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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

UNRELATED ALLOGENEIC CORD BLOOD BANKING AND TRANSPLANT FORUM

Cosponsored by:

Center for Biologics Evaluation & Research, FDA
and
National Heart, Lung, and Blood Institute, NIH

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C O N T E N T S

P R O C E E D I N G S

DR. HARVATH: Good morning. You will have to bear with us in this morning's session because we have many different laptops hooking up to the Power Point presentations.

We will begin with some introductory and welcome remarks from Dr. Jay Epstein, who is the Office Director of the Office of Blood Research and Review at the Center for Biologics Evaluation and Research of the FDA.

Dr. Epstein.

Welcome-Opening Remarks

DR. EPSTEIN: Thank you, Liana. It is a pleasure to be here. On behalf of the FDA Center for Biologics Evaluation and Research, it is my pleasure to welcome the attendees and forum participants to this FDA and NHLBI co-sponsored meeting which will provide us an opportunity to discuss the current procedures in the banking of placental/umbilical cord blood and the data on the use of stem cells in such preparations for allogeneic transfusion.

Since the mid-1990s, CBER has actively engaged in a public dialogue to develop a regulatory framework for cellular and tissue-based products including cord blood derived hematopoietic stem progenitor cells.

The proposed approach to regulation of cellular and tissue-based products was published by FDA in the Federal Register in February of 1997. Since that time, proposed rules have published regarding registration of cell and tissue establishments and communicable disease controls, and pending is a third of a series of regulations that would address Good Tissue Practices.

Consistent with its published approach, the FDA intends to promulgate establishment and processing controls and product standards for hematopoietic stem and progenitor cells. In January of 1998, FDA published a request for proposed standards for unrelated allogeneic peripheral and placental umbilical cord blood, hematopoietic stem cell products.

In this document, the public was invited to submit data and proposed standards to the agency on the notion that such standards might ensure the safety and efficacy of unrelated stem cell/progenitor cell products and that therefore such standards might serve as licensing criteria when the products are brought under the licensing framework.

A workshop was held in September 1998, also cosponsored with the NHLBI, that was entitled Hematopoietic Stem/Progenitor Cells, Discussion of Unrelated Allogeneic

Placental, Umbilical Cord Blood and Peripheral Blood Cell Banking and Transplantation.

That workshop afforded the public an opportunity to voice its opinions and seek clarification of FDA's request for clinical and non-clinical laboratory data for the development of unrelated allogeneic umbilical cord and peripheral blood product standards.

The agency is currently reviewing the public comments to these and other documents to determine whether they have adequately addressed the issues that would be pertinent to rulemaking.

It is understood based on the proposed approach that if sufficient data are not available to develop such licensing standards after a reasonable period of time that was initially proposed as three years from the January '98 notice, that unrelated allogeneic stem cell products would become subject to premarket controls including investigational exemptions.

Currently, cells that are more than minimally manipulated already are subject to premarket approval under existing policies regarding somatic and gene therapy products and will continue to require IND applications for investigational use and licensing approval.

We anticipate that the information obtained from presentations at this meeting will assist the FDA in

developing standards for unrelated allogeneic cord blood cell products, so I would like to thank all of the individuals and organizations who already have responded to FDA's request for proposed standards by submitting data and comments pertinent to the use of cord blood products and the outcome of transplants to replace bone marrow.

I am confident that this scientific workshop will serve a useful purpose both to clarify the state of the art in allogeneic use of cord blood stem cells and to facilitate the policymaking process.

So, I welcome you warmly and I wish you a very successful meeting over the next two days.

Thank you very much.

[Applause.]

DR. ALVING: Good morning. I am Dr. Barbara Alving representing the NHLBI side of the house. I am the Director of the Division of Blood Diseases and Resources, and I would like tell you a little bit about our division and why we are so very interested in cord blood.

Within our division, we have at least four major programs, and these are all representing extramural funding to a total of about $300 million a year.

One of the programs is in thrombosis hemostasis, another is in sickle cell disease and hemoglobinopathies again for which cord blood is used for transplantation.

Another is in transfusion medicine, and a fourth is in transplantation. As you know, we have multiple interests in trials in transplantation, and one of our major trials is in the use of cord blood, the COBLT trial.

We have two program directors within the division. Dr. Chuck Peterson oversees sickle cell disease, cellular hematology, and hematopoiesis, and in fact just last week we were discussing transplantation in patients with sickle cell disease and other hemoglobinopathies. We had a meeting to talk about the ethics and the timing of transplantation with these patients.

Our second major program director is the Director of Blood Resources, and we now welcome Dr. Liana Harvath as our new Director of Blood Resources. She has taken off her FDA hat, we are getting her used to the NHLBI hat, and we are delighted to have her with us.

She will be overseeing transfusion medicine, transplantation, thrombosis, and hemostasis, and Dr. Liana Harvath, wearing her FDA hat until approximately a week ago, worked with our Scientific Research Group leader, Dr. LeeAnn Jensen, who heads up our transplantation efforts, to develop this workshop.

So, you can see we are all one big happy family.

As my boss, Dr. Claude L'Enfant, Director of NHLBI, reminds me, NHLBI is not the FDA. We work on

research efforts, we try to advance the knowledge both basic and clinical, but we work hand and glove with the FDA and we greatly appreciate the opportunity to work together with you, the extramural community, and with the FDA to hopefully provide the safest product that we can and to use it in the wisest way, and to develop perhaps new scientific ways to extend and expand the use of cord blood to the fullest.

So, again, I welcome you to this workshop.

[Applause.]

Introduction and Goals of Workshop

DR. HARVATH: Thank you, Dr. Alving.

Good morning and welcome. On behalf of the Organizing Committee of this meeting, we thank you for taking time from your busy schedules to attend and participate in this cord blood banking and transplantation forum.

Today's meeting is the fifth in a series of workshops on hematopoietic stem cells and that this workshop, as Dr. Epstein and Dr. Alving have mentioned, is cosponsored with the Center for Biologics Evaluation and Research of the FDA, and the National Heart, Lung, and Blood Institute of the NIH.

Since 1995, we have held public meetings almost every year to encourage the exchange of scientific information.

These meetings have included the December 1995 workshop on cord blood banking, the February 1996 workshop on peripheral blood, hematopoietic stem cells, the September 1997 workshop focused on ethical issues in cord blood banking, and the September 1998 workshop that Dr. Epstein mentioned, which was focused on unrelated allogeneic peripheral blood and cord blood hematopoietic stem and progenitor cells.

I would like to briefly just go over the objectives of this forum. First, we would like to have an open discussion of the current status of unrelated allogeneic cord blood banking and transplantation.

This is a rapidly emerging technology with a great deal of exciting scientific data coming forward nearly every year and for certain since our last workshop in 1998, there have been numerous other sets of clinical experience with these products, and we will hear about those at this meeting.

The second is to discuss the scientific issues regarding the characterization of cord blood grafts. As Dr. Epstein mentioned, this is important for the FDA's mission in assessing the data for the safety and

effectiveness of these products with the ultimate goal in developing product standards.

The NHLBI is particularly interested in identifying future research directions both at the basic science level and those that are of translational research natures.

As outlined, a couple of specific goals on this slide, and as mentioned already, we intend for this conference to be an open public dialogue for the collection of information for the development of unrelated allogeneic cord blood product standards, and secondly, to identify areas that are in need of research and further development.

I would like to take a few moments to publicly acknowledge a number of individuals whose contributions were critical to this conference. I have put in quotations "committee," because we did not have an official working committee, however, this loose structure of committed individuals was responsible for putting together the ideas, that is, the topics, the recommendations for speakers, as well as some very specific discussion points that we hope to have addressed by all of the participants who are speakers in this conference today and tomorrow.

There were a number of individuals who submitted data to the FDA in support of product standards. Some of those people, in fact, to our knowledge, most of the people

who have provided scientific data will present a summary of that information at this meeting, and we hope that they will identify areas that are in need of further investigation.

Collectively, the NIH and the FDA believe that useful cord blood product standards will result from the concerted public effort in providing data that established the safety and effectiveness of cord blood for hematopoietic reconstitution.

In that effort, the individuals who contributed to this, I would like to personally thank. They include Dr. Joanne Kurtzberg of Duke University, Drs. Pablo Rubinstein and Cladd Stevens from the New York Blood Center, Dr. John Wagner from the University of Minnesota, my colleague, Dr. LeeAnn Jensen, who has done an enormous amount of work in getting most of the participants here, and she will further that contribution by keeping our speakers on time today; my colleague Dr. Ellen Lazarus, who recently joined the FDA in the Office of Blood Research and Review, and I would like to especially thank our administrative assistant, Kathy Brasier, who is the one who tirelessly works behind the scenes to make things happen for all of us.

In addition, we acknowledge and thank a number of scientists who recently attended the June 2000 ISHAGE, as

we refer to ISHAGE meeting in San Diego. Many of them at that meeting came forward to us with specific suggestions for topics, especially in the area of emerging technologies involving the identification of hematopoietic stem and progenitor cells and the field of ex-vivo expansion.

I would like to end with a few housekeeping details. As you can see from the agenda, we have a full packed conference agenda. In order for all of the speakers to have adequate time to present their data, we ask that the speakers stay within their allotted time.

As I mentioned, Dr. Jensen will serve as the official timekeeper for this conference, and she will indicate when the speaker's time is finished.

We will have all of the discussions held until the discussion panel. This will ensure that all speakers have time for their presentations and it will help us focus the opinions of the collective group of speakers for each of the sessions.

I would like to also mention that this conference is being audiotaped and an unedited transcript will be posted on the CBER web site, so we ask that all participants identify themselves before asking any questions during the panel discussion.

For participants who are anticipating any messages, it is our understanding that they will be posted on a table in the lobby.

Finally, it is a pleasure for me to introduce Dr. Ellen Lazarus who will co-moderate the first session with Dr. Kurtzberg. Dr. Lazarus joined the Division of Hematology in the Office of Blood, Research, and Review of the FDA one year ago.

She comes to this program eminently qualified with a background in pathology and transfusion medicine, and is highly qualified to serve as the new FDA scientific point person for minimally manipulated hematopoietic stem and progenitor cell products.

Dr. Lazarus.

SESSION I

EXPERIENCES OF UNRELATED CORD BLOOD BANKS

Co-Moderators - Drs. Joanne Kurtzberg, Ellen Lazarus

DR. LAZARUS: Thank you, Liana, for that glowing introduction.

It is now my pleasure to introduce Dr. Cladd Stevens, who will be sharing with us this morning the New York Blood Center experience in unrelated cord blood banking.

NYBC

DR. STEVENS: I am going to present some of the experience that we have had at the New York Blood Center in establishing an umbilical cord blood bank and some of the information that I think may be relevant to issues of standardization and a definition of the quality of cord blood units for transplantation.

Actually, what I am going to do is first present sort of an overview of how we have established the bank and how we carry out the collections and gathering of information and testing, and then provide you with some of the data of things that we have learned.

This slide really summarizes the sort of history of our bank starting with a NHLBI grant in 1992 with first collections about five months later and the first transplant in August 1993.

We operated under the NIH grant for three years and then in June of 1996, received an IND exemption from the FDA, and we have been operating under this exemption ever since.

By November of last year, we had shipped our 1,000th unit and opened a second collection site to try to expand the numbers of units that we were collecting.

This summarizes our activities to date. We have collected a little over 11,000 units, carried out almost 10,000 searches for patients around the world. We have

shipped a little over 1,000 units to 1,027 patients. The difference in the number reflects a small number of patients who got second back-up grafts when the first one failed to engraft, or a small number of individuals who got more than one unit at the same time.

So, the basis of what I am going to present to you this morning and what Pablo will present later today and tomorrow comes from the information that we have gathered both from the collection and testing of the units and the evaluation of the outcome of the transplants.

Just to go through first what we do in terms of carrying out the banking itself, this slide shows you a brief outline of our hospital SOPs from collection of the unit from, in our case, from the delivered placenta, so that means that we have a team of trained individuals who are doing the collections in the hospital, procedures for cleaning the cord with the primary objective to remove maternal blood and contaminating organisms that might be on the surface on the cord and could contaminate the unit.

The next steps in this process in the hospital is the collection of the maternal written consent, review of the mother and infant's medical record, interview of the mother, and collection of a maternal blood sample, and in our case, we also have collected saliva from the infant for cytomegalovirus culture.

What is really complex about this donor situation, and I think maybe one of the most complicated donor systems in the world to my view, is that this process takes place, it involves two people, both the mother and the baby and the delivered placenta in this case, but the collection of the associated data over a several day period of time, and all of the stuff--all of the stuff--all of this information and samples must be linked accurately.

We have a limited number of apriori exclusion criteria: known HIV or hepatitis B infection, most women are now screened for these viruses during pregnancy - stillbirth, known genetic disease in the infant at the time of collection, those units obviously are voided.

We initially had no minimum on the collected blood volume, but beginning in August of '93, we had a minimum of 40 ml, and beginning in January this year, we raised that minimum to 50.

Finally, lack of maternal consent, those units are excluded.

The goals of our hospital SOP are several fold. One is to maximize the number of units that we collect and also the volume. As you will see, volume is a critical issue in determining cell dose.

We want to exclude the highest risk units, and that is the reason in particular why we exclude units from

HIV-positive or hepatitis B-positive mothers. We want to minimize contamination of the unit with mother's blood or infectious organisms. We want to minimize identification errors.

As I emphasized on the previous slide, here we have a process that takes place over a period of several days and we want to minimize the chance that we take, for example, the wrong blood from the wrong mother, or interview the wrong mother or review the wrong medical record, and that is a very important aspect of this process.

Finally, we want to assess maternal risks. I indicated that we have a relatively limited number of apriori exclusion criteria, and that means that we want to evaluate the risks that the mother might have either an infectious disease or there might be a family history of genetic disease that we should be alerted to.

Screening strategies. We carry out a medical record review before the collection to identify mothers who are HIV- or hepatitis B-positive, or any prior knowledge of genetic disease.

We assess the infant's health immediately after birth, before the collection, and then, as part of this whole process, we record information about the collection procedure itself, complications during pregnancy,

complications during labor and delivery, family history of genetic disease, family ethnic background, which is relevant both to genetic disease and the HLA typing issues, mother's infectious disease risks, and the newborn's clinical course prior to discharge.

Our testing strategies may be a little bit different from some of the other banks, and I am going to emphasize a couple of aspects of our testing strategies.

First, in terms of HLA typing, we have taken the tack of testing both the mother and the cord blood unit, as well as the segment attached to the unit prior to shipment for any transplant that we release a unit for.

The testing of both the mother and the unit allows us to do a couple of things. First of all, it helps in the HLA typing, as I will show you with some of the data that we have gathered in comparing the typing of the mother and the infant.

It helps to make sure that the sample that we collected, in fact, was from the right mother, and the typing of the segment makes sure that there was no error in terms of the processing itself and that the unit that is in the bag actually is the same as the sample that we tested in the original typing.

The infectious disease testing is pretty standard. The same tests are used for routine donor

screening, and we do this through the New York Blood Center's testing program. Other infectious diseases that we have been interested in are cytomegalovirus, so we are testing IgG and, in the case of the mother, IgM antibody.

We have been interested also in hepatitis B, so we have tested anti-surface antibody in the mother and the cord blood. We have carried out CMV cultures in the infant's saliva, and then we do bacterial culture and sensitivity cell counts in some cases colonies, and more recently, CD34 cells, and then test for hemoglobinopathies.

One of the things I want to emphasize so far is the issue of preventing errors, particularly preventing errors in identification of the mother and infant, and in labeling. So, this slide outlines some of our labeling principles.

First of all, we use bar coded unique I.D. labels, a preprinted set that is assigned at the time of collection and applied to the unit data forms and all specimen tubes, and that I.D. number then links the unit, the mother, the saliva sample, and the data on the forms.

Another principle is that we generate new labels from old labels using the bar code I.D. number, so we do this for the test aliquot, stored specimens, and the final freezer bag, and those are generated by electronically scanning the old label.

We have a one-at-a-time rule, and that starts with the collection process itself. We collect one unit at a time, carry out all the labeling one at a time, and it goes all the way through to the end of aliquotting and processing. So, we have tried to avoid batch labeling and batch processing and batch testing.

Data entry is from scanned-in labels, so we minimize the chance that there is an error in the entry of the data.

Processing also follows a one-at-a-time rule, again, to try to minimize the chance of mislabeling during the processing itself.

Partway through this seven years of experience, we initiated a procedure to reduce the volume of the stored unit to a uniform 20 ml, and this involves addition of Hespan with a soft spin to remove red cells, hard spin to remove excess plasma, and the addition of the cryoprotectant as the final step.

This slide just summarizes the aliquots that are taken at various points in the processing. A total of 7 ml taken pre-processing for test aliquots, the cell counts, HLA, and ABO/Rh and hemoglobinopathies, post-processing test aliquots, again to repeat the cell count, so that we know what actually goes into the frozen unit, and bacteriology samples from the plasma.

Then, we have storage aliquots from the placental cord blood itself, cells, a couple of aliquots, DNA, and plasma. In addition, we have storage aliquots parallel, but not the same numbers from the mother's samples.

Another important component, we think, of cord blood banking is having information about the follow-up on the recipient of these units. The outcome of the transplant is actually the ultimate test of how well the bank is performing. In following this principle, we have asked the transplant centers to provide us with information.

As you will see in data that Pablo presents tomorrow, the transplant centers have been remarkably cooperative in following through on this, but we document the condition of the unit at the time it is received in the transplant centers.

We asked that the centers give us a transplant report, which tells us about the patient characteristics at the time of the transplant, the thawing and transplant procedures, any test results that they might have carried out on the thawed unit, and immediate side effects that the patient might have experienced.

Then, we receive follow-up reports at 100 days, 6 months, 1 year, and then annually thereafter to look at post-transplant treatment, a variety of endpoints that help

us assess how well we are doing in terms of these cord blood transplants, and also chimerism, which either the centers themselves may do or we will accept samples and test free of charge to establish that indeed the cells that came back in the patient are those of the donor, and not the patient's own cells.

Now, what makes a good placental blood, cord blood unit? I think this is the key issue from a regulatory position, and I think that is really the fundamental issue that the FDA has asked us and the NHLBI has asked us to address here.

What I am going to do now is present you with additional information that we think is relevant to this issue. What defines a good unit can vary, as you will see I think, through the presentations that are made here today. I think that this definition actually translates into our ability to assess the risk versus the benefit to the patient.

Here, we have a situation that is very different, for example, from blood transfusion. A patient who has a life-threatening condition, who may succumb without this transplant, and the clinician is in the position of having to assess the risk of any particular unit and weigh that against the benefit that the patient might have from a chance to have a transplant, and this involves both the HLA

typing, the testing of that unit, evaluation of risks that the mother might have reported.

So, our placental cord blood units are defined by a variety of factors. One is the HLA type, cell count and viability, maternal risk factors, infectious disease serology. In our case, we are doing CMV serology and culture, bacterial culture results, and where it is relevant, genetic disease testing.

Now, I am going to show you data which address each one of these issues in turn.

First of all, HLA typing. I made a point earlier about the fact that we are typing both the mother and the placental blood itself. One of the things that we have done is looked for discrepancies between the mother and the baby.

The mother of these cord bloods, in a sense, should match the HLA type of the cord blood with at least one antigen at the AB and DR locus. Now, out of more than almost 8,000 mother-baby pairs that we have examined at this point, most of the mother-baby pairs do indeed match, 96.8 percent but 3 percent, there was a mismatch at one or more locus with the mother, 3.2 percent.

Most of those were a single antigen mismatch, either at the AB or DR locus, 38 had 2-antigen mismatch and 10 had 3-antigen mismatch.

Now, what do we get when we look at this in more detail? Here are the single antigen mismatches, and this is sort of a work in process, but I want to illustrate a couple of points. Forty-seven of these were retyped and we found that we could change the typing and now they were a match. So, this was a matter of definition of the HLA type.

Twenty-four were splits, that is, they were related typings, but the baby's typing had been taken to a higher level of resolution, and so these were really not mismatches. We have some that are pending. Half of those that are still pending retyping are cases in which there is a blank in either the mother or the baby. So, one possibility is that there is an antigen that was missed or there is a possibility that some of these are also explained by splits.

What about the small number in which there are two or three antigen mismatches? Seven of those have been repeated and corrected, so this in a sense is an advantage for being able to look at the mother's typing, and, in fact, having seen this data we have now implemented a strategy in which we don't look at these results in retrospect. The lab does this on an ongoing basis, and so they can make corrections immediately.

Five with 2 or 3 antigen mismatches, the mother had an egg donor, and so you wouldn't expect that the mother and baby would match. Seven were errors, and what kind of errors are these? Wrong mother specimen, we have a couple of examples in which the wrong mother sample was drawn, and one of these, in fact, was an example in which the wrong mother, the so-called "wrong" mother was HIV-negative, but the cord blood unit itself tested HIV antibody positive, just to illustrate the importance of making sure that we have the right mother when we draw these samples.

Other errors are mislabeling. Despite the SOP that we have in place, the do one-at-a-time, we do have some examples where SOPs were apparently violated and labels were applied in batches, and switches in the lab in the testing itself.

We were asked to provide information on the volumes collected, and this just shows you our experience over time. The initial period when we didn't limit the minimum volume, our average was about 60 ml, and it obviously increased when we raised the minimum, and now, with our 50 ml minimum, we are up to an average of 78 ml.

The volume collected is clearly important in terms of the issues of cell dose, and I think every one has the same kind of data. It's an obvious point that the

volume is critical to the total cell dose that you will have available for the patient.

What about processing? I mentioned that we have a process to reduce the volume of the units stored to a uniform 20 ml. That processing can result in loss of cells, and this slide shows you the pre-processing total nucleated cell count compared to the post-processing cell count. There is obviously a correlation, but what we see here is that we do have a decrease on average about 9 to 10 percent compared to the pre-processing result.

Why is cell dose important? The clear issue has to do with engraftment, and the more cells you have in the unit that has provided for the graft, the more rapidly the patient engrafts, and this is shown by a Kaplan Meier analysis here.

This is also obviously the ultimate test of the viability of the cells that you have put away in the freezer. One of the issues that has arisen and will be addressed later by Peter Wernet is the question of nucleated red blood cells in cord blood.

We know that there are nucleated red cells commonly in cord blood, and the numbers vary considerably. One aspect that we can contribute to this is a study that we carried out looking retrospectively at peripheral smears from units that we have collected in the past, and we did

this, both a random sample and all of the units that had been transplanted to patients through the end of 1999.

I am going to show you the data from the transplanted units because it is relevant to the issue of potential impact on engraftment. What you see here is something that may be a little bit surprising, and that is, I just showed you the total nucleated cell dose correlates with time to engraftment, but so does the total nucleated red cell dose.

This is the lower dose, and we see more rapid engraftment when there is a higher dose, so to speak, of nucleated red cells. Obviously, the red cells are not engrafting, but it's apparent that there is a correlation between these immature cells and the more immature cells that are the source of engrafting tissue.

That is shown somewhat indirectly with this slide which shows you the correlation between nucleated red cells and numbers of CD34-positive cells with an r-squared of 0.51.

Now, I want to turn just briefly to issues of infectious disease risk. I mentioned that we have limited apriori exclusion criteria, and so our strategy has been to do the best job we can to try to assess that risk. The infectious disease risk that we are concerned about is

whether the unit itself is infected, and that is also a somewhat complex issue.

The risk that the unit is infected is related to the risk that the mother might be infected or might have become infected, and still test negative at the time of the collection, and how can the unit itself become infected.

Well, there are three possibilities. There can be in-utero transmission. There may be perinatal transmission, and we don't always know what that route is specifically, but we can imagine that perinatal transmission could involve contamination of the placental blood or the fetal circulation during labor and delivery, and there is a possibility of contamination during the collection procedure itself.

This shows you a slide broken down by ethnicity, comparing the history of risk factors for any blood or sexually transmitted disease in Caucasian, Hispanic, African-American, and Asian donors.

You can see there is some variation as you would expect. Nearly 30 percent of Asian and African-American donors reported some risk factor. I am going to skip this because I am already getting the five on that.

The risk factors that account for these blood or sexually transmitted disease risk factors vary by ethnic

group. Transfusion, tattoo, body piercing was more common in African-American and Hispanic.

Contact with blood and body fluids was more common in the Asian, and these were mostly people who were in health care professions. History of hepatitis was more common in Caucasians somewhat surprisingly.

Viral marker seropositivity correlated with a blood or STD risk factor for hepatitis B, HIV, and hepatitis C, not for HTLV, and this correlation suggested about a two- to three-fold higher risk. Now, that's for test positive. Presumably, that also translates into those that were test negative, which is what we are concerned about in asking about risk factor.

Let me skip this with the hepatitis B.

The other issue that is somewhat controversial is whether or not we should reject units from mothers who have repeat reactivity, but who are negative on confirmatory testing by RIBA or western blot.

This is the standard procedure for blood donors, but one of the things that was interesting to us is that despite the correlation with known positivity, there is no correlation with the repeat reactives and a history of STD or blood exposure.

One of the things we did find a correlation in particular with HTLV is with maternal parity, so some of

these nonspecific reactions may be related to maternal parity.

Let me turn just briefly to cytomegalovirus since I think this is one of the aspects that has maybe been unique to our data. The prevalence of IgG antibody in the mothers varies also by ethnic group, about 45 percent seropositive in the Caucasians, but up to 90 percent or nearly 90 percent in the African-Americans and Asian populations.

What about IgM? IgM also varied by ethnicity, but the highest frequency in the African-American and Hispanic populations, and this parallels data that has been reported before.

We have done cultures on a little over 6,000 saliva samples from the infants who have participated in this study. We have only had 12 positives, which is a little bit lower than what has been reported in the literature.

The literature reports about a half to 1 percent incidence of congenital infection. Overall, we have only 0.2 percent, but as has been reported in the literature, it is more commonly occurring in African-American and Hispanic infants.

Culture positivity correlated with the IgM antibody status in the mother, but this also is relatively

low compared to the literature. Most of the literature suggests that about 25 to 30 percent of mothers who have a CMV infection during pregnancy will transmit to the baby, but our rate at least as documented by CMV IgM antibody detection is only about 2 1/2 percent.

There is a strong correlation, as I say, with maternal IgM, but we are now also getting a few culture positives when the IgM antibody is negative.

CMV infection in the patients is strongly correlated, as we all know, with the patient's pre-transplant CMV antibody status. There are a small number of patients who were CMV antibody-negative prior to transplant who we have reports of CMV infection.

What I am going to do is take this data a step further and look at the relationship to the donor's maternal CMV antibody status. So, that takes that previous slide and splits it by maternal CMV IgG, and you can see there is absolutely no correlation with the mother's CMV IgG status.

IgM also is not significantly correlated. There were nine cases in which the patient was IgG antibody-negative and got an IgM-positive maternal IgM-positive unit, and none of those patients reported infection.

This shows a little bit higher incidence in the patients who started out as seropositive before the transplant, but that's not significant at this point.

Just briefly, a little bit about bacterial cultures. We have cultured both aerobic and anaerobic for 14 days with subcultures for identification and sensitivity. Our culture positivity rate has varied over time and this slide shows you those in which there is an organism identified and a small proportion with no growth on subculture.

What we did here was change the technique for cleaning the cord. Initially, we had used a simple process of just an alcohol swipe, and then we implemented a procedure to simulate the procedures for cleaning a blood donor's arm in cleaning the surface of the cord.

That change in procedure had a dramatic effect on our bacterial isolation rate. I am going to skip these issues in case we have to come back. One of the things that I think was interesting is that transplant centers are also reporting to us the outcome of bacterial cultures on the units that they are transplanting the post-thaw cultures.

There were eight units in which we had a negative result, but the transplant center reported a post-thaw positivity. In most cases, these are units that were

stored in bags that had a tendency to crack. In most cases, the centers reported that the bag had indeed cracked, they observed a crack and leakage.

Four of the units that were transplanted that had bacterial culture positivity, of those, two were negative on post-thaw and two had the same organism identified. Of interest are the units that registered positive in our system, but on subculture came back as negative, no organism identified. All five of those that have been transplanted, there was no organism cultured on the post-thaw.

Two more slides. This shows you sort of a summary of the data on both risk factors, repeat reactivity, viral serology, and bacterial isolation by ethnic group, and shows you the potential impact of the criteria that you might use for excluding units.

It's an important issue in terms of our transplants because one of the things that we have observed is even though we have reported this data to the transplant centers, it has had very little impact so far on the selection of the units for transplants, in other words, the transplant centers are weighing this risk-benefit ratio and deciding in favor of taking the unit for transplant.

In fact, in terms of the outcome, there has been no difference in the outcome at least in the first year post-transplant.

I go back to the final point, which is the issue that we are having to address here, which is what makes a good placental cord blood unit, and I think it relates to all of these issues of how we define the unit and how those centers actually carry out the testing and identification of these definitions.

Thank you very much.

[Applause.]

DR. LAZARUS: While we are figuring out the computer hookup, I can introduce our next speaker, who is Dr. John Fraser from the UCLA Department of Medicine, Division of Hematology/Oncology, and he will be presenting the COBLT experience in unrelated cord blood banking momentarily.

COBLT

DR. FRASER: We just have to wait for the Mac to boot up. I would like to thank Cladd for that excellent introduction because, quite frankly, as with most of the other people in this room who are doing cord blood banking, a lot of the work we are doing is very closely modeled on what has been established in New York.

This is not a good sign. I am hoping I didn't have to do 23 slides without any slides. [Pause.]

This isn't looking good. My first slide.

What I am going to do in this presentation is describe the cord blood units we have available in the COBLT bank. [Pause.]

I am happy to go ahead without any slides if you are happy to listen to me. We will give it one more try. Before anybody came in this morning, we had about three or four tries, and it worked on the fourth try. [Pause.]

I feel sorry for the person who is going to follow me, Donna, right? You are going to have to switch it all back.

As I said, I am going to describe the units that we have in the combined COBLT cord blood banks and the processes and testing that was performed on these units in order to determine that they were, in fact, appropriate and suitable for allogeneic transplantation.

There is no way in the time remaining to me that I am going to be able to cover all the details in our standard operating procedures or go through all the questions we ask in the medical history, all the data we extract from the medical records. For those of you who are interested in that degree of detail, all our SOPs and all

the data forms are on the World Wide Web at the Emmes web site, emmes.com.

At the present time, there are two cord blood banks active in the COBLT program, the Carolinas Cord Blood Bank under Joanne Kurtzberg at Duke, and the UCLA Umbilical Cord Blood Bank under my direction in Los Angeles.

A third cord blood bank here in Georgetown University was previously active, is no longer active, and its units have been rolled into the Duke inventory.

As of last Friday, these combined banks have generated a search registry of 4,587 cord blood units. The average volume and collection for these units was 78.8 ml with a range of 40 to 235.

I should note, however, that this is based on a calculation of weight using the rather convenient assumption that 1 gram of umbilical cord blood is 1 ml of cord blood, so what we really have here is the median weight of cord blood of 78.8 grams. This is net of anticoagulant, this is actual cord blood, no anticoagulant in here.

This cord blood yielded an average viable total nucleated cell count pre-processing 11.7 times 10^8, range 3.2 to 65.1. The post-processing cell count was 8.9 times 10^8, 2.5 to 40.1. The average total CD34-positive cell dose in these units is 2.6 million, range 0.01 to 72.3, and the

average total CFU/GM content was 900,000 with a range 0.01 to 13.4.

As I said, we followed very much the New York practice with a couple of significant differences. We don't have, like New York, a whole lot of apriori prenatal exclusion criteria because, for example, we do not collect umbilical cord blood from multiple deliveries, twins or triplets, where there is a known infectious disease or a risk history, and known prenatal diagnosis of congenital abnormality in the child or where the mother is less than 18 years of age.

