relevant good tissue practices as well as other FDA and external requirements, but the effect will be a matrix of quality management concepts that are specific technical requirements hung onto this framework, and the standards will be a document that uses the quality framework to dictate how the standards are met in the collection, processing, storage, and infusion of hematopoietic progenitor cells.

Another advantage is that we have an automatic gap analysis and a continuous process improvement that is built into the process. So let's go through, I know people bat around ISO, and it doesn't particularly mean anything. So let's take a quick trip through how the ISO process is supposed to work and how we hope our standards will work.

Okay. The first thing that you have to do is understand your program needs and improve your understanding of those needs as necessary. For those of you who do better with flowcharts instead of bean men, this is a different way you identify your customers. Your customers here, if you are a laboratory providing these services, may often be the transplant physician.

Your customer is always, I think, the patient, and you have to figure out if you're meeting your customers' expectations. They expect you to show up at 7:00 on Monday morning to collect a patient, and you say, well, it's inconvenient for me to get there until 10:00 and everything in the hospital has already started, you're not meeting your customer's expectation, either your patient or your transplant physician. So you need to work that out ahead of time.

Okay. Then you have to say what you're going to do. That means you have to find out if your processes are well documented. If they are, that's fine. Rework those into your standard format. If you don't have them well documented, if it's, well, Jane's on today. We'll do it Jane's way. Tomorrow it's going to be June, and we'll do it June's way. We can't do that. So if you need outside help, there are consultants available, and of course, the ISO people are always saying we're a consultant for this and a consultant for that. But there are consultants available, and sometimes it will save you a lot of time.

Then you have to do what you say. If you write it down, and you document it, then that's what you have to follow. You have to follow your

procedures and do your documentation the way you said you were going to. Then you have to prove that you've done it. You prove that you have done it by conducting a surveillance audit, and then if you haven't done very well, you have to perform a gap analysis, and then figure out how you're going to fix it.

Then the next step is to improve it. When you conduct the internal audit and perform your gap analysis, then you conduct surveillance audits to make sure that you're fixing the problems that come up. And this may be something as simple as looking at your pheresis collections over time from unit to unit and process to process.

We found out that some of the processes that are very popular and are used don't get very good results simply by looking at the outcome sheets at then ends of the days in our different regions around the country. In the American Red Cross, we have about 18 different places that collect. And if you find that it's different from one place to the other, you know, you need to say, hey guys, you have more collections per transplant of any place in the country. What's going on? We did that recently, and the people changed their mobilization regimen. I think it needed to be changed.

So once these standards are designed, they will have requirements. They won't have guidance recommendations, and they will be included in the body of the quality management standards. And although there'll be other mechanisms to disseminate guidance to members so that won't be left out, but it shouldn't be in the standards. The standards shouldn't be the practice of medicine. That should be a separate document.

Now, let's go through what the suggested ISO categories are, and I'll show you how we have adopted those to blood banking. Now, the warning here is that these may be different. When we get through with the process, we've had one meeting. That meeting didn't include all of the folks that we hoped were going to be there to help with this process. And so this is just an introductory trip through the 20, actually 21, concepts that need to be covered.

And I hope you'll see when we get through why all 21 need to be covered. The facility must define and document responsibility and authority of all individuals involved in collecting, processing and storing. We're talking about our management responsibility. We must identify and provide adequate resources, including trained personnel, and

appoint a management representative with authority to establish and implement the facility's quality policy.

The quality system, the facility must establish, document, and maintain a quality system; prepare a quality manual; and define document, and effectively perform all procedures; and define how the HPC facility will ensure quality in new or modified products and services.

Agreement review. When a facility must have a procedure for reviewing agreements with customers, and again, usually with a collection service or a laboratory, it's going to be the transplanters or the hospital's blood center as to how and under what circumstances, what timeframes they provide these services so that there's a meeting of the minds because you cannot tell if you have met the requirements if nobody ever said what the requirements were.

Design control. The HPC facility must plan and organize the design of each new or modified product and service. This requires that the design meet the requirements for new or modified products, and that the increasing role of research and the evolving nature of the HPC collection really heightens the fact that you need to have some

control over this process, and that you have some minimum documentation as to what you plan to do and what kind of preliminary steps you've taken to make sure that this is not harmful or detrimental before you put it in -- before you start using it on your patients.

Document and information control. The facility must control documents that relate to the requirements of these standards, and document control must ensure that they're clearly designated and available where they're needed, and that the invalid and obsolete documents are not used, and that they're tagged as invalid or obsolete, and that they have history on them to say this was in effect from 1995 to July of 1997. So if you're looking for a result that related to that timeframe, that's where you look, but this is obsolete, so don't use it today.

In obtaining products and services, this is a concept that has two faces in the hematopoietic progenitor cell laboratory because it ensures that the products that effect the final quality of the product or service conform to requirements.

This includes newly collected products from donors, or reagents that are brought in from the outside, and that you must evaluate your

suppliers. If their product effects the quality of your HPCs, you have to maintain lists and records of acceptable suppliers and report supplier failure to your management so that you don't continue to get things from suppliers whose equipment or supplies don't work.

Number seven is control and processing of autologous. In the ISO standards, it's customer supplied product. They must verify, preserve, protect the products received from autologous donors, store them for the donor's future use, and notify the donor in case of loss or damage.

And number eight, product identification and traceability. You must be able to identify the source, the processing, and the final disposition of HPCs units, and create records of identification, and the tracking and tracing of any process performed while it was in your facility so that it's not a black box situation. If you get to the end and something happens at the time of infusion or something happens in the transplant, you have to be able to go back and find it.

Process control. This is usually the largest part of blood bank standards. Two little words, but that's where most of the things fall in. It's the controlled conditions for collection and

processing operations that must be maintained, and it's the use of written procedures, suitable equipment, and suitable working environment, compliance with procedures and external standards, and monitoring and suitable control of the processes and equipment, and the criteria for acceptable results and suitable maintenance of the equipment. So this is most of your day-to-day actual work.

Okay. Inspection and testing. It's when you must define the inspection and testing for incoming product, in our case, that would often be viability and cell counts, and ensure that any inspection or test required as a part of the delivery of service has been performed.

