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FOOD AND DRUG ADMINISTRATION

UNRELATED ALLOGENEIC CORD BLOOD BANKING AND TRANSPLANT FORUM

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C O N T E N T S

P R O C E E D I N G S

Session 3: Transplant Outcome Analysis

Comparison of Related BMT and UCBT

DR. HOROWITZ: Good morning. I am Mary Horowitz. I am the Scientific Director of the International Bone Marrow Transplant Registry, and I will be starting off this session on transplant outcome analysis.

It has been historically true in the field of hematopoietic stem cell transplantation that most allogeneic transplants involve HLA identical sibling donors and, in fact, much of what we know about transplantation clinically derives from that experience, and experience has shown us that most of the principles involved in unrelated donor transplantation are very similar to the principles involved in related donor transplantation. I think it is probably appropriate to start off this session which will, of course, focus on the results of unrelated donor cord blood transplants, with an analysis of the results of related donor cord blood transplants.

Could I have the first slide? Now, that is easier said than done because there really aren't a lot of HLA identical sibling cord blood transplants done, and the reasons are obvious. To have an HLA identical sibling cord blood you need to have a pregnant mother at the time that

you develop your indication for transplantation. For obvious reasons, that is most likely to happen if you are a child with a disease that requires a transplant and, even more so than in the unrelated donor setting, the overwhelming majority of HLA identical sibling cord blood transplants are done in children.

Even so, numbers are small, and to be able to look at this issue it was necessary to combine data from both the International Bone Marrow Transplant Registry and EUROCORD. This was done in a study that was recently published in The New England Journal of Medicine. The senior author is Gluckman. I don't think that in this audience we need to go through the background.

This is a comparative study. It is a formal comparison of bone marrow and cord blood transplants in the HLA identical sibling setting, and the main advantage of that is that we can minimize confounding effects of other factors that might influence GVHD risk, particularly differences in HLA compatibility between the bone marrow transplant and the cord blood transplant setting. We focused on children under the age of 15 years, HLA identical sibling donors. We only looked at unmanipulated cord blood and bone marrow grafts. All these transplants were done in the 1990s.

Our primary question was is graft versus host disease really less with a cord blood transplant versus a bone marrow transplant in a setting where you have the same degree of donor recipient histocompatibility. We also wanted to look at time to hematopoietic recovery of both neutrophils and platelets of severe GVHD, chronic GVHD and survival.

What do we have to consider? And, I show this slide more as a backdrop for our discussions this morning because even when we look at the HLA identical sibling setting we see two groups that have a lot of differences when you look at bone marrow transplant recipients and cord blood transplant recipients, and we have to consider differences like recipient age, sex, weight, CMV status, donor sex, ABO match, conditioning regimen, GVHD prophylaxis, nucleated cell dose which I know that will get a lot of discussion today, and use of hematopoietic growth factors post-transplant. There are a lot of differences in the way transplants are done and in whom they are done when we talk about cord blood transplant recipients and bone marrow transplant recipients, and that has to color our discussions this morning. To try and adjust for these confounding effects we use a statistical approach with Cox, multivariate Cox proportional hazard regression.

Now, despite the fact that we used two large international databases and captured what we think was about 90 percent of the HLA identical sibling cord blood transplants that were done in the 1990s, we only had 113. During that period of time, we were able to identify 2052 bone marrow transplant recipients with the same eligibility criteria that had been reported to the IBMTR. Even though we restricted it to children, the cord blood transplant recipients were still younger. There were some differences in gender distribution. They weighed less. There was not a significant difference in malignant versus non-malignant disease and CMV serum status.

This is just a list of the diseases for which these transplants were done. There are no surprises here. These are typical transplant indications in children. But, the transplants were done differently. There was a trend toward more use of TBI in the bone marrow transplant cohort, and quite a difference in the type of GVHD prophylaxis used, with many more of the bone marrow transplant recipients receiving combined cyclosporine-methotrexate and many more of the cord blood transplant recipients receiving G or GM-CSF post-transplant in a prophylactic manner to promote engraftment. Nucleated cell dose is about a log different, as one would expect. These patients tended to be transplanted later so, despite trying

to select a similar population, they tended to have somewhat more advanced and long-standing disease.

We will just cut right to the results. The primary endpoint was Grade II-IV acute GVHD. In both univariate and multivariate analysis there was a significantly lower risk of Grade II-IV acute GVHD in the cord blood transplant recipients, a difference of about ten percent in absolute terms.

If we look at the severity of acute GVHD even among those patients who got it, it was less severe in the cord blood transplant cohort. Nine percent of the bone marrow transplant cohort had Grade III or IV acute GVHD versus two percent in the cord blood transplant cohort. Chronic GVHD was also significantly less in the cord blood transplant cohort, less than ten percent versus about twenty percent.

A couple of things to notice here -- even in the bone marrow transplant cohort these probabilities are pretty low. Why? These are children. Children do well with transplants whether you are talking about bone marrow transplant or cord blood. Another thing to keep in mind in our discussions is unrelated donor transplants later on this morning.

Now the downside -- the hematopoietic recovery, either measured by ANC, as shown on this slide, or platelet

recovery was significantly slower in the cord blood transplant cohort. Bottom line -- survival was equivalent. This just shows all patients together. Again, notice the pretty good results. These are children. And, if one separates by malignant and non-malignant disease you see that children who get transplants for HLA identical sibling transplant for non-malignant disease have a very good outcome. Those who get transplants for malignant disease have a somewhat worse outcome. But no difference with either a bone marrow or a cord blood transplant.

I promised I would keep this very short because I don't want to get behind right away. So, what does this tell us? It tells us that we have to really consider things other than the graft type when we compare bone marrow and cord blood transplants; that even in the HLA identical setting it seems that graft versus host disease is significantly less with cord blood than bone marrow transplants; that this advantage is offset by a markedly delayed time to hematopoietic recovery. We were unable to look at immune recovery in this cohort; we just didn't have the immunologic data for most of the cord blood transplant recipients. But, the net effect is that the results, as measured by survival, are equivalent with cord blood and bone marrow transplantation.

I think I will stop there, and we will be holding all questions until the panel discussion at 11:15. With that background on the HLA identical sibling setting, I will ask Dr. Pablo Rubinstein, from the New York Blood Center, to present the results of unrelated donor cord blood transplants as facilitated by the New York Blood Center. Thank you.

[Applause]

New York Blood Center

DR. RUBINSTEIN: Good morning. It is always a wonderful pleasure to follow Mary and it is a very difficult yardstick to measure up to.

The data I will present is abstracted from the clinical results reported to us by the transplant centers which performed transplants of cord blood from the Placenta Blood Program of the New York Blood Center.

In summary, there have been 127 patients who received first transplants from our program. These patients have been transplanted since 1993, as you see in this slide. From this slide, there was a very rapid increase in the number of transplants per year, and this increase is becoming slower and then there is a decrease in the last few years for reasons that may be related to the number of alternatives that are now open to the transplant centers as supplies for this material. When we started

that was the only option for an unrelated cord blood graft. There are now 50 cord blood banks listed in the BMDW summary.

The transplants have been done mostly in the United States but, as you can see here, in a number of other countries, mostly in Europe but also in Australia, New Zealand, Malaysia, the Middle East and South America. The patients transplanted have different ethnicities, as reported in here.

Yesterday we went into some depth in the analysis of the ethnicity issue. Just to remind you, in order to provide an appropriate probability of finding a donor it is necessary to manipulate the frequencies of the donors available. That has been done in our program by directing the collections to specific hospitals where the ethnicity of the donors allows us to have proportions approximating those that would yield rather close probabilities of finding matches. You can remember probably that a few years ago Dr. Miti calculated that the probability of finding a match for an African-American patient among African-American donors is only one-third of Caucasian patients from Caucasian donors.

Now, as we heard, there was an early demonstration of people doing related cord blood transplants that graft versus host disease was apparently

of a lower intensity and lower frequency than with bone marrow. This allowed the concept that it would be possible perhaps to do transplants that are HLA mismatched. In fact, the results of these mismatched transplants through the years have been comparatively good, and many people feel that they actually may be of much lower importance, if of any importance, among cord blood recipients than in the case of bone marrow.

The issue of age and size is more complicated since the size of the graft itself is determined accidentally by all kinds of reasons at the time of collection -- that is as much graft as you are going to get. The recipients in our case, surprisingly, belong to all age groups and there are not very major differences in the age groups included, at least numerically.

With regard to the diagnoses of these patients, about two-thirds of the patients are leukemia, and among these, about half are acute lymphoblastic leukemia. Genetic conditions altogether amount to somewhere between 20 and 25 percent of the total. In order to do the analysis, therefore, we have defined transplant-related events in our slides that we follow as three conditions that are common to all the transplants -- the possibility of transplant failure by autologous reconstitution, or backup graft, and death. They belong equally to leukemic

patients and patients with genetic conditions, while relapse is only applicable to leukemics. So, for this talk we will differentiate between the first three which are common and the last one, separate.

Now, the data that we have received is as follows: For myeloid engraftment we have received data from 94 percent of the transplants. Data for 100-day survival is available for 95 percent. One-year survival, for 91; and two-year survival for 93. And, data on graft versus host disease is available on 95 percent of all patients who engrafted.

This is a well-known fact by now. The number of patients is bigger but essentially it is the same trend as was shown in our paper in 1998 using about half as many patients. The clear indication here is that the cell dose is a major factor in the speed of engraftment. The slide also includes the medians for the four ranges of cell doses that we have distinguished in these studies -- for 100 million or over, the median engraftment day is 18; for the next group, for 50 to 99 million cells per kilogram, it is 25 days; for those receiving between 25 and 50 million it is 30 days; and for those between 7 and 24 million it is 34 days. So, the influence of the cell dose is absolutely clear, and it is progressive. It is not a threshold event, but it is a steady improvement with the cell dose.

Now, for HLA this has been well known, the controversial aspect, and here is the data for engraftment. I should say that all of these slides are constructed by Kaplan-Meier statistics. As you can see here, patients with no mismatch or no mismatch within the definition of our program, which means no mismatch at the serologic level with splits for Class I and the titer solution as available at the time for Class II, shows that those without mismatch engrafted faster and the difference is significant. But there is, surprisingly, no difference between those that differed at one antigen or two.

This, in a way, parallels the experience in unrelated bone marrow. The surprising thing that I showed you yesterday, somewhat unexpected, is that when there are blanks so we have an asymmetric mismatch at a single antigen level and the blank is the mismatch, we can have different levels of match in the graft versus host direction and in the rejection direction. Here you can see that while the graft versus host direction, as described in bone marrow, is a little better perhaps than when the antigen is present in both directions, the mismatch, the opposite is not true of the rejection direction.

Now, the explanation given for the improvement in the graft versus host direction by the Seattle group is that that reflects the withdrawal of graft versus host

activation of the graft and that improves the chances for the graft to overcome whatever resistance may exist in the recipient. The other side, the rejection, was not found by the Seattle group and, in struggling to find the reason for why it occurs here it seems to us that the possibility that needs to be explored is the possibility that there is previous sensitization of the recipient and, therefore, a rejection phenomenon, an immunologic rejection.

Now, just as we reported earlier, there is a difference according to prognosis, and there are three diseases that we identified earlier that came out of multivariate analysis as being significantly slower in engraftment. Two of these, severe aplastic anemia and Fanconi's anemia, are perhaps understandable because of the known problems associated with these diagnoses. CML was a little surprising but, as you can see in the slide, the overall engraftment is not all that different from patients transplanted for other diseases but it is a little slower and that effect may be due to the cell dose since the age difference is quite striking. We will see a little more about this later on.

One of the factors that was a little distressing to us was finding, as you see in this slide, that the U.S. centers do better than all the non-U.S. centers considered as a single group. Obviously, this is an arbitrary

decision from our point of view. It is probably not correct to lump them altogether. There is an enormous variation, but in subsequent analyses there are measurable differences in parameters that we know are important in the overall story. One of them will be seen in this series of slides.

The multivariate analysis of the factors that influence the speed of engraftment is shown here. From all of the factors analyzed, and there are a lot of them, these four factors remain significant after Cox regression in more or less exactly the same way as Mary described a little earlier.

Now, another factor that is interesting is age. In this slide you can see that the patients where age and weight are independent variables -- this slide refers only to adults and for adults engraftment seems to be independent of age. It is a good sign. If we did this for all patients, of course, there would be an important influence of age because age would be confounded with weight. In younger patients and children age and weight are distinctly correlated variables.

Besides engraftment, there are a few other areas that I will explore with you today, and one of them is graft versus host disease. As Mary showed us in the related situation where histocompatibility differences do

not exist, there is a clear difference between the frequency and intensity and chance to develop chronicity for graft versus host disease.