In our program, informed consent is obtained from all women prior to delivery, sometimes several weeks before delivery and sometimes just an hour or so before delivery, but under no circumstances is informed consent obtained during active labor or when the mother is under the influence of mood-altering medications.

We obtain a family medical history. This includes parental ethnicity, the family medical history in terms of things like a history of childhood death, if the children in the family, extended family, died under the age of 10 years of age, the mother is excluded from donating cord blood.

This does create some problems. A lot of the cord blood donors we have in Los Angeles, we find we have

Vietnamese families who have come here and children may have died under the age of 10 in Vietnam during the war. They are, under the current criteria, excluded because we do not know the cause of death.

This is not a high frequency event, but it is certainly frustrating for a bank that is trying to maximize minority involvement. We also exclude for a family history of cancer and leukemia.

We screen for bloodborne disease risks using the same questions or most of the same questions that are used by the American Red Cross in screening conventional blood donors. These are largely looking for blood and tissue exposures, malaria, travel to malaria areas and things of that nature, drug history, incarceration for more than 72 consecutive hours, things of that nature.

We also screen. We ask the mother for a history of inherited diseases on both sides of the family. We are looking obviously for blood diseases, red blood cell diseases, white blood cell diseases, platelets, metabolic storage diseases, and other.

Other is obviously a bit of a catchall, and we very frequently get diabetes, hypertension, those sorts of things you might expect, and of course those are not of themselves exclusion criteria, but you do sometimes get something out in left field, and that is always reviewed by

our medical director, Dr. Mary Theresa at UCLA and obviously Joanne can do her own reviewing at Duke.

We are also, of course, very interested in the history of this particular pregnancy. We are looking for consanguinity, are the mother and father related other than by marriage, the genetic relativeness.

We do not collect umbilical cord blood when there is an egg donor unless we can get consent and a full medical history from both the genetic mother and the birth mother, and that has happened on a small number of occasions certainly in our program.

So, we want to get the full set of data and a consent on both the women involved in this delivery, and we have had one situation where the mothers were cousins, and it was a family situation. It is often quite easy to get that.

We are also looking for drug use by prescription and nonprescription drug use during the pregnancy and any positive results coming here other than the obvious prenatal iron and vitamins are reviewed by the medical director of the program.

We get consent to review the perinatal medical records of the mother and child. This includes screening for maternal and/or infant fever. In our hands, that is defined as greater than 38.5 degrees centigrade.

We are looking for application of perinatal antibiotics. That is not in itself an exclusion criterion because women who are group B strep-positive get prophylactic antibiotics and that is not a problem, but if they are getting antibiotics we want to know what they got, we want to know why they got it, and then we can make a decision on whether or not to exclude.

In our program, any congenital malformation, be it as little as a two-vessel cord or maybe even cleft palate, we are not sure about that one at the moment, but some, even cleft palate, have been excluded. Obviously, more gross malformations are exclusions.

We supplement and confirm the medical history information provided by the mother by reviewing her chart for all prenatal testing. We are looking for hepatitis testing and HIV testing which are performed on most, if not all, mothers born into our programs, and we are looking for medical history.

Mothers report histories of smoking and alcohol. That is not an issue, but they also may report differently to their physician than to us, and we want to make sure that if there is another way to get the same data in a different way, we want to get it twice, so we can confirm it.

We also--and this is exclusively for mothers over the age of 35--we want to see in the chart results of an amniocentesis or chorionic villus sampling to make sure that that is normal.

At the present time, if the mother is over 35, and there is no test indicating that that child is chromosomally normal, that cord blood unit is excluded from our bank. In this day, where a lot of professional women are having their babies later, this does create a certain amount of stress in some of the mothers. Two of my children have had their cord blood units banked, and both of them fell into this category.

However, to cover this eventuality and the fact that we do have blood film on the umbilical cord blood which we can use for, for instance, in situ hybridization to look for things like Down's syndrome, we are holding such units in extended quarantine to see if maybe later on we can justify including those units in the inventory and leaving the decision on whether to look for the chromosomal abnormality to the transplant physician, but I want to point out at this time, in the 4,587 units we have, none of them come from a mother over the age of 35 where there was not normal chromosome test.

We have approximately 300 units being held in extended quarantine because the mother was over 35 without this criterion being fulfilled.

As in New York, cord blood is collected after the delivery of the placenta, and is performed by designated dedicated banks staffed with affiliated hospitals. These are full-time staff from the Duke and UCLA programs.

Cord blood is collected into collection bags containing 25 ml of CPD-A anticoagulant provided by Pall Medical. I don't think this is showing up too well, but most of you are probably familiar with the bag. It is sort of a long conical sort of shaped bag to ease the centrifugation, the separation of the units, with two needles, so that if you insert the needle into the bottom of the umbilical cord and it is clotted, you can seal off that needle and use a second needle without having to worry about clots in the needle blocking up.

An ISBT 128 compliant bar code is applied to the cord blood unit and to all paperwork at the time of collection. That is a preprinted, as in New York, a preprinted label set, and those labels follow that umbilical cord blood unit throughout processing and cryopreservation with the same bar code being applied to all paperwork, all samples, all aliquots, all bags. This

is quite a big sheet of labels, and this is just a sample of the kind of label we are talking about.

Our volume limits differ slightly to that described by New York. If the cord blood unit is less than 40 ml or--of course, I am using ml and grams interchangeably here--40 grams in weight, the cord blood unit is not eligible for banking.

If the volume is greater than or equal to 60 grams, it is automatically considered eligible for processing and banking. For cord blood units that fall between these two limits, the cord blood can be processed if the total nucleated cell count is 600 million cells or more, and that is about 60 to 65 percent of all the cord blood units performed in this category.

Based on this, we expect that almost every cord blood unit we have in the bank should have a starting cell count of 600 million total nucleated cells.

Processing must be initiated within 48 hours of collection of the cord blood--as with New York, this is the processing bag--with cell and plasma depleted using Hespan and differential sedimentation with a slow spin/hard spin that was described by Cladd.

We use an integrated system of series of interconnected processing bags, which are sterile-docked onto the cord blood collection bag to allow closed system

processing of the umbilical cord blood right down cryopreservation.

We have certain processing limits, recovery limits based on the cell count at the end of recovery. Cord blood units must have greater than or equal to 60 percent recovery of viable nucleated cells or greater than 80 percent recovery of viable mononuclear cells or again, finally, greater than 600 million total viable nucleated cells at the end of processing.

The average nucleated cell recovery of units in the search registry, the 4,000-odd units I referred to earlier, was 77.2 percent with a range of 24 to 203. Clearly, the extremes in this range reflect sampling artifacts rather than a 203 percent recovery.

As with New York, we cryopreserve in 10 percent DMSO and dextran using computer-controlled rate freezing. However, at UCLA, we use a conventional controlled rate freezer with rack and frame storage, a separate vapor phase quarantine storage tank, and liquid phase long-term storage tank, which are purchased through MVE.

The Duke program uses the integrated system, the BioArchive system provided by Thermogenesis.

Both systems use exactly the same bags, the same cryopreservation bag. It's a final volume of 25 ml, which is thawed into connected aliquots. The bag, shown

wonderfully here, contains two pockets which are filled at the same time and then sealed into two compartments which can be separated, one of 20 ml and a second of 5 ml.

The tail leading into the bag is sealed off into three or four segments to provide samples which can be definitively linked to that cord blood unit for HLA typing as was described earlier.

During the course of processing, we take a number of aliquots for various testing. We do sterility testing, anaerobic and aerobic bacteria. One distinction between the system described earlier is that each of these samples gets approximately 7 ml of cord plasma plus 2.5 ml of packed cord red blood cells.

These are from the post-processing waste bags. In this way, we maximize the amount of sample that can be applied to the sterility test, thereby increasing the sensitivity of the test without compromising or reducing the number of cells which make it into the final cryopreservation product and therefore to the patient.

Viability is performed by trypan blue dye exclusion pre- and post-processing and we perform a manual 200-cell count differential on cord blood units. This is not required on all units at the time of banking. It is required at the time of transplant, but not at the time of banking. This is largely a workload issue.

We also take a small sample prior to the initiation of processing for ABO Rh typing.

Additional graft characterization in terms of the sort of colony assays and things, we examine CD34-positive cells using the Pro Count system from Becton-Dickinson. We use the Multi-Test system to measure lymphocyte populations, again from BD, and colony assays are performed on all samples at this time using the methocult system from Stem Cell Technologies.

The only exclusion criterion derived from these tests is if the colony count is equal to zero. That has never been achieved at the present time, and we are evaluating whether or not to continue doing colony assays because of the expense and the time delay amongst other things that this creates.

Cladd referred earlier on to the time. At the time of collection, you get the cord blood and then you get the data over a couple of days. It doesn't help when you are having to wait another two weeks to get the results of colony assays.

Our infectious disease screening panel is very similar to the serological panel, similar to that described earlier, only we only test the maternal blood specimen. We are looking at antibodies to HIV-I/II, and the HIV antigen, antibody and antigen to hepatitis B, antibody to core, and

the actual surface antigen itself, antibody to hepatitis C, HTLV-I/II, CMV IgM, and we do syphilis. We were at UCLA doing VDRL, but now using RPR. I am not certain what they are using at Duke.

These results here are based on UCLA data with which I am obviously more intimately acquainted, but any initial positive, any initial positive with the sole exception of syphilis is grounds in and of itself for exclusion.

So, there are no cord blood units in our bank in the search registry with an initial positive irrespective of confirmatory test results for any of these tests with the sole exception, as I said, of syphilis.

This creates an obvious problem with again trying to maximize minority involvement. At UCLA, approximately 16 to 19 percent of our collections are performed from Asian-Americans, and a large fraction of those from Chinese-Americans and 49 percent of those test positive, 49 percent of our Chinese moms test positive for hepatitis B core antibody.

That gives us an overall positivity of 10.8 percent, which is extraordinarily painful, to say the least, that we have to exclude these units, and so we are at the moment looking at strategies which might allow us to include those units in the bank in the future, for example,

looking at quantitative surface antibody analysis and HBV NET testing.

However, at the present time, these units are not in the search registry. There are no hepatitis B core antibody-positive units in our search registry. There are approximately 350 such units being held in the banks in extended quarantine pending a decision based on the suitability of these additional tests.

So, the only infectious disease positive units that make it into our search registry are those which test VDRL-positive for syphilis and are confirmed negative using the FDA assay.

We do not perform our own hemoglobinopathy screening assays at either Duke or UCLA. The banks rather obtain the results of testing that are mandated by the States of California and North Carolina on all newborn screening.

Any symptomatic disease state is excluded. That obviously includes sickle cell disease, but some of the other more complicated combinations between sickle trait and major thal. is obviously excluded. Sickle trait is permitted to be held in extended quarantine pending additional testing looking for any alpha chain defects.

In terms of follow-up, post-banking testing at six months and one year. These are approximate dates only.

We do follow-up at UCLA. This is done by a mailing with a reply paid envelope. At Duke, it is done through the computerized medical records.

It is a fairly simple questionnaire we are asking for. It is like a five-point checklist - does the baby have hepatitis, HIV, other problems, and at the bottom, none of the above, and so far we have had, at UCLA, one unit excluded as a result of this follow-up testing. That was from a congenital deformity in the heart which was not diagnosed at the time of collection or discharge from the hospital.

In addition for those units that are selected or at least on hold for transplant, for inherited diseases, we perform enzyme assays to determine the level of the enzyme involved in the disease.

So, for example, in severe combined immunodeficiency, we are doing an ADA assay. These are again only for those units that are on hold for transplant for inherited disease and the enzyme assayed, is that enzyme involved in the disease. We don't do a massive panel of these enzymes, nor do we do it on cord blood units selected for transplant into patients for leukemia or other malignant diseases.

HLA typing in the program is exclusively DNA based using three tissue typing laboratories which are

affiliated with the original three cord blood banks. At the time of banking, HLA typing is low resolution for Class I or sometimes it is done at higher resolution depending on the specific type that is identified, and intermediate or high resolution for Class II.

At the time of selection of the cord blood unit for transplant, the HLA type is independently confirmed at a second site. So, what happens when a unit is confirmed as eligible in terms of all our exclusion criteria and meeting all the criteria, we send away three specimens for HLA typing - one to our home HLA typing lab, which actually does the typing at that time and provides the data to the coordinating center, and the two other specimens go to the other two remaining labs which hold that for confirmatory typing.

That way we are not sending out specimens at the time a unit is being selected. Those HLA typing facilities already have a specimen in the bank as it were. We do not do HLA typing on the mother at the time of release, at this time, at the time of release or at the time of transplant, although it is part of the study. If we have any money left, we will be doing maternal HLA typing. This is, of course, a matter of some discussion within the standards, and we may be changing this.

As in New York, we keep a large number of samples of both the cord blood and maternal specimens for all sorts of purposes, some known, some unknown. We obviously retain cord red blood cells, we retain eight, 1 to 2 ml aliquots, and these are the specimens we use for HLA typing. There are enough nucleated red blood cells and residual white blood cells left total DNA, and these 1 or 2 ml aliquots perform HLA typing.

We viably cryopreserve two, 1 ml aliquots of the white blood cells in DMSO into cryovials. Those are used for the enzyme assays I referred to earlier, and we retain seven, 1 to 2 ml aliquots of the plasma post-processing. The volumes retained depends on how much cord blood we have. Obviously, if we are taking 7 ml of plasma for anaerobic and 7 ml of plasma for aerobic sterility testing, there is not always a huge amount left, and sometimes we cut this down to 1 ml.

We do not do infectious disease testing on the cord blood units at this time, but this is a specimen that would be available for that. In addition to doing the serologic infectious disease tests on the mother, we do store aliquots, 3 aliquots of maternal whole blood for HLA typing, 5 of maternal serum, and 2 of plasma.

Shipping for transplant is performed using the MVE Cryo Mini Shipper. It is designed to hold temperature

for 14 days, and in our program it has been validated to at least eight days.

The weight of the shipper is determined at the time of shipment and again at receipt to see how much of the liquid nitrogen is retained. Temperature is monitored at the present time using the Cryoguard thermal indicators which change color from green to red if the temperature exceeds minus 120 degrees centigrade, however, as I will describe in a few moments, that has been problematic.

At the present time, we are about to evaluate the Cryo-Transit Data Logger, which is supplied through MVE.

Because we are part of the national clinical trial, the COBLT study, we have at the present time only shipped units within the study, and that means within the United States. As a result, we have been shipping unaccompanied using Federal Express, and we have performed no international shipments at this time, and I am sure that when we move to international shipments, we will find a whole range of new problems.

However, as I alluded to a few moments ago, shipment has been one of the areas in which we have had problems. The temperature monitors are unreliable, at least the Cryoguard indicator.

During our validation study, eight out of 10 of these indicators change color, indicating that the

temperature had exceeded minus 120 degrees centigrade even though we had independent monitors in there showing that the temperature at no time exceeded minus 140 degrees centigrade.

It may well be due to the fact that sometimes when you take these indicators out, you have got to see the color, you wipe the condensation off, and the color changes that quickly.

This is unacceptable. We don't want to have a situation which has happened where the cord blood unit is received, they look at the indicator, they check it out, it's read, the transplant facility now does not know what to do with that unit.

So, as I said, we are now evaluating alternatives, and one we are looking at is a product that I found out about at ISHAGE, which has a temperature probe built into the lid of the Cryo shipper, which goes down into the shipping container and with a little computer on the outside which constantly monitors the temperature. It can be hooked up to your PC at the time of receipt or at the time it is received back in the lab, so you can actually monitor the temperatures throughout shipment.

I am told, although we have not yet received one of these beasts, that it can, in fact, show the temperature change. It can show the pressure change when the plane

pressurizes and re-pressurizers during ascent and descent. We don't need that degree of sensitivity, but it would certainly be nice.

The problem with that, of course, is that if we are using that, the transplant center has to have the PC and the software able to tie into it in order to really feel reliable.

At the present time we are using weight and the temperature. The temperature is an unreliable indicator of how much capacity is left in the shipper. What tends to happen, as long as there is more than a few drops of nitrogen left inside the Cryo shipper, it will hold temperature, but when those few drops are gone, the temperature shoots up, so what we prefer to do is look at weight, which as the nitrogen evaporates out, the weight slowly decreases.

We know the weight of the shipper empty, we know the weight of the shipper fully charged, and so we can look, we can have a graph there and give the transplant facilities some indication of how much of the capacity has been utilized.

We have had some problems with Fed Ex in terms of delayed receipt, most usually just an overnight delay, and that's fine because the shippers are validated to more than eight days, however, fine is not good enough, we want to do

better, and we believe we can do better by improving coordination with Fed Ex, calling them up, letting them know the cord blood unit is coming, et cetera, and that seems to be working.

However, we had one rather disastrous, almost potentially disastrous situation where the shipment from my own bank going to Joanne's program, in a shipment that contained not one, but two cord blood units for two different patients where the outer container, the outer plastic domed container of the cord blood shipper arrived safe and intact at Duke. The inside shipper containing the liquid nitrogen and the units did not.

So, what had happened was that somewhere in Memphis, the thing had somehow fallen out of its protective overpack and the people at Fed Ex just put the lid back on and sent it on empty.

Now, when Fed Ex found out about this, they obviously jumped to it and they retrieved the shipper, charted a plane, put it up with the pilots and sent it to North Carolina where it arrived in fine condition and the units were suitable for transplant, but that was by good luck rather than judgment.

As a result, we are now supplementing the security of the shipper protective outer container, not just a matter of padlocks, but we are using duct tape if

that is what it takes. This is not going to fall off again.

But these are sort of foolish things that can happen, and if this is happening within Fed Ex inside the United States, I don't really want to see what is going to happen when we go internationally, and I am glad we are getting this sort of shakedown opportunity now.

The other problem that has been encountered has been in the development of our program. At the present time, the joint banks are performing in excess of 600 cord blood collections per month. In order to do large-scale, high-quality cord blood banking, we have to have a robust, high-quality data management system, and that includes both paper and electronic records.

As we have been developing the program, we didn't obviously start at 600 collections a month, and we have had a chance to develop systems with EMMES, the electronic systems and paper records within our own programs, but clearly, the development of such systems is absolutely essential, primarily for the confirmation of search eligibility.

In our programs, two senior cord blood bank staff members must manually review all the paper records of a particular cord blood unit before it is considered eligible for search, however, that is not good enough for EMMES.

Before they can approve it, they have got to see those two signatures and then electronically, using their data systems, compare the data we have entered to release criteria, and only when a cord blood unit passes all three tests and is actually typed is it eligible to reach that search registry, which as I said contains a little over 4 1/2 thousand units.

Is it important to search eligibility? Very obviously it is. This kind of robust data system is absolutely essential for performing things like data quality audits, allowing us to electronically cross-reference data sources. It is not possible to do this manually when you are doing a large number of cord blood collections.

This can be as simple as comparing the date and time of collections, the date and time of birth. If they are on different days, we have a problem. The systems that we have developed in conjunction with EMMES internally allows us to do this.

In order to obtain the results of hemoglobinopathy testing from the State of California, we have to provide the identity of the mother, Social Security number, things like that, but we also need birth weight, birth time, and what have you.

We get those results back from the State, and that allows us to confirm the identity and birth weight of the mother electronically, and this really gives us a great deal of confidence in the kind of data quality that we have.

The other problems that we have are more in terms of the development of that kind of systemic safety net, the things that protect you from making mistakes.

For example, eye readable versus bar code labeling. I strongly believe that if you are doing any number of cord blood units, you have to be using bar code labels.

We are seeing the problems that can come if you are not using bar code labels, and scanning frequently to check things when you are taking sample A, putting it in bag B, you have got to make sure that the lab bags have the same label on it, and you can't really do that by eye when you are doing as many units as we have been doing or any number of units at all.

The other thing is adequacy of space and other infrastructure, and this is the problem I have noticed at other banks, and I have certainly noticed at our own bank before our new laboratory or while our new laboratory was being created.

That has to include adequate space for processing and for data management.

With that, I will close. Thank you.

[Applause.]

DR. JENSEN: Thank you, Dr. Fraser, for that wonderfully comprehensive and detailed presentation.

Our next speaker is Dr. Donna Wall, who is the Associate Professor of Pediatrics at Cardinal Glennon Children's Hospital, and she will be presenting her rather unique perspective on this experience in unrelated cord blood banking.

St. Louis

DR. WALL: Thank you very much to NHLBI and FDA and colleagues for this opportunity to share our experience in cord blood banking at St. Louis.

We had a long laundry list of what to discuss in this half-hour, so I have taken some editorial license, kind of guessed what New York and COBLT were going to say, and have just focused in on what I think are some central points that we can offer, either a unique perspective on or some data to support regulatory development.

What I will cover in this half-hour is our unique and sometimes argued approach to cord blood collections with a community-based cord blood collection system. We have some data on the issues of bacterial contamination of

cord blood, briefly address minimal volume and cell count standards for banking.

We have a fair amount of information correlating the various measures of hematopoiesis that we have, that I would like to share with you, some data on length of storage and impact on transplantability of units, expected yields post-thaw, and I would like to make a pitch for validation of cord blood products before release for transplantation.

Our program initiated naively with a visit with Pablo and Dr. Gluckman, et al., about six years ago to St. Louis where we said, boy, this would be a good idea to venture on, much the same as many young banks.

In doing so, we took advantage of the Midwest philanthropy and looked at using community-based hospitals and community-based health care providers as our collection team.

To date, we have over 300 obstetricians and midwives within a 150-mile radius of St. Louis, at 30 obstetrical units performing our collections.

To date, over 20,000 units have been collected and out of those, 5,500 units have been banked, are fully characterized and accessible for transplantation.

In doing this, our start in thinking this through was that obstetricians and midwives have, as their

fundamental training, the necessary skills for cord blood collection, the basics. They are able to phlebotomize. They have an understanding of clean, sterile technique. They understand blood transfusions.

We also took the observation that who best knows the mother than the obstetrician and the midwife. We have found that obstetricians and midwives are playing an important role in screening out families who are suitable for donation and who are not suitable for donation.

This, we have coupled with a quality control program which has acknowledgeably evolved considerably over the last five years. We have an up-front ongoing interactive education with the people who are actually doing the collections, with the obstetrical unit who is coordinating collections, with the obstetricians' offices and Lamaze classes who are introducing the program.

We have several important control points in the process. The first is that all expectant parents who are going to deliver contact the bank prior to delivery. With this, we have a bank member, an R.N. of the team, speaking to the mothers, explaining the fundamentals of the informed consent, that this is a donation, that their records will be screened, that long-term linkage will be maintained.

This is done in the third trimester, prior to onset of labor. So, we start, we cannot backload our work

prior to delivery. Additionally, after collections we monitor bacterial contamination, we look hard for mislabeling, incomplete labeling, suboptimal products, and have an active program where we go back to the collecting physician, midwife, and have an ongoing dialogue that way.

Our collections are different than most in that we use third stage labor collections. That means the baby has been delivered and is in the isolette. The placenta is in the process of being delivered. Usually, there is a five- to 10-minute time period between delivery of the baby and the delivery of the placenta during which the placental veins are available for cannulation.

Key to this is that the collections are only performed on uncomplicated singleton deliveries. It is critical that we know that there is only one baby in there, so that we are not draining a placenta that could be a shared placenta with another newborn.

Critical to this, and we hammer with our obstetrical colleagues all the time, is that there is absolutely no change in delivery practice, and we make no attempt to tell obstetricians what is a good practice and a bad practice for delivering the baby, but insist that there be no change in delivery practice.

We have a simple cannulation of the umbilical vein after cleansing with alcohol betadine, a procedure

that looks remarkably similar to the New York procedure, and we are collecting conventional Baxter CPD blood collection bags.

You have heard much this morning about mixup prevention control points. We have a concept which we fondly call "blood bank in a box." Mothers are delivered in a delivery suite. They labor and deliver in the same suite. We have a complete package provided by the bank, so again we have control of test tubes and alcohol wipes and syringes, collection bags, all the rest is controlled from our bank.

It comes in a discrete box, and the collection then occurs in the room. Maternal blood samples are drawn in the room and go into the box. We collect our red top, serum tube from the cord blood. That also goes into the box and the cord blood is collected and into the box, and maternal consent is re-obtained at that time period, and we have that all as a defined unit.

That unit is sealed in the delivery room and is transferred to the cord blood bank. So, that is our approach at getting 90 percent of the stuff that can get mixed up at the delivery room under control.

Key to this, and in talking to colleagues on the East and West Coasts is that their obstetricians, no way

would they buy into this, this potentially adds risk to their deliveries.

It does take a little bit of time on the obstetrician's part, but to be honest with you, it takes time on the delivery room staff's part. The nurse gets everything ready, the nurse hands the bag to the physician who cannulates--physician/midwife--so the work as always is done by Nursing.

We have tracked, we did a formal survey of our obstetricians in 1998, and really fished hard for any perceived or actual risks to mother and infant at time of delivery, and worked hard at getting our responses from the obstetricians, and really could not pick up a risk given that you are only collecting in completely uncomplicated deliveries.

We have had to work hard in our consenting process to make sure that mothers know that their cord blood may not be collected if there is any issue at all with the delivery.

To date, we have had one obstetrician needle stick and we have been tracking over the last five years what happens to obstetricians, and the obstetricians who drop out of the program are the ones who are longer delivering babies, and we don't control that.

As I mentioned, we are tracking positive bacterial cultures over time, similar to others. Initially, we had a relatively high incidence of positive cultures, and we have settled out to about a 2 percent positive culture rate with some seasonal variations.

We have been focusing on trying to figure out what is the best way of doing bacterial cultures. The issues are, number one, cord blood compared to regular blood collections is at greater risk of being contaminated, so that is just a fact, and it can be contaminated with maternal vaginal gut skin flora, which could aerobic and anaerobic organisms.

The other issue that is alluded to is that the cord blood product is limited, and you don't want to put all your product into testing, and the final point is we really don't understand what the clinical significance is of positive cultures, and that is speaking as a clinician who has infused infected platelet products and run that roller coaster in the past.

We have, over the last six months, been working on a project which we thought was going to be simple, and we are comparing the absolute most wonderful product for aerobic and anaerobic cultures, and we have a similar processing procedure where we have a closed system and a direct collection of waste, of extra plasma and red cells,

and we culture that with a 10 ml inoculum into aerobic and a 10 ml inoculum into anaerobic.

Our standard procedure prior to that was 0.5 ml of the end product that was cultured aerobically only. With this process with 325 cord blood units, overall culture rate has remained at 2.2 percent, however, we found a real inconclusiveness in that only two out of the units, 300 units, were positive all the way across in aerobic, anaerobic, and end product culture.

We had the kinds of organisms isolated, were all what we would expect, skin contaminants, air contaminants, vaginal flora.

All three culture points had positive cultures in it, and not infrequently, the positive culture was in our final 0.5 ml of end product, what is going into the freezer culture, and this is done with everything under the hood, optimal culturing techniques, and basically, very similar inoculum between what we are inoculating from the waste product to what we are using as our end product samples.

So, at this point, we are kind of stuck. We were going to try and throw away that final product testing blood culture, but we are stuck. We have data to show that we can't do that, so I would advise not doing this quality control trial.

The second piece of information is that we have had two patients who have received cord blood units out of clinical needs that were bacterial culture-positive. One was a diphtheroids and one was coag-negative staph.

Both of those units on thaw had negative blood cultures, negative cultures of the thawed product. Neither patient had difficulty and both engrafted, so a piece of useless information for regulators.

Switching gears to what should be the minimum cell dose or volume banked, my end statement for here is that the minimum should be decided by the bank because it's a financial decision rather than a regulatory point.

Starting from when we first started the program, we used a minimum volume of 40 ml and a minimum total cell count of 600 times 10^6 cells, and using those criteria, we banked about 50 percent of our products.

We have, since 1997, used a criteria of 50 ml and a TNC of 800 times 10^6. Using that, we bank about a quarter of the products, 25 percent. This move has been a practical move driven financially.

Our mean number of cells in the bank is close to 100 times 10^8 cells, and that was not a rational decision, that was a financial decision.

We have been looking at our approach to measuring hematopoiesis in units. We have been watching with great

interest as we perform searches for programs, and we find programs who make their decision on a cord blood unit based on the total nucleated cell count.

We see programs who use the CD34 count in deciding on a unit. We see programs who use a hybrid of both. So, the clinical practice is clearly in evolution.

We have been performing CD34 analysis and CFU analysis similar to the other speakers, and I would just like to present our first 2,000-odd cord blood units that we looked at, specifically, trying to ferret out can we throw away one measure of hematopoiesis or is one measure of hematopoiesis better than the other.

To do that, we are going to look at the units that we banked who, for a given total nucleated cell count, had either a super-duper high CD34 count or a low CD34 count or the CFU assay in looking at the alternative measure of hematopoiesis.

We, as others, had found there is a good correlation between total nucleated cell counts and CD34 count, between total nucleated cell counts and CFU, and CFU versus CD34.

Pictorially, what I am talking about is looking at cells with a given total nucleated cell count, looking at their CD34, and looking at ones who, for a given cell count, had a lower CD34 count than you would expect, or

ones that would have a better CD34 count, so, ones that you would say are relative duds or could be super-achievers.

We did the same thing. It looks a little cleaner on the CFU assay where we have several here who had a much higher CFU count than you would expect for the given cell count.

When we take a look at the second measure of hematopoiesis for those who had a high CD34 count for a given TNC. We found that they had identical CFUs as the main population. That is the population of cords that were within two standard deviations.

So, we did not find a parallel increase in CFUs for a population, so we were not picking out super-achievers by the other measure of hematopoiesis.

Similarly, the ones that we identified as potentially being underachievers hematopoietically, those with a low CD34 count for a given TNC, actually, had a higher CFU count for a given TNC compared to the norm, and we have repeated this on the next cohort of cords that we have done, and we have the same trend going on, and I have got no smart answers for that. I would be interested if someone else does. The flip also occurred.

The statement I would like to make from here is that at this point, I don't think we can hang a hat on

specifically a CD34 count or a TNC or a CFU as being the sole measure of who is the best cord.

We did find that as part of our meandering around, that if you hold your samples for CD34 analysis, which will happen if you are processing, you are starting to process at two days, you finish your process at the end of the day, it gets held over another day before you run your CD34 assay, that your CD34 assay will go up nonspecifically over time, and this has been enough for us to set up flow in our laboratory just to make sure we don't have this false-positive rate for CD34.

We also have been looking at nucleated red blood cells, and our nucleated red blood cells in our banked cords ranges from 0.2 to 33 percent, and we were worried, as others, whether we were seeing a false elevation in nucleated red cells, false estimation of hematopoietic potential.

This is just a correlation curve comparing manual nucleated red cell calibration in the NRBC index on the XE-2100, which is very nice.

This is a little bit busy, and if you want to just focus here, nucleated red cells per 100 white cells, pre-processing, our mean was 6 cells per 100 white cells for nucleated reds with a range of 0.4 to 48.

Post-processing, we lost some nucleated red cells, but by no means all of them, and we still had a relatively high contribution of nucleated reds. We also found a correlation between nucleated reds and CD34-positive cells, similar to the New York group, the high nucleated red cells counterbalancing its effect within the cell count.

We have been looking at cell yields on thaw. Sorry, this is a little bit jumpy, but we wanted to cover all of these areas. One of the things we have had is that we have had the benefit of transplanting kids at Glennon with the gold standard cord bloods from the New York bank, and so this is our gold standard bar in the middle here.

The recovery that we are getting on thaw of units that have been frozen is about 80 percent. The viability coming out of thaw is about 85 percent, and we have had a very similar experience with the cords both that we have transplanted at our institution and also those that have been exported and thawed at other centers with different cell counters, different trypan blue stains and whatnot.

So, I think we are starting to get a feel of what you should expect for cells coming out of thaw.