Eleven. Control of the inspection, measuring, and test equipment. You must prove that that's in line because if it isn't, all of your measuring and testing may not be valid. You have to know the inspection test status of each unit as it goes through the lab so that you don't have products in limbo that you don't know what's been done on them. You have to control the nonconforming product or service.

Now, this is, I think, a really important area for us because when something turns out not the way you thought it should, you have a nonconforming

product, it must be identified, segregated and documented, to review whether this unit can be accepted or used with special precautions such as a unit with a positive culture you may wish to give with the proper antibiotics. Or you may wish to destroy it if you have plenty of others.

The laboratory director and the patient's physician must confer on whether the product is acceptable and usable for the patient.

Corrective action and prevention plans. The HPC facility must establish procedures for corrective action and prevention and review the relevant information on each event that happens, and ensure that the corrective and preventive actions are appropriate to the magnitude of the problem.

One of the big problems that you have with this area is sometimes a religious belief system is made out of corrective action and prevention if the problem is absolutely minor. And sometimes when major things happen, they say, well, I'll get around to that tomorrow. We need to put that into perspective. And we need effective handling and investigation of the case and determination of the corrective action that is necessary and the application of control so that you don't have to do that as often in the future.

Storage, distribution and transportation. The facility must ensure that the products are stored, distributed and transported in a manner that won't damage them or allow them to deteriorate.

Control of records. You must have a process for handling, storing and disposing of records. Examples include identifying information of cord blood donors associated with the banked cord blood or units or records verifying disposal of components that are from unacceptable donors. So records of the things you have, records that you threw away the things you should have thrown away. Confidentiality is a major component of control of records whether those records are manual or electronic.

Quality assessments. The facility must plan and implement quality assessments on a schedule basis based on the status or importance of the activity that's carried out by personnel independent of those having responsibility for the activity.

Training. The facility must identify training needs and provide adequate training for the qualified personnel on the basis of appropriate education, training, or experience.

Servicing. This is fairly minor, we think, in our construct. Once the products have

been delivered to a customer, the facility must continue to be responsible for their storage, if by contract that's what you've agreed that the facility is going to do. So that's servicing a product after it's gotten into somebody else's control or some of our laboratories go out and help with the infusion, and so that's considered a servicing act.

Statistical techniques. We've had mentioned before. The standards say that you must apply the appropriate statistical techniques to make sure that your processes are up to snuff and stay there.

Safety. This gets into the OSHA requirements and the requirements that employers have to provide a safe work environment, and we think that that's critically important.

So all of these 21 different categories are called the core standards, and they're the backbone, as it were, of your quality policy. So they're also called level one plans, and so in there, you have to begin with your organizational chart and your statement of authority and responsibility.

Then on level two inside of your quality document, you need your purpose or objective. For instance, in training, your purpose or objective is

that all personnel that are providing services or doing procedures have had appropriate training.

And then you need to say there who's responsible for that and what your references are. In other words, look to our training manual or our training plan, and then you have to define terms if those are not obvious or clear. And then you have a general plan for action.

Okay. The next level, if you set your lab up this way, your level three going down are your bench procedures. This is the actual dot-to-dot that people need to use, and you need to verify that work or job instructions are clear and are being followed.

And then your level four forms are your work report forms, your finished documents, and these are your tools for improvement. If you finish those, put those in a hopper and never look at them again, you're unlikely to know what went wrong or how you need to improve.

So in conclusion, the ISO-type standards are program based. They're designed on a general outline so that you can't miss anything. And let me give you an example of something that we think might often get missed. On course standard six, obtaining products and services, many laboratories have

struggled to obtain the proper reagents for performing colony forming unit analyses for progenitor cells.

I know our own laboratory uses Stem Cell Technologies Medium 4434. Now, somebody in purchasing might get us a deal one day and get us another brand that was very much cheaper, but it would shut down the operation because you don't get the same results. We know that. We've qualified the vendors. We have it on file, and that's the kind of thing that this approach would help.

We have embedded methods for minding the shop or continuous process improvement. So we hope that this standard writing effort will be successful, and we offer it to this group as our goal for the immediate future and we hope that it will be helpful. Thank you.

DR. SHPALL: Thank you. If we could have our slides, please, and I'd like to thank Liana and the organizers for inviting the three organizations, FAHCT, ISHAGE and ASBMT to talk today. And if the first slide could come one. Let's see. Do I do it? Yes. With the first, let's see, there we go. Okay.

These are the parent institutions of FAHCT, and constitutes the vast majority of transplanters both primarily all of the academic

centers and many of the community transplant programs, both the laboratory and the clinicians who have basically formed FAHCT for the purposes of inspections and accreditation.

The history briefly, FAHCT was founded in 1996 by those two organizations, and the purpose was solely to establish standards for high quality medical and laboratory practice, and to develop and implement a voluntary inspection and accreditation program which would ensure optimal patient safety.

In 1992, ISHAGE under the direction of Scott Rowley at the time, had a committee which drafted laboratory standards that encompassed all aspects of stem cell processing. A year later, the ASBMT under the guidance of Gordon Phillips and a large community of clinical transplanters drafted a set of clinical standards which actually addresses every aspect of clinical transplantation that would be involved in a program to date, a modern transplant program, inpatient, outpatient, nursing, pharmacy, et cetera.

We then took those standards, merged them into a single document in 1995 and founded FAHCT for the sole purpose of initiating and continuing to carry out an inspection and accreditation program which covered all facets of transplantation.

Our goal has been and continues to be to promote quality patient care and quality laboratory performance. And we believe that a valid accreditation process has to review both aspects, both the clinic and the laboratory aspect of things, and without the end result, the clinical end point, the valid accreditation, the processes of the laboratory are in a vacuum.

Our unique strength, and really this is for us a first attempt to cover a global collection, processing, and clinical transplantation for all stem cell sources. In the transplant world, it's unique. We have not had an inspection or accreditation process that addressed the clinical programs before, and so that's something we're continuing to develop as we get better at doing that.

The standards that we have developed are process oriented because as you've heard from some of the speakers today and as you will hear from us continually, we can't really define the stem cell product yet.