The same happens for unrelated placental blood grafts. You see here, with the number of mismatches of HLA we increase the number of severe graft versus host disease, in the red bars, or medium to severe, Grade II, in the yellow bars, and we decrease, of course, the frequency of those that have no graft versus host disease. But the overall frequency even for people receiving two antigen and three antigen mismatched grafts is not in the same league as that which follows or has been reported following bone marrow transplants.

Now with a larger number of patients and longer evolution, we can tell that there is a significant difference in the probability of having chronic GVHD according to the number of mismatches. Most of this chronic GVHD is of the small variety, so to speak, not extensive chronic GVHD.

About survival, the overall data shows, as expected, that there is a better prognosis for patients with genetic disease than for malignancies, and that acquired disease, meaning severe aplastic anemia and similar conditions, are somewhere in between, but these are the overall two-year plots.

To remind you, we separated relapse and the total number of patients that suffered failure are shown here -- 23 had autologous reconstitution and 43 required a backup graft. Death from all causes amounted to 424 patients. There were 91 cases of relapse.

In general, survival is compared or analyzed in comparison to the cell dose, and we will do that too but we just wanted to show that, as is well known, there is a correlation with the age of the patients. Also as Mary said a few minutes ago, younger patients do better than older patients and this is seen here. Now, for adults the same phenomenon happens. So, this is a little different from engraftment where age didn't seem to make much difference. It does make some difference, but this level is not significant but there is a kind of a trend in which the younger patients seem to do a little better. The numbers are not large.

Now, total nucleated cell dose, which is the conventional way to look at these, says that there is the expected correlation. When we look at the curve, it seems that the major difference occurs very early on. The curves are rather parallel from some point and maybe about three months after transplantation they are roughly stabilized. the major differences imposed by the cell dose occur early on. Perhaps that is not a surprise.

Now, the age group is, of course, correlated to the total nucleated cell dose, much more so at the beginning, in the early age groups, than later on but these are significant correlations between these two variables. Here is a multivariate analysis attempting to look at both age and cell dose even though they are correlated variables. As you can see, if we study the patients from the point of transplant on, we see that both of the are significant. Age and cell dose have an independent influence but after engraftment the cell dose drops off and age remains the single important variable, or I should say a single significant variable. This is the influence of HLA and I think with these numbers of patients there is little doubt that HLA is important. Of course, I will show you later that this is confirmed by the multivariate analysis where HLA is important when you look at it from the beginning of the graft, and is also important after engraftment.

HLA mismatches can be looked in a number of different ways. This is to look at patients with a single mismatch to try to see if one locus is more important than others. We all have the feeling that DR is probably more important but the data do not support a significant difference. Here is the effect of high resolution. It is clear that high resolution is very important. Patients

with high resolution mismatches do as bad -- in this slide it looks as though they do worse than those with low resolution mismatches. So, resolution is important for DR.

Now, a question that lingers in the mind of many HLA people is whether there are effects associated with haplotype. The patients with one mismatch can have a shared haplotype or maybe they have two haplotypes that are different and only one of them results in a mismatch. We would like to see a difference but this is still not significant.

When you have two mismatches, does it make a difference if they are at one locus or at more than one locus? And, the answer is that there is no difference. These numbers are quite significant.

The next two slides are a little complicated. Here is when you have two mismatches at A only, at B only -- the first two lines, two loci, or DR only. DR only seems to be different. In this context, however, there is no significance for the overall group but we have to keep an eye out on DR and, in fact, we avoid two DR mismatches like the plague.

Here, the same lines are kept but, in addition, we have these lines of combinations -- BDR and AD or ADR, and you can see that there is some difference with the ADR being a little worse but this is not significant. When the

two mismatches are Class I or involve one Class I and one Class II antigens, there is no difference.

Another factor that is of importance and that we have been looking at is the influence of the experience of the center in the results. This has been seen for bone marrow in a number of patients, reported in the literature, and for cord blood it seems to be the same. Centers with more than 20 transplants are doing better. There are many factors that go into this, not just the experience and dexterity of the transplanters but there is a great deal of learning that is required to improve this overall prognosis, and this is happening and it is not just those with more than 20 but those with 10 that are doing better.

To remind you, there is also a difference between the United States and the other centers and, again, most of the difference is at the beginning but we will see these in a multivariate analysis in the next slide.

This is the difference between different diseases. Again, CML, Fanconi's anemia, severe aplastic seem to be doing worse but, again, there is the influence of other factors. This is a multivariate analysis. This is from the beginning. As you can see, age, cell dose and HLA match and center experience, U.S. versus non-U.S. and disease are either significant or close. But after

engraftment the multivariate analysis only shows two highly significant variables and those are age and HLA match.

Just to finish, we have accumulated now 18 patients with a second cord blood transplant. As you can see, this seems to have saved some of these patients. Engraftment is not all that different, and survival for this very small group of patients indicates that at least some of these patients will survive. So, it may be a reasonable option to consider.

As you can imagine, there is a very large number of other things. I have some more slides but I think I have exhausted my time and so thank you very much for your attention.

[Applause]

DR. KURTZBERG: We are saving questions until the panel discussion, and we are going to have a change in sequence because John is stuck on the Metro, I understand. Anyway, he is coming but Donna Wall has kindly agreed to go next and will talk about the St. Louis experience.

The St. Louis Experience

DR. WALL: Thank you very much. Thank you again for the opportunity to share our experience. Basically, I would look at this talk as building on the groundwork that has been developed by Dr. Rubinstein and basically a validation of his experience from a second cord blood bank.

I would also like to take advantage of the time I have to paint a little bit of a picture of cord blood experience at a single pediatric center. I think this might be helpful as policy is being developed in looking at the potential impact of cord blood in stem cell transplant with a pediatric focus -- so, kind of a flip.

The St. Louis Cord Blood Bank started in 1996. We explained our basic operations. We have 5500 cord blood units that are available for research and transplant. Our philosophy is that we have a resource for use by centers who are transplanting. So, it has been our approach to make our units as available as possible. In doing so, we have listed our cord blood units with the National Marrow Donor Program, the Bone Marrow Donor Worldwide and Net Cord. In addition, we have recently signed on with the COBLT trial as a transplant center and our cords will be available for use with the COBLT trial for those who do not have matches within the COBLT inventory.

To date, we have released 175 units from the bank to 61 centers and 12 countries. From this, 166 transplants have been performed; 22 have really just gone out recently and are not part of this evaluation. We have not received data on 20 of the transplants, and we have 110 that are evaluable. This is fresh experience and these are not going to be as stable curves as Dr. Rubinstein's data. We

have not included in the analysis cord bloods that were used for second transplants, cord bloods that were used for expansion. So, you are going to see some of the numbers move around a bit.

The diseases being treated are similar to what is being treated with allogeneic transplants, majority of leukemias, and the majority of the leukemias are ALL; immune deficiencies; bone marrow failure; hemoglobinopathies and the metabolic storage disorders. Our median age of patients being transplanted is a pediatric age of 10. We have had 35 adult transplants performed.

We have tried to define engraftment/non-engraftment, and the definition that we have used is a neutrophil count by day plus-42 and non-engraftment would be an earlier documented lack of chimerism or death between day 28 and 42, saying that those deaths are more likely due to delayed engraftment. And, I would be very interested in how everyone else is doing this one. Using that definition, 12.5 percent of the transplants failed to reach ANC by day plus-42. When we used day plus-60 as our cut point, we have 7 percent of the grafts, or 6/86 that failed to reach engraftment. We took a look at these 5 patients who engrafted late and they did come in at variable time points and, interestingly, once they did engraft they were

all survivors. So, I give you that as information -- what you do with it.

Overall, our time to engraftment for the cohort is very similar. Our median time to engraftment is 25 days for an ANC of 500. Similarly, our platelet engraftment -- we are really not seeing much platelet recovery until about day 40 and the median time is about day 60.

What we have done is take a look at cell dose impact on engraftment, and when we do that -- and we did this at many cell dose cuts using total nucleated cell dose, and basically at any time point that you look at you will see that the more cells, the better, which is similar to what Dr. Rubinstein has discussed. For us, the biggest cut comes once you are below 3 X 10^7 cells/kg and that is a recurring theme. Once you are above 3 X 10^7 cells/kg you are pretty reliably coming in with an absolute neutrophil count before day 25. When we look at survival, we also notice that there is a difference in survival once you are above and below 3 X 10^7 cells/kg.

When we take our cut higher -- and actually a number of pediatric patients are able to make this cut -- we do have really very reasonable engraftment times once we are over 5 X 10^7 cells/kg or 10 X 10^7 cells/kg. At this point, our survival curves are really not impacted once we

are in this cell dose range. This is actually the lower cell dose per kilogram curve.

What we have done is taken a look at seeing whether we can define a threshold for which we say we are above a given cell dose; you will almost assuredly engraft quickly. So, this curve is cell dose per kilogram, and that is 10 X 10^7/kg and this is days to ANC 500. This is work in progress. This line, here, is basically our median time to platelet engraftment at 25 days.

If we take a look at patients who received 5 X 10^7 cells/kg or, the lower line, 3 X 10^7 cells/kg, you are seeing pretty much a threshold effect where a majority of the patients are engrafting reliably, and we are sort of fishing for should we be using total nucleated cell counts; should we be using CFU; should we be using CD34 in guiding transplant centers to make a good decision?

Similarly, when we look at CD34 X 10^5/kg for recipients, we find this threshold is a little bit cleaner, where we can define a CD34 cell count above which we are pretty reliably engrafting at a reasonable time period. The same holds true for CFU, which is not rocket science because we know all three correlate fairly well with cell dose. When we take a look at platelet engraftment, it is much more of a scatter plot and there is less of a dependency of platelet engraftment on cell dose.

I would like to go back to the original slide looking at neutrophil recovery and focus on those who have less than 3 X 10^7 cells/kg, and point out that in this population there are those who had this lower cell dose who engrafted very reasonably compared to an equal percentage who were slower to engraft.

One of the questions we asked was whether this population had a higher CD34 count. So, could we, on lower cell doses when we are looking for donors for folks, flip over to CD34 count and say this is a rational approach? That we will be able to predict that you will be a more rapid engrafter? In doing so, when we look at absolute neutrophil count less than 25 days/greater than 25 days for those with a lower TNC dose per kilogram -- that bottom half of the curve, we are seeing that the more rapid engrafters had a statistically significant difference, p less than 0.04, but not a really clinically significant difference in cell dose on the CD34 count.

Similarly, when we did the flip we took a look at the ones who were rapid engrafters versus slower engrafters who had a low CD34 cell count, and we looked at their total nucleated cells per kilogram and we do not have a statistically significant difference between the two groups. So, we can't flip to the other measure of hematopoiesis in trying to predict at time of choosing a

product for transplant, saying that this product is likely to be a better product than not.

Overall, survival for all cords from the bank at this point is a week bit better than 50 percent. When we take a look at survival by cell dose, we have the expected difference for cords that were transplanted at less than 3 X 10^7 cells/kg, but above that we have basically a scatter plot. The highest cell dose is the purple line and then intermediate cell dose. So, basically my read of this is a threshold effect above 3 X 10^7 cells/kg roughly.

We took a look at the same analysis using CD34 cells. We really did not see a difference in long-term survival. You know, our power of the numbers that we have is nowhere near as great as others, but with a sizeable number of transplants we are not getting an absolute sinful number of CD34 upon which not to transplant.

Much the same as Pablo, we have a mix of HLA matching that has been accepted by transplant centers. A majority of patients have a 1- or 2-antigen mismatch, and the matching is defined by serologic Class I and high resolution molecular Class II. All typing has been done molecularly.

With the power that we have, we are not seeing a difference in HLA matching for survival. In our 6/6 antigen matches compared to 4/6, compared to 5/6, and then

a small number of 3/6 matches -- they are not pulling apart at this point, the problem being that we had a very few fully matched and majorly mismatched products.

The question is raised as to how big an impact is cord blood going to have on transplantation as an alternative donor source, and that is a question that is going to drive resource allocation -- how many cords need to be banked; what is the expected utilization of cord blood. So, what I would like to do is quickly paint through for the regulators in the audience our experience at Cardinal Glenning Children's Hospital for the last six years as a pediatric transplant program, with an acknowledged bias towards use of cord blood as the alternative stem cell source.

During that time period, we have performed close to 100 allogeneic transplants. We are a good Catholic hospital so we have close to a quarter of our transplants being HLA matched siblings, and this is a higher match than most transplant programs will have. We have identified 8 partially matched family members, and the rest are unrelated donor and in that pool, roughly 2:1 use of cord blood compared to unrelated marrow.

When we look at overall survival for the program, actually our gold standard is our matched sibling curve, sitting at about 60 percent, and superimposed with the

unrelated donor marrow with cord blood and partial matched family members just above. So, basically no major difference in survival between the different allogeneic groups.