We have now data that is just starting to ripen, and this is meant just to provide some information and

acknowledge it is very fresh data, looking at the impact of the length of storage on recovery post-transplant.

Here, we are looking at three time points post-transplant. The red bar in the middle are cord bloods that were stored for a year. There is a blue bar between one and two years, and the green is greater than two years. The longest is getting close to four years of storage, and we are not seeing any impact on the grafting time and no impact on survival.

We have slightly different time points, but this curve right in here are our oldest cords, and none of these curves have any difference in them, and similarly on platelet engraftment, there is no difference based on length of storage.

The last thing I would like to talk about is the validation of the cord blood viability prior to usage. If we make a pitch from our bank, our pitch is that--and I am speaking for my colleagues--is that it makes a lot of sense before you release a unit that you make absolutely sure that you have in your unit what you think you have.

So, we have made a lot of use of the pigtail on the cord blood unit, taking that off before release of the product and making sure that we repeat HLA typing and making sure that what we say we have we really do have.

So, it is a variation on what the New York team is doing with the maternal samples, but basically, for us, it's a drop-dead, do you have what you think you have that you are giving.

The worry we have is that over time the cords are only going to get older, and there is going to be a concern with transplant units to want to use newer cords or at least to make sure that these cords are really still viable after five years, 10 years of use.

So, this is very much work in progress, but what we have been doing is taking a contiguous segment off the cord blood unit. We have some research cords that have been in for an awful long time.

We have measured. We have about 40 microliters of cord blood product in that segment. We know the cell concentration of that, and we back calculate and perform a CFU assay on that segment, a trypan blue viability. What is left, we spot on paper for HLA typing, and we have been able to show that we can repeat our HLA typing on that section.

We have then gone on and thawed the units conventionally and taken a look and compared post-thaw CFUs between the units and the contiguous segment, and looking at unit CFU and segment CFU, we find that actually--this is probably early days too good results, and will only get

worse with time, but we are starting to get a feel for what you will find when you do a direct comparison.

That has got the potential--this is built into the system--of allowing you to validate your product before it leaves the bank. Make sure that you are releasing the proper HLA type and a viable product to the bank. You know, it is not a complete story, but I think it's an important component.

So, in summary, I would like to say that I think we feel that we are comfortable that we have been able to develop a community-based cord blood collection system that is a feasible way to cord blood procurement, that bacterial contamination of cord blood is more likely to occur than other blood products, and to just simply say we are going to use other blood products' criteria really probably isn't the fairest thing to do, but once you do that, you open yourself into a slippery slope kind of, you know, very difficult to define product.

The easiest thing for the regulatory body to do is to say any positive culture, you are out, but as we have been showing, depending on how you do your bacterial cultures, how rigorously you do them, you are going to start picking up larger numbers of organisms, and then the question comes, as was pointed out by other groups, the

clinical significance is not known, and to echo Dr. Stevens, the risk-benefit ratio has got to play in.

I feel strongly that minimal volume and nucleated cell counts for banking is driven by economics, and this is a different statement than what you use for transplant, but what you put into the bank, if you have got all the money in the world, you can bank small products that may be okay to use for 6-kilo kids or for expansion when it comes in, but the rest is basically an economic issue.

There is a direct correlation between nucleated cell count, CD34 enumeration, and CFU analysis, but no one measure itself clearly defines the hematopoietic potential of the cord blood unit, and there is a lot of room to go in defining this.

Using three years of storage, we see no loss of product potency with that time frame. Our yields are in the 80 percent range, and I have got more data on that, and we are very much interested in developing end product validation.

I think I will stop at that point. Thank you.

[Applause.]

DR. LAZARUS: Thank you, Dr. Wall, for broadening our perspective on this issue. That was most interesting.

Our final speaker is Dr. Thomas Lane, who is the Medical Director of the American Red Cross at the Western

Area Community Cord Blood Bank, and he will be giving us the American Red Cross perspective on unrelated cord blood banking as soon as we can get the computer going.

ARC

DR. LANE: Thank you very much. We are switching computers here and then I have to copy this over to the hard drive.

While we are copying files over, I want to, first of all, thank you for inviting me as a representative of the new kid on the block, the Red Cross system, and I would like to say, first of all, it is a great pleasure for me to be here to actually speak at the same program with Dr. Pablo Rubinstein, who I have known for many years. He is a great scientist, a great humanist, a great human being.

I had the good fortune to spend some time in his lab as a fellow, and Pablo, I think distinguished himself in my mind as one of the more prominent and probably the best teachers I have ever had.

He spent time actually trying to teach me how to think, which very few teachers actually do, and maybe he thought I was particularly deficient in that regard, but I have always had a warm place for him in my heart because of that.

I also want to thank Pablo for helping our program get started and indirectly the Red Cross. I also

want to give a great deal of thanks to John Fraser and to Laura Gindy, who were really very, very unselfish with their time in helping the Western Area of the Red Cross get started and indirectly, the entire Red Cross Program.

I can see already that some of this is going to be washed out, so just like John's slides--so, I am speaking then on behalf of the Red Cross Program and primarily because Becky Haley is enjoying herself in Florence, I think she was invited to speak.

I would like to just go over a little bit about our program. I have the benefit, I suppose, of following three very distinguished speakers in that I don't have to go over the same things that they went over with you before, but what I am going to do, given that we are, as I said, the new kid on the block, I am going to explain our program to you and try to focus a little bit on what running a big program like this is like, it is rather unenviable, I think, and then focus on some differences between the Red Cross Program and others, if I may.

So, first of all, organizationally, we have a national director, Heidi Patterson. The banks consist of the first one, the Ross Cord Blood Bank in Columbus, Ohio, directed by Larry Lasky, and this one was started independently with funding from Ohio State University and the Red Cross was brought in.

The University of Massachusetts started a cord blood bank which was later taken over by the Red Cross, and this is run by Karen Ballen, and most of the data that I will show you, the descriptive data is really driven by her blood bank, which is the largest of the group.

The third was the one I am medical director of, the Western Area Community Cord Blood Bank Program, centered in Portland, Oregon and San Diego, California. I am the medical director, Doug Dooley is the scientific director.

This cord blood bank was started by a generous gift from the Paul Allen Foundation. The Minneapolis, Minnesota Cord Blood Bank, under the direction of John Miller, and a bank, which is just really starting now under the direction of Roy Baynes in Detroit with the Comonis [phonetic] Cancer Institute, and hasn't really started up yet.

Finally, we have great support from Red Cross National Headquarters. We have three individual INDs, but we are going to fold them into a single national IND under the direction of Becky Haley.

So, this gives you a more visual description. I borrowed this slide from Heidi, which gives you a visual perspective on what we are. The babies represent collection and processing sites, as you can see the Western

Area here, San Diego and Portland, Minneapolis, Detroit, Columbus, and Worcester, Massachusetts.

We do have some collection-only sites, one starting up, the former COBLT site at Holy Cross Hospital, and we are just starting now another collection site in Oakland, California, which will feed into the Western Area.

We have planned--I am using the editorial "we" here for the Red Cross--a variety of different collection sites around the country focused to some extent on targeted minorities and other extent on interest.

Most of the light pink here is pie-in-the-sky at this point depending upon budgets and where we eventually go, but this is the total plan.

So, the goals of the program are to collect and store 50,000-plus cord blood units over the next five years, and in particular because of the people we want to treat, to emphasize ethnic diversity in cord blood unit collection because of the underserved minorities in terms of finding a suitable donor for transplantation.

Some of our guiding principles are to have a common IND and as many common protocols as we can, also common listing and searching protocols, but we all recognize that there is a great deal to be done to define best practices, best methods of doing a variety of things, and therefore, research and development, both internally

and external collaborations, of which we already have several, are going to be very important to this program. So, this is very much a work in progress.

Now, what are the features that unify the Red Cross Cord Blood Program? I guess I have gone over some of these. First of all are governance, as shown here, national director. We have an Executive Committee made up of most of the medical directors, scientific directors, and a variety of subcommittees, as you can see here, and then, of course, the invaluable support from National without which we could not function.

So, unifying features in this program, eventually a very large program, will be maternal eligibility criteria. We are moving towards having identical criteria within the next year, cord blood acceptability criteria. We don't like to call them minimal standards, but I suppose that is what they are. We have common standards at least, although there is disparate HLA testing philosophies and locations right now. We are moving toward, if not centralization, at least limitation of the locations and the conditions under which that will be performed, as I said, a common listing and searching mechanism, common outcome criteria.

We have undertaken a nationwide program to develop a common quality assurance program based right now

on the 20 principles, if you will, of the modified ISO quality assurance program that has been used by the American Association of Blood Banks, but keeping a very, very careful eye on other organizations, such as FAHCT.

We don't have now, but will move toward a common data management system. Of course, the Red Cross has an R&D program, and as I mentioned, we will have one IND very shortly.

Now, there are some differences among the various sites, as well, among recruitment strategies including pre- versus post-natal consenting, collection strategies in utero primarily performed at Worcester, Massachusetts site versus post-third stage of labor, which is everywhere else, processing strategies, upright versus inverted spin, although everyone uses the Rubinstein method essentially, storage, differences in storage including bag types, liquid versus vapor, liquid nitrogen, HLA typing, which is going to be temporary, and, of course, there are separate R&D and collaborative arrangements among the different centers, which we feel should help cross-fertilize the program as a whole.

The Executive Committee meetings we have had to date have been--you can certainly describe them as lively, and I think we all benefit from them. Sometimes we scream

at each other across the tables, but I think we all benefit from that.

I wanted to just, since I happen to be from the Western Area Program, I wanted to take this opportunity to highlight this. I think I have already said most of it. We started out--I have to give a commercial here--with a generous gift from the Paul Allen Foundation in August of '98. Our goal is 30,000 units over five years.

We have now three hospital sites. We will be quickly moving to five hospital sites in Portland, San Diego, soon Oakland, and other regional sites, processing in Portland and San Diego.

But the really unique feature I think of the Western Area Program is that this was designed from the ground up to be a regional program, to have a unified management in one location running a variety of collection and processing centers. Again, we owe a great debt of gratitude to people from COBLT for sort of showing us the way on that, but we do have unified management, document control, data control, and an IND, centralized lab testing.

We figured that doing CD34 and CFU testing in one location would diminish the amount of variability, and we are doing that now. We have identical protocols for recruitment, collection, processing, you name it, quality assurance.

To the extent possible our equipment is identical and our supplies are identical. This makes it easier to compare what we do and easier to run a large program like this from one location.

So, we feel that this can also serve as a model for the ultimately unified National Red Cross Program.

Now, I tried to dutifully follow the instructions for what I was supposed to present, and everyone else has presented real data, so I am going to present more descriptive data, and for those of you who manage to stay awake before the break, I just want to highlight some of the differences among the different sites.

As indicated here, there are differences in recruitment. Ross Cord Blood Bank does mostly postnatal recruitment. Although there is prenatal education, most of the actual consents are obtained postnatally, whereas, the Worcester system uses almost a case-oriented approach to obtain consent well before delivery. Of course, they do obstetrician collection.

Minnesota, I don't know how they do it, but they manage to get all their consents prenatally, and that is a pre-admission I think in virtually all cases.

In Portland and San Diego, conditions vary, as you can see. In Portland, we obtain most of the consents prenatally, but most of those are actually in hospital,

many of those are, and San Diego, prenatal consenting in the hospital we are in right now is rather difficult and most of our consents are postnatal. Once again, we do a lot of education prenatally.

Collection is always after the third stage of labor except for the obstetrician-run collection program at the University of Massachusetts. Minimal cord blood volumes for processing are 40 ml, although I think, as everyone else is doing, we are inexorably moving upwards in volume.

The Ross Cord Blood Bank uses a 60 ml minimum volume except for ethnic minorities for which they will use a 40 ml volume, which is interesting. Likewise, we have minimal cell counts times 10^8 for processing, either 6 or 8 or in some cases none.

Shipping of the sample varies and it occurred to me recently, and I was a little horrified at first, to realize that I believe the Western Area is the only program in the country that uses 4 degrees, but we actually had a reason to do this, and that related to the necessity to keep the nucleic acid testing sample from maternal infectious disease testing at 4 degrees, and rather than shipping--because we do a lot of internal shipping--at two different temperatures, we decided to use one temperature.

But people discuss whether or not immediate immersion at 4 degrees may alter the quality of the cord blood sample. I don't think it will, and, in fact, at least our overtime viability is very good, and, of course, there are older data that support that, as well. I am not going to show any of that today.

Likewise, there are differences in the acceptable shipping time before processing, and in the Western Area we are more similar to COBLT. Processing varies, as well. As I mentioned, we all use the Rubinstein method, but one of the banks, the Ross Bank, uses an inverted spin, which I believe may have been borrowed from St. Louis and modified, and they obtain really excellent total recoveries, perhaps better than the rest of us, using an upright initial soft spin. Cryo bags differ from the Baxter cryoside to the Med Sep. Volume distributions differ. Ross uses two, 25-ml bags, and, of course, the Med Sep is divided into a 20- and a 5-ml portion.

Cryo storage methods differ to some extent now between straight liquid nitrogen and vapor phase, which we are using. As I have mentioned, all of use essentially the modifications of the Rubinstein method for red cell and plasma depletion, and we are all using controlled rate freezing and 10 percent DMSO, 1 percent dextran.

The product assessment criteria are all fairly similar, and you have seen all this before, nucleated cell counts, and these are really minimal. Most of us are doing much more than this, but just to put down the minimal data, nucleated cell counts and NC counts both before and after processing.

We also look at nucleated red blood cells, and I would love to show you our data on this, too. We have similar data to what has been shown, viability assessments, colony-forming units. As I mentioned, San Diego's CFUs are done in Portland, and CD34 analysis using AICH2 platform method, which requires a correction for nucleated red cells.

Now, microbial cultures, I wanted to focus on this for a moment. There is quite a bit of variation in how this is done. I wasn't able to obtain the data from Ross, but all this use, at least in an aerobic culture varying from 1 to 7 ml in this case of plasma, or in this case of both plasma and red cells.

Some of us employ an anaerobic culture and in the case of U. Mass, a separate fungal culture although most of the rest of us use the automated systems that are out there, and this is--I am sort of surprised nobody else talked about this because fungal cultures are something that the FDA talks about and we are all very interested in.

We don't really think it is a problem, but what sort of reliability do we have or confidence do we have that we have a reliable fungal culturing system.

We had some discussions with our Microbiology Division about this, and we are assured that for the kinds of organisms that we would see, that yeast organisms grow out fairly well in the automated BAC-TAC or BAC-T alert systems that we are all using.

The duration varies, as well, from five days to 14 days of culture. It doesn't seem to make much difference. Portland is now at 21 days, but will be shifting down to a shorter time period, and we all discard any positive units.

Other infectious disease testing performed on mother is pretty much the same as everyone else does. We don't perform infectious disease testing on baby, although given that most of our banks are modeled after the COBLT system, we save all the samples that they and the New York Blood Center saves on both baby and mom.

We are doing NAT testing in most of our sites although right now we consider this a research procedure, although we haven't had any positives anyway. We are all doing CMV testing either by combined IgG/IgM methods, and if a positive comes up, then, a separate test for IgM. None of us are doing salivary swabs.

All right. So, having said that, what are some of the results we have had? At least in the Western Area, we are really focused on the idea, as John Fraser I think has pointed out very nicely, that we need to obtain ethnic minorities if we are going to do some good in this venture.

This shows you what we have obtained so far. Perhaps a very important line here are the number of banked cord blood units. You can see that the Western Area is really just getting going and, in particular, San Diego. But having said that, there are some fairly obvious differences in ethnic minorities.

I think San Diego will be a very good place to obtain cord blood units from others of Hispanic origin. Our Oakland site is focused on obtaining cord blood units from mothers of Afro-American origin. That site should be up and running very shortly, and we hope to really improve upon this ethnic distribution.

You will note also, curiously enough, that in the Western Area we have a lot of mixed race mothers, and I think what the difference between this and everyone else simply means that we haven't made the judgment calls that others have in assigning race to one versus another.

We actually have a fairly detailed questionnaire regarding race and we are not quite sure what to do with it now, and we have several check boxes showing mom and

partially this and partially that, and dad is partially this and that, and so we wind up having a lot of mixed race individuals. This could probably stand a little bit of data cleaning or at least judgment calls, I suppose.

So, we have a total number of units in this system now of 2,020 as of April 2000, and we hope those numbers will be going up very soon and very quickly.

What do these units look like? In terms of volume minus anticoagulant, that is, anticoagulant subtracted, the mean value is about 75 ml with a mean value of about 9.9 post-processing, total viable nucleated cells.

Mean recovery is again there is a really outstandingly good recovery from Ross Cord Blood Bank using the inverted spin technique. The rest of us are in the 70s. I keep apologizing for San Diego. We are the youngest bank. We are getting better. We got our first 95 percent recovery the other day, so I think these numbers are going to go up.

Viabilities are all what you would expect, again post-processing. CD34s are about what everybody else has been seeing on mean. By the way, this is a weighted mean. The CFU/GMs vary as again you might expect, greater from one side to another, but our mean data is about the same.

Now, since we are asked to focus on problems, I tried to do that, at least in terms of the kinds of

problems that we are seeing in collections, recruitment, and exclusion, and this rather busy slide is an attempt to try to summarize the data that we have now, primarily coming out of these four sites.

If you look at the percentage stored of the units collected, we varied from a low of 41 in San Diego, and there are reasons for that, up to a high of 65 in Minneapolis.

I think that to a certain extent, your ability to do prescreening on mothers will--prescreening well before delivery will diminish to a great extent your exclusions, at least in San Diego where most of our consents are postnatal, and we find exclusions when interviewing moms in the hospital.

So, if you look, then, at the reasons for discard, primarily, the single most common reason in most of the banks is the volume collected was too low. The second most common reason then is the total nucleated cell count once it was done, simply didn't make that first cut.

Now, looking further down the list, you can see some interesting differences between the banks that have different philosophies of collection. I think these are probably not unanticipated. For example, at U. Mass, where the obstetricians are doing all the collections, they

really have problems with labeling, with getting units to the bank in time, clotted samples, and contamination.

Now, Karen Ballen tells me that this contamination rate is a little closer to what Donna Wall represented recently, it is closer to 3 percent, but taking all the units that they collected, it was in the range of 6 percent.

If you look at contamination rates in the other banks, although the numbers are smaller, we have yet to see a contaminated unit in Portland and San Diego where we used exquisite care in collecting the cord blood, on the other hand, whereas, in U. Mass, where they do very careful prenatal consenting in contrast to Portland and San Diego where most of the consents are performed in the hospital, we have a great deal more maternal history exclusions.

This really is a problem area for us. Now, I should point out our maternal histories are based largely on the blood donor criteria, and we are not sure that we ought to be excluding many of these moms, and frankly, it is very painful to exclude a mom who has lived in Great Britain between 1980 and 1996 for greater than six months. I don't think we should be doing that, but right now we are.

We are also excluding moms over the age of 40 who haven't had a normal karyotype. I am not sure we should be doing that either.

So, I hope we see these numbers decreasing on a rational basis in the near future.

One rather disturbing problem that we have seen, it seems to be recently, had been problems with the bags, and it is most distressing for the technologists who end up weeping, going home at night weeping when they have processed a wonderful unit from a minority donor, and then get it into the bag, and it all just leaks out.

We have had bag defects and we are going to be talking to our suppliers soon about that. At least at this stage in our development, certainly, it is depressing even if it doesn't represent a large reason for losing units.

Occasionally, we have postnatal consent refusals on units that have been collected, and a lot of miscellaneous reasons.

So, I think that nears the end of my talk. I will say in defense of the Western Area and the Red Cross, that we have tons of other data that I could have shown you, but I tried to focus on this and others have done a good job of that.

So, where do we need to go? Well, I think we need to recruit more minorities. I think others in this

room are doing a great job of that and we intend to do better.

We need to refine donor selection criteria as I have shown you on the previous slide. We need to do better in terms of education and consent. There are many collection issues that need resolving. I think we can do better collections than we are now.

We need to know more about the best ways to ship, process these units to obtain better recoveries. Product standards really are very important, and I certainly agree with what Donna Wall said, that it is to some extent an economic choice.

We need to learn how to cryopreserve and store these units, and very importantly, I think, an area that has seen perhaps less attention than it needs is how to thaw them prior to transplant.

The Red Cross is addressing many of these issues through its own R&D program, and we hope to address many of these issues as a group program, as well, obviously, not all of them.

I just wanted to say that this is a very complicated undertaking if you look at the structure of a single cord blood bank, so any change you make in one parameter has domino effects on other things you do, and

that is why you have to be very careful when you change protocols.

I guess I will end there. Thank you very much for your patience.

[Applause.]

DR. LAZARUS: It is time for break. We will have to shorten the break time a bit. Perhaps if we could just give ourselves 15 minutes, that will work. There is a small coffee bar in the lobby on this floor, and there is also a cafeteria on the second floor where coffee and tea, and such, are available. So, thank you. See you in 15 minutes.

[Recess.]

DR. LAZARUS: If everyone could funnel back in, that would be great because we are trying valiantly to keep on schedule.

I have received a couple of comments from some individuals that it is difficult to hear from the back of the room, so as you come back to your seats, perhaps you could move as far forward as is feasible, because I am not sure that we can increase the microphone amplification at this time. I will also be asking the speakers for the remainder of this session to try to speak into the stationary microphone in addition to wearing the lapel microphone.

The next speaker for our first session is Dr. Dennis Confer, who is going to speak to us about the National Marrow Donor Program experience. When the last few people file in, we can get started.

Dr. Confer.

NMDP

DR. CONFER: Thank you very much. I would like to thank the organizers for inviting me. I would like to thank LeeAnn for giving me the first talk after the break, so that I could get my slides set up. I would like to thank Northwest Airlines for canceling my flight yesterday. I got to my hotel room at 3:00 a.m., so I am looking forward to a long day of presentations.

I would like to tell you some stuff about the National Marrow Donor Program's Cord Blood Program, and I think I would like to spend some time highlighting some of the issues in coordinating among multiple banks.

The NMDP's cord blood initiative was authorized by the board of directors, and it was really to establish a network of member cord blood banks just like we have member transplant centers, member donor centers to manage our stem cell and bone marrow donors, member collection centers, et cetera, and, of course, it would be to facilitate matching and transplantation of cord blood units in addition to the bone marrow and peripheral blood stem cells.

Implicit in this was that we would collect recipient outcome data, and as most of you know, the National Marrow Donor Program has a highly developed system for collecting outcome data from transplants a series of forms much like was described with the New York Blood Center's follow-up frequency.

NMDP forms are more than 99 percent of all the forms due have been submitted and are error-free, and this is for a simple reason. In order for a transplant center to continue initiating new searches, they must submit their follow-up data, and so we have very good data submission compliance.

The last point here was that this cord blood program would operate under a Food and Drug Administration Investigational New Drug Application. Our IND has been accepted by the FDA and amended now once to describe two arms to the study, and I will get into that in just a little bit.

We currently have six banks that are approved for membership. Among these six, only three banks are currently listing units that are searchable at the NMDP. We have 6,200 units on our registry. The other three banks are working on contractual issues and also data issues that we will talk about later in order to get their units listed

with the NMDP registry. We have five more applications that are in process at this time.

At this point we have only coordinated nine cord blood transplants. I am not going to talk about the shipping process, et cetera, except to say that NMDP uses the cryoshippers, as we have heard described earlier, and we are using a bonded courier service in order to pick up and deliver the cord blood units, and that has worked very well with these small numbers at this point.

Currently, all of our cord blood processes are operating at the NMDP headquarters in Minneapolis, and the reason I say that is because it's important to point out that operating a large cord blood coordinated effort really involves every department at the National Marrow Donor Program, obviously our Information Systems Department and our Search Coordinating Unit Department, but also we get donor services involved in membership, our Communications and Education Department.

This is our Office of Patient Advocacy, which is required under authorizing legislation for the marrow program, but now has to deal with cord blood questions from patients who are calling them on a daily basis.

Very important, and we don't have time to dwell on it, finance issues and contractual issues are clearly important in running cord blood programs and running cord

blood banks. Research and Scientific Services gets involved in the data outcome collection, and also in the quality monitoring of the HLA typing.

Now, our original IND was really based on the structure of our Marrow and Peripheral Blood, Stem Cell Program. In the IND, we described a cord blood bank membership process, how we would receive applications for membership and conduct site visits for the cord blood banks that were candidates for membership.

We also described a data set, a set of data that we wanted to collect from on every cord blood unit, and in there we talked about how these data would be included in the NMDP computer system, the search, tracking, and registry system, and we intended at the outset to develop a supplementary application that would allow the banks to submit their data to the NMDP electronically.

The other network relationships, namely those with the transplant centers, of course, we had already established. One of our goals in setting up this program was to be able to search all potential hematopoietic stem cell sources concurrently, so that a transplant center could submit a search, talk about this, this afternoon, and get back a report, not only of potential matching marrow and stem cell donors, but also potential matching cord blood units.

Now, one of the issues here was that when we developed what we thought was the data set, and then we started having banks come on board, they had already developed what they thought was the data set, and their data set and our data set didn't match, and so we immediately had issues.

Now, this is a listing of the cord blood banks that are currently approved for membership. We have already heard from Donna Wall at St. Louis, Cardinal Glennon Children's Hospital. Bonfils Cord Blood Bank in Denver, Dan Ambruso, the Ashley Ross Cord Blood Bank, interestingly not related to the Ross Cord Blood Bank in Columbus that will soon be joining this list, but this is Ashley Ross at the San Diego Blood Bank, Tony Melaragno.

As we have heard from Tom Lane, the ARC Northeastern Region, U. Mass. Cord Blood Bank, Karen Ballen, Life Cord, which is a collaborative arrangement of several entities in Gainesville, Florida, John Wingard is the PI there, and the most recent member from the Institute for Transfusion Medicine, and Life Source in Chicago, Rich Moldwin, is the PI and Joe Kiss is his co-PI on that.

So, these are the member banks and one of the things we had was get people together, discuss what the issues were, and try to build consensus about how we were going to address the differences in what we thought the

data set was and what each of these individual banks thought the data set would be.

So, we met several times with the bank PIs to understand their recruitment procedures, their processing, and testing protocols. We did some fairly extensive analyses of differences, particularly comparing maternal risk factor questionnaires and family history questionnaires, and from all of this we established a baseline set of minimum criteria for listing units, so this was the minimum criteria for listing units, and we have adopted, referring to this as Version 1.

So, these are minimum, this includes minimum electronic data collection that then is listed in the STAR system, and when these units come up on a search, then, it is necessary to supplement the information with paper, paper and facsimile.

So, these are basically units that were collected prior to reaching consensus and on which we have agreed to have a certain amount of data, but we certainly don't have all the data.

Then, going forward, we wanted to build consensus to establish uniform data collection for each of the banks and all of the data, and that would include not only the data collection, but the testing that would be done on future units.

Now, we have yet to implement this although some of these things are moving forward with the implementation. This is Version 2 of our data set, and under this scheme, all of the information would be electronically submitted to the STAR system, so it could be electronically submitted and also electronically retrieved from the STAR system, and we would eliminate the need for facsimile communications or E-mail communications to find out the details on the various units.

So, what are some of the differences between Version 1 and Version 2? Well, if we look at maternal risk factor questions, in Version 1, we honed that down to 24 questions that we thought were the bare minimum that needed to be asked and have data on them in the computer system.

If a bank didn't ask the question, the unit was considered an exception unit, in other words, the transplant center would be notified that if you decide to use this unit, then, the transplant physician is going to need to sign off on it and agree to accept the unit, recognizing what the issues were, and the transplant physician would also have to secure the informed consent of the patient who would be transplanted with that unit.

Under Version 2, this number of 24 is expanded to 41 questions. These were based in large part on the blood

donor questions, and we started with the AABB uniform questionnaire, and we added after this initial set some additional CJD-related questions. So, that is how these two differ.

What about the family medical history? For Version 1, we say simply you have to do one, you have to do some kind of a family medical history. We know that among the various banks, the contents of this family medical history vary. If questions are not asked, then, it becomes an exception unit, and basically, this is something that has to be handled by facsimile during the search process.

Under Version 2, the family medical history is a standard content that each of the banks has agreed to. It has now expanded to 70 questions, but they are actually in nine separate categories, so it is possible to deal with all 70 questions by asking the umbrella questions without having to ask the detail if the answer is negative.

Again, all of this is largely based--there is a lot of similarity between the banks--and much of it is based on the process developed at the New York Blood Center.

IDM testing didn't change for Version 1 and Version 2, and our tests are the same as those you have seen presented earlier this morning. We have come up with a process for anti-HBC-positive. Repeat reactive units, if

the bank chooses to list them in the NMDP STAR system, then, these are exception units, and therefore, the transplant center physician would have to agree to accept the unit and would have to obtain the recipient's informed consent. We haven't yet had one of these units, had this be an issue or have one of these units come up.

For anti-CMV, IgG, or IgM-positive, we collect these separately. We will accept a repeatedly reactive. These are not exception units in our system at this time.

So, what about these some 6,000 units that are in the NMDP STAR system? I already told you they were from three different cord blood banks.

This slide shows the average volume prior to processing, so this is the volume collected without any additives plus and minus the standard deviation, and you can see that among the three banks there is a fairly remarkable consistency, and these numbers are fairly close to what you have seen presented earlier. All of this seems to suggest the pregnancy and delivery is somehow some sort of a fairly well characterized event at this point in our evolution.

What about post-processing? This is post-processing total nucleated cell count times 10^7, and this is a histogram unlike what was shown earlier, so it shows the number of cord blood unit in each range down here, and what

you can see is that, just like Dr. Wall said, the median, the mean is somewhere right around 100 times 10^7.

We have a few units less than 500 that have very high nucleated cell counts, and just a handful that go way up into the high ranges of nucleated cell counts.

The same is true of CD34. We should have broken this category down a little more, but this is the histogram. Zero to 10 million CD34-positive cells frozen. That is the vast majority of the units in this system. You will see on the next slide that the average is right around 4 times 10^6, so again very consistent with the data that you have seen presented earlier today.

We have a few units that have higher doses of CD34-positive cells, and just handfuls again that go above 20 million CD34 per unit.

This then looks at our three banks and points out that there is a remarkable similarity in CD34 content between bank A and B. Bank C has a routinely higher CD34 content and a higher standard deviation. We have yet at this point to determine whether this is a difference in the way CD34 cells are measured because that is bank dependent or whether it is a difference in the way that this bank selects its units for storage, and, in fact, they do have higher CD34 content.

So, these are some of the things that have to be addressed as we go forward to try to figure out what causes variations from bank to bank.

Now, the way the NMDP system is set up for confirmatory typing, we have already heard people talk about retyping of the units. Any unit that comes up on a search, before it can be transplanted needs to be retyped. At our confirmatory typing stage, we will send a sample from the unit to a central laboratory that is under contract with NMDP for intermediate level HLA AB typing and high resolution DRB1 typing.

The transplant center, in turn, sends a recipient sample to the same laboratory for typing. So, both the cord blood unit and the recipient are HLA typed in a single laboratory to confirm the degree of match between the two.

We have decided that confirmatory typing only needs to be done once on a cord blood unit, and this will help conserve aliquots, the precious aliquots on these units. The results will be available for another transplant center, and then they would need to confirmatory type the recipient.

Now, the one caveat on this is that if there are new alleles described at a certain locus, it may in fact be necessary to retype that unit in order to take account of

those new alleles that have been described before the original confirmatory typing and the subsequent search.

All this also says that HLA matching, though, is not the only thing that is being considered here. There are many other factors that are considered, like how was the unit typed, was it typed by serology or DNA, what is the cell dose.