It's not a product. It's a graft in evolution, but we want to set our standards, address the issue of producing this product in an optimal and quality way. We want to foster excellence in

the lab and clinic. We want to manage all aspects of stem cell transplantation, and we want to continue, and this is a major goal of ours, the development of rapidly evolving technology which has been very, very successful in producing lifesaving treatments for patients over the past decade.

Our standards require that all clinical transplant programs as well as collection and laboratory or processing facilities evaluate and report clinical outcomes. That means time to neutrophil engraftment, time for platelet engraftment, GVH, and death.

All accredited programs must have in place the quality management program much like Dr. Haley just described. This is a key and critical part of our standard program. It includes quality audits, system for detecting, evaluating and reporting errors, accidents and suspected reactions and obviously safety provisions.

And this is how the process works. We have a standing Standards Committee. It's comprised of basically the ISHAGE and ASBMT members who developed the initial standards as well as the FAHCT Board, Chaired again by Scott Rowley, and basically, we are continually evaluating our standards and planning for revisions as needed.

We're a very reactive group and a very responsive group and actually get together very often either by telephone or in person and evaluate new data, update and integrate new data, and quickly respond to changing technology much more quickly, we believe, than the bureaucratic or perhaps more governmental approach.

The process for actually incorporating a new standard is shown here. Basically, a new standard is drafted and revisions are proposed by the membership, our constituents who are ASBMT members, almost 1,000 member physician transplant group, and ISHAGE, 1,000 members of laboratory Ph.D.'s and scientists, and basically whoever wants to come to us, gives us the revision or the proposed revision.

The Standard Committee evaluates these in a timely fashion. We look at the medical and scientific data, and we revise as needed. We publish the proposals at both in our ISHAGE journals, Journal of Hematotherapy as well as the ASBMT journal, the Biology of Blood and Marrow Transplantation for public comment of our members.

Each comment is then reviewed very carefully, taken very seriously, and the standards are revised based on the comments from our members,

and then they're reviewed by our legal counsel before they're adopted. New standards are then adopted by the Committee and approved by the Board of Directors and published in our journals again.

It's very fortunate to have journals for both societies because it again fastly transmits new thoughts or thoughts that need to be commented upon in a very easy and straightforward way.

The qualifications to be a FAHCT inspector are outlined here. The inspectors must have a minimum of five years experience performing the activity, be it clinical, collection, or processing for which they are going to inspect, and for the clinical transplant facility, these are all physicians who have been clinical transplanters in a program for five years.

For the collection inspectors, these are M.D.s or Ph.D.s who again have been five years involved in the field, or we do have a small cohort of nurses and technicians who were supervising collection facilities, similarly, supervisors of stem cell laboratories for five years or more, and those people have been allowed to become inspectors of facilities. Otherwise, it's an M.D., Ph.D. whose run a lab for five years.

We have a standard inspector training course which is required for any inspector before they're allowed to go out in the field, and in order to be an inspector, you must be affiliated with a FAHCT accredited program or have applied for FAHCT accreditation.

As of last week, we have 170 inspectors fully trained and ready to inspect, many of whom have begun the inspections. Another 50 will be trained by the end of this year. We have 123 facilities who have applied for accreditation and new applications are coming in every week. We performed 20 inspections. Another 30 are scheduled and will be completed by Thanksgiving, and the approvals are coming in as the inspections are done.

About 70 of these institutions, it's a very lengthy application that has to be filled out before we assign an inspection team. And so more than half of the applications are now back at the centers as people are working on filling them out.

So where does that leave us. We believe we have a very successful albeit young inspection program, but it looks to be, I can say to a man, for anybody who has applied for FAHCT inspection and/or who's been accredited, everyone says it's been a royal pain in the neck, but the programs have

improved as a result. And I think that it's very gratifying to know that it does look to be the case, that people believe that patient care has improved by this process.

The docket which was proposed mentions registration, and I think that we do understand the need for oversight in the field, and we want to work with the FDA as closely as we can, but I have to say honestly, and you'll hear about this more from my friend, Dr. LeMader, the constituencies of our groups are worried and concerned about it.

And their concerns are outlined here. As you heard from Fred earlier, it's not necessarily true that registration will improve safety. And I think we heard phase in, first step, et cetera from FDA, and what comes next is unknown to us. And although registration on its face and listing some products doesn't sound terrible. But it's not clear to us what the real long-term agenda is, and I think that's what's making people a little concerned about even agreeing to the first step.

FDAs ultimate intentions, granted, we don't know, and I'm not sure they do either, so I'm not sure that can be answered, but I think it's something that should be discussed as we try to work together to meet everybody's needs. Obviously, our

concern being the people who developed marrow transplantation, peripheral blood transplantation, rapidly exploding technology with cord blood have offered our patients grafts every two years that weren't there a year and a half and two years before. And we know that in many cases, not all, certainly not even most, but in cases, these have improved their clinical outcome, and perhaps, saved their lives.

And the thought that by adding regulation which may not improve safety but could potentially impede the technologic advances that we've made over the past decade and compromised really what's optimal patient care because we need to cure these people with fatal diseases, that is an issue that we have to deal with and come to terms with and come to an agreement with as we move forward because we're the ones who have to look the patient in the eye and say we can't do this because that, et cetera. And it's a very serious issue that we hope to be talking to the FDA about.

What would be the solutions? There are many potential solutions, and we just offer you one here, and that is, perhaps, if our FAHCT inspection and accreditation program after it's review and approval by FDA met its expectations, we would be

very interested in a deemed status relationship where we could, in fact, accredit the transplant programs who voluntarily agreed to request that accreditation.

We obviously have the transplant expertise through our parent organizations, and we have a vested interest in making this work in a collegial and collaborative manner with FDA rather than as a fight. We believe we have an effective operational inspection and accreditation program. We obviously can learn from other organizations how to do it better. We'd be certainly willing to work with FDA and alter our procedures if there were others that made things more comfortable, but we hope that we can begin this dialogue.