Out of the cohort of kids that we did treat with cord blood, they ranged in age from 2 weeks to 15 years. All were done on IRB approved protocol. All did not have an unrelated marrow donor available in a timely fashion. They represent very much the major advantages of cord blood.

We have a fairly active immune deficiency program, and the metabolic disorders and, if I may just cheat a little bit as a pediatrician can do, this little one had RSV pneumonia, was on a ventilator, in terrible shape; was able to have a cord blood identified and be ready as soon as she came off the ventilator for treatment for underlying immune deficiency, and is a long-term survivor.

This little one had a necrotizing pseudomonas pneumonia going into transplant and was transplanted within 2 weeks of diagnosis. This kind of movement and the availability of an alternative donor stem cell source can only be done with cord blood.

Similarly, our little ones with leukemia are in a similar clinical state where they have either

myelodysplastic syndrome evolving into leukemia, an untreatable disorder with conventional therapy -- very hard to keep these kids in anything kind of condition to get to an unrelated donor marrow transplant. This little one failed autologous transplant. We were able to get her aplastic and then take her into a cord blood transplant.

This is my soapbox opportunity, that in developing cord blood utilization strategies, they really have to be flexible and rapidly available to transplant programs.

The other major group of disorders that we treat are the bone marrow failures. The majority of the children who are transplanted receive 3-6 antigen matched cord blood units. There were only a few 6/6 antigen matches. Most had major mismatches. Our bias, as a program, has been to try and match for DR although I know the data doesn't support that. The cell dose was in general a healthy cell dose with several of the kids getting well above 1×10^8 nucleated cells/kg.

What I have done here is compare engraftment between all our marrow recipients and all our cord recipients. So, this is our matched siblings. This is our unrelated donor marrow and our partially matched family members as the marrow curve, which is in peach. The engraftment time for the patients with cord blood is

basically superimposable in our pediatric population. There is what looks to be a little bit of a shoulder of more rapid engraftment with the cord bloods and that is completely attributable to use of steroids in the cord blood and methotrexate in the marrows. But you notice that the time to engraftment is very quick, with a median time in the range of 15 days.

Our platelet recovery is slower, as reported by everybody where we compare marrow coming in roughly at 25 days, and the cord bloods coming in significantly later at closer to 60 days.

When we look at survival, we are again echoing our larger experience with the cords that we sent out where we are really not picking up a survival difference between any of the groups. The anti-leukemic effect looks like it is real. Of the ten patients transplanted for leukemia, we have had only one relapse.

Graft versus host disease, given the degree of HLA mismatching accepted, is well within what we are used to seeing in unrelated donor marrow settings. So, if we look at our overall patients, 18 of the patients had Grade 0-1 graft versus host disease and 7 had Grade 3-4 graft versus host disease and, as Dr. Kurtzberg has mentioned in the past, this tends to be very treatable graft versus host disease. I don't want to under-sell it but it is

treatable. Our experience also is that we are seeing very little in the way of long-term chronic graft versus host disease, and the majority of it being limited in severity.

So, our conclusions are that cord blood is a very robust alternative stem cell source, especially in pediatrics. With a good cell dose, engraftment time is very similar to that of bone marrow but is delayed compared to platelets, and graft versus host disease does occur. In our hands, we are not able to pick out a difference between the degree of HLA matched but our patient numbers are smaller.

I would just like to thank the many people involved in this: my home team, the obstetricians who are involved in the collection program, the Stem Cell Transplant Program, and the National Marrow Donor Program. Thank you.

[Applause]

DR. HOROWITZ: Thank you Donna. Just to remind you that we are holding questions till the panel discussion. Now Dr. John Wagner, fresh from the Metro, will present the combined Duke and University of Minnesota experience. Thank you, John.

Consolidated Data of Duke and Minnesota

DR. WAGNER: Sorry for the delay. I finally made it but, unfortunately, I actually missed the first couple

of presentations. So, I am at a little bit of a disadvantage but, nonetheless, I am sure we will have a chance in the discussion section to talk about the differences in the analysis that I present versus what some of the others have already presented and perhaps in the future as well.

Going back now to old-fashioned slides, what I am going to show you is basically sort of a thought process of why individual transplant centers may have something in addition to offer from the larger registry presentations. Certainly, there are a certain number of advantages in the data that is coming from the umbilical cord blood banks, but there is also something to be said about the individual transplant centers presenting their own transplant data from a variety of points of view. Certainly, the umbilical cord blood banks are sure to have a tremendous potential for evaluating the cellular composition of the umbilical cord blood graft; certainly a variety of infectious disease testing issues, and genetic disease screening issues as well.

Obviously, the transplant centers are particularly interested in hematopoietic recovery engraftment, acute and chronic graft versus host disease and opportunistic infection. The umbilical cord blood banks certainly have a large database and certainly there

are a number of things that we cannot do at individual transplant centers. As I am sure Dr. Pablo Rubinstein has already shown, there are certain issues, particularly with regard to HLA, that we may not be able to demonstrate because of inadequate patient numbers.

On the other hand, there are certain advantages of transplant centers: There is greater treatment homogeneity with a transplant center or group of transplant centers, and there are standardized eligibility and assessment criteria which do not take place in a registry database.

Certainly one of our goals, as addressed yesterday, is to come up with information that will help standardize the product in terms of its characterization and also standardization of clinical endpoints, something we will probably want to address a little bit later this afternoon.

There are a number of demographic issues that we have already demonstrated in bone marrow transplantation that are key in understanding what will happen to the patient after transplantation, and we don't want to forget that there are certain things that we already know in bone marrow transplantation. Whether or not they hold true for cord blood remains to be determined, but I think that we have to focus at least on those demographics that are

clearly important within bone marrow transplant biology. And, all of you who do bone marrow transplants are certainly well aware of these important issues. But there are also aspects to the graft parameters that we also know will be important based on bone marrow transplant literature, and a number of these things we have already addressed this morning, I am sure, and I will address in this presentation as well.

But all of these issues are to bear in mind, I think, while we are trying to come up with product standards and we have to keep reminding ourselves of the similarities and differences between the different presentations that these are things that we need to focus on in terms of all of our presentations in trying to come up with these product standards.

Now to the data presentation, as many of you have heard before, Joanne Kurtzberg and I have put together a series of patients both at Duke University and University of Minnesota, and this most recent analysis that has never been presented before is now an updated version of 257 patients with 50 eligibility criteria for this analysis. The patients must have had at least 42 days of follow up, and in this case it is actually longer than that. They must have also not had a prior allogeneic transplantation. They had to have a graft that was 0-, 1-, 2- or 3-antigen

disparate between donor and recipient. There were several patients that had a 4-antigen mismatch graft that were not included. And, they must have had a preparative therapy. There were several patients that did not receive a preparative therapy for the treatment of immunodeficiency syndromes.

So, with that in mind, there were 257 patients that were eligible for this analysis. I should include one other group of patients that was not eligible, and that was those who were enrolled in the COBLT study which I believe was about 24 patients. So, other than those patients, all patients were eligible for this analysis. As you might expect in a primarily pediatric program, about two-thirds of the patients had malignancy, with the majority having acute lymphocytic leukemia but also a variety of diseases, as shown here. About one-third of the patients had a bone marrow failure syndrome, again, pretty much in line with what has been presented before but with larger patient numbers. And, you see there is a high preponderance of patients with a metabolic disorder because of the interest of our type of transplant at these two institutions.

With regard to HLA mismatch, clearly the majority of our patients have either a 1- or 2-antigen disparate graft. So, as Donna Wall just presented, clearly, when we talk about the influence of HLA we are really talking about

differences between 1- versus 2-antigen mismatched grafts because there were too few patients in the 0- or 3-antigen mismatched group to be discussed in great detail. Nonetheless, we will report the results in all the outcomes for each individual degree of HLA mismatch.

When we had 9 that were unresolved, Joanne Kurtzberg and I went through the data and there are a couple of patients for whom we could not determine, for sure, what the degree of HLA antigen disparity was, and we will discuss that in our discussion session if you would like. Recipient serum status is shown here. About 42 percent were CMV positive, again, not particularly surprising because this is a pediatric population.

In comparison to what Donna Wall just presented, at her institution where she has indicated that there is a preference to using umbilical cord blood, that was also true at Duke University but was not true at the University of Minnesota. So, the way we select donors is actually somewhat different between the different institutions. Nonetheless, as it turned out, the majority of patients both at Duke and at the University of Minnesota would have been considered high risk on the basis of criteria that are used by most transplant centers. That is, the patients were either in relapse or in third remission, and there were also patients -- which I guess I did not show well

here -- who were in accelerated phase CML or patients who had high risk cytogenetic abnormalities perhaps in CR1 or CR2, but they would be the standard high risk patient population for most transplant centers.

GVHD prophylaxis was another issue. As many of you know, initially both at Duke and University of Minnesota we both started using cyclosporine and methotrexate which would be considered the standard practice in bone marrow transplant, but because of our concerns about the delay in neutrophil recovery, rightly or wrongly, we very quickly moved on to using cyclosporine plus methylprednisolone. As many of you know, at Duke there was use of high dose methylprednisolone, starting at a higher dose with a slow taper, as compared to the lower dose methylprednisolone initially developed actually at Sloan-Kettering which we adopted and then modified at Duke. Prophylactic G-CSF was not used at the University of Minnesota originally based on the results in sibling transplant using umbilical cord blood. However, based on analyses at Duke, we began using G-CSF at a dose of 5 micrograms/kilogram and at Duke it remained at 10 throughout the study period.

We looked at a variety of demographic factors, graft parameter factors and treatment factors that might have potentially influenced the outcome after

transplantation. Certainly, our goal was to be able to identify those types of patients that would benefit most by using umbilical cord but, alternatively, those patients who would actually do worse using umbilical cord than might have been expected with a bone marrow transplant. Were there certain graft parameters that would help us predict outcome, or were there certain treatment factors that may or may not be important in the outcome after umbilical cord blood transplantation? At least, that was our initial design of the study.

In terms of overall neutrophil recovery, this is not too different than what you have already observed multiple times. The median time to neutrophil recovery is 25 days with this rather large cohort of patients, with a range of 10-59 days. Neutrophil engraftment occurs equally between those with malignant disease versus non-malignant disease. I also should have pointed out in the prior slide that the overall probability of neutrophil engraftment is 92 percent.

We looked at the effect of neutrophil recovery by age and, as you might predict because this is related to the cell dose and size of the patient, there is a correlation between age and neutrophil recovery. But, as you can see there on the bottom curve, those patients are over the age of 17 and certainly a high proportion of

patients have neutrophil recovery but it is both delayed and ultimately inferior to what would be observed for the very youngest patients, around the ages of 0-1 or up to 2-9.

We also looked at the effect of recipient weight. But, nonetheless, all these things really correlate with the overall cell dose, and I apologize because you will not believe the fact that I have actually left out that slide.

[Laughter]

I can't believe that that has occurred but, nonetheless, that one slide is missing. What I can tell you is that we divided the patients into quartiles and basically what we found is that those patients receiving a cell dose of greater than 1.5 X 10^7/kg had an overall probability of engraftment of approximately 90 percent. It was those with a dose of less than 1.5 that had the inferior engraftment. So, nonetheless, perhaps we are fine tuning, now as patient numbers are increasing, where that cutoff might be but we can discuss that further. I apologize for that slide not being present.

Nonetheless, we also were interested in terms of what is the effect of G-CSF on neutrophil recovery. It would appear in the univariate analysis that G-CSF may be of importance in predicting ultimate recovery. As you can see in the original cohort at Minnesota, not using G-CSF

appeared to be associated with delayed recovery. Whether or not differences in ultimate grafting occurred is not clear but, nonetheless, as we have started using G-CSF it appears to be improved but it would appear that there is no clear-cut difference between a dose of 5 versus a dose of 10.

As others have already reported, those patients with Fanconi anemia or severe aplastic anemia appear to have a lower probability of ultimate engraftment as compared to the other patients, as shown here. The reason that we pulled out CML individually was that there was some early data that suggested that CML patients might actually be doing worse but, in fact, as we have accrued patients in this population it does not appear to be any different. The numbers are still small.

Now, certainly in our own experience we are going to focus on HLA disparity because our results differ somewhat from what has been reported by Dr. Rubinstein. What we see here, at least in terms of looking at neutrophil recovery, is that we find no clear-cut difference between those with a 0-, 1-, 2- or 3-antigen disparate graft but, again, the bulk of our patients are 1- or 2-antigen mismatched and, clearly, between those two there is no difference.