We have already alluded to the critical role or cell dose in CD34 content. What is the information on the donor history, and answers to the maternal risk factor questions, and so on, and so forth? How was the unit processed since we have seen that this clearly differs from one bank to the next? How was the unit stored since this also differs from one bank to the next? What is the availability of additional samples for testing?

We heard it alluded to that if the unit is going to be used for metabolic disease, then, most people will want to do an enzyme test on a sample from that unit to determine whether the donor was, in fact, affected or a carrier of the disease for which the transplant is being done.

Of course, there is other stuff that people get concerned about, some of it real and some of it in their imagination. Nevertheless, it's a concern.

So, in order to try and deal with the massive amounts of data and the questions that would be asked by the hundred transplant centers participating with NMDP, we developed a software program to allow cord blood banks to submit data to the NMDP. This is called CORD LINK. It allows for the collection of data on individual cord blood units. These data are then formatted and transmitted to the NMDP STAR system. This currently does not occur over the Internet, but over phone lines.

Future releases will include on-line forms and forms due data, and also additional assistance with inventory management because currently, this software does not meet the needs of a cord blood bank in its quality control processes and its overall inventory management, and so we are going to try to work with the banks to build in the functionality that they need going forward.

This software is provided under a license to the cord blood banks, it is provided free of charge. It is actually provided to any cord blood bank that would like it and is willing to sign the license free of charge, so that a bank that is developing, if you wanted to start off with CORD LINK, we would like to make it possible for you to do that.

I am just going to show you some screens. I don't expect you to be able to see all this stuff. But

CORD LINK consists of a whole bunch of tabs, the identification tab, blood volumes, delivery information, cord blood unit antigens, maternal antigens, maternal demographics. This is the only one that doesn't come to us because this would have mothers' identifying information if the bank chose to put it in, history and notes and forms.

This particular identification screen really contains I.D. serial numbers, dates of processing, registry status, whether the unit available for searching at this time or whether it is on a search, et cetera.

It also contains down here--and I don't know whether you can see it--the race data. We collect the race data on the infant, and so you can identify as many different race codes as you want for that infant, and presumably that is the combination of the parental codes that have been collected at the time the unit was stored.

This is the blood volume screen, and it just starts about cell counts and blood volumes during processing and CD34 content, and CD3 content. Down here is an interesting part of the screen that is blown up here.

It includes the ABO, but also we have heard people allude to already the different segments that are being stored or different samples that are being stored on the cord blood unit, so this has number of segments that are attached to the bag, whether there is filter paper

available, whether there are red blood cell pellet cells available which would have DNA in them, extracted DNA aliquots, nonviable cell aliquots frozen just straight away, plasma, viable cell aliquots again, and then over here is where maternal aliquots go.

We have had to put in all these different classes of aliquots in order to accommodate what the banks are storing, because different banks are storing different things, and so we had to provide enough fields so that they could put what they have on their units into our computer system.

This is HLA typing. This is called the CBU antigen screen. You can see that we can collect data on all HLA loci. Required data are HLAA, B, and DRB1, C is optional as is DQDRB3, et cetera, and you can specify the method, whether it was DNA typed or serology typed by putting a "D" or an "S" in this column before you entered the data.

All of the banks right now are typing by DNA, so every bank that is working with us is typing by DNA at both Class I and Class II, and I think that is very encouraging, and then you put in the HLA type--O2AEK, the famous NMDP originated allele codes.

This will only accept valid antigen or valid allele codes in each of these fields, and so you enter the

first antigen, second antigen, and so on, and so forth, the date that it was obtained.

This is maternal information, I won't go through this. It has got mom's birth date, how many infants were delivered during this pregnancy, and then whether there were any complications during the delivery.

This one, processing protocols is something that has been really difficult for us, and here what we want to do is be able to describe the protocols for how the units are frozen, how their volume reduced by red cell removal and/or plasma removal, what anticoagulants are present at collection and in the final frozen unit, what cryoprotectants have been added and at what concentrations, what additional diluents or additives are present in there, and we are still working on refining this, but we want to identify for each bank how their units are processed, so that you can assign a single protocol to the units from that bank and be able to determine what is in the bag in the freezer and its component contents.

This is our forms tab. We will accept forms. Maternal infectious disease markers are required. Cord blood unit infectious disease markers are optional. The maternal at-risk questionnaire is required. The family medical history is also required. I am speaking here in Version 2.

In Version 1, these things are expected to be held at the cord blood bank that are available for sharing with the transplant center, and all of this data accompanies the unit when it is transported for transplantation to the transplant center.

Just going into one of these forms only, I will just show you what our family medical history area form looks like.

This is a maternal-at-risk questionnaire. We have broken this down into tabs for high-risk behaviors in the past 12 months, in the past 3 years, and then some additional questions. So each tab has questions in that order, and this first tab, what you see here, is the "have you ever" tab. These are our Version 2 questions, had AIDS or a positive test for AIDS virus, had sex even once with anyone who has.

You have to enter the value here. There is no default value in any of these fields. You have to enter Y or N into each of these fields.

This is the family medical health history questionnaire. I told you these were broken down into nine categories. Up here is information about prior pregnancies, miscarriages, whether any children died before the age of 10, et cetera, that we have heard talked about earlier. Here is where we collect that information on our

forms. Then these are the actual family medical history forms. This shows the cancer and leukemia tab here, which I blew up here. So then if they answer yes, there is a history of cancer or leukemia, it goes into the various organ systems and asks the brain or nervous system, you must enter a Y or an N in each of these columns.

If you enter a Y in any of these columns, then you have to specify who was affected, baby's mother, baby's father, baby's mother's sibling, baby's grandparent, baby's sibling or baby's father's sibling, if the family can even figure that out.

Here we have that they check other cancer or leukemia, and then a pop-up box opens here and you have to enter what the disease was and who was affected.

So this is how we are working forward to try to collect data, try to bring some organization to this. Once of the issues, of course, is that we have brought six banks together and tried to reach consensus on this. When each new bank comes in, then they say, "Well, I wasn't part of that consensus-building." So then they want to have the opportunity to relook at things.

They have only got one vote, and the other six people have six votes. So maybe we will just limit it to five additional banks, and then they will never have a

majority, but I do not think that will be a feasible solution.

What I have tried to show you is that I think data management is a major challenge, that there will always be differences between banks, and we have to figure out some way to take this massive amount of data and collect it in a way that can be shared with the transplant centers in a feasible fashion and in a quick fashion so that these searches can perform, as we all expect them to perform, in a very rapid fashion.

The software in the systems need to accommodate changes as we go forward in regulatory and other requirements. So this will not be static. It will be changing, and there will be a Version 3 and a Version 4 and a Version 5, et cetera.

What we are doing now is working toward recruitment, collection, processing, and storage standardization, recognizing that that will be ongoing and it will involve really multi-bank collaboration.

So I would like to thank you for your patience. That is all I had to present today. Thank you.

[Applause.]

DR. LAZARUS: Now that we have a very broad perspective on the U.S. experience, we will move to European data. Dr. Peter Wernet from the Institute for

Transplantation and Cell Therapy in Dusseldorf will be giving two presentations, one immediately following the other. He will first present NETCORD data, and then he will segue into a discussion of nucleated red cells.

NETCORD

MR. WERNET: Thank you very much for this introduction. I would like to thank Dr. Liana Harvath particularly for inviting me, and as you will soon realize, I am going to present to you not European data, but data of an international network of cord blood banks and the two major goals of this group of international cord blood banks have addressed a high uniform standard of quality of the units and the solution of a network system to achieve the best possible allocation of a given and available cord blood unit in any of the banks worldwide for any given patients at any time.

In 1998, at that time existing larger cord blood banks in the world had realized that they were facing, more or less, similar problems, and one was standardization of their product in terms of quality and validation and the other was communication between the banks and the transplant centers.

So I would like to present you after a brief introduction of the organization some examples of the experienced gains thus far, and since I was asked to

present problems, problems are coming with experience and you will be amazed at some of the problems we have encountered. I hope to present to you also some aspect of the solutions, particularly a very recent success which I think has been made by NETCORD in that we now have, together with the North American organization, a real thoroughly worked through standards booklet. As you see at the end, this is resulting now in a joint accreditation process for cord blood banks.

This gives you an overview of the present 14 banks in NETCORD. In 1998, the NETCORD was founded in Italy, in Milan, where Geronimo Silkia [ph] is our acting director and Pablo Rubinstein is the vice president, and I serve as the main receiver of all the complaints. So you see these banks have had the requirement to enter with an inventory of at least 1,000 units.

We have, as I just pointed out, developed together with FAHCT, a joint quality standard and international accreditation system, and we share an allocation system which at that time, November of '99, it contained 48,000 units. As you will soon see, this figure is increasing.

Here we have units involving access to transplant centers, and this afternoon, Peter Hakenberg will present to you the detailed story of this internet-based search

system and allocation system. So I will just briefly touch on the basic problem.

So we have the focus on these different things, and it is a little difficult to present you with something new after all of these detailed presentations you have heard this morning already. So I thought I would try to present some special aspects.

This gives you the actual picture as of May this year of the 14 NETCORD banks. The inventory, there is now 46,397. The number of transplanted patients is 1,641, and the majority was 1,246 in children and 376 in adult patients.

As I just pointed out, the major goal this year has been the completion of the international standards for cord blood collection processing and testing banking selection and release, in its first edition jointly with FAHCT, and for someone who would like to look at this later, Dr. Spall [ph] from Denver has kindly given this to me here. As you will see also, there is an inspection checklist already available which you may want to look at also maybe a little later.

If you look at the basic structure of the standards, you will see that the Part A encompasses terminology and definitions. Part B is the banking requirements. This is quite detailed and elaborate and

contains the combined experience of the NETCORD bank together with the FAHCT organization which had gained, as you know, considerable experience in stem cell processing laboratories. Part C concerns donor and collection standards. Part D is the cell processing standards. The E part is dealing with selection release and shipping which is just the inventory, but it in fact goes into exact details of the standard operational procedures.

The other major goal of the international NETCORD organization is the coordination of the allocation process because optimization is possible only with complete information concerning all cord blood units listed and available and all patients requesting a cord blood unit.

To that end, you have to realize that quite distinct from the search process in unrelated bone marrow transplantation, because of the larger window of flexibility with regard to tissue typing and actual incompatibility, there is frequent situations that you have two patients for whom one definitive unit in the inventory is the best matching and similarly the largest unit, but at the same time, you have a number of other units available which could also be acceptable to this or that patient, but is not the optimal unit.

Then you have patients in the background who also would like to maybe receive this unit, but for whom also

other alternatives may be available. So how to deal with this fairly complex situation is the key element of the paramedics working group in NETCORD. As I said, Peter Hakenberg will address this, this afternoon, to you. This gives you a preview of the internet program.

With regard to the critical final part, how to evaluate the cord blood units listed in that NETCORD, we have in Europe utilized so far the EUROCORD Registry which was set up by Ilia Glukeman [ph] who, as you know, performed the first cord blood transplantation at the end of 1988 in Paris. The EUROCORD Registry collects clinical outcome data from many countries worldwide. For the European arm of NETCORD, this is presently our operations basis to assess the clinical outcome of the units of NETCORD from mainly Europe.

You see that the registry as listed here, 505 units from NETCORD banks here, are the detailed figures. Always New York is leading, as you can easily imagine.

When you look at individual developments, so I take the opportunity to do a little PR for Dusseldorf bank, you will see that the number of units start and are preserved, ready for transplantation, and has increased in the NETCORD banks quite substantially. This is the number of units released. We presently have released 117 units for clinical transplantation.

The basic logistics of the program in NETCORD is fairly standardized. The transfer after the obstetrics mod to the GNP facility is followed by processing standard volume reduction following Pablo Rubinstein's protocol, preservation in liquid nitrogen, and these are the accompanying test systems in the quality standards. You will find that accreditation is required for histocompatibility testing, for example.

This is the basic infectious disease testing you have seen several times, and if you have a request for a unit report, then certain measurements and laboratory testings will start, will be implemented within or by the bank, and in case of a transplant request, definitive transplant request, actually typed confirmation is followed by confirmation of the maternal haplotype. You have seen the potential problems which can result. If you do not do that this morning in Cladd Stevens' presentation, we do a second virology testing on the mother. In the shipment, we control the temperature in the dry shipper electronically by a so-called hot dog element. The thawing and washing and preparation procedure at the transplant site before infusion into the patient is again followed by Pablo Rubinstein's protocol. Critical is the clinical follow-up which primarily has been conducted by EUROCORD, but we now

also discuss alternative and more international data assessment programs.

To come to some of the problems in cord blood banking, this presents the critical relationship between the volume and cell number you want to transplant to the patient. This is the body weight of a given patient from zero to 120 kilograms. This is the frequency of a cord blood unit which can be found in the inventory, and you see three lines, red, green, and yellow. The red line is if you are aiming at a transplant cell count rate of one times 10^7 nucleated cells per kilo body weight, the green for two times 10^7, and the yellow for three times 10^7 nucleated cells per kilo body weight.

You can easily see when you are wanting to transplant a modest cell rate, you have a body weight of 70 kilograms and you have a 70-percent chance of finding a donor unit available in your inventory, but if you are aiming at three times 10^7, then if you look at 70--you see the enormous limitation presented by units which have been collected in earlier years which were 40, 50 ml, in that area.

One of the consequences of this experience has been that we tend to collect volumes not below 80 ml, and the clinical result, which is telling us the absolute need to go for the highest possible cell counts and volumes in

cord blood banking is presented here. This is an analysis from the EUROCORD Registry in Paris.

If you look at adults with malignancies and their overall survival according to nucleated cells infused per kilogram, you see how dramatic the difference is in clinical outcome.

There is one possibility to overcome that potentially beyond the cord blood primary selection and banking, and that would be an expansion technology which would encompass different components as depicted here, and within NETCORD, we have distinct activities. We have working groups. For example, when it comes to ex vivo expansion, we have the Barcelona bank, the Dusseldorf bank, and namely the Denver bank has gained some experience in working with expanded cord blood units or only partially expanded cord blood units. Of course, the rationale is clear. The cell number given primarily for an adult patient is usually too small. We need expand early progenitors to the patients over the early plastic phase because of the obvious infectious disease dangers, and we have to, however, not forget the goal of long-term hematopoietic ingraftment.

We face presently in the ingraftment data a prolonged time to platelet ingraftment. So we would like to expand possibly more mature progenitors to obtain short-

term ingraftment. In order to do that, the idea is that we have frozen units not in one bag, but in two bags, or in the modern volume-reduced small bags, you have a 5-cc compartment which can be taken off on the frozen bag and the major 20-cc unit stays frozen. So you can 10 days ahead of the schedule, transplant days, you can expand these progenitors in order to transplant at day zero the component which has been expanded for short-term ingraftment and the non-expanded component of the same unit for long-term ingraftment.

I cannot go into that, but we have an example here where we in fact use three cytokines with the aim to inhibit expansion of the cells and T cells and K cells. We had a 16-fold expansion of CD34 cells, 4.34 of CFUC, and 7.34 of LTC-IC. This is the control. We transplanted a 3-year-old girl with acute leukemia, and now the patient is doing fine and alive 3 years after the transplantation which was performed under dramatic circumstances with aseptic pneumonia of that patient.

Some very trivial things which you may be surprised about have come up which we could evaluate because we share an inventory of all the units in terms of volume, nucleated cells, HLA type, infectious disease markers and so on.

If you look at a very seemingly simple parameter as nucleated cells, you see that the nucleated cell count per unit reported to the NETCORD inventory is varying considering. If you look at the larger differences, which is sort of magnified in this slide, you will see that the seven NETCORD banks analyzed here give an enormous or surprisingly large variance in nucleated cell count.

Taking into account the critical importance of nucleated cells per unit, this is not a physiological variance. We think that this is a technical variance, and that tells you how important is international collaboration and standardization in this area. If you look at the mean and standard deviated related to date of reporting data into NETCORD, you can easily imagine that, again, here there is some individual and overall variance going on.

The other example which has been addressed this morning by Dr. Cladd Stevens to you already is that of the surprising large percentage of nucleated red blood cells in cord blood units, particularly after volume reduction.

This gives you the median value of nucleated red blood cells in before-volume reduction and this after-volume reduction. If you simply consider again the major parameter for the use of a unit in a patient with regards to his body weight, you can easily imagine that a clinician who receives a unit with a defined nucleated cell count

containing such a larger number of nucleated red cells will thaw this unit, and in the thawing process, most of these nucleated red cells will die and be washed out. The resulting counting before the infusion into the patient will be a disappointment to the clinician because he will encounter at least maybe 20 to sometimes 40 percent less nucleated cells than he had been told to be in that unit by the cord blood bank.

Therefore, I would like to address that problem in a separate presentation after this, kind of as a technical explanation how we can understand this. You have heard this morning already by Cladd Stevens that behind this is, however, luckily, hiding the elusive stem cell quantity which is compensating for this loss in nucleated cells.

Another peculiarity with regard to cord blood transplantation is the immune reconstitution, and one of the possible bases for this is the special phenotype of the dendritic cells present in cord blood which is shown here, namely that cord blood contains the vast majority of the dendritic cells, 95 percent, something like that, as Lin-negative DR-positive.

You can isolate these specific dendritic cells and subject them to functional analyses, and you can assess the frequency which is seen here. This Lin-negative DC is,

as I said, the vast majority. Then you have a Lin-dim DC population in maybe 5 percent median in the cord blood units which would be the type of dendritic cell which leads to an enzyme-specific T-cell response. I do not want to go into the situation here, but we believe from our experimental data that the peculiar composition of the dendritic cells may be one of components that the frequency of occurrence of acute and several engraft disease after cord blood transplantation is reduced.

This shows you a EUROCORD analysis of the probability of Grade II to IV acute GVHD after age-identical sibling transplants, and this is the figure obtained with bone marrow, and this is the figure compared with cord blood which is clearly lower. This data has been published recently in the New England Journal of Medicine together with the IBMTR, and this is the same figure for chronic host disease. This brings out a really critically important distinctive difference of cord blood over bone marrow.

Those of you who have seen patients with acute severe host disease or even worse chronic hose disease know what I am talking about.

Our recommendations based on this experience is that we need a tight cooperation between the transplant center and the cord blood banks for graft selection. We

need fast procedures. We need reservations of cord blood units limited in time and number in order not to block a sizeable proportion of the cord blood banks inventory for other patients. That means we need cord blood selection based on a joint report showing all suitable cord blood units to a transplant center, and we should ask the transplant centers to collaborate and, if possible, establish a scheduled date so that the reservation is defined.

Last, not least, I can tell you that in order to obtain and really guarantee a unique uniform high quality of standards in the NETCORD-based banks, we would start the accreditation program of the NETCORD banks in the next months which will be operationally run out of the FAHCT office in Nebraska with the help of Dr. E.J. Spauld.

I think that brings me to the end of my first presentation. I guess I can go on without any questions or interruptions.

Nucleated Red Cells

MR. WERNET: So then we come to the second presentation which will address this peculiar problem of nucleated red blood cells, and maybe I can just briefly show you some slides.

When you look at the correlation of white blood cells with nucleated cell counts of unprocessed cord blood

in this sample size, you have the possibility to measure the white blood cells with an optical counting procedure and the nucleated cell number with an impedance count procedure which is the standard procedure on most automated cell counters. You put the linear regression through this. You willi observe that here is a deviation. Biology deviates here.

When we saw that, we thought that this is not an artifact. We have to follow possibly this lead of technical differences. If you analyze the recovery of nucleated cells after thawing with the optical count shown before freezing here and the impedance count before freezing here, you see that there is a divergence occurring. When you look on that basis into the details of the correlation of many nucleated cell count with white blood cell nucleated cell count in fresh and thawed, not volume-reduced, cord bloods, here is the fresh, here is the thawed cord blood units. You see that the impedance count, the yellow curve, is clearly diverging from the optical count curve in blue in the fresh cord blood units. In the thawed units, this divergence has disappeared.

So I do not want to bore you with too many of the details, but I want to remind you that the optical measurement of white blood counting is using these depolarized system. You can clearly detect very precisely

white blood cells of all and any type, including nucleated red cells, and the principle for the impedance measurement, which is shown here, the measurement is based on the size of the nucleus. When you look at cell concentrations in cord blood units before and after volume reduction, you see these figures turning out before volume reduction and this is the data after volume reduction, from 89 to 27 ml, and the white blood count, the mononuclear cells, nucleated red blood cells, platelets, and hemoglobin--you have an increase depicted down here which tells you that it is not trivial whether you measure with impedance or optical counts.

The yield of hetastarch volume reduction leads to an increase of certain elements in this cell composition. The yield after volume reduction is in blue, white blood cells, 89 percent, mononucleated cells, 84 percent, nucleated red blood cells, 95 percent, platelets, 58, and 27 hemoglobin. So, from that, you see that there is in fact an increasing concentration of nucleated red blood cells in the process of volume reduction of the cord blood unit which explains why after volume reduction the content of nucleated red cells in the cord blood units is more than twice or much higher than in the beginning before volume reduction.

Here, you have a scattergram of the fold increase of nucleated red blood cells. If you go per 100 white blood cells before volume reduction--and this depicts the fold increase of nucleated red cell concentration after volume reduction. So this, we think, is the technically based explanation for why in most cord blood units the dramatic frequency of nucleated red blood cells has been missed initially, and on the market, there are only a few, to my knowledge to instruments, which can deliver the distinct measurement of nucleated red blood cells which then can be deducted from the nucleated white blood cells to be given to a transplant center.

This is a technical control just to show the linearity of measurement is in fact available for platelets. This control has been done, and I think the difference of cell count between undiluted and 1:2 diluted samples of volume-reduced cord blood is also something which one can utilize to assess the quality of these measurements. I think I don't want to bore you with all these figures.

Then another point is that you have to be aware that if you use the standard counter for nucleated cell counts, this number may contain a sizeable proportion of nucleated red blood cells which will be missed by countings done on the thawed units immediately before the infusion

into the patient, and that can cause insecurity in the clinician, and that may cast doubts on the quality of the cord blood banks delivering these units.

Thank you very much.

[Applause.]

DR. LAZARUS: Thank you very much for providing an international perspective and also for sharing the data on cell counting which is of significance clinically.

Our next speaker is Philip Coelho from ThermoGenesis, and he will be presenting some information on the effect of transient warming events on cryopreserved stored cord blood cells.

Transient Warming

MR. COELHO: This was a study taken in response to several anecdotal comments about cell losses, perhaps due to the invasion of heat into the units. It is hard to compete with my own computer here for sound.

In addition to what Peter has presented, it occurred that there may be some other reason why transplant centers report lower cell recoveries rather than the cord blood banks have reported.

Also, when were visiting Coriel [ph] Institute, we found that they had made the decision a number of years ago to store all of their cell line samples underneath the liquid nitrogen, and apparently they had done a study, or

so they told us, where when they used to hold them in vaporphase, they found a repeated loss in cell viability of those units at the very top of the nitrogen freezer.

Since every time you open a nitrogen freezer and you see all the fogs and that is actually a thermodynamic event, it is an infusion of heat down into that environment. It occurred that there may be some effect to these altering temperatures that occur.

A transient warming event occurs routinely through normal practice. We call it "transient" basically because the unit begins at a low temperature and then must move through the ambient air into the subsequent freezer. Typically, in cord blood banking, you may see a procedure like this where a unit is frozen down to some temperature. It could be minus-80. It could be lower. It is then transferred through the air into typically a quarantine dewar. At some later time after the infectious disease testing is in, they then transfer to a storage dewar. Then, over a period of time--it could be years--it may be a rack that may contain more than one unit--would be taken out to retrieve a single unit and then placed back in the nitrogen. How many times that occurs just depends on the probabilities of the draw there.

Then, at a later time, finally the unit is retrieved for transplant. It is transferred to a cryo

shipper. That arrives at a transplant center where it undergoes it one more time. The last time, of course, it is taken out and thawed for transplant, but there is clearly a number of occasions when the units are exposed to the warmer air.

A transient warming event is a number of things. Clearly, it is at a warmer temperature. So there is radiant heat invading into the frozen unit. The convection heat is a little more complicated because it carries with it a water moisture that is in the air.

Just to give you some quick example of this, if you have a gram of water vapor which condenses down into a gram of liquid, you are basically extracting the latent heat evaporization, and that is 580 calories per gram. If that same liquid then freezes, you are adding an additional 80 calories of heat that had to have been absorbed or it would not have occurred.

Heat runs to cold. So, to that extent, if 1 gram of water vapor condenses and then freezes on a frozen unit, that alone is 660 calories, not counting the heat that is transferred as a result of dropping from the condensing temperature down to freezing and then on down to the temperature of the unit, which would add to this. But that alone would take a 25-ml unit and jump it up 53 degrees Centigrade essentially. The question is how fast does that

occur, and we will see some data in that regard, but the answer is it occurs as fast as you see the frost. That is the evidence that it has already occurred.

This rate of warming increases obviously with ambient temperature and in two ways. As air gets warmer, it will retain more moisture. So the ambient temperature is important. The humidity is quite important. Also important is air exchange rate in those locations where you are doing this.

As it turns out, of course, you need to evacuate the nitrogen from the rooms where you have these liquid nitrogen dewards. So, typically, they have very high air exchange rate. They are required to. When you have these high exchange rates, it is hard to scrub the moisture out of the air because of the sheer flow. So, typically, they tend to be of a higher humidity than the air-conditioning may make available elsewhere in the building.

I have been to centers where they have humidifiers right in the room, and sometimes on more than one occasion a day, they will dump a gallon of water out of it and put more and start collecting again.

So this particular experience was basically taking 3 cord blood units, volume reducing to 20 ml standard, DMSO, a percentage, divide it into 1-ml aliquots. We set up basically three temperatures to explore, minus-

25, minus-40, and minus-80. The control was maintained in liquid nitrogen.

They were frozen in a standard; in this case, control rate freezer, 2 degrees per minute, down to the final temperature. We basically set these up with ethanol baths so that we would know that we achieved that temperature and no higher.

So, basically, we tried to go from the minus-196 control temperature up to one of these three temperatures and then back down. That would be a single transient warming event condition, and then with other units, we repeated at five times arbitrarily. The total recoveries were measured in ways that you are familiar with here, both CD34 and assays.

Looking at some of the results here, we will do three tables for the raw data and then some summaries. Basically, this is then unit one, the first of the cord blood units, the second unit, and the third unit. You can see that with one encounter--so this is by the control here, 68 percent--one raise to minus-80 and back down again showed a small loss. Five times showed a larger loss. When we went up to minus-25, there was a relatively small loss, but then a considerably larger loss.

The second unit showed similar or no loss here with one time, but a loss showing up on five transient

warming events. Minus-40 is kind of a similar pattern. The third unit, again, repeated the same basic pattern which was very small loss, once to minus-80, but a significant loss showing up with repeated times, and at minus-40 essentially getting worse.

If you look at the CD34 selected cells, you find basically a similar position. In this case here, 4 percent rising to 10.6 percent and repeated exposures, 11 rising to 23, no loss, but rising at minus-5--rather at five occasions. Anyway, it is fairly similar.

The colony-forming assays again followed a similar pattern, once a little higher than the others have reflected, higher yet, and repeated exposures. You can see at minus-40, the conditions seem to be more significant.

By the way, this is unit number two and unit number three. We did not do this with unit number one, but, again, the same basic pattern, some loss, greater loss, some loss, greater loss.

So, if you look at a summary of these--in this case, just looking at number of transient warming events, for the leukocytes, these would be the medians, zero to 4 and 9 to 11. With this particular unit number one here, we didn't compute the range. CD34 cells, again about the same, CFU-C about the same, but basically the same pattern

in that the higher the temperature you go to, the loss appears to be greater.

Here is looking at five times. The main thing to be noted here is that the losses are on each occasion with each different cell examination worse than they were with a single exposure.

So basic conclusions, these TWEs can cause measurable decreases of cell viability, including colony-forming activity. The damage incurred appears to depend on the magnitude of warming. It appears to be cumulative. At least when repeat, the TWE is repeated five times.

I think what this demonstrates is that we should be looking more closely at this and looking forward to establishing optimal stoppage and transportation conditions that will allow the patients to have a little better chance here.

I have put down four quick methods to address this TWE damage. This is based around our system, but essentially all of these things can be accommodated with any system, not just thermogenesis system, but the photos will reflect ours because that is what we had available.

First of all, just in general, it is good to have a small bag rather than a large bag, whether it is the Pall one or another one. Basically, the surface area is the conduit in which the heat uses to get inside the unit. So,

if you have the same volume and you shrink the surface area of the bag, you have given it less chance to come in, less surface area to introduce the heat. You can demonstrate that here between the Baxter bag and the Pall bag rising up from nitrogen temperature.

Note here, this is 22 degree C., 46-percent humidity. What we didn't capture at this time, and not realizing the importance of it, is the air exchange rate in the room. It is also important to know where to measure these bags when you are doing this.

If you were measuring a plasma bag freezing, you would want to measure it right in the geometric center because what you are interested in is the location in the bag that gets to that temperature the very latest.

When you are thawing and you are looking for the elevation of temperature, then what you want to do is measure it just inside the skin a little bit, just inside the bag, because that is going to be the onset of the heat, heat coming from the outside in. So both bags are measured just under the surface here for these particular tests.

In fact, that is a little hard to see here. There is actually another line here. This here is the Pall bag outside of the canister, and here it is with the stainless steel canister which does retard it to a certain extent because stainless steel doesn't have a very high

thermal conductivity. Aluminum canisters have a higher one, and they do not really provide insulation.

The second way to do it is to reduce the incidence of TWEs. In our case, basically we are taking these three dewers and located them into a single one. So what that allows is basically when we integrate the control rate freezer into the storage freezer, then there isn't a transfer through the air. The unit is frozen here, and then once it is down to, in this case, minus-50 degrees Centigrade, it is simply transferred down to the nitrogen. So there is no transit through the air.

We eliminate the quarantine freezer or the requirement for it by overwrapping the unit in another bag, and you need to use a plastic that has a glass transition point below minus 200 degrees Centigrade so that you can actually validate it that you really have a unit well protected.

The third way is insulate the PCB unit upon removal. This happens to--when our units are removed, they are removed up into a special retrieval cartridge. So it basically deposits the unit into what we call an insulated sleeve. Again, this is a little hard for you to see here, but the canister is not inside here. You can read the barcode, but you can handle it with your hand. The purpose of that is it clearly stops the moisture from getting to

it. You can still identify the unit by its barcode, but you can handle it with your hand. So you have not lost the dexterity that you have when you are in those big thick gloves. I have actually been in centers and seen units dropped.

Also, if you do drop it, you have some insulation, but this basically can be done for any site out there, and I strongly recommend it. Again, this is hard for you to see, but this right here is the unit. This is now higher temperature, higher humidity, but this is the unit without the insulated sleeve, and this line right here connecting these dots is the unit with the sleeve. What that does is it jumped from a couple minutes out to around 12 minutes to accommodate a transfer before you get to minus-80. So it is all common-sense stuff.

The final thing is this is, I think, a real phenomena. The cord blood banks, I think, should be looking to record them and running their own tests and their own validations, and that information should be provided to the transplant physician along with some sort of understanding about the effect that they may expect in a unit.

Thank you very much.

[Applause.]

DR. LAZARUS: I will take this opportunity, this technical break, to introduce our next speaker in the interest of time, and we are doing very well.

Dr. Akabutu is the executive director of the Alberta Cord Blood Bank. He is a professor of pediatrics, and he will be presenting the Canadian perspective on this subject.

Alberta

DR. AKABUTU: Well, I would like to thank the organizers for the privilege of presenting before this workshop. My presentation is going to cover what we have tried to do at the University of Alberta in Edmonton, Alberta, in Canada, the frozen part of the North American continent.

The scope of my presentation is to tell you about the aim, what we have been trying to do, describe some of our banking activities, evaluation of our activities, and some new insights that we have recently made. It is sort of a difficult act to follow when your presentation has been mostly given by the people before you, but, anyway, I will do my best.