And we acknowledge, obviously, we don't want to be the police. We're peer reviewing each other, and the FDA obviously will always have a role in those centers that would choose not to participate in a voluntary program. So that's all I'll say about the FDA.

Liana asked us to talk about where funding should be in the next couple of decades, and I think you've heard from a couple of people this morning that it's one problem to define a product, and if we had the right antigen or assay to define this

product such as CD34, that would be terrific. But currently not only do we not know what the right parameter is to make it, it is really difficult to standardize these across laboratories.

CD34 analysis in Los Angeles doesn't necessarily relate at all or compare at all to the one in Denver, and so ISHAGE has, over the past several years, developed a or had several studies where we've sent multiple samples out to multiple groups and tried using the Sutherland method to standardize the 34 analysis.

More recently, we've been trying to do this with tumor detection assays, and actually, Adrian Gee and his group, we've just completed the first phase of an immunostraining standardization study for breast cancer detection, and again, this is the kind of thing that it's expensive to do this, and ship them out, and buy all the reagents, and this is something that we believe.

It's not as glamorous as gene therapy, but it is a serious need that needs to be funded if we're going to make any of the data from center to center interpretable. So that I'll stop and introduce my colleague Scott Rowley, who's going to give you the ISHAGE perspective on stem cell regulation.

DR. ROWLEY: Actually, thank you, Dr. Shpall. I'll be talking about the FAHCT standards also and not specifically about ISHAGE. ISHAGE, as you saw, is one of the two founding members of FAHCT.

First off, I do want to thank Dr. Harvath for the opportunity to be here as well as the Organizing Committee. I've had many interactions with Dr. Harvath and people at the FDA, and I know that the development of regulations as well as the development of standards can be sometimes painful and political, but I do believe that we're doing our best to protect the health of our community.

Now, this morning, Dr. Harvath briefly reviewed the reason for this meeting, and that is the regulation of unrelated cord blood and peripheral blood stem cell components. And although she didn't go into it in as much detail as this slide here, FDA, in their January 1998 publication, requested that the field, the industry, if you will provide published standards for establishment control such as personnel and facilities, controls for donor selection and informed consent, and finally, also proposed product standards that would be applied to the acceptance of a unit.

Dr. Shpall has already introduced to you the existence of a comprehensive set of standards for hematopoietic stem cell collection and processing, and that the standards form the basis for the FAHCT inspection and accreditation program.

It is our contention that these standards are appropriate to meet the concerns and the interests of the FDA.

My task this afternoon is to review these professional standards published by FAHCT, and I'm not going to go in detail, the book has over 400 individual standards in this, but what I will do is briefly review some of the philosophy behind the standard document that we have.

Our document has four chapters in it as outlined here, and I'm not going to say much about section A except that it does have a requirement for quality assessment, quality improvement that's applied to all aspects of hematopoietic cell processing including the donor collection activities, the cell processing activities, as well as the transplant activities.

Then we have three other chapters here, chapter B which is the clinical transplantation standards, our donor collection standards, and the

laboratory or progenitor cell processing standards, which is section D.

Now, I'm going to go through these individually. The clinical transplant standards. The philosophy behind this is that we, as clinicians, believe that the level of expertise, staffing, and facilities that allow the delivery of appropriate medical care can be defined in a standard document, but that medical practice itself cannot be prescribed. This is the role of the clinician, and the clinician's colleagues.

Examples of these in section B are definition of a clinical program, a definition of what we believe is a minimal size of a program to be accredited by FAHCT, the requirements for Institutional Review Board review of all investigational procedures, requirements for data management, quality management plan as we mentioned, physician as well as nursing staffing requirements, not only the transplant positions, but also other ancillary positions such as the infectious disease positions are important to quality medical delivery, clinical unit standards such as the air handling systems and units, and then other required services such as dieticians, and social works, and a variety of other aspects of a clinical program that we think

are necessary for the delivery of quality medical care.

Similarly, our collection centers standards have the philosophy that, again, the level of expertise staffing the facilities allowing for appropriate collection activities can be defined. We do believe that standards can be specific to the tissue being collected, so there will be different standards for cord blood as there might be different for peripheral blood stem cells, some differences.

The standards do not vary according to the intended use of the collective cells beyond the differences between autologous and allogeneic, so we're not going to say if the cells are being used in a myeloblative setting that they have to be collected in this way. If they're in a nonmyeloblative setting, they have to be collected that way. We're not going to be talking about standards that components collected for the treatment of any particular disease have to be collected in a particular way.

And again, our examples in section C are that we have standards for donor evaluation and selection. We have standards for the facilities in which the component is being collected, and then we have standards for the collection procedures also.

And just to pick on the two subjects of this meeting, cord blood and peripheral blood stem cells, to show you what we're dealing with, we have collection standards that call for informed consent, of course, for both, and that there shall be medical director and adequate facilities for either activity.

Products standards start actually at the time of collection. There will be donor health screening including genetic diseases as appropriate for cord blood. There'll be testing for viral diseases, the ABO/Rh cell count and volume, and we're also calling for clinical outcome as a part of the quality control of the component that's being collected.

Now the laboratory standard philosophy, again, I'm going to repeat myself in that we say the level of expertise staffing and facilities allowing the appropriate processing can be defined. But again, we can write standards that are specific to the complexity of the processing technique, but again, the standards are not going to vary according to the intended use of the tissue that we feel that one set of standards is applicable whether the tissue is used for related or unrelated settings.

And the chapter subheadings in section D include general policies which define staffing and facilities, policies for hematopoietic progenitor cell processing, the cryopreservation, again quality management which is throughout our document, labeling, storage conditions, and so forth.

What we specify when it comes to component standards, something that the FDA is asking for is again, we start off in the collection, the donor evaluation and testing. But we continue into the laboratory processing that there shall be testing components such as cell counts, the microbial cultures, the ABO/Rh. We think that time to engraftment, the outcome of the transplant is an important aspect to the quality of your component.

And of course, the component is labeled to include things like volumes and additives, but what we're not specifying because we don't think it could be scientifically justified because of the many different clinical settings in which these cells would be used.