This is just one slide and it was meant to show you the correlation between neutrophil engraftment and a nucleated cell dose. You can see here that you might get the impression that there is a correlation. Indeed, there is, however, I wanted to point out to you that when we looked at those specifically who had graft failure, they really were well in the range of those that engrafted and so, therefore, we can't necessarily predict who would have had a graft failure on the basis of their cell dose. I am not sure if the New York Blood Center experience is any different but that was clearly an important outcome that we were looking to find out whether or not we could predict a graft failure and, at least based on cell dose, we cannot.

In the multivariate analysis, again, we find no effect of HLA disparity in terms of neutrophil recovery and ultimate engraftment. We do find a significant effect on the base of cell dose. Not surprisingly, CFU-GM also fell out as being a significant factor, but the two are inter-linked with one another.

Then, diagnosis was also important. Those patients who had aplastic anemia or Fanconi anemia appeared to have a lower probability of engraftment as compared to those with non-malignant diseases. Then, patients with growth factor appeared to have improved recovery as compared to those that did not.

Looking at overall engraftment, 51 percent achieved engraftment by 6 months after transplantation. The one thing I wanted to point out to Dr. Wall, seated back there, you made a comment that it appears to be delayed as compared to bone marrow transplantation. In an analysis that we performed at the University of Minnesota on a case-controlled comparison and also looking at the data from the NMDP there really is no difference between umbilical cord blood transplant versus bone marrow transplantation, though the reasons for the delay may be somewhat different.

When you look at age, once again, there is a clear-cut effect of age. Patients who are older tend to have a slower rate of recovery. Again, this is shown here in terms of weight, not surprisingly, and then here in terms of cell dose where you see once again that those patients with the higher cell doses appear to have a better recovery than those with the lower cell doses.

The reason why we were interested in looking at the effect of GVHD in terms of platelet recovery is that this is clearly a factor that delays recovery after unrelated marrow transplantation and perhaps with larger numbers of patients we might be able to detect a difference but, so far, those with graft versus host disease appear to

have a modest delay in platelet recovery that does not appear to be statistically significant.

When you look at the effect of HLA disparity, here you do find some interesting trend in that you find that those with matched or 1-antigen mismatched graft may, indeed, have improved platelet recovery as compared with those with more mismatched grafts -- not quite statistically significant but you are seeing a separation between the two which probably reflects patient numbers and may change over time.

So, in the multivariate analysis, again, we cannot detect an effect of HLA mismatch, although there may be something that will develop over time. Again, cell dose appears to be important. Age is important and diagnosis is important in terms of predicting platelet recovery.

Acute graft versus host disease is certainly one of the major potential benefits of umbilical cord blood transplantation, not only because we are hoping that there may be reduced alloreactive response but also may allow us to cross HLA barriers. So, even if we do find that there is an effect of HLA disparity which will be important, certainly what it also shows us is that despite HLA disparity so far we have not observed a high rate of acute graft versus host disease. Overall, at this point it

remains stable -- the probability of a Grade III or IV GVHD of 12 percent.

When you look at the effect of age which would have predicted increased risk of graft versus host disease in the bone marrow transplant setting, thus far we find no difference between the various age groups.

When you look at the effect of CD3 cell dose in the bone marrow transplant category, you would have expected a positive correlation between CD3 and acute GVHD, and here you find no such correlation and, in fact, though it is not statistically different, those receiving the lower cell doses actually had the highest graft versus host disease. Clearly, there is no effect of CD3 cell dose thus far.

We were concerned that perhaps using a lower dose methylprednisolone might be, indeed, associated with a higher risk of graft versus host disease but, as you can see, all three regimens were virtually overlapping with each other. Grade II-IV acute GVHD was not influenced by HLA match, and I should point out, because of the discussion yesterday, when we are discussing the effect of HLA match with graft versus host disease we are referring to the degree of mismatch only in the GVHD vector. When we are discussing grafting both for platelets or neutrophils we are only talking about HLA mismatch in the engraftment

vector. So, we separate those out. This is not just overall mismatch. So, if I showed you the numbers under each degree of mismatch they would differ between the two presentations of engraftment versus GVHD.

Chronic graft versus host disease is what remains dramatically low, at least within our own experience. Overall probability of chronic GVHD is only 7 percent. This is dramatically different compared to what we would expect with bone marrow transplantation even with a pediatric age group. When you look at the effect of age here, also considering that maybe we will find a higher rate in the adult patients, thus far we have not. In this analysis there are practically 50 adult patients or at least 50 over the age of 17. When you look at the effect of HLA disparity, you find that there is no clear-cut effect of HLA disparity once again.

The one thing that we do find is perhaps a trend towards increased graft versus host disease with a lower dose of methylprednisolone with very little in the other regimens but, again, the patient numbers were small but it does approach statistical significance based on this univariate analysis.

Something somewhat interesting that was not expected was that there may be an association with the use of melphalan, at least high dose melphalan. We compared no

TBI, that is chemotherapy prep alone, to those that received TBI in combination or without low dose methylprednisolone and TBI plus high dose melphalan. As you can see here, there seems to be an association between the use of high dose melphalan and the development of chronic graft versus host disease. Why that is the case is not clear.

When you do a multivariate analysis, again HLA mismatch does not fall out in the multivariate. Cell dose appears to be correlated but I should tell you that the cell dose is actually inverse to what you would have expected. That is, the lower the cell dose, the higher the GVHD. CD3 cell dose, again same thing -- there is inverse relationship of what you would have expected so it is not explainable or, at least, I can't explain it those far. Then, the use of melphalan with a relative risk of 16.

When you look at overall survival at one year, we have a survival that exceeds 50 percent, and at four years it approaches 41 percent.

When you look at those with malignant disease versus non-malignant disease, it is not particularly surprising that those with a malignancy have a poorer overall survival but, nonetheless, quite respectable considering that the highest proportion of patients had a

high risk disease, and also the majority of patients having 2-antigen mismatched grafts.

Looking at age, clearly there is an effect of age. Those that are youngest did the best but then, again, those are that are youngest are predominantly with non-malignant diseases. Nonetheless, you see that adults also do respectably well and that will be discussed in greater detail in the next presentation.

The one thing that we previously did not observe, we found previously no effect of CMV on outcome but, as you see, as we increase the numbers of patients we are now being able to delineate an effect of CMV seropositivity in a patient having a poor risk in terms of overall survival -- not particularly surprising in view of the bone marrow literature.

The one thing that we also wanted to look at which we have never done before was to look at the impact of race. There were 49 patients that were in the minority category, and you can see here it appears in this univariate that minority patients have tended to do worse overall. However, this is a variety of diseases and so it requires looking into in greater depth but, nonetheless, in this first pass we do detect a difference and we never evaluated this previously.

Survival is clearly impacted by cell dose. In this lowest quartile you find that the survival is low, and it appears that somewhere in this range of around between 1 and 2.9 there may be an area where we want to cut off our cell dose as being an acceptable graft. Certainly, those with higher cell doses have a better survival.

But look at the effect of CD34. I want to point out this is just the University of Minnesota. The reason for segregating the University of Minnesota here is to make two points. One is that CD34 cell dose seems to be quite important in predicting outcome, and there almost appears to be a threshold effect and above 1.5 the outcome appears to be satisfactory; below 1.5 it appears to be very bad.

On the other hand, certainly if you combine the University of Minnesota with the Duke University data set, there remains a correlation with CD34 cell dose, but the CD34 cell doses at Duke were higher than at Minnesota which is a reflection, I believe, of the methodologies that were used. So, for this analysis we will probably be forced to separate the presentation into two slides because there are differences in the methods that are used -- an important point in using this parameter as a means of selecting a graft.

Graft versus host disease, although it appears clinically not to be dramatically different, it has

approached statistical significance and those with GVHD have a slightly poorer outcome as compared to those that did not, and what you find here is that once again we do not detect any clear-cut difference in terms of degree of HLA disparity and overall survival. As you can see, in the green are the results of 2-antigen disparate grafts and you can see in yellow the results with 1-antigen disparate graft.

In the multivariate analysis you see, again, that HLA disparity does not fallout; cell dose does; diagnosis does once again. This time, for the first time we are showing CMV seropositivity as having an impact on survival, as does graft versus host disease. I would point out though in this particular analysis race did fall out of the multivariate analysis.

Just to touch on the only slide that I have in terms of what was the impact of umbilical cord blood cell dose on those who engrafted, we detect an influence of cell dose in those after engraftment. What this might mean remains to be determined, but it suggests that perhaps cell dose may have an impact above and beyond just engraftment itself -- in terms of immune recovery, or what-have-you, is not clear. But this is something that is worth following, though I did see at the very end of Dr. Rubinstein's presentation that in your analysis I believe only HLA fell

out as being important after engraftment, if I saw that part correctly.

Now, what does this mean in terms of relapse? I mean, certainly if we find that we have a lower risk of graft versus host disease, does this mean that we have a higher risk of relapse? Though many of you in this room are interested in cord blood, there are many people who do not believe that umbilical cord blood will be of benefit in the long run, particularly because of the concern of the graft versus leukemia effect. It appears that everyone has bought the idea that graft versus host disease is lower frequency after cord blood, but everyone is concerned about the risk of relapse. Certainly, as time goes on this will be declaring itself one way or the other, but despite the fact that 75 percent of malignancy patients would have been in the high risk category, we only have a 20 percent overall probability of relapse within the malignancy patients. So, it is quite respectable thus far.

When you look at the individual diseases -- again, certainly greater detail needs to be displayed which is not yet available, but you can see that even in the patients with acute lymphocytic leukemia the probability of relapse has remained low with a median follow-up in excess of two years.

Even when you compare standard risk versus high risk no clear-cut differences have yet shown up. We do not yet discern impact by graft versus host disease other than that maybe something may occur with time as numbers increase, and we have not yet determined impact of chronic graft versus host disease.

The one thing that has fallen out, and I have shown to you before that we cannot yet explain, is an impact of the use of G-CSF. Not using G-CSF appears to relate to relapse. This has fallen out every time we have done an evaluation and looking at other factors that may have had an impact upon that. But, as you can see here, those that have the higher risk of relapse are those that did not have G-CSF, and you can see that those who had G-CSF tended to have a lower risk of relapse. In the multivariate analysis it was the only factor that fell out as being significant. How this is explained remains to be determined.

So, I guess in summary, we believe -- that is, Dr. Kurtzberg and I believe that umbilical cord blood should still be based principally on cell dose when trying to select which graft is the optimal graft. That is also with the caveat that we are talking about choosing grafts that are between 0- versus 2-antigen disparate.

The other thing that we have not yet pulled out, which is clearly going to be important, is really whether or not the types of mismatches make a difference. We have actually done a preliminary analysis looking at those with one mismatch and looking at class of mismatch, and we have now detected a difference but, again, the numbers are quite small. Looking at those with a two-antigen mismatch where we have compared those with two Class I versus a Class I/Class II versus two Class II mismatches, again, we do not yet detect a major difference between the three categories. But in those with two Class II mismatches there were very few patients to make any real conclusions. So, clearly we are worried about that group of patients, nonetheless, with our own analysis we have not yet detected a difference. But for the most part, most of the patients have one Class I and one Class II mismatch, and in that situation it appears that we would still want to base it on cell dose as a primary criterion for selection rather than specifically on HLA disparity.

What the minimum cell dose will be remains to be determined. I believe it is somewhere above 1, maybe 2. It is going to be somewhere between the two. That remains to be further defined.

In terms of what other things we need to be looking at, I think we need to focus on the standardization

of CD34 analysis. I think it has an important impact upon graft selection but because different banks and different transplanters use different methods for CD34 analysis, right now I don't think that should be used to choose a specific graft.

In terms of banking, certainly it appears from the discussions yesterday that everyone is moving to banking larger grafts. Clearly, more is better in this circumstance and, certainly, we are going to hear more about the expansion potential of umbilical cord blood hematopoietic stem cells this afternoon, and I think the data that we have all presented in terms of cell dose and its impact upon survival is the greatest reason for looking at this ex vivo expansion strategy.

Clearly, I have to acknowledge all the work that has been done at Duke University as well as Todd DeFor who has done the statistical analysis. Thank you.

[Applause]

DR. KURTZBERG: And now the final speaker before the break is Mary Laughlin, who will give us analysis of a pilot adult experience with cord blood transplantation.

Adult Experience

DR. LAUGHLIN: I appreciate the opportunity to be here this morning to present, as Dr. Kurtzberg mentioned, a

focused study on outcomes in adults grafted with umbilical cord blood.

The background is, as you have already heard in the past two years, of the use of allogeneic transplant for patients with hematologic malignancies in marrow failure syndromes. The patient description for myself, an internist, will differ from that which has been presented to you of primarily pediatric patient populations.

We face the same problems, however, of graft availability given the demographics of American families, and the higher instances of graft versus host disease when we turn to matched unrelated donor or partially mismatched family members as sources of stem cells for these patients.