The aim of our banking is to really collect as many cord blood samples as possible across Canada, and the reason we chose that philosophy is that we are trying to reflect the genetic diversity of the Canadian population.

In addition to that, also, we have decided that we will have a public unrelated bank with equal access for all Canadians and hopefully for the rest of the world. Also, we have decided to store as many cord blood samples, and we do not know what the optimum number as, that will permit us to provide samples for transplantation for children and small adults who need this form of therapy.

The scope of the operation is huge, and if I had known this, believe you me, before I started this thing, I would not have done it.

Our enthusiasm for cord blood banking started after I attended the first FDA meeting here in 1995. So I have to really put the blame at the feet of you guys who encouraged me to do this.

Our program starts with the recruitment of the patients, and this is originally done by sending registration material to the potential donors. We do not advertise our services because we are not financially very sound. So we do not want to be inundated with cord blood samples.

The registration has to take place before the thirty-second week of gestation. This allows us to have dialogue with the potential donors to answer all their questions before they donate.

At the present time, we have about 1,500 samples stored and a total of 25 of these are for families in which there is a member that may benefit from cord blood stem cell transplantation. We do that also free of charge.

We have 1,000 samples, HLA type using the DNA, for both Class I and Class II. This is done on contract drugs by a firm in the United States.

We have about 10 searches so far and have performed one transplant which was a related transplant successfully. This was in a child with MDS who is now alive and well 2 years later.

The next series of slides will just detail for you the scope of operations. These are donors according to province, and my own province, the Province of Alberta, of course, has the highest number of donors, but we get donors from right across the Nation.

Up front, we have exclusionary criteria which are administered for us by the obstetricians and midwives, and they do a very good job. Those issues that you are not able to solve are referred to me, but interestingly enough, the two frequent questions that I get asked about, the exclusionary criteria for cesarian section and maternal age--and some of our donors get quite irate with me when I explain the reason why, especially maternal age--the reason why we exclude them.

We have had very enthusiastic participation by physicians and midwives across Canada, and I should say we have physicians in every province of Canada collecting cord blood samples for us. The institutions also have been very, very cooperative with us. In fact, most institutions do their own IRB before they start collecting the samples for us.

This slide just shows you the various areas in Canada from which we have collected so far.

With the kind of philosophy that we are using, collecting right across the nation, transportation becomes extremely important, and in Canada, it is fairly expensive, about $40 per sample. The problems that we have had have been very minimal. We have only had two samples frozen, and one was because of the snow storm that we had, but other than that, we have had very little difficulty. The greater difficulty, of course, it is sometimes the couriers are not very prompt in the delivery of the samples, and we try to educate them to that.

The next series of slides are just to tell you about what we have done in looking at the samples that we have collected over the last 18 months. We had 3,000 patients registered, and we collected from 1,700. I do not know if you can see this, but the out-rate volume is around about 63 mls, but our process volume issues about 80 mls.

We have had very little contamination of our samples. We have had 7 cord blood samples that were contaminated during the collection process and 11 samples that were contaminated during our processing. We have also had the TD markers done for us. This is done by the Canadian Blood Services. So the standards are the same for blood transfusion in Canada.

We have also looked at the availability of these samples for transplantation based on the weight of these individuals. As you can expect, the largest number between four children is between 11 and 19 kilograms of body weight.

Our preferred method of collection is during the first stage of labor. In the beginning, we also collected using delivered placenta. When we look at the volumes, there was a significant difference between the delivered sample and the in utero sample. Therefore, we have switched completely to the third stage of labor. This is easy, also, for the institutions and professionals that have been helping us.

If you look at the collecting institutions that we have over here, the differences in the volumes that are collected for us are very, very minimal. They are all about the same.

We have difficulty hesban [ph] in Canada. So we have moved to the use of pentaspan [ph]. We find no significant difference between the use of the two agents.

We have also looked at the number of samples collected by an institution and the volume, and as you can see, there really is not very much difference between them.

We did a series of investigations to take a look at the birth weight of the donor versus the volume of cord blood we obtained, and although there is a trend, there really is no significant effect of weight of the donor on the volume of cord blood sample that you can collect.

We looked at the birth weight of the donor versus the leukocytes, the same story, no something influence, and we looked at the birth weight of the donor and the yield of leukocyte, and again, you can see that there does not seem to be any difference.

We looked at the volume of cord blood that we have collected and the number of leukocytes that we have. This is important for us because of the fact that our volumes tend to be a little bit smaller. So we want to make sure that we do not discard cord blood samples that may contain enough cells for transplantation.

In about 50 percent of cases, now there seems to be some form of a correlation between the volume of cord

blood and the number of leukocytes. Again, yield of leukocytes, there is no degree of correlation there.

Because we are collecting right across the nation, we decided to take a look at the influence of time of receipt and processing to the yield of leukocytes. Up to about 48 hours from the time of collection, the yield really stays about the same.

We also looked at the viability of the cells up to 48 hours after collection when the cord blood samples are maintained at room temperature, and, again, it does not seem to be a major influence on that.

Recently, because of the fact that we have been trying to improve on the yield of leukocytes, we decided to experiment with a different method of processing the cell. In the beginning, we used a single method of centrifuging the cells to yield the leukocytes that we would cryopreserve, but recently we have gone to a process of double centrifugation. In other words, we reconstitute the blood back and then centrifuge a second time and then proceed to freeze the sample.

In this slide over here, we are comparing the preprocessed sample to the double-collected sample, and you can see for sterility which would be a major issue, we have not contaminated any of the samples, but as you can see, we

have a very good yield to the double collection methodology.

Again, this slide compares the pre-single collection and double collection, and in all parameters, you can see that the double collection technique seems to yield a lot more cells. This is a similar story in the slide over here. So we now routinely use the double collection method with sterile docking procedures to minimize contamination.

The final series of slides that I wanted to present really follows very neatly on the last presentation that you heard. We worried that the method of labeling cord blood for transplantation usually is the preprocessed and the value that I used. What we wanted to do was to take a look to see if this is really the best thing to do.

In the first instance, we validated our methodology that we can actually use the single platform technology, that we can actually accurately measure CD34 cells, and you can see the linearity of that.

The second one is about the viability of the cells. Again, we can measure them.

What we did in our experimentation was we took cord blood samples and we froze them to different sub-zero temperatures. You can see minus-10, minus-20, minus-30, minus-40, and minus-50.

In one group of samples, we thawed them slowly after freezing. The other group, before thawing, it was plunged into liquid nitrogen to simulate the cryopreservation process, and then they were thawed.

The first slide over here shows you the recovery of mononuclear cells after being plunged into liquid nitrogen. This is done in the absence of DSMO. As you can see, there is about a 25-percent loss of cells right off the bat when you do that. The survival of these cells are dismal, and the cells just about die when there is on protection.

The next slide, now with 10-percent DSMO, shows that there is really no difference in the recovery of these cells, but survival is better.

We did the same studies with CD34 cells, and again, there is a major loss in the beginning. This is without DMSO. The yield is lower than nucleated cord blood stem cells. Of course, the survival of CD34 cells is really very low. There was an interesting phenomenon over here. So that, at the intermediate temperatures, we seem to have an increased survival, but at much lower temperatures, the cells all died.

Then we added DMSO to the samples, and again, the recovery is about the same, but the interesting thing is that we began to see an increase in survival. The critical

temperature that we found was minus-20 degrees. That these cells are cooled slowly to minus-20 degrees before being cryopreserved, you will have at least 75 percent of these cells survive.

So that, say if you are a processing laboratory and something happens and you don't cool the cells to minus-20 before you freeze them in liquid nitrogen, you are going to have a lot of dead cells. However, if you reach minus-20 before freezing them, you can estimate that at least 75 percent of your CD34 cells will survive. We would urge that this kind of study be repeated by the other people to see if it is entirely true.

We can conclude from the last observation that indeed the DMSO is protected with preservation. Pre-cooling of the cord blood samples to a minimum temperature of minus-20 degrees Celsius is protected when cells are plunged into liquid nitrogen, and double centrifugation of whole cord blood during processing results in more efficient sedimentation of red blood cells with improving the yield of cord blood.

Thank you for your attention.

[Applause.]

DR. LAZARUS: Thank you to this morning's presenters, including Dr. Akabutu for distilling a year's worth of interesting data for us.

Now I will let you all know, the participants, that it is the lunch break. We have an hour. In case you are a unfamiliar with the situation at the clinical center, in the B-1 level, there is a quick cafeteria for takeout stuff, and then on the second floor, there is a more functional cafeteria. So I recommend that you all make a dash for it and try to come back at 1:30.

[Whereupon, at 12:32 p.m., a luncheon recess was taken, to reconvene at 1:40 p.m., this same day.

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

[1:40 p.m.]

Discussion: What differences, if any, are important?

DR. KURTZBERG: Let's get started with this afternoon's session. For the panel discussion, would the speakers from this morning come join me on the podium.

I think there are going to be some stragglers coming in, but I would like to get started. I would like this session to be pretty informal because it is important to discuss issues about what we heard in the session this morning.

From my understanding, one of the main focuses of this whole workshop was to find out whether or not we could identify what a good cord blood unit is--well, "good" may be the wrong word, but one that ought to be in a bank and to see if we can agree on some common criteria to define that unit.

In listening to the talks this morning, I think there were a lot of similarities between the practices of banks, but also a lot of differences. I would like to use this session to focus on the differences.

Some of these things that we could talk about are at the level of very technical or minutia level organization. I do not think we should get stuck in those

kinds of things, but I do think we should try to decide how we sort out some of the important differences between the different banks and the different practices.

I would hope that on some of the bigger issues, we could reach a consensus, or at least define where we need to reach a consensus.

I also would like to say that if you sit in the audience as a transplanter and listen to what is going on, you feel--or at least I feel--a lot of people are trying to do the same thing. I do not think of it as a race, but I do think of it as a program that ought to have quality associated with it.

It would be nice to join resources and have different people working on different things instead of the same people working--or different people working on the same things because that is not a good way to use resources. Those of us who are banking know that this costs a lot of money to do properly, and it is not cheap. It takes a lot of people. None of us have enough money to do it the way we would like to.

It would seem that if we could combine efforts rather than kind of be working on parallel tracks, we might get further more quickly. I also think that it would benefit the transplanters and the patients to have more unified searching available so that you didn't have to

remember the names of 12 or 14 banks in order to find out if there were units, and that putting all of this together, my goal would be to come up with some kind of program that was unified enough to allow combined searching and to provide some kind of product standards that would be affordable. That is easy to say and kind of hard to do.

What I think I will do is go down over here and let people participate in discussions and just bring up topics that I heard differences in, unless people have comments right now.

The first topic I thought we should talk about is cell counting, cell counts. I am struck by the fact that when you hear about a cell count on a certain unit, it could be a nucleated cell count. It could be a viable nucleated cell count. It could be a nucleated cell count with nucleated red cells, a nucleated cell count without nucleated red cells. I am not sure that all banks report the final count before it goes into the freezer versus the count that was collected, which is different than the count after processing, but in the transplant community, if we are going to be selecting units with any attention to cell dosing, we have to be talking about apples and apples and not apples and oranges.

So the first thing to put on the table is can we come to a common definition about cell counts and what it means.

Any comments on cell counts?

Cladd.

DR. STEVENS: Cladd Stevens, New York Blood Center.

I think your point is well taken about the post-processing count as being the one that is of interest to the transplant centers.

One of the things that is important in that regard is most of us, I think, are doing some kind of processing where we are removing excess plasma and red cells, but how you accomplish that has a tremendous impact on the numbers of cells that you are able to retain.

So, even though we get a strong correlation, the relationship--for example, in our bank, we have lost about 10 percent on average of the cells on processing, but that may vary from bank to bank depending on their technique. So the real thing that is important is what you have in the bag at the end.

One of the points I tried to make in showing the data on the nucleated red cell counts in the units that we have used is that in terms of the outcome of the transplants, it doesn't matter. In fact, it is just as

good as the white cell count when you correct the total nucleated cell count for nucleated red cells, the correlation with the ingraftment is just as good as it is with the white cell count. Again, that is a reflection of the correlation with the progenitors, but I think Peter Wernet had an important point which is you may lose those cells when you thaw them. So that information may be useful. I do not think it is essential, but it may be useful information for the transplant center, just in terms of knowing what to expect on post-thaw counts.

DR. KURTZBERG: I think it goes a little further than that because if you are in an institution where if we set a minimum cell count that we say is appropriate for transplant, then you may exclude a unit that could be included or vice versa based on differences and the way people count.

DR. STEVENS: If cell count is one of your exclusion criteria, that is correct.

DR. KURTZBERG: Maybe we should put that on the table.

DR. STEVENS: I do not know if you noticed. We were excluding up front based on the volume collected, but not on cell count, and we have elected to take that strategy because some of the smaller units have been

perfectly okay for smaller patients who might require them. So we did not exclude them.

DR. KURTZBERG: I do not mean excluding on the banking end. I mean when you are selecting for the patient.

For example, my IRB right now will not let me transplant a unit that does not deliver 1 times 10^7 per kilo, and whether that is right, wrong, or indifferent, that is their rule.

DR. STEVENS: It is good to have the IRBs setting standards.

DR. KURTZBERG: But they set the standards based on the data they saw from our outcomes. It is not unreasonable to set a standard like that when you look at our outcome, but if you are going to have a rule like that, then how you count the unit may make a difference in terms of what really is in that product you put into the patient.

Yes.

DR. RUBINSTEIN: The problem is that your IRB-based recommendation of no--10^7 as per kilo--on data, that did not correct for the red cells, and those units from which that count was derived lost the same number of nucleated cells on thawing that the ones that you are going to get now will lose. If you just base your

transplantation minimum on the post-thaw account, then you are wide open to these problems.

DR. KURTZBERG: No. You cannot base it on the post-thaw account because you have already selected the unit at that point, but my point is that if different banks count differently, then you are not getting the same data from each bank and it could affect your decision.

So what is really on the table is not whether you count nucleated red cells or not, but just can we define some common rules we all follow for counting that either does or doesn't include nucleated red cells and does or doesn't correct for viability, so that when you get a report from Bank X, you can know that the cell count would be the same in Bank Y if they counted it.

DR. WAGNER: I guess to address this point, we are going along with Pablo from the transplant perspective. What you need to provide to us is the total nucleated cell count, but also provide to us additional types of blood counts like mononuclear cell count or the number of nucleated red cells. Then we can in the future assess what is the appropriate cell count, but for right now, we have to have at least total nucleated cell count because that is all the data we have on which is a good unit and which one is not.

DR. FRASER: We should be polling the banks at the table. Peter is not here, but at UCLA in the COBLT studies where it is total viable nucleated cells, it therefore includes the nucleated red blood cells. Is that what is presented to transplanters from New York?

DR. RUBINSTEIN: The current suggestion that has been approved in NETCORD for reporting the cell count is to identify the numbers of both, total nucleated cell counts and nucleated red blood cells. This is part of the FAHCT NETCORD standards.

So I think these particular problems will become- as the cell regulations become implemented.

DR. FRASER: Does that correct it for viability?

DR. STEVENS: It is not corrected for viability.

Most of our units up until recently had had colony counts done which is a measure of viability. One of the issues that you might raise in the viability is how do you assess that assay.

DR. WAGNER: I guess to address the question that John is bringing up in terms of reporting viability, at least the majority of transplants that had been done to date had been done through the New York Blood Center, and if the total nucleated cell provided to us pre-cryopreservation does not include viability, then that is the number we still use as our standard by which we compare

everything else in the future. We may find something that is a more active prediction in the future, but right now that is our gold standard by which we compare everything else. So it is the total nucleated cell count in the pre-cryopreserved and in the post-thaw specimen. We have both of those pieces of data now which we can make some conclusions. Both correlate with outcome.

DR. FRASER: So, if you were looking at a search from the COBLT study, you would want to see it uncorrected for viability so you would have a direct correlation?

DR. WAGNER: I think we have to. I think that we have to be able to compare things similarly. Otherwise, if you take a harder-line approach, then you will not be accessed as frequently as other banks. That is not to say that it is not a better approach. On the other hand, we will determine that over time.

DR. LANE: From our standpoint, there is variability in the way counts have been recorded. It is very tempting for me to speak only for the western area, but I cannot do that. So there is some variability in the way counts have been recorded.

The Red Cross IND right now does not require a viability correction. However, I believe that all of our banks have that data available. So my inclination would be to provide you with all of the above data, since I do

believe all of us have it, that is to say, the total nucleated cell count, the percent of nucleated red cells, the total viable nucleated cell count, the total mononuclear cell count, corrected or not, for viability. We can actually provide you with all of that if you wanted. So it is a matter of your selecting your decision and telling us which one you want.

DR. WAGNER: My point of view is that people will be making decisions based on information that they believe might be correct, but don't necessarily have data to confirm.

What I mean by that is if you tell me that there is more nucleated red cells, I could have taken then the position saying, "Oh, well, then that nucleated cell count is not as good as I thought it was," and then make the wrong decision. We do not know the answer yet. We can make guesses.

There was another point that really concerned me which was the presentation by Peter Wernet which indicated, if I read it correctly, it looked like that different banks cannot even get the same types of nucleated cell counts which I thought was an easy thing to do, but apparently that may be one thing that you as a panel who do this all the time might want to address why is it that you cannot standardize even getting a nucleated cell count.

Thirty-four, I can understand, but the nucleated cell count, I thought it should be a given that that is doable.

DR. KURTZBERG: To be honest, I think even in the same lab, it can be difficult sometimes to get two different techs to get the same count.

DR. MARTI: Gerald Marti, CBER, FDA.

The last, Dr. Wagner, who mentioned about essentially trying to get an accurate WBC or nucleated count on cord blood, you can rest assured that all of the automated cell counters that are in existence and approved probably did not get their approval on the basis of doing their determinations on cord blood. That is number one.

Number two, there has been a post-phenomena with the automated cell counters independent of flow cytometry to pull out this further information that can be had from the scatter profiles that is with the automated counters.

I would not be at all surprised that with the proper standardization that the nucleated red cell count could become a count rather than a percentage that is being estimated. It is certainly being done for a proptosis on automated cell counters in Japan.

The other thing that I wanted to comment upon is that if we look at the drift or the trend in non-corded blood allogeneic transplantation, we see historically

nucleated counts and WBCs, but we see this drift toward an absolute count, an absolute CD34 count. Then we see the interjection.

One of the speakers this morning used the term of a single platform rather than two platforms. That is why he has all that data that he is talking about because, if you have a single platform rather than an automated hematology counter and a flow cytometry percentage, a lot of your standardization will come into focus.

So, if you could do a single platform, which means you have to have some counting particle to standardize, I think that would help a lot.

DR. STEVENS: Just to follow up on that, we just recently evaluated the SYSMEX XE 2100, or whatever it is called, which is one of the automated counters that has claimed to count nucleated red cells. We did this in a manner in which we tested the samples in duplicate on the instrument and then also did manual counts of nucleated red cells with three different technicians, all under code, blinded, and the technicians doing manual counts--we got about an 80-percent r-squared, which was surprisingly good. Of course, I trained them. So whatever they were counting, they were counting the same thing I was.

A correlation with the machine, which was just as good--the machine obviously counting the duplicates had a

99-percent r-squared. So the machine agreed very nicely with itself and also with our manual counts, which I thought was reassuring in both directions.

DR. MARTI: It might be more expensive. In the flow cytometry, it is certainly done routinely in bone marrow aspirate analysis to use a red cell marker like luciferin to estimate red cell precursors.

DR. LANE: First of all, I want to agree with that. I think that is a very good idea. We have talked about doing that in the western area.

One of the things we wanted to do was to have a single lab doing CD34's in the western region. We thought that that would diminish some of the variability in that assay.

The first thing I need to say is I am not a flow cytometrist, but when we evaluated the single platform versus the dual platform methods, at least at the time we did that, there was no single platform method that we felt confident that you could ship cells without fixing. In fact, they do not permit you to do that, at least the ones we looked at. So the dual platform methods had the advantage of permitting you to fix cells right after processing ended and then ship them up to your counting site. We evaluated the number of days at which the counts were stable and have validated our system, but the single-

platform assay did not permit that, and we do correct for nucleated reds.

DR. AKABUTU: I would like to address the issue from about three standpoints.

The first standpoint is that the different classes of cells have different responses to cryopreservation. For example, at the end of cryopreservation, when you thaw cells, there is a marked difference between recovery of mononuclear cells and, for example, recovery of CD34-positive cells. However, the survival of CD34-positive cells is higher than the nucleated cells that are recovered.

So, if you depend upon nucleated cells as the measure for the cell dose, you may be actually underestimating the number of true progenitors that might be present in the sample.

The thing that worries me is the fact that as we continue to manipulate these cells, we may end up losing that relationship that exists between our various cell compartments, the CD34 cell going onto the true multi-important stem cell that we all want for transplantation.

So I think the issue of what do you label the post-thaw product as--is it total mononuclear cells or CD34 cells?--it is a very important one. From where we stand, I

think that most of the labeling right now probably underestimates the potential for transplantation.

DR. KURTZBERG: I want to back up because we need to simplify this. Right now, there are 38-, 40,000 units, supposedly, in banks around the world available for transplant, and they have all been collected and processed in different ways. There are transplanters out there who would use them, and there are patients out there who need them. There is a lot of theoretical and esoteric kinds of things that can get sorted out in the next 5 to 10 years that may refine this, but if we want to use these units right now and a transplanter needs to make a decision about a unit from Bank A and Bank B, how are they going to do that? Do they have to know the code and the processing methodology and exactly how counts were measured, et cetera and so forth, from each bank, which isn't really possible?

I think different things will happen. Some transplant centers will choose not to use certain banks. Other transplant centers will not even realize there is a difference, and they will just be blindly going along. Then there might be a problem that could have been prevented.

So, if we were playing a game right now where we had to use what we have now, what could we settle on that everybody could agree on? Is there something that

everybody could agree on that could be further refined over the next 5 years?

DR. STEVENS: The easy part obviously is the post-processing total nucleated cell count. The issue relates to the other elements of this breakdown of different types of cells, nucleated red cells.

The one thing that we have not talked about very clearly here is the issue of viability. I did not see any data presented that tells us about the techniques that the banks are using or the variation in viability and what factors influence viability, and that, I think, would be critical rather than just an up-front decision that you have to have some sort of measure of viability, what is the technique and what is the experience of a bank.

The other point I would like to make, again, is a plea for the banks to gather their data on outcome of the transplants. I think that is the ultimate measure of how well the bank is performing.

DR. WAGNER: In response to that last comment, I agree that certainly the banks should have outcome data. On the other hand, it is going to be difficult to assess, especially early on, whether or not the bank is doing a good job because it could all be patient selection.

The patients themselves influences the outcome, nothing to do with the graft itself. So unlike the New

York Blood Center, which has a large database, the newer banks up front are going to have potentially more problems. It may appears that the results are negative when in fact it had nothing to do with the graft itself.

DR. STEVENS: That is an important point where you have to take into account the complexities of all the factors that influence the outcome.

Nevertheless, I think that is the ultimate way to assess, and eventually we will have that information. I think you certainly would worry about a bank that provided 10 transplants and none of them engrafted, just to take the extreme.

DR. WAGNER: But if I had 10 patients with CML or if I had 10 patients with Fanconi anemia and they didn't engraft you, it said, "Well, it probably is the underlying disease." But what you could agree on--

DR. STEVEN: Not all 10.

DR. WAGNER: Maybe not all 10. I exaggerate a little bit.

Like the NMDP is doing in trying to evaluate how transplant centers do across transplant centers, you may find up front what diseases which we consider perhaps good-risk diseases in which you could compare one place to the other, one transplant center to the other, or I should say one bank to the other. So you could actually choose your

diseases which you consider perhaps as model diseases from which to compare.

DR. CONFER: John is right. It is very difficult to do these center-specific analyses whether you are trying to analyze differences in transplant center outcomes or differences in cord blood bank outcomes.

I do agree, though, that we have to collect the data and that the data have to be collected and audited. We have to provide definitions for each field that are clear and consistent, and we have to ask people to try to follow those. The problem you run into is that no matter how much instruction you provide them in a book that is about 3-inches thick, they won't read it, and then they will end up taking the number off the machine and doing something to it because they think it makes sense to do it and they come up with a data field that does not contain the data that you thought were in it.

These are just some of the issues that we have to address going forward.

DR. KURTZBERG: One thing I was going to bring up is what if we could define a simple certification program that banks could voluntarily participate in, just like CAT for a pathology lab where you would share samples and whatever we decide goes on the list, but you could do counts. You could do CD34's if you thought that was

important, viability, whatever, and see if 10 banks or whatever could get within 10 percent of the same answer.

I am not sure in some of those things that will happen, but it would provide some validation for each bank and at least define the ranges that would be reasonable and that would correlate with one another, and it wouldn't hold you to a methodology. It would hold you to getting an answer within a certain range. You would still have the problem of what the right answer was.

DR. RUBINSTEIN: Speaking for Dr. Wernet, I would say that that is extremely reasonable. It is a very good proposal and something that can be done, and it would provide the answer that you describe.

DR. KURTZBERG: Good.

DR. LANE: Speaking for the Red Cross, I would have to agree. Our quality assurance program has discussed internally doing something like that, and I think we would be delighted and pleased to participate in either our own internal or somebody's outside program.

DR. CONFER: The only cautionary thing I would add is that these things are extremely expensive, elaborate, and difficult to orchestrate, and you have to take that into account. So I think it is a good idea, but it is a good idea to put on the table and to evaluate and determine how successfully it can be done and at what cost.

DR. WAGNER: Sort of in line with all of this, I guess I took it for granted that, again, total nucleated cell count was an easy thing to accomplish, and that would be standard between the different banks. Perhaps that is a wrong assumption, but I thought that was the easiest thing.

The thing that I was most concerned about as a transplanter was really the reliability of the HLA typing. So, before we finish this in part of the certification program, it is how can I be assured that indeed the HLA typing is indeed correct, especially in view of the fact that sometimes I cannot get a sample in which to verify myself.

DR. KURTZBERG: There is going to be a session on searching which will cover some HLA things this afternoon, but I still think John's question is a valid question.

One approach, if I understand it right, that FAHCT and NETCORD took to this was to require a sample for duplicate testing that could be sent out to the transplant center.

DR. RUBINSTEIN: Absolutely.

DR. KURTZBERG: You are still going to, I think, at some selected instances be in a situation where you do not have that sample. Then it is a question of is it up to the transplant center to decide if they would still use that unit or not.

DR. RUBINSTEIN: With the advent of molecular technology, at least we will have lists and lists because there is now an obligation for the banks to have DNA prepared and ready to ship. So the amounts that are needed are so comparatively minimums that that should not be a difficulty in getting you the sample.

Of course, in some cases, particularly older units may be different, but going forward that should not be a problem.

DR. WAGNER: Just from my own experience, and you would know this better than I do in terms of the HLA typing, what is required, I can tell you that at least at our own institution from the New York Blood Center, we have not always been able to get enough DNA to be able to do all of Class I and Class II. We have to select what we want to do. So at least from the older specimens that have been collected, there may be that problem that continues to arise.

I would have to say it depends on the bank itself and our level of trust. Obviously, we have a long experience with the New York Blood Center where we do not have that necessarily with all other banks, in which case there is that trust-building. So it is imperative that we have that data.

MR. COELHO: I just wanted to say one quick note. I visited a number of cord blood banks around the world and have seen recoveries lower than 50 percent and the building up to New York Blood Centers over 90 percent. Since everyone knows that cell dose helps determine that a unit is chosen, there is the big financial incentive to being ahead of the curve there. So I don't know how well really all of these calculations are done, but I think to ignore that as a factor is not wise. There has got to be some way of enforcing how all that stuff is done.

DR. LAUGHLIN: One of the issues that I am very concerned about in the cord blood banking discussions--and for myself having been an individual who participated in characterizing traditional allogeneic stem cell sources--I think there is aspects of cord blood that are unique.

I am an internist, and I transplant primarily adults. So I am already heading in with tenuous cell does, and I would advocate as a person in the trench that the individuals in charge of making decisions about cord blood banking somewhat follow a straightforward and simple at the outset analysis because my concern is that it seems that there are some unique aspects of measuring CD34 that we cannot jump from an adult blood stem cell graft to a cord blood graft may have to do with epitope density of 34 and

galsion [ph] distribution and immaturity of CD34 progenitors in newborn blood.

The other concern that I have is the focus exclusively on CD34. In our hands, we have preliminary data that accessory cell populations in cord blood grafts as we know in traditional allogeneic grafts are important ingraftment and particularly in circumstances where we are heading in with tenuous cell dose, 34 dose, that role of these accessory cells may take on greater importance.

In the analysis that Dr. Wernet presented this morning, I did not even see CD56 included, and that would be one of the populations of importance, just to add more complexity to what is already complex enough.

DR. KURTZBERG: I am going to get on the soap box again and say one other thing which is we cannot price ourselves out of the business. You wear a transplant hat some days, as I do, and if you have a global contract that gives you whatever fee to do the whole transplant, get your donor, follow up your patient, we start putting in multiple layers of requirements for testing and certification and we're not going to get anywhere because we're going to price ourselves out of the business. So we have to keep that in mind, too, and prioritize.

I think for today what we really want to come away with is what is really important that you have got to

have, and then there are other nice things that may be proven to be important later, but we do not know yet. That is my 2 cents.

DR. CONFER: I think you have to have a total nucleated cell count, and that is all. I think it would be nice to have some measure of viability. I think it would be nice to have nucleated red blood cells. I think it would be nice to have CD34 content, but that is going to be all over the map right now. So that is what I throw out.

DR. KURTZBERG: Does anybody on the panel have a problem with that?

DR. AKABUTU: Total nucleated cell count at which point, pre-cryopreservation or post-thaw?

DR. CONFER: I think we have got--what did you say, Joanne?--38,000 units, and we don't have post-thaw data on any of them. So this is pre-cryopreservation total nucleated cell count which is uncorrected for viability, uncorrected for nucleated red cell content. It is just all the nucleated cells that are present in the graft.

DR. AKABUTU: I will have to disagree with that because we are going backwards. If we want to move forward, I would think what we should be doing is looking ahead to see what is more reasonable than trying to make rules so that we can use what we already have. I think that is inappropriate.

DR. MARTI: Again, if you have a single platform method in flow particularly, then you can get all of those numbers, those measurements, and even the person before who spoke about another subset, in three- or four-color flow cytometry, it's about $50 to add another reagent.

Also for your standardization of viability, in listening to you this morning, there are at least four methods that have been discussed. Some of you used trypan blue, that very ancient marker that should be red within 10 minutes of less of when it is put on the hematocytometer. Some of you are very sophisticated and use ethidium bromide. In flow cytometry when people say they use ethidium bromide, it usually means they have not rewritten their protocol from 10 years ago. They are not using it anymore. Acridine orange was the third one, and I did not see it, but I suspect some of you use preputium iodine.

DR. KURTZBERG: 7AAD.

DR. MARTI: 7AAD. So that is the fifth one.

I shudder about the standardization of five reagents, but you will have fun doing it.

DR. KURTZBERG: Well, it goes beyond that because you may not run your flow cytometry within the same period of time. Different labs have different operating hours and different ways that they function, and that affects viability.

So the all tried-and-true trypan blue, do it right when you have the unit in your hand, is not so bad.

DR. MARTI: In the adult samples that we hear about, the minute those are thawed and you see the last crystal dissolving, you want to have that drawn up and injected. What little bit is left over, you might do your viability testing. How far away the laboratory is where you are going to do that testing--and this morning, something that I have always been suspicious of, but it sounds like even if you have a dead-appearing CD34 cell, it has some kind of recovery process. Anyway, that is what I interpreted the data from Alberta to show.