What we're not specifying is that there's any defining quantity of nucleated cells or hematopoietic stem cells, whether defined by culture or flow cytometric analysis, or even the quantity of accessory cells which are important for engraftment

in the allogeneic setting because an increase of this may allow a decrease in this.

And so the decision about whether a component is appropriate for use for a particular patient belongs in the clinicians purview not in the laboratory's purview.

So in summary for my talk, I want to just end up saying that the FAHCT standards define and infrastructure required for the safe collection, processing and use of stem cell derived tissues. We require an ongoing quality assessment of these activities. But FAHCT standards do not prescribe the use of these tissues.

I'm going to stop at this point and turn the podium over to Dr. LeMader who will speak for the American Society of Blood and Marrow Transplantation.

DR. LEMADER: For those of you who are die hards, the hour is late. I have five slides. It will go quickly, and I would like to echo our appreciation for the opportunity to speak here.

I'm Chair of the Public Affairs Committee of ASBMT and will be speaking in that capacity today.

ASBMT was incorporated as a 501(c)(3) professional organization in 1993 to promote education, research and clinical affairs in stem

cell transplant. There are about 900 members, and it's important to point out that over two-thirds of those members are involved in either clinical practice or clinical research.

ASBMT has taken a leadership role since its inception in trying to define standards in transplantation. As Dr. Shpall mentioned, in 1995, guidelines for training of clinicians involved in transplant were published to establish minimal cognitive abilities and skill sets that are necessary to perform these complex procedures.

Also in that year, guidelines for clinical centers were published to establish minimal proficiencies that are necessary to assure quality care, and as you heard ASBMT participated in the cofounding of FAHCT.

Now, as you heard today, stem cell transplants are well-established as a potentially lifesaving therapy. They may be collected for marrow, blood or umbilical cord, and any of these sources can reconstitute hematopoiesis after high dose chemoradiotherapy.

They are collected on individual patient use, in other words, on a patient by patient basis. The only possible exception to this perhaps is cord blood, and this is somewhat different from blood

banking. The collection procedures are well-defined and well-tolerated.

There are established standards for donor selection that consider both donor and recipient safety. Now, we had some discussion this morning about long-term follow-up of donors, and I don't have long-term follow-up data for the following observation, but the last time I checked, it was a whole lot better to be a stem cell donor than a heart donor. It's late.

The safety issues for the recipient are well-defined. The risk benefit considerations that go into evaluating these safety issues are also part of what the patient goes through in evaluating whether to undergo the transplant itself and are part of the clinical care of that patient.

And as we've alluded to several times today, the stem cells themselves are an integral part of a therapeutic process, in other words, sometimes these cells do more than just reconstitute hematopoiesis.

Now, the ASBMT is somewhat concerned about proposed regulation of this field. If regulations are promulgated, they must recognize that stem cell components cannot be differentiated by use, to reiterate a point of Dr. Rowley's. They must

recognize that the principles of transplant are the same regardless of the component. They must differentiate commercial development and advertisement from clinical care of patients.

They must facilitate research. It is imperative that such regulations improve safety if what we're really after is a public health consideration, and they should not be immune for plans for validation in process and improvement themselves.

If these considerations are not heeded, we fear that regulation of stem cells has the potential to jeopardize an otherwise lifesaving therapy, the potential to impede development of new therapies, very importantly, and I think this was well-illustrated in Mary Horowitz's presentation, the potential to slow dissemination of lifesaving techniques.

There is the potential to interfere with the quality practice of medicine, and I think there can be little argument that regulation will increase costs and in a very heavily overburdened health care system in which our case rates have been cut to bare bones now, it's important to consider cost.

And I think as a sidebar comment to evaluating G-CSF in normal donors long-term thinking

about the large numbers of normal donors that have to be followed and the long-term expenses of that, I'm not arguing that it's not a noble effort, I only question if performing that kind of study would be as useful as investing those monies in other areas that might have a little bit higher yield.

The ASBMT does support responsible, basic and clinical research, the development of appropriate standards, and we welcome this forum and would like to have continued discussions with the agency in regard to development of these standards. And we strongly support the voluntary accreditation of stem cell programs through the foundation for the accreditation of hematopoietic cell therapy. And with that I will close and thank you for your attention.

DR. HARVATH: Before beginning the discussion, I'd like to check with our staff to make sure we can stay in the auditorium until 5:30. Is Joe Wilczek out there? I don't want them flashing the lights on us and kicking us out. I think we're okay until 5:30. So if we -- it's okay. Great.

So just so we set our time parameter before we begin the discussion.

I would like to thank all of you very much. I mean many of us have gotten to know one

another over the last five years, and I have to say that I've really learned a lot. And my colleagues at FDA have learned a lot, and the one thing I heard, particularly from the transplant perspective, is your incredibly real concern about being overburdened by what the FDA has proposed.

And I think you have seen us modify some original proposals in response to comments you have made. The one question that I have for you is that how do you see the entire professional community working together to derive a single consensus and whether you will recognize one another's voluntary professional standards.

Let's say there may be a cord blood bank. That's established. And they may do incredibly outstanding work and have a fabulous track record, and they may choose not to be accredited by FAHCT. Now, if you as a FAHCT accredited transplant center and professionals in that area need to select a unit, would that influence your decision?

And would it influence your decision if they were accredited, let's say, by the AABB or some other professional -- this is one question I would like to pose to the representatives of the professional groups. And also to mention the position we at FDA have to frequently face.

We have to answer to numerous inquiries from Congress. How many tissue banks are there in this country? We don't know the answer because we don't have a registration system. How many stem cell transplants are performed, and we posed these questions to you before, and you know as well as we do, it's impossible to get those actual numbers.

So short of, and we've asked some of your Societies for those numbers too. Those are some of the realities of things we get asked at the FDA. How do you propose that we could collectively work together as a body of diverse professionals who all care about the same thing, which is the quality of the products the people are going to be given?

How do you propose we all work together to achieve that goal because we really are here to listen to all of you?

DR. SHPALL: Well, to answer your first question, I think, if you can't tell, we're very adamant about a few things that we think will reflect optimal quality, and that is the tracking of engraftment, and I would say a priori, any volunteer organization that would be comparable in the depth and breadth of the inspection, and if we were convinced that it was comparable to a FAHCT

inspection, we would not have a problem recognizing each other.