That background sets the stage for this new stem cell source and most of the data thus far has been generated in pediatric recipients, and has included proportions of adults but has not focused simply on that patient population over age 18. These reports have noted delayed hematologic engraftment and reduced graft versus host disease.

The focus of our study was to outline cord blood transplant outcomes in 68 adult patients that were transplanted consecutively at five centers during the time period of February, 1995 to September, 1999.

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Patient eligibility included patients with high risk or recurrent hematologic malignancies. I must emphasize that these are Phase I studies -- patients at very high risk, multiply relapsed; patients transplanted with umbilical cord blood. Second protocol includes patients with severe aplastic anemia, inherited metabolic or immune disorders. Patients must be under age 55, have normal organ function, no available HLA matched sibling donor. Protocols stipulate that they must not have an available unrelated donor by the National Marrow Donor Program, and for many of these patients, their disease status would preclude the time necessary to identify and mobilize a donor.

In this study, preferred units were those matched for HLA at 3 of 6 or better, with a minimum cell dose 1 X 10^7 nucleated cells, whatever that means to the bankers in the audience, recipient weight. No graft manipulation was performed, no ex vivo expansion, no T-depletion other than hetastarch red cell depletion. Preparative regimens included TBI-based or busulfan-based and serotherapy was provided to all patients, ATG at dose 30 mg/kg, day minus 3, 2, 1 prior to infusion of the cord blood unit on day zero.

Graft versus host disease prophylaxis varied among centers but included cyclosporine and the variable

was the dose of steroids. Steroids were tapered generally by ten weeks post-transplant and cyclosporine was tapered anywhere between 6 and 9 months post transplant, depending upon whether the patient was exhibiting symptoms of graft versus host disease.

Supportive care included use of G-CSF. The dose of G-CSF varied among centers between 5-10 mcg/kg/day, starting day 0 and continuing until full neutrophil recovery. None of the patients were supported without growth factor.

Our statistical analysis was a retrospective analysis with follow up as of August 2. The endpoints in this study included kinetics of hematopoietic recovery, event-free and overall survival. Univariate analysis was performed using log rank or Wilcoxon. Multivariate analysis incorporated the Cox proportional hazard regression model. The variables that we studied included age of the patient, gender, HLA and ABO matching, weight, disease entities, CMV serology status of the recipient pre-transplant, and graft characteristics.

A few definitions -- myeloid recovery was defined as the first of three consecutive days after transplant when the absolute neutrophil count remained above 500 per mcl. Platelet and red cell recovery were defined as the first of seven days when the platelet count remained above

20,000 and the hemoglobin level remained above 8 g/dl without transfusion support.

Chimerism was evaluated in all patients using one of three techniques -- in situ hybridization for patients with sex mismatched grafts; DRB1 in cases in which the donor and patient differed in HLA DR; and use of quantitative PCR for microsatellite DNA markers.

In this study, primary graft failure is defined as absence of donor-derived myeloid engraftment by day plus-42.

Further definitions -- overall survival was measured from the date of transplantation to the date of death, and censored at the day of last follow up for survivors. Event-free survival was measured from the date of transplant to the date of relapse or death, whichever occurred first, and was censored at autologous hematopoietic recovery or date of last follow up, whichever occurred first.

With those definitions, grafts were characterized for HLA using serologic typing for Class I and high resolution DRB1. In only two patients was a 6 of 6 matched graft infused; 18 patients received a 5 of 6 matched graft; 37 patients received a 4 of 6 matched graft. And, 48 of these patients, 71 percent, received cord blood grafts that

were disparate at two or more HLA antigens and half the patients approximately received Class II mismatched grafts.

The median weight in this series was 69 kg and ranged from 41 kg to 116 kg. The median age in this series was 31 years, and patients ranged in age from 18 to 58 years.

What I think is unique about this data set is that as opposed to some of the reports from pediatric programs where a few patients with pediatric diseases may live into their 20s, this patient population does represent the typical adult patient population that an internist would see and refer to an oncologist. And, 50 of the 68 patients were considered high risk, as I mentioned, using IBM-TR criteria; 13 of the patients were non-malignant; 7 of the patients had failed prior transplants; 15 patients had ALL; 19 patients were diagnosed with AML; 15 patients were diagnosed with CML, of which the majority of the patients were either in accelerated phase or had had blast crisis and had received induction chemotherapy to reattain chronic phase. One patient was diagnosed with CMMl; 2 patients had refractory recurrent Hodgkin's disease; 1 patient had lymphoma; and 1 patient had CLL.

In the patients with non-malignant disorders, one patient was diagnosed with Blackfan and Diamond anemia and had been transfused for a period of 20 years. One patient

was diagnosed with ALD. There were four Fanconi's patients in this series; two patients with myelodysplasia that had not demonstrated evolution to acute leukemia; four patients with severe aplastic anemia; and two patients with myelofibrosis.

The graft characteristics in this series -- cryopreserved cell dose, the median cell dose was 2.1 10^7 per kilogram and ranged from 1 to 6.3. Infused cell dose was 1.6 10^7 per kilogram and ranged from 0.6 to 4. CFU was infused and CD34. The take-home point of this slide is that these numbers are fully a log less than that which we would normally infuse using a traditional allogeneic graft. CD3 infused dose was similar to what we would see with traditional bone marrow grafts.

Here we have plotted cryopreserved cell dose versus infused CD34 and with superimposed regression, the correlation coefficient here of 0.5. I think this is an important graft to emphasize because for the clinician many times this is the piece of information that they have with the HLA typing of the graft that allows them the opportunity to make the decision whether to proceed in treating their patient. I think it is reassuring that this 34 surrogate analysis correlates well with cryopreserved cell dose.

Further graft characteristics that we analyzed -- CD34 reinfused versus CFU, again, correlated with one another and, finally, cell dose cryopreserved with infused CFU.

The kinetics of hematopoietic recovery in these patients receiving non-expanded grafts median day to ANC greater than 500 was 27, and ranged as early as day 13 and as late as day 59. The actuarial probability of neutrophil recovery in this series was 92 percent, with 95 percent confidence interval as outlined here. Median day to platelets greater than 20,000 was 58. Median day to platelets 50,000 and 100,000 is as outlined. In our MUD transplant patients at our shop, these numbers are also similar as far as kinetics of platelet recovery, again, likely for different reasons. Median day to hemoglobin greater than 8 g/dl was 60 days. In all patients who engrafted there was complete donor chimerism, and there had been no late graft failures observed.

A Kaplan-Meier curve of day ANC 500 with 95 percent confidence interval, median here day 27 and ranging from day 15 to day 59. When we broke out the Kaplan-Meier curve kinetics of neutrophil recovery versus the cell dose cryopreserved, breaking it here at the median, we saw faster kinetics of neutrophil recovery in those patients receiving the higher cell dose.

Also, when we broke out infused CFUs into these groups, as outlined, and analyzed the Kaplan-Meier kinetics of neutrophil recovery, again, we saw faster neutrophil recovery in those patients receiving a higher CFU cell dose. This is the Kaplan-Meier plot of day to platelet independence -- I apologize for the misspelling. We did not analyze, either by univariate or multivariate analysis, kinetics of platelet recovery because in this series that comprised 32 evaluable patients and we are awaiting further numbers of patients in this analysis.

Outcomes in this series -- there were 8 early deaths prior to day 28 and those patients were censored. In the remaining 60 patients, 5 patients demonstrated graft failure. The day 100 survival in this series is 50 percent. This is very high I think and is reflective of two important issues -- patient selection and delayed hematopoietic engraftment.

Grade II-IV acute graft versus host disease, despite infusion of grafts mismatched at more than one HLA loci, was 60 percent, with the confidence interval as outlined here, and the incidence of Grade III-IV graft versus host disease in this series was 20 percent, which is representative of 11 patients.

In our series of adult patients the incidence of chronic graft versus host disease, 12 of 33 evaluable

patients, was 38 percent. This was limited in all patients and non-progressive, except one patient.

In our analysis there was no association between graft failure and infusion of HLA disparate grafts, infusion of Class II disparate grafts, CMV serum positivity in the recipients, whether the patient was diagnosed with a malignancy versus a non-malignant disorder, and breaking out their diagnoses.

There was no association between the grade of acute graft versus host disease and the graft HLA disparity, whether or not the graft was disparate at Class II with the recipient, CMV serum positivity in the recipient or whether the patient was conditioned with a TBI-based versus chemotherapy-based regimen.

Further outcomes in this series, the median follow up of our survivors is 22 months, and ranges from 11 months to 51 months. We have observed four relapses. There seems to be maintained graft versus leukemia factor in this series of very high risk patients. Three patients were diagnosed with ALL and relapsed with their disease. One Hodgkin's disease patient relapsed at these time points post-transplant.

Event-free survival at four years is 26 percent, with confidence interval, and this represents 18 patients; the Kaplan-Meier of event-free survival with the confidence

interval as indicated, with follow up out to four years. The Kaplan-Meier here of the risk of relapse in this series is 16 percent. When we analyzed event-free survival by CD34 infused there was improved event-free survival in those patients receiving a higher than median dose.

Also, when we analyzed cryopreserved cell dose versus survival, there was a trend towards improved survival in those patients receiving the higher cell dose, however, this did not attain statistical significance. There was no impact of receiving an infused higher cell dose versus lower cell dose on event-free survival.

When we analyzed event-free survival versus age in this paper, an analysis focused on adult patients alone, we found no significant difference in event-free survival when comparing patients less than age 25, ranging in age 25 to 40 versus those patients over age 40. There was a trend towards less improved survival, however, not statistically significant.

We further analyzed event-free survival by HLA disparity and we did collapse this data to combine those patients who received either a 5- of 6-matched graft or a 6 of 6 matched graft to attain equivalent numbers of patients in each study group. There was no significant difference when you compared HLA disparity among the groups. There was a trend towards an inferior outcome in those patients

receiving a 3 of 6 matched graft. Somewhat surprising and I don't want to overstate it -- this is 69 patients, but here you would expect an improved event-free survival in patients with 5 of 6 or 6 of 6, but this curve is actually those patients receiving a less well matched graft -- again, a trend and not statistically significant.

When we went on to analyze whether this observation was attributable to disparity at the Class I loci or Class II loci, again, we observed a trend of improved event-free survival if you received a graft that was mismatched at Class I rather than better matched at Class I. I don't wish to overstate this observation, this is 68 patients and it is not statistically significant. It may represent the selection by the transplant clinician who, many times, will choose a less well matched graft of higher cell dose, over a better matched graft of a lower cell dose.

When we broke out event-free survival versus patient disease entities, comparing patients with ALL, AML and CML versus others, there was a trend towards improved survival in the CML patients compared to ALL patients but this did not attain statistical significance in the univariate analysis. This differs from observations in larger data sets. My only explanation is that this cohort of CML patients had perhaps different characteristics.

They tended to be patients in accelerated phase or blast crisis and, therefore, a good proportion of them had received chemotherapy at a time period prior to transplantation. I don't know but that may have had an effect.

When we analyzed event-free survival versus CMV in the recipient, here negative versus positive, we did see a trend towards improved survival but, again, this difference was not statistically significant in this series of 68 patients.

Further univariate analysis included whether comparing 6-month survival and using log rank, whether the unit was hetastarched, whether the patient received a busulfan-based versus a TBI-based regimen, whether the patient was diagnosed with malignancy versus non-malignant condition, and none of these variables were statistically significant.

Further analysis -- whether the graft was matched at Class II, whether the patient developed chronic graft versus host disease -- the caveat here though is that this was a small number of patients, 36 of these patients were non-evaluable due to death prior to day 100. Whether they were high risk versus low risk using IBM-TR criteria, there was no significant difference using log rank analysis.

The multivariate analysis in this series, comparing event-free survival, included CD34. We used relative risk 0.8 associated with an improved event-free survival. The observations that we had made in the univariate analysis emerged in the multivariate analysis with a relative risk of 2.5. The diagnosis of CML compared to patients with ALL trended towards an improved survival but was not statistically significant. Female patients fared worse compared to their male counterparts and advanced age was associated with poorer outcome in event-free survival in the multivariate analysis.

So in summary, cord blood can be successfully engrafted into adults with refractory hematologic malignancies and marrow failure syndromes. Despite a high level of HLA disparity, cord blood from unrelated donors is associated with a low incidence of a severe acute and chronic GVHD in these adult recipients. There is delayed time to hematopoietic recovery observed.

I guess like my pediatric colleagues, I do want to take at least one step out of the statistics to describe a number of the statistic. This person, Chris, is 28 years old, likes to ski in Colorado, was diagnosed with Hodgkin's disease, and attained complete remission status, however, presented to our transplant program with thy-related AML 14 months later. For this individual, no sibling match was

identified and the pace of his disease precluded the necessary time to identify and mobilize a MUD donor. I think as we learn more about this new stem cell source, we can be assured that even in adult recipients a proportion of patients can derive direct benefit from use of this allogeneic stem cell source. This patient is now two and a half years out from his cord blood transplant.