DR. STEVENS: Of course, the other problem is what is the influence of nucleated red cells.

DR. CONFER: I think John was getting ready to say any time you add a cost to processing a single unit, you only recover that cost on the unit that is transplanted. So, if you had $50 to the cost of processing each unit and you transplant one out of every 20 units, that is $1,000 increase in the cost of one unit that gets transplanted. So the finances here are very concerning in this nascent industry.

DR. KURTZBERG: I think we figured out a cost of about 90 bucks to do just really two tubes which gives you a 34 and a CD348 and 56, and that per unit.

DR. CONFER: Most banks would be delighted if they were transplanting 1 out of every 20 units they have collected. In fact, it starts off around probably 1 in 100 and then slowly goes down over the years.

I am not sure that anybody, except perhaps New York, has reached close to 1 in 20 at this point.

DR. RUBINSTEIN: I think that the finances are truly a very important aspect of it, but we have to keep an eye on the ball.

The reason to do all of this stuff is to provide the clinicians with something they can use with a certain degree of assurance as far as possible, and if this $50 were really necessary, it should be done. That should not be a deterrent.

The problem is there are a number of ways in which you can determine an approach to estimate the really important factor which is the hematopoietic potential.

Viability is the same as estimating the number of red cells when it is done on total leukocytes from the cord blood. Of course, if all the nucleated cells are dead, then you have a problem. Viability, per se, is a difficult thing to estimate quantitatively in a way that can travel across laboratories. It has always been.

We can do it in a quantitative way simply by fixing some parameters, for example, all using the same

technique, same--and it will still be valuable across laboratories, even if we use a counter, cell flow counter. It is not possible to improve or imagine that.

But the question is what is necessary. We heard Dr. Confer suggest that what we really need are total nucleated cells. Dr. Wagner has said that at the moment that is a piece of information that most accurately represents what he needs to know. We should move forward to develop further things, and we should do this with an eye to the cost, but we should do it--we should keep moving closer and closer to the element that we really want to know which are the stem cells.

DR. KURTZBERG: I want to spend a few minutes talking about HLA again and what level of resolution a transplant center would require of a bank or a bank should minimally put into a registry because different banks are using different HLA typing methodology and reporting out typings on the units at different levels of resolution.

You could argue that if there is always a sample to cross-check that you could take that sample of DNA and do whatever level of resolution you felt was appropriate at your transplant center, but for searching, there has to be some minimal level of information in the registry. Otherwise, it doesn't make sense to list the unit. So I am looking for comments.

DR. RUBINSTEIN: If I may, the question is a little more complicated in the case of a cord blood bank than in a registry because, by and large, there is a historical development in HLA that is reflected by the level of typing of the units that are in storage. So we have a problem that we try in our own bank to address by updating continuously as we can the typing.

We would like to suggest that the future should hold that the unit should be tested at the level--at least at the level of high resolution for Class II for the RB1 gene minimally and at the medium resolution level for HLA-A and B.

The use of molecular techniques for A and B has an advantage in that it does not require viable cells for testing over cytotoxicity methods, but at least in the case of cord blood, the superiority of high resolution in Class I has not been shown. It has not been demonstrated.

We fully expect that it will be, but at the moment, even the current state-of-the-art, I think a medium resolution DNA-based method for Class I should be implemented for all the new units.

DR. STEVENS: One of the issues here obviously in terms of the level of resolution relates to the question of whether it matters in terms of transplant outcome. I think there will be data tomorrow to look at transplant outcome,

and clearly, I think we are beginning to get convinced that HLA matters, but the question is at what level of resolution.

DR. CARTER: I have two questions. One is: At the New York Blood Center, what is meant by medium resolution? The question is: At what level do you type the B15's?

DR. RUBINSTEIN: You choose your questions well.

For Class I, we use mostly SSP methodology, the best we can get, and for B15, there is a limited resolution there.

DR. CARTER: Does that mean you do not cross the 15-70 boundary and you do not get to a allele level?

DR. RUBINSTEIN: We do have 70, 71, 72, and so on, but not all of the alleles of B15 group are separated by those methods.

We have on occasions and sequencing when we really need to know for specific reasons. One of those reasons is precisely related to evaluating the role of these minor substitutions, so to speak, allele-level substitutions of Class I alleles. This is work that is currently going on in the lab, but not for routine. We do not do it in the routine characterization of the unit.

DR. CARTER: In COBLT, we have the same experience. It is problematic, the B15's. Plus, some of

the alleles, you do not even know what the serologic equivalent is to date, as they are being added to quickly.

I do not think I heard an answer to the question of what do you mean by medium resolution for Class I.

DR. RUBINSTEIN: Well, medium resolution really is a problem like anything in the middle. It is not allele level and it is not serology level. It is somewhere in between.

DR. CARTER: Like an O1XX. Is that medium to you, or is that low? An 01XX, would that be medium or low, as you call it?

DR. RUBINSTEIN: That is probably low.

DR. CARTER: So you are distinguishing like just small strings of alleles, like 01010102 or something. That is medium?

DR. RUBINSTEIN: Yes.

DR. KURTZBERG: Any other comments on this topic?

DR. LAUGHLIN: Much of our discussions at our shop have focussed on this debate between added benefit of moving towards high resolution versus that of medium resolution, and knowing that in patients exhibiting chronic graft versus host disease, that their T-cell clones are directed against minor histocompatibility antigens, not major loci.

I was wondering comments of the panel on this--that perhaps some of the confusion in the data is that further and further resolution of HLA may have diminishing returns because much of the alloreactivity is not necessarily against that expressed in chromosome 6.

DR. CONFER: I do not think we know the answers to these questions. I think there are some alleles where mismatching for particular alleles is going to turn out to be an issue. I think there will be others where mismatching for alleles is not an issue.

I agree with Dr. Rubinstein that for Class II, we need allele-level typing. For Class I, we need an intermediate-level DNA-based typing that is at least sufficient to classify the groups of alleles into their serologic equivalents, if known.

The good example there is B15. You have to go farther than B15XX. You have to go down to where you can start putting it into at least the serological equivalents. Otherwise, you might as well type by serology because it is a hell of a lot better than a B15XX. I think that is what we know we need at this point.

One of the values of cord blood is the ability to have antigen mismatches at certain loci, and you do not need allele-level typing to know that it is an antigen

mismatch. If it is an 01 versus a 7, that is an antigen mismatch. You do not need any more data than that.

DR. KURTZBERG: So we did not really answer the question, although I think what Pablo said was a modification that it is really low or medium typing at A and B, depending on what antigen you are talking about, and high resolution of DR beta one is the general practice. Is that something that everybody could live with? It doesn't mean you cannot go further. It just means you cannot do less.

I want to switch gears because we have about 15 minutes left, and there are two other topics I would like to have time to discuss.

One is, as you listen to the different presentations, there are different kinds of exclusions that people use and different practices in different banks. I listed a few of them on this slide, but this is not meant to be all inclusive.

I want to ask the question: Are there any definitive exclusions that we could read a consensus on that everybody would exclude; for example, an HIV-positive unit? Is there anybody who would not exclude that unit?

[No response.]

DR. KURTZBERG: Is there anything else that would be in that category that anybody can think of?

DR. STEVENS: If you have a known genetic trait like sickle cell disease, you probably would not use it.

DR. KURTZBERG: Yes, I agree. Other genetic red or white blood diseases, which you would hopefully pick up by family history unless it was a new case in that family.

I listed some of the more controversial ones that we do not have common practices about on the slide. One that is a particular favorite of mine is maternal age. You saw numbers up there that said over 35, without an amnio was excluded over 50, without an amnio was excluded, no ages excluded. If you have follow-up with baby, it is not excluded. I do not know what the right answer is, but we do not even get the information about the maternal age when we select a unit from a bank. So it is not factored into the decision in the listing, and if different banks have different practices and if we think it is important, then we obviously--

So the first question is: Does anybody think it is important?

DR. STEVENS: I don't think it is.

One of the points I was talking about in presenting all the data that I did in my talk, the epidemiologic data, is clearly in our bank, we have taken the strategy of not excluding up front. In particular, it relates to the point that Tom Lane made about our

obligation to try to get adequate transplants or adequate grafts for ethnic minorities, in particular for the African-American population where we have the hardest time trying to find suitable grafts with good matching.

One of the points I was trying to make is when you add in all of these potential exclusion criterias, you end up with very few suitable units, particularly from some ethnic minorities.

One of the things that we have tried to do now in modifying our strategy for collecting risk factor data is to get much more detail about potential risk. For example, I mentioned a number of Asian-American women would be excluded because they have a risk of exposure to blood or blood products working in the health care industry primarily, but when you ask them in more detail about whether they had a specific accident or a specific exposure, the answer is generally no.

What we have tried to do is to try to gather as much of that kind of information to provide to the transplant centers so they can make some kind of a ball-park estimate of this risk-benefit ratio.

One of the things that we feel relatively pleased about is that about 30 percent of the units at our bank have come from African-American donors, and we have been able to provide grafts for almost 140 such patients, which

is about 14 percent of all the transplants we provided, which in fact is about the proportion of African Americans in the population. So it is providing a resource that is not otherwise available, and I think that is an important issues in making a priori judgment that we can exclude these units up front.

DR. WALL: I would like to agree with Cladd.

Our approach has been to collect as much information as possible and to fully disclose it to the transplant center after the initial search as part of the comprehensive search report. That would include all the information, material age. That will include travel history. That will include all the soft stuff that we could think anyone could imagine being important.

At this stage, it is a slippery-slope thing, but it is a reasonable approach.

DR. STEVENS: To quote you, Joanne, from the early days, Joanne has made the point that at some time we may have the luxury of being able to exclude such units. It may be that when we have 100,000 units or several hundred thousand units that we will be able to exclude, but I think now we are in this balance, trying to achieve a balance where we give patients who have a problem now a chance.

DR. WAGNER: It still somewhat bothers me because, on the other hand, I do not disagree that we are pushed on the one hand because we want to provide a source of stem cells for our transplant patient who is in need of a transplant immediately. On the other hand, I might not be in the best position to evaluate this relative exclusion criteria or this risk factor that I do not really know how to judge.

So, for example, I am not saying that the New York Blood Center would have done this, but if you had told me about the hepatitis core antibody, I would not have known what to do with it. I do not know if it is significant, if it is not significant. How do I judge whether I should push ahead and do the transplant because this is the best graft that I can find available? I do not know how to assess that.

The other thing that has actually occurred from the New York Blood Center is that I have been given information that this graft was from a mother or from the pregnancy where the mother may have had an exposure to HIV, but I do not have the opportunity to go back specifically to ask that mother really what that exposure was. Though I believe the information was minimized, I do not know what to do with that information because then it creates this level of anxiety that I do not know what to do with.

So the fact that you disclose everything which is the right thing to do, it does not necessarily make it easier for me as the transplanter to decide do I use this, does it have a little bit higher cell dose versus this one at a little bit lower cell dose.

DR. AKABUTU: I think the exclusionary criteria depends upon what type of program you are running. The more remote you are from your collecting center, the more you are going to depend upon others to administer these criteria for you.

I could see where you have a single institution, collection process, that it is a lot easier to be able to validate those things, but if you are not there yourself, then your criteria has to be a little bit more expanded, I think.

DR. WAGNER: It just occurred to me. Then the NMDT makes me sign a form not saying that I have accepted this exceptional status. So now the onus of burden is on me when I do not know how to assess it to begin with.

DR. STEVENS: Yes, but nobody promised you a rose garden.

DR. KURTZBERG: Whether you sign the paper or not, it is your patient.

DR. WAGNER: No, but there is another level now of administrative things that my name is on that I have agreed with it.

DR. STEVENS: There is another issue. You are raising the problem of hepatitis B core antibody. I sort of skipped over this data in my presentation, but the vast majority of the marker positives are in fact related to anti-core antibody. If you test for anti-surface antibody as well, 95 percent of those anti-core positives also have anti-surface antibody, which is an indicator of recovery from past hepatitis B infection.

In fact, in the early days, in the late '60s, there were studies of blood transfusion where units of whole blood transfused into patients that were anti-core positive with anti-surface antibody that did not transmit hepatitis B. Now we have an issue. Here, you are talking about the risk of the mother being infected, which those studies would suggest there is no risk, and then that there might have been transmission to the baby. So, at least when there is anti-surface, I think that issue is negligible, if nonexistent.

Part of the issue is that we are stuck in a sense with some of the blood banking criteria where anti-core has been used as a surrogate for high-risk behavior, but clearly that doesn't apply in many instances with hepatitis

B because a lot of this represents childhood infection in particular for populations where they have emigrated, especially the Asian-American population. This is something that we are going to have to consider also if we are starting to think internationally.

In particular, when we have problems of trying to find units for our Asian-American citizens and banks are coming to the fore in Japan and China and all over Southeast Asia, what do we do about finding units for those patients? Most of those donors, the mothers are going to be anti-core positive.

DR. KURTZBERG: It sounds to me like you need an RFP for a chip that will detect actual particles of these viruses being done on a drop of blood. I mean, I know people are working on that, but that would settle the issue and would be a lot less labor-intensive than all the things we are doing in the mother and the baby.

MR. BRUNOEHLER: In addition to all of the clinical reasons that have been pointed out and as Dr. Wagner pointed out, some of them very confusing to interpret those results, I think reality also has to set in terms of financial considerations in this.

Having more exclusions up front would theoretically give us a better base to delve into, to look for transplants to, and I think having--and it would also

save money and keep the price down by not having to process all of those extra units. So I think that is a factor that needs to be considered as well.

DR. KURTZBERG: I want to take 5 minutes, because that is all we have left, to put up a couple of slides.

One, I listed as I was hearing people talk and also put down a couple of extra things we didn't talk about--that these would be four minimal pieces of information you would need to have a unit for it to be listed in international conglomeration of banks.

What I did not put up there was virility testing which is commonly on people who have serologies on mom, all that kind of thing. So I am calling for comment.

So it is total nucleated cell count, ABO/Rh testing, hemoglobinopathy screens and HLA typing.

DR. RUBINSTEIN: I think they are all necessary.

In some cases, when you can be sure the State hemoglobinopathy determinations are sufficient.

We had some difficulties in our own experience through which we have had to develop our own HBLC facility for these determinations, but in one way or another, you should have--particularly if you are aiming to have a ethnically diverse population in your bank.

DR. KURTZBERG: How about sex? As a transplanter, it is nice to know. It helps you.

DR. FRASER: At what point are you asking for this? Is this on the preliminary research form?

DR. KURTZBERG: This is information you would need to have, to have the unit in your bank and to list it, whatever search engine it would be listed in.

DR. FRASER: So this is just stuff we have to know. It is not necessarily material the transplanter is informed of on the first pass through a search.

DR. KURTZBERG: Right, right. Yes. This is things you have to know.

Are there comments on sterility? Because we have heard different practices, and it is certainly true that in the related or directed donor setting where units may be contaminated with vaginal flora. They have still been used for transplant successfully. In a public banks, should units be sterile?

DR. LANE: If I may comment. I have had lengthy discussions with our microbiology people who I think are very good, and they have corrected me by saying that we are not certified in sterility because we know that is something they cannot do by the limited testing. They are just saying that under the conditions that we test that nothing grew out. Therefore, clearly, the more samples you test, the higher the volume you test, the more likely you are going to come up with a positive result.

We have had some interesting abstracts presented, as you know, in meetings to that regard, but clearly those kinds of tests should be done. I think the more difficult questions from my standpoint are what do we really need in terms of fungal testing. Most of the bacterial testing you do right now is capable of picking up yeast forms that are commonly seen, but not the rarest of the yeast forms that you might need special cultures in 21 days to figure out or to grow. Again, my microbiology consultants tell me those are of no consequence in the kinds of testing we are doing.

DR. WAGNER: I was going to add something else to the list, but in terms of sterility, I am not saying it has to be sterile to be listed. On the other hand, yes, I think it should be tested, and then we know what the sensitivities are. Results have been presented which say it did not impact upon survival. On the other hand, some of those would have been treated prophylactically with the antibiotics at the sensitivity assay provided to us. So I would want that information.

I guess I have somewhat of a concern about the way that fungal cultures are being done currently because they are not really being done as fungal cultures. Certainly, candida theoretically would be a contaminating agent from the vaginal flora.

As a transplanter, I would like to know it, and probably I would consider it relatively as a contraindication to using it, though I do not have any data to say that is indeed true.

DR. LANE: I cannot vouch obviously for every type of culture that is done, but of the kinds that are done that I am aware of in our system, they will pick up candida and other yeast forms. We have data which I did not show at UCSD that shows that they grow out relatively quickly.

DR. WAGNER: However, I can tell you that in the presentations this morning, I do not think I saw anyone who said that they had a positive candida culture. Is that just by luck, or has it really been tested for?

DR. LANE: We have seen positive candida cultures, not in this setting. Of course, our bank is relatively new, but certainly using the same kind of culture system, culturing autologous transplant patients, we have certainly seen that.

DR. KURTZBERG: I know in COBLT, we made the decision if the particle bottle is positive, we do not subculture. So we do not have any information about what is there.

DR. WAGNER: Sure, because it is excluded.

DR. KURTZBERG: Correct.

DR. WAGNER: So, for those banks that do not exclude it, then we would want to know that information.

I guess the one thing I was going to add to that list--are we not going to include genetic history? Yes, I know we are doing hemoglobinopathy screen, but there has already been experience at the New York Blood Center that family history has actually been helpful. So I think that should be part of the criteria, but what I do not know how to best do is to validate that the genetic history is being done properly at each of the given banks, but that would be part of the FAHCT or NETCORD accreditation, I guess.

DR. KURTZBERG: We are going to run out of time. Let me ask if anybody has any burning issues. We cannot finish everything today, but are there any other burning issues people might have that we did not address at this point?

DR. WAGNER: Are we going to address this someplace else, and that is, what are the criteria that are going to be required before it is released to a given transplant center? Will this be addressed anywhere, and is this the place? Are you going to give this unit to anyone? Similarly, as we talked about in the past, we are talking about what are the banking standards, but also I think at some point, maybe not here, but we also should decide what

are the minimum criteria that a transplant center should be allowed to get a graft such as the NMDP proposal.

DR. KURTZBERG: I think that is a point well taken, but I do not think we have to discuss that here. We can do that in the transplant session tomorrow.

I was listening to us talk and just listed some of the more burning questions that we all still have that would help us out, but we do not have answers to right now.

One is we still need a true marker for standard maternal cells, or markers. We need to define further the best methods for processing and storage. HLA is a moving target, and it is going to remain a moving target and we just have to keep up with it. We need viral DNA testing and maybe even bacterial and other organisms as well which could address the contamination issue more definitively than culturing.

Other questions?

[No response.]

DR. KURTZBERG: How about how many banks do we need? How many units do we need? Let's do the last one first. Do we have any idea?

DR. RUBINSTEIN: We do not know yet. We depend, I think, to some extent, on whether hydrosolution is as important in Class I as it is in Class II. That will determine whether you will be able to, so to speak, get

away with a couple hundred thousand units or you will need to go to an equivalent number as in the NMDP.

DR. CONFER: I agree with Dr. Rubinstein, but let's hope we don't have to go that high.

DR. KURTZBERG: Any other comments? Otherwise, I am going to close this session.

[No response.]

DR. KURTZBERG: Thank you all to the panelists.

[Applause.]

SESSION II - PERFORMING THE SEARCH

Dr. Carolyn Hurley, Moderator

DR. HURLEY: Good afternoon. My name is Carolyn Hurley, and I am from Georgetown University here in Washington.

If I can have the first slide. So this session is entitled "Performing the Search." In this session, we are going to focus on those elements such as HLA matching and cell count as well as the procedures that are used to select the optimal cord blood unit for a patient.

The goals for this session are to review the search procedures from a number of banks, to talk about the criteria for units in the bank, to discus the search algorithms and the matching criteria within banks, additional testing post search, and then to finish up with a panel discussion talking about just ways in general that

we can improve communication between the transplant centers and the banks in regards to the selection of units, the testing and the receipt of optimal units.

I am going to start, though, by covering some of the HLA issues for cord blood registries, and some of these were touched on in the panel discussion, and I am just going to cover a few of them in more detail.

HLA, Search Determinants, and Finding a Match

DR. HURLEY: I would like to talk about, first of all, the method of HLA typing and quality control that should be used for HLA typing, the HLA loci that might be defined for banks and the resolution of that testing, matching of HLA assignments in terms of matching the patient with the prospective units, and an issue which, of course, impacts on all of this, the role of HLA and outcome is actually the topic of another session which we will cover, I guess, tomorrow.

So, in regards to the method of HLA typing, as all of you know, the field has been making a transition from serologic testing which uses alloantibodies to test the HLA proteins and the surfaces cells to DNA-based methods, but look at the nucleal-type sequences of the HLA genes that encode those proteins.

The reason that the field has been making this transition is that DNA obviously offers a number of

advantages in terms of the testing, and some of those advantages are listed here, and that it has been shown to be very accurate and very consistent in terms of defining types, very robust with an unlimited reagent supply, the synthetic DNA reagents that are used in the testing, that we can apply the testing at very high or allele-level resolution, and that for large-volume testing of HLA, such as one might do in a cord blood bank, this allows the banks to manage their sample flow using repositories and potentially allows the banks over time to automate that testing which will cause the cost of HLA testing to decrease.

I wanted to first cover the reasons in terms of the accuracy and the consistency of HLA testing and why we are making the transition from serology to DNA, and just review for you just one study that the National Marrow Donor Program has just carried out.

As you may know, the NMDP just began testing their adult volunteer donors by DNA-based methods for HLA-A and HLA-B, One of the ways of validating that testing was to do a parallel study in which over 42,000 individuals were typed for HLA-A and B by both serology and by DNA. Almost all of those individuals were from U.S. minority populations which are the groups of individuals obviously

that the cord blood banks are seeking to serve and which we feel are not well typed by serology.

When those results were compared between the serologic and DNA-based testing of those 42,000 individuals, the discrepancy rate between the typing was found to be 24 percent, so a very large discrepancy rate between the HLA assignments given by the two technologies, and you can see the breakdown between HLA-A and HLA-B in terms of that rate.

Then those discrepancies were retested by DNA-based typing using a stratified random sampling. So 650 of those individuals with discrepant types were retested by DNA, and the study was able to show that the DNA typing correctly typed over 97 percent of HLA-A and B typing. So this really nails down for us that DNA-based typing of the HLA loci is the way to go.

Some of the things that we addressed in the study which hopefully will soon be submitted for publication is the issue of null or non-expressed alleles in which out of the 650 donors, only 1 null allele was found. So we suspect that they will be quite rare.

Potential problems with the DNA technique in terms of failures to amplify and so forth--but those were uncommon, and the majority of the discrepancies were due to

known limitations in serologic typing, new antigens, undetected antigens, cross-reactivity and so forth.

I wanted to address the issue of quality of the DNA-based testing and just describe for you the method the National Marrow Donor Program has been using for their adult volunteers, and perhaps in the panel session, we can discuss what might be a good QC approach for the cord blood banks.

This is the NMDP is using blind QC testing of their adult volunteers at recruitment; that is, that they insert in the sample shipments samples of known HLA types, and those are used to monitor the quality of the HLA typing for their contract laboratories. The whole procedure has been described in these manuscripts. The laboratories are working at much less than a 2-percent error rate, and laboratories are dropped from the typing project if they exceed that error rate over 3 months in a row.

There is also other issues related to quality control in the way that other registries monitor quality control that are reported in this publication here if you want to look at that.

Next, loci defined in the resolution of testing. We actually just covered that in the panel discussion. Of course, this is going to depend a lot on the impact of specific loci and outcome. Right now, I think it is fair

to say that we want to be typing for HLA-A, B, and DR at the minimum. The resolution, again, will depend on the outcome and the match criteria, and the availability of donor samples to do retesting or extended or higher resolution testing. So, in terms of the level of testing at the time the unit is banked--actually, I was glad that you came to the same decision I put on my slide which was A and B at the equivalent of the serologic split and DRB-1 at the allele level.

I want to bring up this issue that has to do with the impact of new HLA alleles on assignments. The number of known HLA alleles has increased dramatically as we have looked at larger and larger sample populations, and this is just showing you the number of HLA-B alleles that we knew about in 1995, about 120 or so, and today we actually know that it is getting close to 350 HLA-B alleles that have been described.

This rapid increase in the number of alleles that have been identified has a dramatic impact on the DNA-based HLA typing that you record on your cord blood units, and that those typings become outdated.

If we take the NMDP DR typing that was performed in 1998, we now know that 82 percent of those HLA types are outdated in the year 2000, and let me describe for you what this problem is. That is that when we identify HLA types

by DNA, we test for a specific limited set of sequence polymorphisms. If we just pretend in a very simple world there is only four different DRB-1 alleles, the HLA laboratory will be testing for nucleal type polymorphisms that distinguish allele 1 from 3 from 4 from 7.

If an individual is positive for this red-striped polymorphism here, we would then interpret this result in the HLA typing lab to say that this individual carries the allele 0101. So that is what you would enter in your cord blood bank.

Next, your new allele is described, and those new alleles often share these polymorphic motifs that have been tested by HLA typing with other alleles that we already know about. So now when you do the typing and you have this new allele 0102 which is identical to 0101 in this region we are testing, but different in some other part of the allele, we get the same result for the test. The person is positive for this red-striped polymorphism, but now our answer is different. Our answer is not just 0101, but the person carries 0101 or 0102. So that means that the DNA-based HLA types that you recorded in cord blood banks and bone marrow registries that are used over large, long periods of time, become outdated as new HLA alleles are described.

The National Marrow Donor Program has been working on an informatics project related to this and have been trying to address this issue by collecting the primary HLA typing data; that is, the polymorphisms that are tested for by the HLA typing laboratories as present or absent, and that in-house they have interpretation software that can take that primary data and now reinterpret it on some kind of routine basis in which they can incorporate the new HLA alleles that have been described and therefore update their DNA-based HLA typing.

They are now working on approaches to incorporate and compare all testing results. That is, we are doing it now with a set of laboratories under contract, and now we would like to extend it to HLA laboratories using a variety of DNA-based testing techniques.

These are just four papers that you might want to look at. I can only just touch on this issue, but these have been published and describe the issue in much more detail if you want to read more about it.

The final thing I want to cover is matching of HLA assignments and how we take a patient and how we match that patient to the HLA type of the cord blood units in the bank.

As we have been making this transition from serology-based typing to DNA-based typing, that transition

has also been involved, a change in HLA nomenclature, the names that we give to HLA types. The naming system used for serologic typing is a different naming system than that used for DNA-based typing, and that, of course, creates difficulties.

So now what we are doing is we are trying to match individuals that are typed by different methods, by serology, by DNA, by different DNA methods, and at different levels of resolution, and all of a sudden, we are talking different languages.

So we are taking a very large number of individuals, many of the individuals typed by cord blood banks in the past and in registries that are typed by serology. So we might have an individual typed as B62. We are taking an increasing number of patients who are typed at allele level, so B1501. We are starting to have an increasing number of units in banks and adult volunteer donors in registries that are typed at intermediate resolution by DNA.

Now we are faced with the prospect of taking these different types of serology, DNA at different resolutions and different testing schemes, and knowing and understanding how this type B62 relates to this type and this type in order to select matched donors.

This is a significant problem because of a number of complicated issues about the HLA system, and those issues are that there are some HLA alleles that have no serologic assignment associated with them. There are times when the name of the allele does not reflect the serology. There are examples of single alleles which have been assigned several possible serologic types. There are examples where a cluster of alleles, that is, a type that is an intermediate resolution type, comes up as associated with several serologic assignments, and then we also have the problem of comparing assignments from non-standard DNA-based testing.

The final issue, actually, that I forgot to add in here is that at the end, the end user, the transplant physician or the search coordinator has to understand what comes out of the cord blood bank and has to understand how all of this matching was done and what this different nomenclature means and whether individuals are matches or not.

We have been working on a tool at least to get some of the serologic allele associations. The World Marrow Donor Association has an ongoing project to do this, taking data from a number of cell exchanges, from the National Marrow Donor Program registry and individual laboratories. The last report was published in 1999, but

it is going to be an ongoing effort and should be published every year or two.

So that is sort of in a nutshell all of the HLA issues that you probably did not want to know about, and I want to just acknowledge the collaborators in the studies that I have describes, the NMDP Research and Scientific Services and Information Systems and the DNA typing labs that are under contract to the National Marrow Donor Program.

Thank you.

[Applause.]

DR. HURLEY: So what we are going to move on to next is having discussions form various banks and registries that will basically discuss some of the matching issues and the search algorithms and all of those issues.

The firs speaker is from the New York Blood Center, Pablo Rubinstein, the most famous cord blood banker.

New York Blood Center

DR. RUBINSTEIN: To begin, I would like to tell you that the experience I will relate in this session is based on the activities of the blood center program so far.

We have research for 9,816 patients. Our inventory at this time of the first search had 590 units, and currently also 731 this year had 11,000. We have

shipped, 1,059 units, and patients receiving these units have been 1,027 for reasons that Cladd gave this morning. Some patients receive twice, and there are a few other reasons.

In performing the search, there are a number of valuables. Some depend on the donors. Some are evident, and some not quite so evident. Some are from patients. Another one relates to patients and donors.

The number of graft greatly influences the mechanism of the search. It is not the same thing to do a search when you have 10,000 grafts, as we do, or 4 million grafts. Techniques for performing the search are different, and it is surprising, but as you increase your numbers even within this range of 1 to 10,000, these change and forces you to change your mathematical approach to this.

Another course of change is the phenotype resolution. I do not think I need to belabor it after our previous talk, brilliant talk in my opinion, which pinpointed all of the aspects of these problems. This is a continuing problem, and it is an improving situation all the time, but from the point of view of the data, it means the data are changing continuously.

Not only are the data themselves, the characteristic of the units changing, and not just for HLA,

but for other characteristics such as, for example, definition of some risk factors, but the allocation system is changing.

I am not going to go very much into this from the point of view of search because this will be addressed by my colleague, Dr. Hakenberg, a little later, but just for the purpose of this introduction, allocation is a situation that occurs when a unit is requested by more than one patient.

There is a potential conflict, and there has to be ways of handling this. Even if you report on a second match of the same unit because the first transplant center has not decided, you have to let the second transplant center know about the existence of the conflict so that they can gage the urgency with which they need to resolve their situation and the potential for a conflict eventually developing.

For the patient, the HLA type and resolution has exactly the same situation except that from the point of view of the banks, there is no evolution. We just get the typing from the transplant center.

There is a problem, however, in that we have had the good fortune of having the collaboration of the transplant centers in sending us not only the recent HLA phenotype, but a copy of the HLA typing laboratory report,

and there are, as you would expect, a certain number of discrepancies which we can resolve on this basis.

There is an element here of urgency. Urgency is important for the search because it enables us to edit the output file of our search algorithm to remove potential or morally certain conflicts and not overload the transplant center with problems that they do not need to have.

Finally, ethnicity which works in a way that you would anticipate from existing knowledge, but which you can see demonstrated here. In our bank, there are basically four populations represented. As you can see on the top, the patients have asked our help in finding matches--are also from these four populations. If you look at the probability of providing matches of different types, as you can see on the right hand, the red circles are zero mismatches, yellows are one, and black are two mismatches. You can see that for every ethnic group, you move from potential donors of a different ethnic group to the same ethnic group. The percentage of good matches goes up.

Consequently, you have to tailor your donor population to your patient population, a problem that has been wonderfully approached by NMDP. Here you have the consequences of the way we have approached it.

As you can see, even though our clients are patients asking us, 65 percent of the cases are Caucasians.

The units that we have acquired, only 37 percent Caucasian. These units in a certain way is a disadvantage in an environment in which there is abundant competition, but it is very important in a different sense if we look at something that is not in this slide, but I promised to show you. You will see that the probability of finding matches now is more or less equivalent to the ethnic distributions in our population, and you can do this by tailoring your client donor population.