But I think that's the first and major hurdle that has to be overcome, and I think it has to be a substantive agreement and a substantive assessment of true quality from the various societies. But of course, we'd be willing to move forward.

To answer your second question about how we work with you, we understand, I mean particularly the Boards understand that you get asked these questions by Congress, and it would be nice for you to know how many centers there are doing this, and that registration on its face is not necessarily an onerous thing.

The problem is if you look at your documents, it's a phase in, the first step. And we go back to our constituents who say well, what comes next, and that is not clear from our discussions today, and I would hope that as we meet both informally and formally with you over the next couple of years or preferably months, we'll begin to talk about that so that we can go back to the members and say this is truly what it's about, and there isn't a hidden agenda or another agenda coming

next year with the next Federal Register which will take everything we're doing to a screeching halt.

And I think if we trust each other and we move forward that way, I think it's imminently doable.

DR. HALEY: My comment on the FAHCT, here's this thing again, on the FAHCT accreditation standards for clinical centers is I must express my great relief when those finally came out because since I was trained as transplanter and then wound up providing cells and services, I've always resisted, although we're in the position of having the technical capability of supporting a bunch of people.

But I don't think it's ethical to support people who can't really carry on the program, and I've carried this message back many times. So in the professional cooperation, I think that we have a duty to each other to support the programs that serve the patient's needs, and to try to have professional accreditation and professional agreement in the areas where we really think it's necessary, and that it improves medical care.

So I would like to hold up that there is so much to be gained by this kind of cooperation because each of us has skills. Some of us are

heavier in one area, and others are heavier in others. But I would just like to throw into the mix that I was relieved when I saw those standards because I said, "Yes, somebody knows what I'm worried about."

DR. SHPALL: Thank you. And we couldn't agree more with you, Becky.

DR. ROWLEY: Yes, I'm going to not address the question about the different organizations working together. We've dealt with that before. But in terms of our interaction with the FDA, it was in the winter of 1995/1996 that the FDA proposed that cord blood and peripheral blood stem cells would be regulated under the existing models of the ELA and the PLA process, and I think that the industry, specifically the transplant programs, strenuously objected to this because of the impact that this would have on research, that you could not go out and modify a license everytime an investigator changed a protocol and do that in a timely fashion.

And I think the FDA heard us. But there were still some concerns about your talk this morning when you talked about -- you mentioned the term licensure, and we still hear the word requiring IND so that we can look at outcomes, such as you

mentioned GVHD. And we would differentiate and say that, not the licensure but the GVHD aspects of using cord blood versus peripheral blood versus a marrow or any other stem cell component is in the purview of the clinician and not in the purview of the FDA.

DR. HARVATH: Yes. I heard you say that. You feel that the agency is going to step into your decision-making as physicians and health care practitioners as to which graft you're going to choose. Absolutely not. I don't think the agency has ever said they're going to regulate how you perform transplantation.

In fact, it has been focused on. Those things that are already in your existing standards which you already, both groups, have addressed those processing controls and establishment controls. It's the -- I think, when I hear you speak, it's those product standards that really --

DR. SHPALL: You said GVHD. You said it yourself.

DR. HARVATH: -- that really sets -- and that's why the request for data, what is in that Federal Register notice is literally verbatim taken out of that proposal of February 28, 1997 when we had the public hearing. Nothing has been changed in

that proposed approach to sell tissue based products that came out.

If you read through it, it's about a 32, 34 page document. If you read through that, you will see the section there on hematopoietic stem progenitor products from peripheral and cord blood. And in that proposal, it outlines verbatim what is in this Federal Register notice. It's just that this is the official call for data for that.

So what you have is the comment period of two years. So what I would like to say in explanation for what I have heard that there are these concerns that the agency is trying to slip something else in, and that eventually it's going to erode more and more of a practice is to say that in that original document of February 28, '97, it maps out the things that registration and listing. It maps out good tissue practices.

It doesn't go into detail, and it says that the agency would come forward with proposed rules which is what it will be doing. You've seen the first one with registration and listing. The one calling for data, for standards was already discussed in that document in which the organizations had responded to the approach and said

in general that they didn't have a problem with the concept of registration.

But what we would like to hear is if you feel it will not be possible to develop product standards because of what you're saying of concerns for the cellular product and the constantly moving field. Then that is a response to the docket.

If on the other hand there are studies being done such as multi-center studies that are trying to get at minimal criteria, let's say for cord blood units, those are also equally valid data as a starting point. So this open dialogue process, we view, as constantly taking a pulse.

We know that our original proposal, we got your feedback on that. Now, we have this next one, and we're putting forward all of those pieces from that proposal that come forward. None of those pieces have changed. They're exactly following up on what was outlined in that document.

I would just like to explain that because it sounds like you have a concern that we're trying to add more to it without having given public opportunity to comment.

DR. SHPALL: Well, first of all, I want to say, we didn't necessarily agree with the first

document. So your assumptions that everything is okay in that first document --

DR. HARVATH: No, we heard your comments, and in the proposed rules that come forward, we actually reraised those questions based on comments we got to the docket.

DR. SHPALL: So for example, and we need to understand where you're coming from. So your slide today that said product standards and you had graft versus host as number four, what do you mean by that? How is that -- what does that mean to us?

DR. HARVATH: In the proposal for request for data, and also if you look in the Federal Register notice which everyone got a copy of, you will see what are your criteria for determining the quality of a product, the quality of your graft. We know you monitor graft versus host disease. We know that's part of your medical practice as well as your scientific.

So what kinds of data would be unacceptable, and I think you already are answering those questions through your scientific peer review journals. It's just that if you come together as the professionals and say we know that this level is completely unacceptable because we've moved way beyond that, we're asking you to set the minimal

criteria as a group of professionals, not the optimal because we know that that's not possible yet.