I would like to also credit my collaborators, Pablo Rubinstein and Cladd Stevens, Joanne Kurtzberg and David Rosari of Duke University, John Wagner and Dr. Barker at the University of Minnesota and Mitch Carro at Lombardi, and my colleagues Hillary Lazarus, Stan Gerson and Omar Koc at Case Western Reserve University. Statistical analysis in this study was performed by Pingfu Fu of our epidemiology and biostats department. Thanks.

[Applause]

DR. HOROWITZ: Thank you very much. Amazingly, we are ahead of schedule. So, we will take our break now before our final presentation and we would ask you to come back a half hour from now, at 10:30, and hopefully we are going to have a little extra time for discussion.

[Brief recess]

DR. KURTZBERG: I would like to invite everybody to come on back in so that we can get started again and

keep our advantage for being ahead of time so we have time for discussion.

It is my pleasure to introduce Dr. Takahashi from Japan to tell us about the experience with the Japanese cord blood banking and registry.

Japanese Experience

DR. TAKAHASHI: Thank you, Dr. Kurtzberg. I would like to thank the organizers of this workshop for giving us the opportunity to report our experience in Japan, and I would like to talk about the kind of situation of cord blood banking and transplantation first, and then to report the clinical outcome.

In our country, about 1500 patients need allogeneic hematopoietic stem cell transplantation every year. The number of bone marrow transplantations has been increased but we can see that they reached a plateau in recent years. On the other hand, shown in green and red, particularly the cord blood transplantation increased very rapidly. We don't have the statistics of 1999 but half of the hematopoietic stem cell transplantation in children are done by cord blood transplantation.

This is a brief history of cord blood transplantation and banking in Japan. The first related cord blood transplantation was done in 1994, and the first unrelated cord blood transplantation was done at the

Yokohama City Hospital, University Hospital, in 1997. Since April, 1998 the cord blood transplantation was covered by the national health Insurance which was approved in 1998. So, that was very good news for the patients. Now, 270 unrelated cord blood transplantations were done until the end of March of this year.

The first cord blood bank was set at the Kanagawa Prefecture in 1995. At that time the concern about the safety of cord blood transplantation and then the quality of the cryopreserved unit. The government research group made a standard for the cord blood banking and also the indication for cord blood transplantation in 1997.

Also, in the history in Japan was that we have a very good group which supported to establish the National Cord Blood Bank in Japan. They are so active that because of that Health Ministry got a lot of pressure by the Japanese people and they assembled the Cord Blood Transplantation Study Group in 1997, and we are the technical members for this group and we established clinical guidelines for cord blood transplantation in 1998 based on this preliminary standard. The guidelines are almost similar to NETCORD, COBLT or New York Blood Center standards. Finally, Japan Cord Blood Bank Network was established last year, last September. Now in the Japan

Cord Blood Bank Network there are 3000 cord blood units cryopreserved in nine local cord blood banks.

The objectives of this network are to promote unrelated cord blood transplantation patients in Japan through collaborative management and joint ownership of information. The establishment was on August 11, 1999. The financial support comes from the Ministry of Health and Welfare, but the financial support is not enough to cover all the expenses. The support covers a part of HLA testing and they also give us the equipment, such as freezer, cell counter and so on, but still the support is not enough. But the basic idea is that the government supports the banks but there should be a competition among the local banks to serve better the patients and hospitals and also the quality. So, there should be competition among the banks, and also every bank should get their own financial support from other places.

The main office is in the Japanese Red Cross in Tokyo, and then there are two committees, the administration committee and evaluation committee, and there are main working parties, such as internal system working group, infectious control, distribution of transplant centers.

Now, the big problem for us is that there is no price for the unit which was transplanted. That means the

units are given to the transplant centers free. So, we have to solve this problem as soon as possible. The administration of cord blood banks -- there are nine cord blood banks registered after government inspection.

The projects of the Japan Cord Blood Network are the standardization of collection, separation testing, cryopreservation shipping, transplanting etc., evaluation of management of local cord blood banks and quality control of cryopreserved cord blood units, collection of 20,000 cord blood units in five years, the construction of Internet access system for cord blood search, international collaboration in banking and transplantation of cord blood, and the registration of transplant centers.

This is how we get to the national goal of 20,000 units. This is based on Prof. Juji's and Akaza's report. They analyzed HLA type among Japanese, and you can see this is the donor size and this is compatibility, and this shows 6-antigen mismatch serologically and genotypical. Here is 1-antigen mismatch. If the cord blood transplantation is acceptable in 1-antigen mismatch, we can say here if we have 20,000 units of cord blood 19 percent of the patients should be covered, could find their matched unit. So, therefore, 20,000 units is our first national goal.

The government inspected nine local cord blood banks and they have made an inspection team and we helped

them and we made a checklist, and two of the banks were approved without any change, and others were asked to include several points, such as improving the facility and better document management and centralized processing center. There are nine banks but there are fourteen processing centers in Japan, but several processing centers are now centralized.

I would like to introduce our bank as an example of Japanese local cord blood banks. We started collecting cord blood in our department in 1996 following New York Blood Center's protocol. Then we joined the NETCORD. We are the founding members of the NETCORD. The first cord blood transplantation was done to a 28-year old woman and that was the first success patient in Japan. In 1999, in September, we joined the Japanese NETCORD and they asked us to collect 800 units last year and 1200 units this year, and this is the largest number in the network.

I would like to report briefly about this patient later, and now we have 1667 cryopreserved units, transplanted to 47 patients, 17 adults and 30 children. Our institute focused on transplantation of the cord blood to adults. So, we have the experience of 20 in our institute and 17 others and 3 are children.

This is our process room. We have a clean room P-2 level and the cleanness of the air is NASA 10,000. The

clean room is divided into three booths and each booth has a hipofilter and purity is going up to 1000, and the benches also, of course, are just 100. We use bio types. In Japan all the banks use the Hiss method now and two of the banks and three processing centers use bioarchive.

I want to just briefly introduce our experience of this patient. There was a nuclear accident in Tokai, near Tokyo, last year on September 30th. The three workers at the uranium convergent front were exposed to radiation where they were mixing too much uranium with nitric acid.

This is a picture of how our patient poured into the tank. This is completely out of the SOP.

[Laughter]

The three workers were exposed to the radiation and a 35-year old man was in serious condition and he received stem cell transplantation. The 30-year old man was in less serious condition. He received cord blood and he didn't have any HLA matched sibling, unfortunately, but we decided to do the cord blood transplantation for him. We found one unit in a bank and this is the patient and this is the donor of cord blood based on the DNA typing, 1-antigen mismatch, and 2 X 10^7 nuclear cells were there and as preconditioning ATG was administered for two days. Then GVHD prevention was cyclosporine-A and growth factor was

administered to the patient expecting rapid recovery of neutrophils.

This summarizes all the treatment. You can see the rapid drop of the hematological cells, and on the day of the transplantation, on day 16, the neutrophils came out and reticulocytes on day 22, and the platelets on day 26. The patient survived for 7 months but, unfortunately, he passed away last May because of severe burn of the radiation and at the last multiple organ failure, he passed away.

So, I think this is a hard case for the Japanese people but I think we can find a good advantage of cord blood banking on transplantation which used our system in such an emergency situation.

Now, back to the Network, this is our system. We have a main server, two of them. One is in Tokyo and one we put in Kokkaido. We are afraid of an earthquake. Each of the nine banks send cord blood information to the main server every week, like HLA typing, all the family information to the main server. And the patient and physician can search through this main server. Everyone can see if they know their own HLA typing, and if they find the matched unit -- 1 mismatched and 2 mismatched antigens are mismatched unit in the order of their dose. After they find their matched unit they now contact with transplant

centers but now they have the ID number and the password. They search the second one and then they can find out more deep information about the unit. After they decide to use the unit, they talk to the local cord blood bank because the bank's name of the unit is put in the computer so they can find it. Then they send by fax or they telephone and decide on the final decision. After the decision, they keep the unit for three months and then the local bank submits to the main server the results. Only one patient just has the right to reserve only one unit.

All the banks send all the data to the main server. We just have a minor program. If we are fortunate, we can start the search within that week. We are also preparing our English version, and we hope that foreign country people can access the Network.

This is the first page of the website, linked to other banks also, and this is the screen on which the patient can type the patient HLA and the body weight and other things.

Now the clinical outcome -- the clinical data are summarized by the Basic Clinical Study Research Group Cord Blood Transplantation, chaired by Prof. Saito. These are members of the research group, but there is a strong argument on who should collect all the clinical data -- should it be the bank, or should it be transplant centers?

And, there is a strong opinion that academic societies, such as Hematopoietic Stem Cell Transplantation or Tokai, such a neutral society should collect the data and analyze it. So, our information is still limited but we have all the data sent to the research group.

Until the end of this March, 207 transplantations were done for 203 patients. There were 121 males and 86 females. The age was 0 to 49 and the median was 6 years old. Body weight, from 5.3 to 69, the median was 18 kg. The total transplanted nuclear cells are 0.62 or 16.6 X 10^7/kg and the median was 3.4, 7 X 10^7.

The indication of disease categories of unrelated cord blood transplantation -- acute leukemia, immunodysplastic syndrome, lymphoma, solid tumor, bone marrow failure, congenital immunodeficiencies, etc., and metabolic disorders, and the nuclear accident. So, more than half of them were leukemia patients.

Engraftment -- the number was 170. Recognize, myeloid engraftment was in 136 patients. The rate is 80 percent. Autologous reconstitution was observed in 9 patients, 5.3 percent. The transplanted total nuclear cell dose and engraftment -- there is no strong association in these two factors. HLA disparity -- more than 70 percent were antigen mismatched and 10 percent were true match and 25 percent were 2 anti-mismatched.

The severity of acute GVHD -- the variable number was 145 Grade 0, or 32 percent; 1, 39 percent; 2, 24 percent; and Grade III and Grade IV, 3.4 percent. Moderate acute GVHD total was 39.2. Severe GVHD was 50.1 percent. There is no association between HLA disparity and severity of acute GVHD, as shown in this slide. You can see that 85 percent of the patients showed no chronic GVHD; 5 percent were positive; 8 percent as indicated here; and extensive case was 2 percent.

This is Kaplan-Meier univariate analysis that we have used in the study, and overall survival was about 58 percent, and the follow-up median is 158 days. This is disease-free survival, shown here, and it is about 50 percent, and engrafted patients are shown here. HLA disparity and clinical outcome, disease-free survival. There are two haplotype identical transplantations that were done in our institute. This is those two, and we did data analysis by log rank test and the p value was 0.5 but we see good survival here, but there is no statistical difference yet.

This is nucleated cell dose disease-free survival. It is complicated. So, we divided into higher than 4×10^7 and lower than 4×10^7. You can see the clear statistical significance here.

Age -- if we divided the age to younger than 3 and -- sorry, 16 years old and the others, we see the statistical difference. Yes, age is also an important factor for clinical outcome. And, disease categories and disease-free survival -- we divided this into malignant disorders, congenital disorders, MPD, MDS, SAA, and there is no difference.

This is still on colleague outcomes among the acute leukemia patients, the total of acute leukemia, and you can see that it worse on CR1 and CR2. But for AML patients we still couldn't see the difference, but in ALL patients we can see the status on CR1 and CR2. The cause of deaths, 23 of them, in 79 patients MOF, VOD, GVHD, relapse others and unknown cases.

So in summary, we have established the Japan Cord Blood Bank Network and the computer search system will be operational this month. The patient and physician can search the patient's matched unit through the Internet and the decision to use the best unit for transplantation will be made by the transplant center physicians, not by the banks.

In summary about the clinical outcome, it is still early to analyze the data more statistically. We have only 207 cases. The following factors may be considered as a favorable disease-free survival in cord

blood transplantation a total nucleated cell dose, age and disease status and HLA match.

This is my last slide and I would like to thank the many American scientists, physicians who helped us a lot from the beginning when we started, such as Pablo Rubinstein, Dr. Carro, Joanne Kurtzberg -- so many people, Wagner and Cladd Stevens, of course, and then the help to establish guidelines, and because of that help I think we could progress our cord blood banking and transplantation much faster than we expected. Thank you very much.

[Applause]

DR. HOROWITZ: Thank you, Dr. Takahashi.

I would like to ask all the previous speakers to please come up for the panel discussion. I know there are a number of people who have had some questions that they wanted to ask in the morning, so why don't we start this session with just opening up the discussion with questions from the audience?

Discussion: Can we Define an Acceptable Unit for Transplant?