In addition to these basic problems, there are other problems that may be important in determining the quality of the match. I call them possibly important, although there is some evidence that most of them are indeed important.

There is a problem of allosensitization. This problem has been approached particularly intensively by the Seattle group in the case of bone marrow and by a number of others, and there is very good evidence that if a patient is sensitized, that transplant will not do well.

We do not have similar evidence in the case of cord blood, and from informal inquiries with our colleagues, it appears that many groups are not very concerned with this aspect. We feel that this needs to be addressed, and I will show you data that suggests that it should.

Other aspects are obvious. Patient's weight is obvious. If you have at least a potential donor at a certain match level, you do not need to send and clutter the transplant center with all of these that are unnecessary, if the donor has a weight that would make many of these units unusable.

The primary diagnosis is also perhaps important. We know that for certain diseases, the cell dose considerations are more important than for others, and so this is stage. One that is very interesting is the search for a bone marrow donor.

We do not see the world as being split down the middle into bone marrow and cord blood donation. I think both systems have their place, and the clinicians know very well how to use one or the other, and they are learning as we learn how to improve this.

There are two aspects about a unit prior to going into the search. One is the availability, and we have defined by hand that what we have been doing up until now, the available status of a unit on the basis of these criteria. There has to be documentation of the mother's consent and lack of withdrawal of the consent because sometimes the mother repent. So there is some evolution here.

There is a need to be sure that the typing has been completed and the testing has been completed. There is a need to make sure that we have a method to catch flags in the testing or the typing, et cetera.

One of the things we have learned is the consistency of the HLA typing between mother and child. It is a very good way to exclude the possibility that all of your data comes from one lady, and your sample for transplantation comes from the child of another lady.

I will not go back into this. The lesson was clear this morning.

We have defined a certain number of excludable features that have been commented upon.

We also use a certain proportion of our units for quality control studies and quality assurance. Any one unit in the inventory will be subject to a certain probability of being picked up for these studies at any time. So that, we have to verify at least prior to the search.

Finally, there has to be demonstration that that particular unit has not been requested definitively by any transplant center. We can do a little better than by hand. By hand, there is always a possibility that the person who has to physically enter into the computer and tell the computer this has happened, that person may forget to do

all that he or she has to do, and it has happened to us. So we are looking into a way to do this process automatically.

Now, there is a different proposition which we name allocation. Availability depends on features of the unit itself and nothing else. Allocation depends on the status of the unit vis-a-vis the recipients, the potential recipients.

We have basically two categories, unencumbered and units in preliminary reservation, as we call it, or you could say in potential allocation conflict status.

Why can this happen? Because there are units that are still in search even though they are being considered by a transplant center. The reason for this is that transplant centers, many of them, will select three of four units, for example, for increased resolution typing when those units are not fully typed yet, or because we need to still wait for confirmatory typing or because we are performing genetic testing for a potential match to a patient with an enzyme deficiency disease and so on.

There are also troubles sometimes that the transplant center has in securing the approval of the insurance companies for some of these procedures.

The patient and the transplant center has to approve these, and we require in each case a confirmation

that the patient has signed informed consent. We have the consent form signed by every one of the patients. So all of this takes time and makes potential trouble.

With that, I will just refer briefly to the algorithm, how do we do a match. It needs concept. In testing the algorithm, it is very simple. It can become more and more complex, depending on the degree of sophistication, and this is mandated largely by the number of potential donors.

In its essence, the first step of the matching algorithm requires you to unify the level of resolution at which the unit, the donor, and the patient will be studied for compatibility. This refers back to what Dr. Hurley told us about the levels that each one can catalog and the various names. The computer algorithms does not know the equivalents. So we must have a table that tells our computer how and at what level we will do these, and this depends on the moving target that the typing level resolution that we receive indicates and also of the units that we have in our storage.

We have such a table that has the advantage of an economy of not going back all the way to the minimum resolution possible, but for the best for that patient.

We define then, as you see in red at the bottom, the HLA antigens as will be used for that match. We do the

same for the units. In our case, because the numbers are sufficiently small, we still cannot afford to do all patients. We do not need to do any tricks in ordering or sorting out patients to facilitate this arrangement.

Then the match grade is simply a process of adding up the antigens that match per locus and the total number of antigens detected per locus, and this is done for each locus and for all loci combined.

There is a problem in HLA which we largely sweep under the rug, and that is the existence of blanks. Even molecular techniques, we sometimes can define only one antigen at the locus, and this is understandable. In the majority of those cases, we are dealing with a homozygote situation.

But there are some exceptions. In any event, we do not know and we have to deal with the situations, and we have chosen to deal with them in the sense of considering them asymmetric matches.

When there is a blank in the patient, then it means the donor has one more antigen than the patient, and this mismatch--if there is a single mismatch, that mismatch is only in the rejection direction, and conversely when the blank is in the unit.

Here is what I promised to show you. This asymmetry has a clinical consequence. When you select

transplants where there is a single blank and that is the only mismatch, then you have the black here--is a conventional case in which the single mismatch is an antigen that is different in the donor and the recipient.

When the mismatch is a blank and it is in the graft versus host direction so that the graft is--the blank is in the graft, then the outcome is the same or a little better, something that in bone marrow is known to occur and has been defined quite well.

But when the blank is in the rejection direction, the mismatch is towards rejection, the consequence is much worse. That has not been described in the case of bone marrow, and that can most likely be a consequence of a sensitized recipient.

I think we need to look very carefully at this aspect. Although the number are relatively small, only 23 of these and only 36 of these, it still is statistically clear as a significant problem.

This is the multi-valued analysis. Because my clock here is saying I should stop, I will skip this and go to the end of the search. We now have the facility to produce an output file that can be edited easily by our personnel before submitting to the transplant center. So that, we maximize the usefulness of the proposal, and we do the proposal in the form of a preliminary match report

which is organized by match level and the units within each match level are sorted by cell number.

It describes the HLA type at the level of the matching that took place, but it gives any additional information that we have about resolution. In our bank at the moment, all of the new units are typed for DRB1 by sequence.

Now, after the search has taken place, we do confirmatory typing. The transplant center decides on the unit and sends us a sample of a patient. We send DNA from the unit to the transplant center, and we get a blood sample from the patient. We try as hard as we can to convince people to do the typing of our unit, and we will do their typing at no cost to them because we consider this is the last time that we have a chance to stop a mistake.

We do our typing from the unit, from the segment. So this is integral. The other testing that can be done is the enzyme activity, and this was described earlier for genetic disease.

Then we also want to encourage all transplant centers to document by chimerism studies that the ingraftment is donor type, and we offer to do that if people do not have a facility that will do it for them, again at no cost. All of these costs do not come out of our pockets. They come out of the reimbursement fees that

the FDA calculated for our bank. Of course, it calculated when the ratio between units in the bank and units given was much better than it is now.

I wanted to show you a proposal on how we should do these or we could do these units in the future in a collaborative manner.

We have discussed in the context of NETCORD a method for handling all of the telematics and the communications that are necessary to make optimal use of our units and permit the banks to continue to learn and to continue to apply what they learn to the benefit of the patients.

So the purpose is to maximize probability, to find the best unit for the patient that is available at the time the patient needs it, and make the optimal use of the units in all accredited inventories worldwide and accelerate the process of reporting.

Of course, you should know that no one is accredited so far. We will all be accredited by the FAHCT/NETCORD standards. This is going to be starting within a month or two at the most.

The idea works, more or less, like this. We would develop a virtual office. The idea is that the transplant center would issue a search request. It would go to a virtual office that might reside on the web. This

virtual office would then distribute directly to all the banks that are accredited. The banks would perform their search, would produce preliminary match reports according to a strict protocol that would be agreed upon, and report their individual results back to the virtual office within and a standardized throughput time, and the virtual office will make it available to the transplant center within, at the most, 24 hours.

If the virtual office will do one other thing, the input is rather easy. It just mirrors the search request to all of the transplant centers. The back way from the banks to the transplant centers is a little more complicated because the virtual office will automatically combine the output from all of the banks into a single report for the transplant center.

There is a little arrow here that represents our hope. You see on the far right? We would like to develop with the NMDP a joint approach to handling situations in which we do not find a good unit, and to avoid wasting time, we could communicate these to the NMDP in some mutually accepted form or to any other bone marrow transplant registry to get the wheels in motion and accelerate the performance of tests in the benefit of these patients. That will, of course, require a hope, but not a totally silly one.

What is required for each bank to be able to product match reports, and this is not so difficult as it may seem initially. The banks already are doing 90 percent of all the work. They have to have their own database and their management system. Without it, they will be dead before they reach 1,000 units. So they have the wherewithal. All they need is this last thing which is probably much less than 5 percent of the total job.

We are not handling millions of units yet. So each bank can do this comfortably, and there are programs that can be shared to do these effectively.

As a last thought, even the assistance of this virtual office, the creation of a virtual office is not essential. We could do it differently. We could just have the transplant center issue a request to any bank in the accredited group. The bank would then do the first part. It would mirror to all the other banks.

All the banks would produce search requests, and their standardized match reports would go straight to the transplant center separately, and the transplant center would have a very simple program, the same one that we are postulating for the virtual office, which would take care of receiving all of these match reports, assembling them into a specific format that the transplant center could determine for itself, and could continue to receive the

information they need until all of the banks have been able to report not subject to these such stringent requirements of being able to do everything within 24 hours.

Now I just have to apologize to you for extending my talk beyond the time. Thank you very much.

[Applause.]

DR. HURLEY: Thank you, Pablo, so much for giving that overview and presenting us with something to think about and discuss related to the joining of all banks together to facilitate the search.

So the next speaker is from the National Marrow Donor Program, Dennis Confer.

NMDP

DR. CONFER: Thank you again. It is my pleasure a second time to be up here speaking.

What I would like to do is tell you a little bit about how the NMDP has evolved its cord blood searches, and I will be showing you some data about how we are currently producing our cord blood searches on paper and what the different match categories are and what the match grades mean, and then I am going to show you a preview of some new NMDP software that we hope will change the way that searching is done and I think may lead in the direction that Dr. Rubinstein has suggested this whole process needs to go, and that is real-time searches for HSC donors.

Let me just tell you that the way we are conceiving of the search process, we know that we have cord blood units, a small number listed alongside a large number of bone marrow and stem cell donors. Currently, we have 4.1 million bone marrow and stem cell donors. About 57 percent of those have been typed for HLA-A, B, and HLA-DR, in most cases DRB1. So those are DNA-based typings.

The cord blood units, as I mentioned this morning, have all been typed by DNA at the present time. We require DNA typing at DRB1. We will accept serologic typing to HLA-A and B.

When a transplant center submits a search request to NMDP, they can either submit a preliminary request in which case the next day they will get back a listing of potential bone marrow donors and cord blood units. So they get back two search reports whether they asked for two or not. They get both bone marrow donors and cord blood units that are potentially matching.

If the transplant center wants to move further in the search, then they have to issue what is called a formal search. That is where they actually ask for confirmatory typing either on one of the bone marrow or stem cell donors or on the cord blood units.

We cannot do searches for free, unlike Dr. Rubinstein's program where the searches do not incur costs.

However, we are doing things to try and look at search costs and try to streamline things and remove hurdles for searching patients.

One of the things that we have encountered, very similar to what Dr. Rubinstein mentioned, is that these searches have gotten so complicated that the physicians and the coordinators at the transplant centers really have a hard time working their way through them. So we are having to provide increasing software solutions and also services in order to help them get through the search process.

The main computer system is called STAR, the Search Tracking and Registry system, and we have developed a series of products to link STAR to the various centers. STARLINK links to the donor centers. As I mentioned this morning, CORDLINK links to the cord blood banks. I am not going to talk about the repository software which is CRISLINK. LABLINK is in development. The newest software piece is TRANSLINK that provides a link between STAR and NMDP transplant centers.

These are our data on preliminary searches per month. These are data from the last year. 650 preliminary searches a month, just over half of those go formal, about 394 a month.

Interestingly, you can see that more than a higher proportion of our searches are for Caucasians than

what Dr. Rubinstein just showed you. These are about 70 to 75 percent of the searches are for Caucasians in contrast to his 65, and this shows that I think the transplant centers are already going to the cord blood searches in an effort to try to find suitable units for their minority patients.

This, I already told you about, that we do simultaneous searches. However, there is only one activation fee, whether the first choice made, whether it is marrow or cord blood initiates the activation fee, and you can search for both donors and cord blood units simultaneously.

In order to prepare transplant centers to do cord blood searches, we had to train them to interpret the cord blood reports. We did a series of audio conference training sessions for the transplant center coordinators. We began the training and the searches in September of last year. Once they were trained, we could turn on the search reports for their center.

We confirm that the cord blood transplant center--that there is a cord blood protocol that has IRB approval at the transplant center before we release a unit. So they must have an IRB-approved transplant protocol.

The current paper reports come out on these, the 110C which is the initial search result, the data

supplement which I will tell you what is on that, and then a search progress report is called a 114.

NMDP changed its match categories just about 10 months ago, and we used to say we had the 123 match categories. The best match was a 111. With the proliferation of DNA typing, this was no longer adequate, and you can see what we did. We split the one category now into five subcategories.

Basically, this is recipient donor typing. If both are typed by DNA, there are four possibilities. Either an allele match which is an A, a potential allele match--we call it allele potential match which is a P, an allele mismatch which is an L, or a true antigen mismatch which is an M. If one or both is typed by serology, however, the best you can do is a serologic type of match which is either a split match, S, a broad match, B, or, again, an antigen mismatch, M. So this has really added additional complication to the search report results that I will show you in two slides.

This is just the front page of a 110C. I am going to show you the very top part, and then I am going to show you this part in the next two slides. So this top part has the recipient information. So this is a recipient who is Caucasian, actually a 35-year-old recipient. This is a real search. This recipient was typed by serologic

methods at Class I. This was a serologic 1, a serologic 3, a serologic 7, serologic 8, and then corresponding out here are the search determinants. These are the numbers that are used now to go into the computer and look for donors for this individual.

At DRB1, the person was typed by DNA, however at the lowest possible resolution, 01XX and 07XX, corresponding search determinants 01 and 07. This person was ABO--A-positive and CMV-negative.

When you run this search, you get back now the listing of potential matched summary of matched cords. The NA means that you cannot evaluate this category, and that is because this recipient was typed by serology here. So there is no way you can look for an allele match or an allele potential match at AB. Because they were typed at DRB1 at the lowest resolution, you also cannot look for an allele match at DRB1. So several of these categories are simply not applicable.

When you go down the list, you see that the very first match comes out. Here there is one cord in our registry that is a split match at AB and an allele potential match at DRB1. The no cords, no cords, no cords, these are all zeros if you cannot see it. Here are eight cords that are at least a one-antigen mismatch at AB, and we will allow up to two mismatches, a mismatch at A and an

mismatch at B, and an allele potential match at DRB1. There are eight of those, and here are five here. There are 51 where there is a known antigen mismatch at A and a known antigen mismatch at DRB1. This would be an A or B, but not both, a known antigen mismatch at DRB1. So this is what the thing looks like.

The next page then starts providing the detail. This is the recipients typing repeated here, and here is that first cord that showed up. It is a split match at A, a split match at B, and a potential match at DRB1.

Here is the typing on that cord. You can see it was typed by DNA, 03XX, 01XX, 0702, 0801. So this is allele-level typing at B. Then, at DRB1, we have one that is an intermediate typing, an AE, and one that is an allele typing, 0701. This cord is 12 months old. Its donor was A-positive. It is CMV-negative. It is a Caucasian baby, and the baby was a male. This is the status of the cord over here. This cord is available for searching. This cord has a total nucleated cell count of 75 times 10^7 and a reported CD34 count of 2 times 10^6.

The next cord is a split match at A, a mismatch at B, and an allele potential match at DRB1. So here is the mismatch. It is a B37 versus the B8 up here. This is allele-typed at DRB1, 0101, 0701, very similar demographics on this cord. It is 18 months old. It is from a girl

baby, O-positive, CMV-negative. So this is what this search report looks like, and it is up to the transplant center to search through it and figure out if there are cords in here that they are interested in.

The supplemental report they can request, this will have HLA history. If the cord has been typed more than once, it will give all the previous typings. It will also give the maternal HLA typing if that is available, as it would be from New York Blood Center, for example. Collection data, processing data, availability of additional samples--these would be aloquots for more testing--the infectious disease marker results. The high-risk questionnaire results are on this, and the family medical history results are on this supplemental cord blood report. So you have got to request that for each of the cords you are interested in.

Let me tell you about TRANSLINK which now is our attempt to link transplant centers real time to the NMDP STAR system. This is an internet-based application that will send and receive data from the transplant center to NMDP. It provides a very interesting acute tool which is allele code look-up. So you put in AE, and it tells you exactly what that means. You put in 01AE on that previous one, it tells you exactly what that means. You can also

put in a series of alleles, and it will give you the corresponding codes that contain those alleles.

Patient data can be entered, and it is sent electronically to NMDP to begin the search. Transplant center can view individual donor demographics, the infectious disease marker history, and the search activity history for the individual donors, and the transplant center can automatically request DRs which won't apply to cord blood, confirmatory typings which will apply to cord blood and marrow stem cell donors with TRANSLINK. This is a little pop-up screen that says CT donor with this ID number.

This is a real-time search. This search currently takes--Version 1 of this is out and in the transplant centers. We are continuing to train transplant centers every 2 weeks. We bring a group in for training. When they get it installed at their transplant center, they enter the HLA data on the patient. They push a run-search button, and the search takes anywhere from about 30 seconds to 4 minutes to run.

We can add all the cord blood units in the United States, another 50,000 units, and be running against 4.15. The cord blood component of this is still being developed. It will be in the subsequent release, but we expect to have that out in the next few months.

We think that this few-minute turnaround can be sped up by increasing the horsepower of our servers, but it still is a dramatic increase in the turnaround time compared to the usual day-or-more wait to get the printed reports back.

So we are real excited about this. They get match grade summaries, just like are on the 110C. They will also get strategy notes. This will be information about particular HLA types that may be mistyped or they may want to use a different strategy. You can run a search button. I guess you need one of those.

These are just those same categories I showed on the 110C repeated here on the CORDLINK screen. One of the most interesting things about TRANSLINK is the ability to subset donors based on many different characteristics. For example, you can specify the level of HLA typing. You can subset them on the basis of race, sex, pregnancies, donor weight, donor age, ABORHCMV, current donor status, whether the donor is a peripheral blood stem cell potential donor, whether we have samples in our repositories, whether the donors are at an international center, et cetera.

For cord bloods, we will be able to subset cords based on several different characteristics, again, including HLA typing, but also the race of the cord blood unit donor, the sex of the cord blood unit donor, the

volume of the cord blood unit, total nucleated cell count, ABORHCMV, and whether the cord blood unit was previously CT-typed potentially in the case of a very urgent search.

This is what this subsetting screen looks like. This is a mock-up of what it will look like in the production versions of TRANSLINK for cord blood. This is very similar to what it looks like for the bone marrow and peripheral blood stem cell donors currently.

You can see that it lets you specify kind of minimum criteria at HLA AB and DRB1. You have the additional ability to search at HLACDQ and RB3. Here, you can select as many baby race codes as you want to identify those ethnicities, as Dr. Rubinstein mentioned, the cord blood unit ABO typing, the mother's CMV results, the current status of the cord blood unit, whether it is available or whether it is on another search or active on this search, whether you want to have multi-race, a bunch of other stuff, whether the cord is in an international bank.

This would be the initial cord blood unit volume. So you can push this little bar over, if you are only interested in looking at cord blood units that started out with an initial volume of 70 or 85 cc's, and you can also look at the minimum total nucleated cell count frozen times 10^7. The minimum here is 12, and you can push this little

slider over to whatever level you want. If you know you want 2 times 10^7 for your recipient's weight, you can push it over to the level that is necessary to achieve that.

So we think these filters are going to make it dramatically more easy for the transplant centers to subset their potential cord blood units and provide one of those components that Dr. Rubinstein mentioned.

Then they get a cord blood unit list that meets their criteria. It will show all the donors and cords that meet their selected criteria in order of the match grade, and it will provide all the key information. For CB use, that would include obviously the nucleated cells per kilogram of patient weight because, on our patient screen, we have got a spot for them to put in the patient weight.

The cord list allows them to go through it and remove cords from consideration or add them back in to the list. They can also enter comments on each individual cord if there is some reason they are not interested in it. They can record that on the screen and it stays stored there.

They can go to details where they get the supplemental information, and then they can request activation either for confirmatory typing or they can put it into State AG which is held or OR which is on order. They can save this entire list, go back, go away, come back

to it a day or weeks later, and it will still be there when they log in the next time.

So we think this online searching provides the ability to see all the stem cell sources, as we have talked about how critical it is to integrate these stem cell sources, the ability to manage this complex HLA data and bring tools to bear that might help in the evaluation, compare the demographics of cord blood units with those of the adult donors in the same kind of software setting, to apply consistent search strategies that are tailored to the transplant center's needs because, if they know what their search strategy is, they can save that strategy as a custom search strategy and then reapply it over and over again to subsequent searches, and it will also maintain a history of all the searches from that transplant center.

I had to run through that quickly in the interest of time, but thank you very much for your attention.

[Applause.]

DR. HURLEY: Thank you, Dennis.

I think what we will do is take a break at this point. Let's take a 15-minute break, and we will come back at 10 after 4:00 and start again.

[Recess.]

DR. HURLEY: I am very happy to introduce next Shelly Carter from the EMMES Corporation who will talk about the COBLT trial.

Shelly?

COBLT

DR. CARTER: Thank you, Carolyn.

I am happy to have been invited today to speak on behalf of the COBLT search process, and I would like to thank NHLBI for their continued support of the COBLT trial.

I will present some brief search and transplant data summary. I will also discuss the criteria for acceptance of units on the COBLT study, describe briefly our search algorithm in the COBLT search process, go onto some special issues encountered while we designed the search process, and then I am happy to demonstrate the web-based search system that we have almost completed for the COBLT study to enable transplant centers to search directly the COBLT bank.

Preliminary searches started in November of 1998, and as of August 8th of 2000, 976 preliminary searches had been performed. Of those, 401 are currently active. We have had formal searches submitted on 135 patients. Formal searches basically mean the initiation of confirmatory typing on the cord blood unit. 68 reservations have been placed, and to date, 49 transplants have been facilitated.

The search registry as of last Friday contained 4,587 units. This slide shows the diversity of the ethnicity that has been collected due to the significant efforts of the cord blood banks. We have 56-percent ethnic minority representation in the search registry. Of that, the Hispanic group makes up the largest proportion at 21 percent. We also have a little over 11 percent that have come from mixed heritage.

By mixed heritage in the COBLT study, we mean that we crossed sort of the major bounds here so that you have crossed perhaps black versus Hispanic. This does not mean there has been a cross within the Hispanic group like a Caribbean versus a South American Hispanic. That is a mix over the major categories.

Of the 401 currently active searches, about 5.5 percent find a potential match at 6 out of 6. For COBLT, we define match as potentially low-resolution DNA for HLA A and B and high resolution for HLA DRB1. So what you can see is about 8.5 percent of the patients do not find at least a 4 to 6. So that, 92 percent of patients are finding a 5, 6, or better HLA match with the current registry size of 4,587.

The HLA typing for the COBLT study--the COBLT study had the luxury that the NMDP didn't--that we got

started years later. So all units have been typed using DNA technology.

There are three COBLT contract laboratories, UCSF led by Leanne Baxter-Lowe [ph], UCLA led by Elaine Reed, and the Navy Medical Research Center and Georgetown University led by Carolyn Hurley and Jennifer Ng. All the labs either use SSOP or sequencing-based methods for the typing of all the units.

For preliminary typing, HLA A and HLA B are typed at low to high resolution depending on which allele it is. For confirmatory typing, the requirement is at least low resolution, and for some types, such as B15, most transplant centers are requesting higher resolution typing for confirmatory typing.

HLA DRB1 is typed at intermediate to high for preliminary, but at confirmatory typing, it is typed at high resolution. Point of fact is that the bulk of the DRB1's are typed at high resolution, although that is a moving target.

For the criteria for acceptance of units on COBLT, all data is double-reviewed at the cord blood banks, as John Fraser, I think, mentioned today. Those are hand-reviewed by two senior cord blood bank people to make sure that exclusion criteria are met.

At the medical coordinating center, we have also written a computer algorithm that verifies that no exclusion criteria are met based on the data that is entered via the internet data system. We have not gotten a chance to present the internet data system. So I am going to make a brief aside to tell you a little bit about that.

The COBLT banks both have access to the internet data system where they enter all data related to the cord blood units directly. This includes collection information. It includes donor and delivery information, the processing information, and in fact, the processing form is used real time in that as they go through the processing stage, they can enter the data as they get it. The processing form is calculating for them automatically cell recoveries. It also has a feature such that as they are labeling samples and aloquotting out samples, as they label it, they can press the re-scan to ensure that the label they are putting on the samples is for the unit that they are currently processing.

The medical history data is also entered through that. So what the algorithm at EMMES does, it draws the raw data and verifies it against any exclusion criteria, checks every and all exclusion criteria to make sure none are met. So there are three checks on the data to make sure that the unit is acceptable for the search registry.

Finally, of course, HLA typing has to be completed before we can put it in the search registry.

The search algorithm itself, the search determinants are the linkage between serologic typings and DNA typings, and also the list of valid alleles is all table-based. Making it table-based makes it easy to maintain. It makes it easy to add alleles as alleles are discovered.

Also, a feature of the search algorithm is that we have an audit trail and that the search histories are maintained. The audit trail means that any data changed on a recipient is tracked and recorded from HLAs to weight to birth date, whatever is changed. We have a record of what the change was, who made the change, and when that change was made.

The search histories, we track. We have ready access to the previous 10 search histories for all patients. Point of fact, since we back up our server every day, we have histories for every patient from day one, but we have ready access to the previous 10.

A feature of maintaining these search histories is that when a search is updated or when new units become available in the search registry and that the search registry is updated, new units will present on the search report with a double asterisk to signify to the transplant

center that they have not seen that unit for that patient before.

Also, if HLAs get updated because of confirmatory typing or for some other reason, a single dot will appear by that unit to say something has changed since the last time you saw this report on that unit.

As part of this search process, we have internal quality control summaries that are produced. The internal quality control summaries currently are reviewed by EMMES staff to see what has changed on every search before it from the previous search to this search. It just gives us a way to constantly check the search algorithm to make sure it is performing as we think it should be.

In terms of the software itself, it is unique in that the matching process is controlled by a dynamically generated SQL. Also, the system is scalable, scalable meaning that we can maintain the current performance of the search system by adding hardware and servers to the system and then making duplicate copies of the search engine. So it doesn't matter if we have 4,000 units in the bank or 100,000 units in the bank. It doesn't matter if we have two users or 100. We can still maintain search performance so that the search reports are received in real time over the internet.

I should say that currently we are not using an internet-based system for the search except at EMMES. So the search report is still on paper. The policy right now is anything that comes into our office by 2:00, a report is produced by 5:00. Confirmatory typing is requested. Reservations are processed within the day.

Also, the search algorithm is a first-in/first-out queuing system. Currently, everybody is created equal. So all users have the same priority, but by adding more pipes and more queues, we can change that prioritization.

The matching, as I have discussed already, is done at low-resolution DNA for Class I A and B and high-resolution DNA for DRB1.

This is actually developed in conjunction with a lot of discussion with the original COBLT steering committee in that we score the number of potential matches at each locus. For example, a 212 means that you have a potential of 2 match for A, a 1 match for B, and a 2 match for DRB1. For A and B, that means that you have at least a low resolution, and for DR, that means you have high-resolution matching.

We had entertained or discussed with the steering committee what we called a confidence level which is really related to what Dennis presented about is it an allele level or is it antigen match or whatever, and we have that

capability to add that in, except that in discussions with the steering committee, they asked us not to when it was brought up. So I am not sure what will happen with that in the future.

The sorting of the reports is done currently by best HLA match and within HLA match category, highest cell dose. So all 6 out of 6's will appear before all 5's, will appear above all 4's, and within each HLA match, the highest cell does will be first. So it will be in descending order of cell dose.

We are in the process of changing that slightly in that on some of our units, we know we have high-res typing for both Class I and Class II. So it is going to be modified slightly so that if it is a 6-out-of-6 match with all high-res typing, that will appear above all other 6-out-of-6's, regardless of cell dose. So I guess it is more like it is high-res typing 6 out of 6, not high-res typing 6 out of 6 by cell dose, and so on for the 5's and 4's.

The current search reports that are on paper only show a minimum of 4 out of 6 on the internet system for release one. You do have an option to extend that report to see 3 out of 6 matches, also.

For the COBLT study, we only show units providing at least a minimum of 1 times 10^7 nucleated cells per

kilogram. That is viable nucleated cells because, after today's discussion, that might also be modified.

I know you cannot see this very well. I am not going to talk about it in detail because I have to do a demonstration later of the web system. This is the current search report that is produced. It has the recipient information on the top and all the unit information comes below with a score on the left. I will talk in more detail about the contents of that later.

The status of the cord blood units--our search registry is updated at least twice weekly. It depends how often we get in HLA updates. It can be updated every day if we have new information to update it.

Preliminary searches, formal searches for the initiation of CT, confirmatory typing, and reserved units are updated daily. What that means is if a person puts a unit on request, as of that day all search reports that are run will have an indication that that unit is on request. That also means that if a reservation is placed that day that the reserved unit will not appear on anyone else's report from that day forward, with one exception. If we have a unit that is requested by two centers and a reservation comes in, we are notified right now by a paper report, soon to be an e-mail, that tells us there are two

patients that want that unit or have requested that unit for CT typing.

The process is that the coordinating center contacts the second center to see if they are still interested. If they are still interested, they have 3 days to let us know. If they are still interested, a subcommittee of the steering committee has been developed to review the need and the urgency for the patient to other available units. So far, that has not come up.

It has come up that we had a unit that was reserved that someone requested, but the second center relinquished the request at the same time we called them.

Methods for guiding selection. The transplant centers are provided with a confirmatory HLA typing result and other basic information on the CBU. That includes the stuff that is on the search report, including CD34 count, total nucleated cell does, total CFUGM, race of donor, and a few other variables. In addition, at the time of reservation and at the time of reservation onto the study, they receive supplemental reports. Included on those supplement reports include collection weight, total CD3 count, maternal infectious disease results, basically all the medical history information. If there was history of maternal serious illness, it will appear on the

supplemental report. If there is any serious diseases diagnosed in the relatives, that will also appear.

Many things are exclusion criteria, but what that supplemental information is designed to do is if it is not a precise exclusion criteria, there is some discretion within the banks for the medical directors to decide whether or not to exclude that unit. So the supplemental information is designed to provide that to the transplant center so they have the ultimate say in whether or not they want that unit.

Another example is just if there is a history of recent tatoo or piercing.

The unit selection in COBLT--COBLT is a little different than everybody else just because we are a controlled clinical trial, controlled by NHLBI, not a registry as the NMDP or other banks are. So the unit selection is guided by study policies.

The current policy is to select the best matched unit with the highest cell dose. However, there is some leeway within the rules for choosing a lesser matched unit if a substantially larger cell dose is available.

For example, if there is a 5 out of 6 with a cell dose of 2.2 and a 4 out of 6 with a cell dose of 5.3, it is the transplanter's option to select that 4-out-of-6 unit.

Post-search. All units and patients are HLA-typed by a COBLT contract lab prior to registration on the COBLT transplant study. Actually, a number of our units are already confirmatory-typed. The Navy Medical Research Center types a good portion of the units. So a lot of them have already been typed twice. Also, like the New York Blood Center and NMDP, units for patients with certain genetic diseases are screened prior to the release of the unit for transplant.