DR. SHPALL: But that minimal can change. The minimal marrow number of CD34 has changed, the minimal peripheral blood. You saw today, the minimal degree of GVH. Depending on the patient population, pediatric, adult, there are so many clinical issues that if we were to give you a number, a CD34 number or an MNC number today, and we had a patient that had to go with a lower number and it worked, then the bureaucracy of having that minimal number and having to justify, that's what's making everyone uneasy is that we've moved very quickly and I believe responsibly in terms of moving the graft technology to the clinic quickly and safely.

I don't believe any of us in this room at least want to compromise patient care. But you're asking us to give you numbers that will change in a very short period of time, and I think then the official bureaucracy of having to respond to why they changed scares people away from being innovative and creative.

DR. HALEY: Let me give you an example of something that came to me last week. We have -- I

had a five-year-old child. We've collected stem cells a number of times. This child is very difficult to mobilize. The child has ALL. We have about 1.5 times ten to the sixth per kilogram CD34 cells in the first collections that we had.

Then we remobilized the child, and got again about two times ten to the sixth per kilogram CD34s. But the child relapsed the next week. Maybe that is not the best graft to use. So we were trying to -- we were talking with the clinicians and with the center physician trying to figure out what's the best graft for this patient.

Now, if we have product standards and it has to be at least three times ten to the sixth, we're probably going to kill that child. There certainly are data available saying that anything above one times ten to the sixth in an autologous transplant is probably going to recover in time. I mean there are no guarantees, but that's a pretty safe assumption.

And so that is the difficulty of this field. It's the difficulty of saying minimum product standards is great when you have a red cell, and it doesn't work, and you can throw it out. That's great. But I think that we do all have to be responsible in keeping our outcome data, demanding

our outcome data, even if it's uncomfortable. And then having professional standards and professional review so that all of this works. I think that's where our fear comes.

Somebody's going to come in and say you can't use this for this group, obviously, medically best graft because you don't meet minimum standards because there are so many influential elements. I mean it's very difficult.

DR. SHPALL: And then the difference between that and blood banking is just that, is that AABB has done a beautiful job of reproducible quality management in something that, you know, is the outcome tracked? You go see if the crits go up when you give a transfusion? The vast majority do not. It's a different issue. It's a whole different ballgame, and that's what we're worried about.

DR. HARVATH: Dr. Snyder?

DR. SNYDER: Yes, I think the comments that I'm hearing are all expressing concerns that people don't want to have too much regulation in what is going on in the medical practice, but I think pragmatically, the FDA responds to Congress, Congress has questions that have to be answered, they come to the FDA, and they're going to come to

us. And I think the comments that have been made by FAHCT, ISHAGE and ASBMT are appropriate.

As I mentioned this morning, and I'll reiterate, I think the relationship that certainly the ADD, and I'm sure the Red Cross have had with the FDA over the years has -- that there are difficult times. But things have been worked out. I think that, as I say, the public health has been served, and the agency has provided us with a framework that we are currently using, and I think to the betterment of what's happening in transfusion field.

Transfusion is part of what ADD does. We also do stem cells, and there are collection facilities and blood centers, hospital transfusion services that are doing stem cells, and I think the interest that the association has in looking at outcomes are exactly the same as are shared by FAHCT and its parent organizations.

I feel, speaking for myself, that we do better if we attempt to work together, all of the groups as has been espoused by everyone here to varying degrees, with the agency which has clearly has stated that they don't intend to come in and steamroll over our ability to practice medicine.

But I think that they still have an obligation to established standards and get a single set of standards that I think we all would do ourselves a lot of good if we got together and worked collegially.

One question I would like to specifically ask the people from FAHCT to answer, when the question was asked or when your response was that FAHCT would accept any set of accreditation, any accrediting organization recognized if it had the same degree and depth as FAHCT. Was it referring to depth within, for example, the laboratory setting in vacuo, or were you referring to laboratory and clinical as being a unit which is indivisible?

DR. LEMADER: The -- we live in -- as the FDA deals with Congress, these organizations like ours that deal with other liability issues as you understand. And the standards that we set up were developed by people in the area. They were reviewed by people who work in the area. They were sent out for comment.

One of the key issues, for example, is that we keep reiterating is since we can't define a test to tell how good a graft is, we have to look to the patient. And so we require that laboratories, the clinical programs only get their stem cell

products from FAHCT approved processing and collection facilities.

It's been an area of continued discussion and contention, and so the answer to your first question was no, right now we can't do that, but it's not because we couldn't work out a schema to do that. It's just that we have to assure ourselves that for all these reasons that I just mentioned that the standards were sufficiently similar in their degree for the laboratory and collection process that, in effect, and the inspection process too, that's another issue, is what is the quality of the inspection itself in addition to what the standards are that we then could afford being statused, if that's the correct term to another organization.

There are liabilities associated with that that we've been advised by counsel not to do that until we've assured ourselves in these other areas. I think it's another area that we could work on outside of our discussion with the agency. And we'd be committed to doing that.

DR. SNYDER: This concept of mutual recognition is exactly what we've been talking about and that's why we're all working together to develop a set of standards that would be common to both

organizations. I mean the current standards that AABB has and FAHCT has are quite similar.

There are some areas, I know the VDRLs, the CMVs and so forth as examples, but these are things that can be worked out, and I don't think the AABB, for example, would say that we must have a definition of what an acceptable stem cell package looks like as we do with platelets. And I don't think the agency would expect us to do that. So I think there's much more room for discussion and give and take than some people might feel.

And there are certain concerns related to what the certain branches of government, the people who are the Justice Department, for example, and restraint of trade, that we're in the big leagues when we do stem cells and say who can collect what, and who we won't recognize as collecting what. So we all have to be aware of these issues so we can work together.

So I think this is a very fruitful area for lots of discussion. I look forward to us working together in this area.

DR. LEMADER: The other area, it seems to me, is that I'm not so sure we're all so far apart, really.

DR. HARVATH: Yes. I don't think we are. I think we're much closer than we think.

DR. LEMADER: I think we're not understanding what each of us are saying. For example, I was very pleased to hear Dr. Haley with the ISO 9000 presentation today, and we were talking about not -- about some of the same issues of defining process and how you look at the process of collecting these stem cells and so on and so forth.

It is very likely that we're not understanding parts of, they're going to hit me, but I'll just take care of patients in San Antonio, and I don't live in your world, and maybe I don't understand exactly the processes that you go through.