DR. KURTZBERG: Please remember to identify yourself.

DR. LANE: Yes, Lane, San Diego. Actually, I am going to start out the questions by asking a question we talked about yesterday, and that is a number of speakers

today talked about cell dose once again, and specifically CD34 dose. John Wagner showed a very nice correlation between CD34 dose and engraftment, and Mary Laughlin, I thought, had just some extraordinary data relating CD34 dose to, I believe, not only engraftment but overall survival.

So, what will it take us then to move from total nucleated cell count to using a CD34 dose as at least a very important measure of evaluating these grafts? I showed you data yesterday that at least within the Red Cross system of cord blood banks the variability in CD34 dose is really not very high, not much more than one would expect. So, how can we get there?

DR. KURTZBERG: I would like to take a stab at answering that. I think we have to standardize methodology between centers to be able to answer that question because even the Duke-Minnesota databases are a good example. We have relationships in both databases but the absolute number is different. And, I think until the methodology is standardized or we have some key that sorts out how, you know, Japan correlates with New York, correlates with COBLT, correlates with San Louis, etc., we are not going to know how to use the numbers themselves.

DR. WAGNER: So, just to follow up on that, I mean, basically for both Duke and Minnesota, both

individual institutes showed a correlation but the absolute numbers were different so, therefore, I came up with a slide that specifically stated -- or maybe a statement in there that the middle number should be 1.5 X 10^5/kg. That may be true for Minnesota but it may not be true for anyone else because of the way we do the analysis. So, it has tremendous implications for people in the way they choose a graft. Until we have standardization we can't use CD34.

DR. KURTZBERG: The other thing to mention, and I think this is true of Minnesota too, but all of our data is post-thaw, and although we say we have a method that correlates pre-cryo and post-thaw, it is only going to be in the COBLT study that we really have a prospective to look at that.

DR. MCNEICE: Joanne, may I make a comment on that? Ian McNeice, from Denver. I am not as convinced from the data I have seen that what people are calling correlations certainly aren't convincing to me when you have an ANC grade of 0.5. I would like to see it be a lot more rigorous before we make that sort of comment. I am still not convinced the data suggest that there is a good correlation that you can predict from the 34 number what is going to happen with engraftment.

DR. WAGNER: Well, just to follow up though, there is a suggestion by at least one analysis to suggest

that there may be a threshold. So, there may not be a correlation that is continuous, but it may be that it does provide us with a threshold dose beyond which we find no real difference between CD34 doses but there was a lower limit.

DR. MCNEICE: And, I would question how rigorous the analysis can be for those numbers. Then when you look at the actual grafts, the majority of the grafts run between 2-4 million total 34 cells --

DR. WAGNER: Right.

DR. MCNEICE: So, there is not a lot of play to actually say this is a much better graft than this one. So, I think we need to be careful before we make such a bit leap.

DR. WAGNER: We are almost ready to identify a number below which it is an unacceptable graft. That is the goal.

DR. MCNEICE: Well, I still don't think that we have the data that supports that that is a true indicator of the engraftment potential of that product is.

DR. KURTZBERG: Let Pablo make a comment.

DR. RUBINSTEIN: It is just to alert you to the fact that this afternoon there will be some discussion on the hematopoietic stem cells and precursor cells. So, part of this answer may be clarified.

From our own data, it is clear that the colony-forming assays predict a little bit better -- not much better but a little bit better than the total nucleated cell dose, and the CD34 count done by Dr. Fisher, with some modifications from the standard procedures, do correlate extremely well at the level or r-squared of 0.88 or 0.9 -- I don't remember exactly -- with the colony-forming cell dose. So, basically, the total nucleated cell dose remains largely enough to account for the quality from the point of view of these two other parameters. They are better, but not very much better.

DR. KURTZBERG: Cladd?

DR. STEVENS: I would just like to make a comment to help clarify what seems like an apparent disagreement between the Duke-Minnesota data and some of the data that Pablo showed earlier about effective cell dose post-engraftment on survival.

If we do a univariate analysis on the total blood center data, you also see a correlation with cell dose. I think the point that Pablo was trying to make was that when you do it in a multivariate analysis the effective cell dose drops out and the only thing remaining is the effect of age. So, it is clear that we have a situation here where we have confounding between age and cell dose, and I

think those are issues that need to be considered in a little more detail.

DR. KURTZBERG: Carolyn?

DR. HURLEY: Carolyn Hurley, from Georgetown University. I, of course, wanted to ask the question about the role of HLA which seems to still be unclear, and I wanted to ask the panel about what their plans were in terms of using high resolution methods to look at matching because, obviously, underneath the issues of are things matched to the serologic level there is extensive disparity that is not being detected. So, that is one question.

The second question is the issue of whether transplant centers or banks are making some planned collection of patient and donor samples in order to go back or be able to go back and address these issues of histocompatibility?

DR. RUBINSTEIN: Thank you very much. That I think is a very important question. There is further heterogeneity that we have been able to analyze so far. Class II is fairly okay, but for Class I all we know is about the level of resolution equivalent to serology with all splits. There will be additional information coming out from that. It is a retrospective analysis. We do have samples on all of the units and most of the patients, but

not all of the patients. So, that retrospective analysis is being done to the extent possible as we speak.

DR. WAGNER: Just to complement what was just said, you know, we also agree that it is one of the things that needs to be done and we, in the institution, don't have a large enough series to be able to answer any of those questions. However, we have samples on all the patients but not all the donors.

DR. KURTZBERG: In the COBLT studies there are samples on both.

DR. WAGNER: Just a comment on trying to determine the role of HLA versus cell dose. I mean, I know that you are well aware that even in the bone marrow transplant setting where we have thousands of donor-recipient pairs to analyze, it is very difficult to separate out the independent factor of specific HLA mismatches, number of HLA mismatches in a setting where you have so many confounding variables affecting transplant outcome.

In this setting we don't have thousands of donor-recipient pairs. We have inadequate typing on many of those donor-recipient pairs, and we have confounding cell doses, and an issue in trying to separate out whether it is cell dose versus HLA disparity is, I think, confounded by some of the selection pressures on whether you do a cord

blood transplant that is mismatched. If you have a patient with very poor risk, very poor prognosis with conventional therapy, you are more likely to take what you might consider a marginal graft.

So, although we would like to be able to definitively say this is the contribution of HLA and this is the contribution of cell dose, I don't think the data exist right now. I think the most that you can say is cell dose is important; HLA is important. The relative contributions remain to be determined.

DR. KURTZBERG: I guess the one thing I would say is that I think this is much more complicated than just engraftment and you are okay versus engraftment immune reconstitution and a bigger picture. If you look at everybody's curves, most of the deaths are in the first 100 days and at least half of those deaths are infections. I know in my experience there are equal numbers of infections occurring in engrafted patients, meaning patients who achieved an ANC of 500, as there are in people with delayed engraftment. So, there is not the same protection from engraftment that I think we are all used to seeing with the bone marrow transplant patients.

My personal theory is that some of this may be related to immune reconstitution which may be more delayed in the HLA mismatched setting, or may have some other

factor contributing like age and capacity for immune reconstitution. But, there is something else driving the fact that these people are getting infections, and that is their main reason for failure. Maybe it is the cell dose threshold; maybe it is immune reconstitution; maybe it is HLA. Probably it is all of the above. But if we could sort that out this would be a lot more successful as a transplant source, and I think it is most important to sort that out for adults because there the cell dose options are less generous.

DR. BROXMEYER: Hal Broxmeyer, from Indiana University. Two short questions: How many cord blood transplants have actually been done? Because that is not clear from anything I have heard or read. Are we in the 2000 range? Does anybody really have a handle to be able to at least estimate how many cord blood transplants have been done?

DR. KURTZBERG: We have done 290 unrelated.

DR. BROXMEYER: That is not helping me.

DR. HOROWITZ: We are in the 2000 range.

DR. BROXMEYER: You think so?

DR. HOROWITZ: Yes.

DR. BROXMEYER: Good. It is not that easy to get that answer. The second question deals with time to neutrophil engraftment, and this is kind of important and

what I heard today changes some of what I was thinking in the past. Is it your feeling that if there were enough cord blood cells given that there would be no real difference to time of engraftment compared to bone marrow transplantation? This is kind of important because it may reflect on what the stem cell is like in cord blood versus bone marrow.

DR. HOROWITZ: Well, I think that the honest answer to that question is we don't know because no one would ever give those few bone marrow cells to a person to be able to test it, and there are very few, if any, cord blood transplants done with cell doses that are in the range of bone marrow transplants --

DR. BROXMEYER: But you wouldn't have to go that high.

DR. HOROWITZ: -- in similar patients, same age, etc. But I think that probably more cells -- that the rate of engraftment is not that different that increase in the number of cells would likely speed it up.

DR. KURTZBERG: But if you look at the babies -- Donna presented data and we, in the pediatric setting, have data of babies getting doses that are between 1 and 2 X 10^8 cells/kg, and engraftment is faster, and that is more striking for myeloengraftment than platelet engraftment. Platelets are still delayed compared to what you see with

bone marrow but not as much as they are in the adults. I know that in our series the ANC to 500 is in the 15-20-day range in that group of patients on average, and the platelet recovery is between day 40 and 50 compared to people getting a lower cell dose where it is, you know, day 60-70 for platelets.

DR. HOROWITZ: The actual times to engraftment are quite similar to T-cell depleted bone marrow transplants. So, that is another way of getting at the question.

DR. BROXMEYER: Yes, but I don't know if that would be a legitimate way of looking at it. I am still trying to figure out -- I mean, the closest I have seen is what Donna presented --

DR. HOROWITZ: Right.

DR. BROXMEYER: -- where it was pretty quick, I mean, between 10 and 20 days, it looked like.

DR. HOROWITZ: The problem of comparing, and the reason I said what I said is that the problem of comparing infants is that the infant cell doses with bone marrow transplants tend to be higher than that, and then age is an independent prognostic factor with bone marrow transplants for time to engraftment, even independent of cell dose at least in our data. So, you don't have exactly a comparable group but I think within the limits of what we know,

probably increasing the number of cells will bring the times to engraftment to be very similar. The question is whether immune recovery will be the same, for better or for worse.

DR. DYKELET: Claire Dykelet, CDC. I have a comment and a question for Dr. Takahashi. First of all, I would like to compliment you on looking at the different causes of outcome in your patients, looking at the different causes of death, and I am interested in the fact that you found that infection was the second most common cause of death. I think that is a really important thing to be looking at. The only way we can really start to prevent death in these patients is to figure out what the causes are and then we can start to target them individually.

Can you tell me, please, what were the infections that were most common? Was pneumococcal disease a problem?

DR. TAKAHASHI: I am sorry, I don't have the data in my hand, but I will let you know.

DR. DYKELET: Okay, I will give you my card after.

DR. KURTZBERG: I can make a comment about that. In our patients where infection is the leading cause of death it is pretty equally split between fungal, viral and bacterial. It is not pneumococcus in this early 100 days.

It is gram-negatives and gut organisms for the infections, enterococcus etc., and for the viruses it is adenovirus, CMV and in our series it is flu too that has been a problem but I think that is environment -- what shall I say? -- I think that is just related to the time of year we happen to be transplanting and what is in our community. For fungal it is both resistant Candida and Aspergillus.

DR. HOROWITZ: Do you think that that is different from bone marrow recipients? It is the same spectrum of infections, though perhaps a higher incidence.

DR. KURTZBERG: Right.

DR. WERNET: Peter Wernet, from Dusseldorf. What is your impression concerning the CMV story? The cord bloods are in all likelihood negative for CMV when donors are positive for CMV. What is the chance for increased reactivation of that latent infection of the cord blood transplantation as compared to bone marrow transplantation?

DR. RUBINSTEIN: Could you rephrase --

DR. HOROWITZ: CMV reactivation in CMV positive recipients? Is that what you are saying?

DR. WERNET: Yes.

DR. LAUGHLIN: I will take a stab at what I think is your question. When the European group made their observation of the prognostic value of recipient CMV serologies on transplant outcomes -- and this is simply my

personal conjecture or hypothesis -- I think it has to do with perhaps a hidden linkage with particularly acute graft versus host disease and then that higher incidence leading to higher incidence of chronic graft versus host disease, i.e., patients who are transplanted with positive serologies are at increased risk of reactivation and with that reactivation known interactions between reactivation of CMV and exacerbation of acute graft versus host disease. That is somewhat my interpretation.

DR. WERNET: Yes, because there have been, as you said, these discrepant results without it.

DR. KURTZBERG: I also think CMV is a moving target because we have managed CMV differently over the years. We used to prophylax anybody who was serologically positive. Then, I know, we went to not prophylaxing those people but following first antigen and now DNAs and only treating people when their DNA converts. I know in our whole group we have three patients who are the way Mary is describing it, the kind who have this interaction between CMV and GVH and all three of them eventually died, although one of them lived for a year and a half before she died.