Under special issues or things that we came up with, I think a lot of this has been discussed. Basically, it is the linkage, how do you link serologic types to DNA, how do you rank the units, what is more important, HLA typing, cell dose. Should CD34 counts be a part of that algorithm? Should unit age be part of that?

In COBLT, also, there was a discussion on how large the report should be. Of course, ranking the units becomes very important when you decide that the report size should only be 25 units. Issues of which variables to appear on a report and are we biasing selection by putting certain variables on the report were also discussed.

Then there is the ongoing issue of HLA typing, the quality control of HLA typing. Because the Navy Medical Research Center has typed a number of the units, we have ongoing conference calls with the labs to discuss any

kinds of discrepancies. These occur about quarterly, and that this helps us have an idea if there is some problem or some type that is being missed by a lab. We readily can correct that problem if it happens.

This was on my outline. It is free under a contract to EMMES.

Now I would like to do a demonstration of the web-based system. This is the log-in screen. This is for the system that will be on the internet in early fall. It is user-name and password protected.

I will just log in. On the first screen when you enter, it gives you a summary of the activity for your center. So, at this center, for instance, they have 95 active searches. 18 formal requests have been submitted. They have one unit on reservation, and it also gives the overall study result that currently the search registry is 4,587 units.

The main menu for the internet system is up here. So that you can add/update recipient data. You can search for a particular recipient if there is already one in the data. You can view all searches. You can check the status if you submitted a number of searches. Summary reports are available, and then the log-out.

What I would like to do is just walk through adding a recipient, show you some other features of the system. So let's first add a recipient to the data.

This is what we call key information. So you enter the name code in COBLT. It is the first three letters of the last name. You enter the date of birth of the patient. The date request submitted is brought in by the system. You can change that if you had to.

You have also underneath the add/update, you still have access to any--you have validations. So if I don't type in the date right, it rejects it and tells me to fix it. Let me try to add it this time.

Like I was saying, from this screen you can pull up all existing recipients. There is a fine that allows you to find it other than with the ID.

This brings up the initial screen. You see the COBLT recipient ID will be assigned after we saved the record and give some more information. It tells you the center. It tells you the date of birth, and it echoes back the date request submitted.

There are two search options, like I had told you earlier. There is a standard 4 out of 6 which means you get the top 25 units that are 4 of 6 or better. You can extend that to be 3 out of 6 so that you would also see 3-out-of-6's.

I am just going to leave it at the 4-of-6 for now and enter some data. These are all drop-down boxes. You click mail. You click A positive or negative over here. The weight is in yellow because it is required, and we will not perform a search unless it is completed. That is due to the restriction that we only want to show 1 times 10^7 per kilogram units for any one patient.

Primary diseases is also a drop-down box. You can specify ethnicities. Those are not required. Basically, the only things required are HLA and weight.

You can enter either DNA information or serologic typing. For the purpose of this, I am just going to make it easy and show some serologic types--I mean DNA types. There is also checks on this to validate that you are inputting validate HLA type, and if you say it is serology, you are inputting a valid serology. If you say it is DNA, you are inputting a valid DNA.

For instance, if I enter 62 here and try to go to the next field, it will tell me it is not valid because I called it a DNA type and it lets me reenter.

If you have intermediate-resolution DNA, you can enter that. We allow space for that. We do allow for the name to be entered. I am not going to do that here. It is encrypted before it is transmitted to maintain the confidentiality of the patients.

Once I save this record, it will be initiated. It takes a few seconds, and the search will be run.

I can go to the check status, but I was not fast enough. So the average search time, it tells you at the top. The recipient ID was assigned as 101040G and the average time to complete a search, 10 seconds at this point.

I can show the results from this screen, but in the interest of time, I want to show another feature and I will show the results from that other screen, the next screen.

This is a screen that shows all searches, and we mean all for the centers. They are sorted in decreasing ID order because the higher number IDs are the more recent searches. There is a number of features on this screen I would like to show you.

First of all, under recipient ID, this provides a link to the search, to the search result. So you can click it from here. Very soon, this will provide a link to a very nicely formatted PDF or possibly HTML file. You can run the search from here. You can update any recipient data by clicking this. You can cancel the search if the patient has gone to transplant or died.

It also shows under status some very nice features which says that this is an active search for the

second patient. This particular patient has requested two units to be put for confirmatory typing, no reservations. Also, this patient below does have a reservation.

I am going to scroll down a ways to show you a few other things. For COBLT, preliminary searches last 6 months. That is renewable, but it lasts 6 months. So we have included here for this patient that the search is going to expire in 15 days. He has been searching for 5-1/2 months, and the transplant center can re-initiate the search or extend the search if they want, but this gives them warning that it is about to expire.

Also, it gives them a list of all patients ever. These are outpatients. This does not mean they died. This has to do with the search status, not patient status. This means that the preliminary search timed out for whatever reason. They can re-initiate it from this screen.

This is the patient I just entered, as you can see 8/14 at 4:30. Let me go in and show you the results.

At the top, what we see is all the data that we entered on the initial add screen so you get the ID, the transplant center, date of birth, ethnicity, ABO/Rh, gender, and then all the HLA information and weight. Again, from this screen, if you saw that you missed something, you can update recipient data. You can rerun the search.

Our plan is actually that the searches will be run every night, sometime between midnight and 6:00, so that except for new patients, there will not really be a need to come in and run searches. They will always be current. The registry is changing daily. The searches will be current daily, but in case you wanted to rerun it for some reason because you had to update it, that option is available.

So these are the units that were found. We see for this patient, the best unit we found was a 6 out of 6, 222, and the cell dose was 2.28. Then there is a number of 5 out of 6's, and we continue to scroll down, we see that we can get into some 4 out of 6's down here with higher cell dose.

So we show the nucleated cell dose. We show the HLA categories of the units. As I told you, most of our units are high-resolution type by DR, and most of the A and B's are typed at low resolution, although you do see some exceptions, for instance, 0702 and the B's.

The confirm column indicates whether or not the unit has been confirmatory-typed by the COBLT contract labs. This means that two or more labs have typed the unit and they agree on their typings. It gives the ABO/Rh of the unit. It gives the total CFUGEM and the total CD34.

If the transplant center was interested in this unit, they would click here for request formal. It comes up to confirm that you want to initiate confirmatory typing, and you can click okay.

At the top, you get the message that the formal search has been requested. In the background, what has happened is an e-mail has been sent to EMMES to tell EMMES--EMMES is the coordinating center that has to tell the contract labs that they are supposed to get permission for the HLA typing so that we can continue to track what the contract labs are doing. So, in the background, EMMES has gotten an e-mail to initiate the confirmatory typing, and has forwarded that e-mail onto the HLA lab.

So, on this section of formal search request, we see that this unit has now appeared. So, for any other searches run this date, this patient will appear, but there will be an indication by the patient that it is requested, as we see below on the search report. We see the word "requested."

COBLT allows up to three units to be requested at any one time. The formal search request can last for 60 days. There are extensions possible. So now we see that two of them have been added.

You also have the option of reserving the unit. Once a unit is reserved, there is also action that goes on

in the background to notify EMMES that a reservation has been placed so that we can send them the supplemental information on the unit.

Reservations are good for 4 months, but are also renewable, and you will see now that that unit has both the indication of reserved and requested. As I said, this unit will no longer appear in other patients' reports until that times out. You do have the option to cancel formal searches or cancel the reservation.

Just briefly, I will show you that we have run under check status--it tells you every unit, every recipient that has run a search for the day and what the status of that--this provides a link to the search report, again. You can find based on ID or other information.

I think that was about all I wanted to show of the search system.

There is a few things to summarize about the COBLT search system and COBLT banks and search registry. All our HLA typing is performed by DNA methods. Cord blood units undergo three reviews, two at the cord blood banks and one by validated computer algorithm at the medical coordinating center.

The search system is the link between the COBLT banks and the transplant centers, and the better we can make that link, the smoother things will go.

The web-based search system that I just showed is currently being tested at EMMES and will be available for the COBLT transplant centers in October. Future releases are already planned to extend the functionality of the search system to meet the needs of the users. We already have a number of things on the list that we want to add and a number of things that are almost ready, but are not being tested so that we can get the first release out. We believe that the features that are currently available will make life a lot easier for the transplant centers, at least for the COBLT banks.

Thank you very much.

[Applause.]

DR. HURLEY: Thank you very much, Shelly.

I would like to move on now to the next speaker who is Peter Hakenberg from Dusseldorf, and he will be talking about the NETCORD search algorithms.

NETCORD

MR. HAKENBERG: First, I have to thank the organizers for being here, and my task is to present NETCORD in this session entitled performing the search, and this addresses the search component of NETCORD, but in fact it is not only one component of the NETCORD activity, and I want to tell you some thoughts about education, some technical concepts, and the current status within NETCORD.

I will shortly mention again what is NETCORD. If you look into the statutes, you find two goals NETCORD wants to achieve, and one is to promote the availability of high-quality cord blood units and, second, the establishment of a network for the search, for the allocation, follow-up and so on. Here you have something like the mission of NETCORD.

I mention this first part, the quality issue here, because this is a very essential prerequisite for this second goal. Quality and comparability must be given for an effective cooperation, for an effective--I think this point was stressed several times today that we see how when we combine data we have a comparability problem.

This leads me to the first of a couple of points I want to address very shortly which are helpful to understand the complete picture of cord blood allocation and to try to understand our vision of an optimal cord blood allocation for which tight cooperation is necessary, especially--and this is the point--if it becomes a more generally used tool, generally used stem cell source also for adults.

This is my first point. It is compared to adult. Stem cell sources, cord blood is different because there are many critical parameters. In the bone marrow setting, you are essentially selecting by comparing HLA and looking

at IDMs, gender, and all of these parameters are compared to the cord blood setting, well understood and determinable, but in cord blood, you have parameters involved which are not so well and homogenic determined worldwide.

Some of them are really critical, as we have heard in the discussion. It makes definitely a difference whether the expected cell dose available after thawing is available or you have only half of it.

This means compared to bone marrow, cord blood is different because there is a much higher intrinsic probability for huge differences in the quality, but for physicians, it is essential that they can really compare data from different cord blood units.

It is not acceptable that physicians have to learn by experience whether data from cord blood units from different banks are direct comparable or not. It is not acceptable to learn this at the cost of the patients. This important issue was addressed together with FAHCT by the development of the common standards.

An important milestone in the history of NETCORD is release of the first edition of these standards 2 months ago, and if it comes now to the search, if you want to allocate the best available transplant in a very important--it is very important that cord blood units are really

comparable and accurate according to common standards. It will help to achieve this necessity.

Let me explain a difference. You have seen a similar slide. Currently, about 50,000 units are available worldwide, and it is my impression that there is no problem in finding a suitable cord blood unit for most of the patients.

Because the cell dose is a very significant parameter with increasing body weight, the usable worldwide inventory is decreasing rapidly. Here, you see the correlation between the patient's body weight and the useable percentage of the inventory for different minimum cell dose criteria.

You have heard an example here. For a 50-kilogram patient, you can only use about 12 percent of the inventory if you want at least 3 times 10^7 cells. If you want less, okay, then you will have a substantial number available. But for this example, this means you do not have 50,000 units. You have 6,000 units worldwide available.

That is my third difference. Cord blood is a limited resource due to the fact that there is a limited volume of blood in the placenta and also due to the fact that it produces costs in the forehand.

Of course, even if the patient's body weight is smaller, the biggest units are the ones the physicians want for the patients. This is the reason why most of the cord blood units selected by physicians are coming from a subset of the complete inventory. The competition increases with the body weight of the patient, and this means adult patients, for example, compete in a way with all patients, also all patients with lower body weight for suitable cord blood unit within this subset. So, when it comes to adult transplantation, you will have more competition.

I have tried to quantify this problem a little bit, and I have classified three conflict types. This is the classical conflict. You have one unit, and two people or more are interested and this is the only one for these people.

Another type of conflict, I call it the 1:N allocation conflict, there is one patient in need for a special unit and another for another patient. This is also the best available unit, but this patient has acceptable alternatives and there could be more patients involved in this.

Then the general case, you see the N:M conflict, and this means there are two or more patients. All of them have alternative cord blood specimen, but very best one is the same for each of them. Only one of them can get this

unit; for example, the unit with the highest cell dose. Of course, this kind of conflict can be solved if it is possible to find an agreement between the two transplant centers and the cord blood banks involved.

In a simulation with 1,000 patients and two pools of cord blood units, I have quantified how often these conflicts are occurring, and this is the situation when you accept one HLA mismatch and this is the situation when you accept two HLA mismatches.

Here, you see that with increasing inventory, of course, the frequency of really hard conflicts are reduced to several percent, but for inventory size which might reflect a little bit this current world situation for adults, so about 8,000 units, you see in more than 20 percent of the cases, you have the same best unit for two or even more patients. This is a simulation of only 1,000 patients. This means exactly that units reserved--exactly the units which are reserved for a patient are also of interest for the other patients and may become available later on in a general search strategy, but perhaps too late for the other patients.

What are the trends? What do we have to expect in the future? When cord blood search becomes a common part of the stem cell transplant search strategy, the cord blood per patient ratio decreases. More patients are

interested in the same number of units. So we will have more conflicts, especially for adults. There are many patients competing for only a part of the units due to the restricted number of cells, and, again, this means a bad ratio and the competition increases. Of course, if you increase the inventory, you will have less conflicts.

So this is the fourth point. There is kind of a competition problem when it comes to adult cord blood transplantation, at least then in a non-negligible way.

So another problem will occur during a validation phase of the transplant allocation process. The validation phase is what I call the phase between search and transplantation. Most of the patients will have alternative units to choose from. So you see a transplant center will reserve some units for validation purposes, and that is what they used to do with bone marrow donors. There are always active searches for many patients simultaneously.

At the NMDP, I think there are 3,000 searches active at any time, and if you assume two cord blood per patient reserved, this will exclude 6,000 units at any time. Of course, this will be exactly the units with the high cell doses. Most of the specimen will be available later on. So the match situation of the patient may change rapidly.

This means there is some dynamic in the match situation of a patient. Transplant physicians do not notice from the bone marrow setting in this degree of--in this size. It could be outdated very soon.

I am nearly at my last point. There is a dynamics problem in cord blood allocation. That is why we need a cooperated allocation process for cord blood. You will need comparability. You will have competition. You have to face the problem of the dynamics. Cord blood is a limited resource. You have to choose from and you have competition for--and this means this is in the field of optimization. It could be a field for optimization. It is the mathematical truth if you want to solve this problem optimally, you have to have complete information. In this case, this means you have to have the knowledge about all units available and all patients. So you have to cooperate.

Coming to my last point of cord blood differences today, cord blood units can be transplanted very quickly after identification, and it is very unlikely or much more unlikely than in the bone marrow setting that you lose an identified cord blood.

In a bone marrow setting, you can lose it because the donor withdrew the intention to donate or is just not

able to donate because of medical reasons or is not contactable any longer.

During the phase of the search for stem cell source for a patient--or preparing a patient for transplantation, it could be in future a good idea to keep your eyes open whether the match situation for the patient changes. It might be possible that there is a better transplant available during the 1 day or so between initial search and transplantation, and this is possible with cord blood without any problem. This is at least a direct difference compared to bone marrow.

All these points in my opinion feed the vision of a comprehensive, fast, modern, electronic cord blood allocation system, and you have the possibility of fast transplantation and the benefit of this advantage should be given to the patients.

Second, a fast and modern allocation system helps solving competition problems. It gives you the means to do this, but, first of all, it helps you avoiding competition because it enables faster decisions. This all results in more and faster communication between transplant centers and cord blood banks because due to the dynamics, you have to work with brand-new reports. Longtime reservation limits the chances of other patients and makes only sense in exceptional situations, in my opinion.

Finally, a modern system can assist the effort in making cord blood data comparable. These are some basic aspects.

A little bit about the history. NETCORD in 1997, I think--the three banks of Barcelona, Milan, and Dusseldorf decided to start such a cooperation with the aim to build a common search system. Initially, this means very easy and pragmatically exchange of cord blood unit data between these locations via encrypted e-mail. Thus, a common report could be generated and faxed when a search request comes in at any of these locations. Later, the association of NETCORD was founded with the partners you have seen, and the search system was, of course, extended by introducing forms to submit patient data and some other details.

Until now, this system is a simple system. With this concept, we have searched together with Milan more than 10,000 patients and without any fee, but this very pragmatic approach is no longer manageable due to the growing number of partners, if you want to implement this vision just explained.

Therefore, we have thought about the modern concept to bring all the partners together into a network, and just for the reason of abstraction and modernization, there are several roles defined within which the partners

play. So you have TCs, transplant centers, CBBs, cord blood banks, SU, search units. This is the place where metrics are produces, transplant registries and other entities. Technically, of course, each partner can play several of these roles, and this will be naturally the way that is the case.

Technically, we need a solution which fulfills several criteria. It must be globally available. There must be flexibility concerning necessary--it must be cost effective and, in one word, it is the internet.

When we look at the demands at the interface, at the point of access, how to connect the partners to this common net, what are the demands here? So you need secular communication which also enables privacy issues. I think this should not be forgotten. There are rules. If it comes to international data transfer, there are privacy--there are rules. I do not know the rules of the United States, but Germany and the European Community has rules which must be fulfilled when it comes to international data transport.

You need a web server. You need a work flow, too, which enables us to develop and maintain all of these procedures in a cost-effective way; for example, to route a request through the defined states, and, of course, a database interface to local database systems.

The general message is here. You do need to develop--you do not have to develop software for this. You juts buy what is available and concentrate on your specific needs. For example, we decided to lose Lotus Notes which has all of these components, and by using this system, we are just in the phase. You can build this virtual office in a cost-effective way.

Just to mention the components you need, you need the cord blood data management. You need conventional search management. You need electronic search request management, and you need the result management and all the communication between transplant centers and cord blood banks during the validation phase, plus other components like follow-up reporting and so on.

Due to the features of software, you can simply buy, for example, these Lotus Notes. You can simply make this the central system with several servers or clients over the world.

So, in principle, it is easy to connect a transplant center directly to the system without an external web server. In addition, this gives us for free scalability and redundancy, which makes the system 100 percent available on the net.

Again, the point is here. You can buy it. You do not have to develop proprietary software for this. You have the possibility of decentralization.

For example, if you have many places--one search unit can take over temporarily the work of another search unit if there are technical problems on one side.

Finally, I want to mention that there are already prototypes developed for several components of this system, for the user management. So a transplant center can reduce up to four search coordinators with personal passwords. The interface to local databases is prototyped. The cord blood data management, transplant center address management, and what you see here, two screen shots, very similar of the patient data management. Here, after the transplant center has locked in it, can select the patient or add new patients or change patient data.

Here, you see a small screen shot of the patient data entry form, which is exactly the NETCORD search form, which makes an almost complete automatic error checking.

Remember, this all can be done, deployed completely, decentralized in several places of the world. Other components are still missing, and we are in the phase together with the University of Colorado, the Denver Group, and New York Blood Center to define and to deploy and to test these things. So that perhaps we can this year reach

the second milestone of NETCORD by having such a common system.

Thank you.

[Applause.]

DR. HURLEY: Thank you, Peter.

In the interest of time, Joanne has volunteered to forego her presentation, but still ask questions during the panel discussion. So I would like the members of this afternoon's session to come up to the front.

We are going to try to finish by 5:30. So it will be a fast session. Can I get everyone to come on up?

Discussion: Bridging Banks and Transplant Centers

DR. HURLEY: There were a number of issues that we covered this afternoon, and I was trying to focus in on a few of them. So I came up with two that I thought we could start with.

The two issues maybe I thought we would focus on is the criteria for release of a unit to a patient. A couple of the speakers mentioned specific level of HLA match that was required before the unit would appear on the search report, and the presence of the IRB protocol. So that was one thing I thought we could talk about.

The second thing, I thought we could talk about the matching algorithms, the differences between the banks. We have heard some of the speakers describe their

algorithm. It sounds to me like all of them are based on HLA matching, but there may be other elements that play a role, and so how we could talk about that, how results are sorted, what kind of expertise was used in putting together the search algorithm for the bank and what might be a way that we could address different searching algorithms in different banks.

So any of the speakers would kind of like to take a stab at any of those issues?

DR. CONFER: I would just say that we talked with the COBLT people about level of HLA match required, what they were doing, and really pretty much adopted what they were doing, which is a maximum of two mismatches, a DRB1 and allele level mismatch counts. At A and B, it is a serologic equivalent, and you cannot have both mismatches at a single A locus or a single B locus or a single DRB1 locus.

Given the state of our knowledge about the requirements for HLA typing, this is probably as good as we can do at this point, and I think it is very reasonable, and I am happy that we are applying--I think we are applying the same criteria, and if we are, I am very happy about it.

We felt, running a cord blood registry under an IND, that the transplant centers needed to have an IRB-

approved protocol to do the transplants; that if a transplant center said, "Oh, this is standard of care," when we were operating under IND, that that was not going to make sense. So we require IRB-approved protocols at the transplant center. The transplant centers have to be members of the NMDP network in order to get access.

At the current time, we are figuring out how we would deal with the cooperative registries because we must have transplant outcome data, and we currently don't get that from the cooperative registries. For example, if we send bond marrow to Anthony Nolan Bone Marrow Trust, we do not get recipient outcome data, and we have to have that under the cord blood protocol.

DR. HURLEY: Peter, would you like to talk about the NETCORD?

MR. HAKENBERG: NETCORD--what we have developed in these first pragmatic ways--this is the cooperation between banks, and most of these criteria for release and reservation are left to the banks. So this is in the moment not much more than a common search report as a first step.

All of these things are at least partly addressed in the standards of NETCORD, and what the future means, there are several ideas which must now be combined a little bit. We have proposals from Denver, from New York.

The old way--NETCORD is doing this with--is just nearly the same. So I think it comes from us. We mentioned HLA ranking, categorizing by HLA match, and then ranking by cell dose. So this is pretty much the same, but I think the point is that the data you have in this report is really comparable.

DR. HURLEY: What level if HLA match do you include on your report? Is it a limited number of units or a certain number of matches?

MR. HAKENBERG: It is limited up to two mismatches, and actually it is categorized in broad Class I matching and generic Class II matching. This is the categories.

DR. HURLEY: Anybody else? Pablo.

DR. RUBINSTEIN: We essentially follow our IND specifications. We believe that the selection of the best match has to be a cooperation between the clinician and the bank.

We will release for them the best units we have for their patient according to HLA typing and cell dose.

There are, of course, a number of other criteria that the clinician will take in making a decision. If a patient can have a transplant that is a 5-out-of-6 match or a 6-out-of-6 match, clearly that clinician does not need to go very far into the 4-out-of-6's, but the decision if

which one they are going to use finally rests with the clinician.

We will provide information, and we will disagree sometimes with some vigor, on the precise unit that has been selected, but we will always honor that clinician's wish after they have listened to our point of view. We will provide data for supporting our contentions.

For example--and I will show tomorrow some of these things--on the point that Dr. Confer cited, whether a unit with two mismatches at one locus if preferable or is less desirable when compared to another unit that has one mismatch in each of two loci, we prefer to give the clinician the data. In fact, that makes absolutely no difference if you have an AB mismatch at both A and B or both of the mismatches are at one locus.

The transplant outcome does not support the precedence of one or another with these two systems. So then the clinician can be free to choose according to parameters other than these in the way we prefer, but we consider our obligation to have them understand what is--then beyond that, it is their decision.

DR. HURLEY: Thank you.

Shelly, did you have some comments?

DR. CARTER: I think Dennis already spoke for COBLT. Like I said, we show 4-out-of-6's. They are

allowed to have a 3-out-of-6, currently no more than one mismatch at any of the loci. That is changing based on a committee meeting recently. So we will start to show double mismatches in the near future.

I will say that impacts dramatically performance on search algorithms because you cannot parse out as many units quite as quickly, but we will deal with that.

Cord blood is a controlled clinical trial so that we have all the centers that can search the COBLT units have a single protocol that they are following.

DR. HURLEY: Thank you.

Pablo?

DR. RUBINSTEIN: Yes. With respect to the release, before a unit is released, there is a very specific ceremony, so to speak. The unit is reviewed by all the members of the staff that participate in retesting, rematching, controlling, reanalyzing, et cetera, all the different factors, and they have to sign. At the end, after all the signatures are in, including that of the receipt of the informed consent forms and so on, at the end of that there is a final review of all of these reviews and a signature at the bottom that says we agree to release this unit.

We cannot claim that we have missed a point here or there. If we missed it, it was not because we did not

have a chance to pick it up, but there is this last part of the releases.

DR. HURLEY: Thank you.

Shelly?

DR. CARTER: I think the main difference between the New York Blood Center and COBLT is that we preemptively do that. They do not get into the search registry unless they have been double-reviewed at the cord blood bank and also passed a computer algorithm for eligibility. So, at the time of reservation, that is already done. The only thing we have left to do is to make sure that the unit is doubly typed by the COBLT contract labs and that the patient has also been typed by the COBLT contract lab.

DR. HURLEY: Does anybody else have any comments they would like to make? Transplant physicians, here is your chance to say what it is you would like to have the banks provide to you.

Joanne.

DR. KURTZBERG: To me, the issue that we haven't spoken--but that is really on the table is whether the bank ought to control release by cell dose.

I think that the banks are providing the transplant centers in general with similar information and the proper information. I do think it helps to get it early rather than the day you order the unit, to find out

that there is some risk factor in the mother or whatever, because then you might make a different decision, and there are so many other balls rolling down the hill at the same time that it can be very difficult to sort of stop gears at that point.

Even simple things like reimbursement get into it because if we order a unit that we have to pay for and we do not transplant that unit, the hospital still has to pay for it. They cannot bill the patient, and they are not very happy with us.

In these days of managed care contracts, that does not do well for your program, but I think the issue we have, we sort of skirted around, which COBLT has addressed by not listing units that are delivered below 1 times 10^7 cells/kilo, right or wrong. It has not been addressed in other banks that I am familiar with, and the question to me is whether that is something that should just be left t o the transplanters or is that something that there may be good reasons why you would go with a smaller unit or should you have a stipulation that says you would only go to a small unit if you have nothing larger that is at least a 4-out-of-6 match or should there be criteria set up to address that.

Obviously, I do not know the answer, but I think that should be addressed.

DR. HURLEY: Does anybody want to respond to that?

Dennis?

DR. CONFER: Well, I agree with you. I think there is a potential escape mechanism for almost any unit that does not meet criteria, and it would be that everybody agrees that under the circumstances that it is the best unit available for that patient's condition and this risk-benefit ratio goes in the direction of benefit over risk, and that the transplant physician basically acknowledges the limitations of the unit and deals with the recipient in terms of explaining those and securing informed consent. I think that was reflected in the FDA's donor suitability document that there would be that mechanism under appropriate circumstances to use a donor who otherwise "did not qualify."

DR. KURTZBERG: I do not disagree with that, but I think that none of us can honestly define for sure what the "best unit" for a patient is.

At the 4-out-of-6 level, you have 12 units to choose from and the CD34 counts can be all over the place. If you believe that is important or it may be a Class I versus a Class II mismatch or a double focus mismatch--I mean, we don't know the answer to any of those questions.

We all make decisions, but it is more based on soft signs and subjective opinions than it is data.

DR. CONFER: I agree. All I am saying is when the transplant physicians says here is the one I want, that I think there is a mechanism to say okay, put it in writing, and you can get it.

DR. HURLEY: Let's have one last question or comment.

DR. LAUGHLIN: Can I break it out to two?

One is I like the rigor of the transplant physician forwarding a copy of the signed informed consent to the bank. I think it is an accountability issue, and it might be something to consider and continue in the dialogue, rather than just the transplant program generating their IRB protocol to make that hoop. It demonstrates accountability for that patient, for that graft, for that transplanter, for that bank.

The second issue that I wanted to bring up is that many of these IRB protocols, including our own, request of the transplant physician identification of a backup source of stem cells in the event of primary graft failure. Many of our patients have marrows that are affected with their disease. So they are not a good source of that backup stem cell source.

I have not seen this afternoon any capability to identify a backup cord blood unit for a patient, and I guess I am the person that transplants adults. So it is very much on my mind. It would be very nice to see incorporated into the searching capability somehow an algorithm to allow you to identify having perhaps identified the optimal graft for a patient to have the capability of identifying a backup cord blood unit to use in the event of a primary graft failure.

It will have to have a flip capability because obviously that transplanter's center reserving that unit is somewhat a low priority while they are infusing that first unit, but that priority flips 180 degrees if it is day 30 and that institution is facing primary graft failure. So I wanted to bring that up for discussion, not necessarily decision-making.

DR. HURLEY: Can we have just a quick comment from one of the members of the panel?

DR. RUBINSTEIN: I think the idea is a very important aspect of cord blood. It is possible using cord blood to have access to that backup. It may not be quite as easy in the case of a living donor, but in the case of cord blood, it is easy.

I would like just to say that we have had two occasions in which the first transplant that we shipped to

a transplant center, in both cases it was in Europe, one in France, one in Italy. The Customs stored the dry-shippers upside-down, and therefore, by the time the grafts arrived at the transplant center, although within the appropriate time, they were completely thawed. In both of those cases, we were able to supply an alternative unit within a very short time, and one of those patients is Madam Bubhall [ph], who is the first long-term unrelated adult recipient of the cord blood in France, and she received a graft that had less than 10^7 cells per kilo. In fact, it was 7.8.

DR. HURLEY: Dennis?

DR. CONFER: Can I just make one final comment about informed consent?

I think it is critical that informed consent is obtained on all sides, but I think we should not go in the direction of exchanging consent forms in the interest of confidentiality.

If a cord blood bank is insisting on seeing the signed informed consent from the transplant center, the transplant center is going to say okay, you send the signed informed consent from the cord blood donor's mother, and you can't do that. We don't want to go there. We don't want to start exchanging that kind of information.

So what I think you do is everybody assures everybody else that you have got informed consent, and then

you document that by periodic audits. You go out and you say okay, you said you had it, do you have it, you got it on these, and then we can assure ourselves that we are all operating according to the same game rules.

DR. HURLEY: Quick comments because I understand there is a bus leaving to the Hyatt and I do not want everybody to have to walk.

DR. CARTER: I would ditto Dennis' comments on the signed informed consent. I think there can just be assurances sent just in the interest of patient confidentiality.

On the second issue of a backup marrow source, we are talking about that at COBLT. You can also already put multiple units on formal search, and I think we are just talking about extending that formal search or having another designation that this is a backup for our patient. So I think it is easily implementable on the internet system.

DR. KURTZBERG: I want to make one plea for transplant centers to have either an HLA lab director or the transplant physician or both check the searches themselves. I do not think it is fair to the search coordinators, to expect them to be able to sort through these kinds of searches. I think that things are missed, and certain nuances of what might be good for one patient

or another will not be appropriated. I think different centers have different practices, but I think that is really important, and it is more important than when you are looking for an NMDP living related donor where the choices are much more limited.

DR. STEVENS: I just wanted to mention one more issue in the allocation problem and conflict, and that is that patients are often shopping around for transplant centers. We frequently get multiple requests from the same patient from different centers. So one of the things that we have done, not using an encrypted name or initials, we use a full name and birth date to try to make sure that we do not have patients in twice and then reserving the same unit for the same patient at different centers.

DR. HURLEY: Thank you.

DR. CONFER: We do that, too, and I agree with you entirely. It is just that you cannot get that information out of us very easily.

DR. HURLEY: I would like to actually call a halt to this afternoon and thank all the speakers for the very interesting session. I thank the audience for hanging in there so long today.

Thank you very much.

[Applause.]

[Whereupon, at 5:32 p.m., the forum recessed, to reconvene on, Tuesday, August 15, 2000.]

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