A lot of what's written in that Federal Register is very scary to me, and what concerns me is not that you're a bad person. In fact, I'm not concerned that you're a bad person. You seem very nice, but unintentionally you may define things that will limit my choices when I sit down with a patient because of an unintended effect maybe because I'm not communicating very well what my issues in the clinic are.

And my decision about whether or not to use in a high risk patient, we have minimal

standards of CD34, but we also have a process by which if we decide to use that minimal standard in our facility, it's a medical decision, but it's part of our process improvement. It will trigger an alert. We'll review what happens in particular with those patients and so on and so forth.

Another example I'm dealing with right now is choice of donors. I read the standards -- I've got a lady who's got lymphoma. She's a young lady, and her sibs don't match. And it's what I think and allogeneic transplant would benefit this patient. Her sibs don't match. Her cousin is a complete match. As I read the standards, not being a first degree relative, if I want to go and do that transplant, I've got to have an IND for that, and I think that's ridiculous.

And so maybe we need to have some more working type meetings where we can understand -- be more sensitive to some of the issues. And I know there are issues well beyond the clinical realm of actually doing stem cell transplants relative to some of the commercial and advertising issues and representing these stem cells can do a variety of miraculous things that they may or may not be able to do. I know you have to deal with that as well.

We are sensitive to that, but I would agree with Ed, wherever he went, the potential is for us to get together and maybe understand each other's problems.

DR. HARVATH: Yes. And I think whenever we receive the letters to the docket, and we see an area where people have expressed concerns such as your concerns about the definition of first degree relative and wanted that expanded. That's why when the proposed registration rule came out, we said comments were received in this area. We invite you to comment further.

And because we did get people saying actually the opposite. We have letters to the docket who felt everybody should, whether they're a first degree relative or not, should conform to the same set of standards.

DR. LEMADER: Some people can't be helped.

DR. HARVATH: But I mean we do have different groups, but the fact is we do have those kinds of comments, and Dr. Stevens has been standing at the mike. Could I let her ask her question and make her comment first, Scott?

DR. ROWLEY: Yes.

DR. HARVATH: Okay. Thanks.

DR. STEVENS: Just a comment from the cord blood perspective which may be a little surprising in view of the discussion. And that is a comment in support of the concept for product standards. Pablo and I have gone around to a lot of sort of fledgling cord blood banks around the world, in fact.

And some are doing quite well and some it's a little bit scary. Here we have a product, in a sense, in a bag that's frozen, but what is that? And how do you know what it is, and how do you describe what it is, and how well are you describing what it is? I think -- we don't know for sure, I mean, we have to be really sure that the people who have frozen this material really have frozen viable stem cells, for example. So what I'm saying is I think there are some issues that can be addressed from a regulatory perspective that do relate to the product.

It's different from some of the concerns that you're raising about your decision-making process, but in terms of the quality of that product, I think in a sense I'm supporting some of the things that Ed Snyder said about there's room for discussion here.

DR. HALEY: Dr. Stevens, I think if someone met the standards that either of our groups

have promulgated, you will be able to look at, you will be able to ask them, they would be obligated if they were approved by either organization to test and tell you exactly what is in the bag, how it was done, what the process control was, what kind of work they did to show that they're going to be alive when you get them out, and I think that you could trust that unit pretty well if they pass either one of our processes. That's exactly what professional standards are about.

DR. SHPALL: We love viability. We like microcells. We'll support that.

PARTICIPANT: I just wanted to make a comment about the issue of cooperation and remind the FDA and I guess all of us that I'm hearing a lot of people being asked to trust implicitly people that it's not intrinsically obvious that that's a good idea because trust is not always that easy a thing.

And so I guess I wanted to say from the now FAHCT point of view, we have been working with AABB and ASBMT and the rest for many, many years. In fact, the first edition of standards that was published as a stand alone edition for the AABB did have representatives from ISHAGE, ASBMT as well as the regular AABB members on that group.

At the time that was published was about the time the FAHCT first edition of standards were published, and we actually had had many mechanisms to all drawn out about how we might work together or how we might have mutual inspections, or go together, or do different things.

Then life happened. ISO 9000 came. We set up our accreditation program. Everybody got busy with developing kind of their own thing, and it really never rose to the top. But I think that the core message is that we've done it before. We've worked with these organizations through the National Task Force, and we worked with the organizations through the development of standards.

And certainly there's no reason that we wouldn't consider doing these things again. You asked us if we would give deem status to somebody. We've not seen standards. We've not seen an accreditation program. We've not seen qualifications for their inspectors.

And so from our point of view, we, and we would not expect AABB to take the FAHCT either without its looking at the standards, the process, and qualifications, and so on. So I think it's a, you know, it truly is a working together kind of

thing that there's no intrinsic reason we can't pursue that.

DR. ROWLEY: Speaking as the President of ISHAGE, we'll say that many of our members do want that there be deem status or a very collegial relationship with other organizations, and that has been a theme that's been repeated to me time and time again by members of ISHAGE.

Writing standards and writing regulations is a political process, and we do keep coming back to, Liana was very brave to be up here with the four of us, that we don't discriminate on the use of components. And a first degree relative versus a third degree relative, to me, a stem cell is a stem cell. A stem cell component is a stem cell component. There's a way of collecting those cells and processing those cells.

But it's up to one of us physicians to decide how we're going to use those cells, whether we use it for a cousin or a sibling or somebody unrelated, we believe that that's the practice of medicine and we'll continue to reiterate this in the political process as the FDA does develop these standards.

DR. HARVATH: Well, on behalf of FDA, I would like to thank all of you very much for your

very insightful comments, for your continued dialogue with us, and I really believe that we can all move forward to accomplish and accommodate your real concerns and to explain any of the concerns that people may have about proposed rules in more detail, and have more dialogue.

And I think this is the sixth meeting since 1995 that we've cosponsored, so I think, a public meeting, that that sort of says that we're very willing to have this discussion and continue it. Thank you, and thank you to all of the attendees for your very useful questions.

(Whereupon, the workshop went off the record at 5:39 p.m.)