But I think that because we detect it differently and we manage it differently it is very hard to look at a conglomeration of data over the last six or seven years and make a cohesive story.

DR. WERNET: So, would you recommend on that basis perhaps a more rigorous preventive treatment in patients receiving CMV negative cord blood units where the patients are positive, right from the beginning just because of that?

DR. HOROWITZ: I don't think that there is any reason to have a difference in the policy towards CMV infection prevention in bone marrow transplant recipients versus cord blood transplant recipients, and in CMV positive recipients who have CMV negative versus CMV positive donors.

DR. WERNET: Okay.

DR. HOROWITZ: I think in all of those instances an aggressive policy towards early detection and preemptive treatment or prophylaxis is indicated.

DR. WAGNER: It is a good question though, but we don't know the answer to it. We never did a comparative study in CMV incidence or disease between cord blood versus bone marrow. So, we don't know the answer specifically to your question but my gut feeling is that there is nothing different about the disease. Once it occurs it is very similar --

DR. WERNET: Yes --

DR. WAGNER: -- but we haven't really formally looked at that but I am writing it down!

DR. KURTZBERG: I mean, the other thing to say is there is a price to pay for prophylaxis.

DR. WERNET: Okay.

DR. KURTZBERG: So, it is not without cost. I don't mean monetary.

DR. LAUGHLIN: I would also add to that. I think that the moving target is going to move more if the experience in our program reflects that of other programs, the financial constraints, particularly in the administration of intravenous immunoglobulin as prophylaxis because of fiscal concerns.

DR. RUBINSTEIN: I am not sure that this speaks to the question but yesterday Cladd showed what the situation between the donors and the recipients with respect to CMV detection is in patients --

DR. WERNET: Yes.

DR. RUBINSTEIN: -- and, from that analysis it is clear that it is much more likely to be a reactivation of an existing situation.

DR. COELBO: I have a question at least initially targeted to Pablo but then anyone else can jump in. One strategy for increasing cell dose is transfusing simultaneously or soon thereafter -- simultaneously I guess -- multiple units. There has been no data submitted in regards to that. Could you comment a bit on your

experience with that? I know that has occurred to a certain extend, and on the basis of what you have learned, do you plan more multiple unit transplants?

DR. RUBINSTEIN: That is a difficult question. There have been several multiple donor transplants. We have been involved in a few of these. There are others in which we participated retrospectively in analyzing what happened. But the data we have is on five such cases. The results of these were not very good. There are two cases in which engraftment occurred, one additional case in which engraftment occurred but it was a case which we learned through the press really, and it would take too long to detail all of that one situation. I believe that one of all of these patients or recipients remains alive out of the five cases.

The problems of why this approach has been used perhaps are because of the evidence of the early successes in bone marrow transplantation with pooled bone marrow donor material. You may remember the first successful transplant was obtained in France from a pool of relatives of the patient by Dr. Mati in '64. That patient survived for almost two years. He engrafted with single donor, and this seems to be the same pattern with the cord blood situation.

I believe that it would be profitable to make an effort to find out but it would have to be a very well thought out and well supervised trial at all levels.

DR. KURTZBERG: Pablo, I have a question that is related. Do you think that if we infuse multiple grafts we can have competition between the grafts and actually more graft failure overall?

DR. RUBINSTEIN: I don't believe that. It was not the experience with the bone marrow where competition would be expected to be far more severe given that the marrow is an immunologic organ fully developed. So, I would expect less competition here than in the bone marrow. On the other hand, the number of cells are much lower so any degree of competition might be lethal. So, I just don't know the answer.

DR. HOROWITZ: Getting back a little bit to the focus of our discussions in terms of how to pick a graft and how to pick patients for cord blood transplants, there have been between 1500 and 2000 cord blood transplants done. They only account for about 3-4 percent of the allogeneic transplants in the U.S. right now. Yet, the early results suggest that engraftment can be achieved even in adults; that most of the problems have to occur early and are related to infection, perhaps related to slow engraftment and slow immune recovery but that long-term

there is what appears to be good disease control and very low rates of chronic GVHD.

What do each of the panelists, and perhaps everyone could comment, consider to be the most important characteristics in evaluating patients to receive cord blood transplants? What do you think should be the appropriate eligibility criteria, and is there a minimum cell dose and to what degree, from each person's personal perspective, should HLA versus cell dose be considered in choosing between units? Anyone like to start?

DR. RUBINSTEIN: About the patients, I think that question should be addressed by the clinicians. About the donors, from the point of view of the data we now have, as Mary, indeed, summarized a few minutes ago, we know that cell dose is important. We know that HLA is important. I don't know that we can make an absolute definitive threshold for anything at the moment. However, it seems reasonable that we heed the advice of the data in that they show that if you transplant a few cells you are going to have more chances of not engrafting. Once you don't engraft, then the game is over largely. So, cell dose seems to be a critical thing, and all analyses show that it becomes really bad if it is under, let's say, 20 million cells per kilo. But I don't think that one should

necessarily establish an absolute arbitrary lower threshold at the moment.

About the HLA, the situation is a little less clear because the data showed that for engraftment the only difference is noticeable, at least at this moment, between full matches the way they are currently defined and any mismatch. So, for engraftment at the moment the chance probably does not exist to exercise a choice in the vast majority of cases, at least in Caucasians and other non-Asian populations. In Japan the situation may be different.

For later indices of success, after engraftment, it seems that HLA is important and there the data are softer and we need more time to know for sure how much it costs. But even with the mismatched grafts the likelihood of success is quite good. So, it is just a matter that they could be better. And this is a problem that will take care of itself as the number of units available for treating patients increases, similar to what has happened in the case of the bone marrow. There are a few other variables that could be considered but I think these are the main ones.

DR. LAUGHLIN: Comments from clinicians particularly focused on adult recipients would be, for lack of a better word, improved confidence that the results of

these Phase I trials demonstrate that the use of this stem cell source -- the outcomes for these patients is fairly equivalent, certainly not significantly inferior to the use of unrelated graft stem cell sources from adult donors.

I personally have greater confidence now to enroll patients into cord blood grafting. I do not have compelled reason to change criteria as a clinician in choosing an umbilical cord blood graft for a particular patient. I think certainly points have already been well emphasized about appropriateness of cell dose and HLA matching.

DR. KURTZBERG: From the pediatric perspective, I think it is very important to look at the younger kids as a separate group, and I am willing to say in my own experience, doing both unrelated bone marrow and unrelated cord blood transplants, that I think the outcome for the unrelated cord blood patients is better. I think many of the pediatric diseases in infants which are not cancer but inborn errors and genetic diseases have time as a very important factor, and cell dose in those patients is easy to achieve, and the fact that you can end up with a much lower incidence of chronic GVHD means that the overall quality of life for those patients in the long run is improved. So, I personally, in that group of patients, would go out on the limb to say I would favor cord blood.

In our own center, our referrals are skewed towards people who don't have bone marrow donors. So, I can't say that we are really in the process of electively choosing cord blood over bone marrow, but in the rare instances where we can make that decision we usually do.

In the older patients, and those are going to represent a population of mostly kids with leukemias that are either high risk or have relapsed, I think we have to ask the question which is better, cord blood or bone marrow. The data would suggest, without doing any kind of randomized trial, that they are at least comparative and that that question should be asked. But, again, to me, the possibility of having a lower risk of chronic GVHD in the long run means that the overall quality of life may be better using cord blood.

DR. WAGNER: I just want to make a comment. I think that the point of the meeting, at least as was presented yesterday at the very beginning, was that we wanted to create product standards and we also wanted to know what new avenues of investigation should be in the area of umbilical cord blood transplant. So, I think going back to what Pablo was saying is, you know, not to say that we necessarily know what the lower limit is but I think that we have to get back to what do you know now that would be considered acceptable routinely? So, you know, can we

get back to what would be routinely acceptable, not to say that you can't do it in a trial within a given institution, but what would be a blanket, you know, acceptable graft? Is that identifiable yet? Because I think that is what at least one of the goals of this meeting was, and maybe the answer still is that it is unknown but, if it is, then, you know, should the whole field be regulated or should we have some area that is saying, well, this is really, you know, a graft that is considered to be routinely acceptable?

DR. HOROWITZ: So, what do you think is acceptable?

[Laughter]

DR. WAGNER: Do you have time?

[Laughter]

No, you know, Joanne and I have been discussing this ourselves, and at least our opinion based on the data set that you saw that was presented was that we were going to suggest that a lower limit or a routinely acceptable cell dose would be on the order of 1.5 to 2 X 10^7 nucleated cells/kg. We weren't prepared to give a CD34 dose because in part, as we have heard today and as Ian pointed out, we are still in the formulation stage of figuring CD34. So, we were going to stick to nucleated cell doses.

Then we thought, well, the bulk of the experience is with 0-, 1-, 2- and 3-antigen mismatched grafts and, not

to say that we know all of the answers but, you know, we really have no data that has been really presented on more mismatched grafts. So, maybe it should not be considered routinely acceptable to do 4-, 5- and 6-antigen mismatched grafts. So, that is why we were saying that we probably have enough data to be able to support at least doing 0, 1, 2 and perhaps 3 mismatches used in the degree of mismatch that we defined today and that may change as we get more sophisticated.

So, again, I think we should focus on cell dose. We should focus on HLA typing, and maybe other issues but can we not make some statement today?

DR. STEVENS: I just want to make a follow-up on the same issue. In terms of a product definition of an acceptable product, I would be surprised -- I don't know how the FDA is thinking but I would be surprised if they are thinking about making that kind of a definition of a minimum cell dose or an HLA definition of an acceptable graft. I would suspect they would be more interested in looking at issues like how you do the testing, how you do the collections, what are the techniques, how do you define those doses rather than dictating to the clinicians what units they might find acceptable. And, I would be reluctant, from a bank perspective, to do that and let the clinicians make those decisions.

DR. WAGNER: Just so that you know, I am not saying -- let's say we said a cell dose of 2 or 3 or whatever you want, it is not to say that clinicians can't use a cell dose less than that. It is just that there may be a different type of monitoring for those types of transplants as compared to other types.

But you are also asking a very good point. I mean, really then maybe the FDA can tell us specifically what it is that they would like to hear.

[Laughter]

DR. KURTZBERG: I would also like to put in a plug that from the clinical side and the reimbursement side, if the FDA were to say that this is routine, whatever definition we would do, which did relate to HLA matching cell dose specifically, that is what the third-party payers are looking at. So, if the FDA made a statement that this is now licensed for cell dose over X and HLA mismatch up to a disparity of You, that would help in the reimbursement setting. Even though there would still be other things that would be done and investigated, etc. that would make a lot of people's lives simpler in terms of getting patients to transplant.

DR. HOROWITZ: We have two people who are waiting. One person over here has been waiting a long time.

DR. HIROSE: Thank you. This may be a little bit off the current topic that we are discussing, but since it seems that cell dose is a paramount variable in consideration of engraftment and since multiple manipulations may contribute to the decrease in the viability of stem cells in a unit, what is the opinion of the panel on using whole blood umbilical cord units in patients, particularly in adults?

DR. RUBINSTEIN: There is no problem in using whole blood, except that it has a lot of unnecessary material. It occupies a lot more room. It is more difficult to ship without breaking the bags, and there is no advantage other than eliminating the losses that invariably can occur, no matter what, during the processing. When you are trying to do volume reduction, no matter how careful and how effective methods are used, the only way that the count can go is down. So, you are going to end up with fewer cells. But if that is done carefully, then you can probably limit that loss substantially.

DR. MCNEICE: Ian McNeice, from Denver. I would just like to make a comment to John's comments about minimum cell doses, and would request that if we are going to suggest cell doses we have to be very careful in how the products are defined. For example, if you start doing volume expansion, I think we have a very different product

that we can't evaluate in the same way and there should be careful consideration to how you put restrictions like this in place. So, I think if we are going to recommend cell doses, we certainly have to very well define what we are talking about and in what context. I think an expanded product is very different. The quality of those cells differs. So, it can't be evaluated in the same way.

DR. HOROWITZ: Good point.

DR. MARTI: Gerald Marti, FDA. Two comments and a question. The inter-laboratory variation of CD34 determinations in the U.S., Canada and Western Europe is plus/minus 1000 percent.

[Laughter]

That is published. With a little bit of standardization you can get it down to plus/minus 20 percent, and in a good two-color assay on a single platform you can probably get that down to plus/minus 5 percent. That is comment number one.

Comment number two, I find great interest that Duke and the University of Minnesota have a two-fold difference in what appears to be, if I see the slides right, their CD34 determinations. A very similar, if not identical, situation to that occurred here, at the NIH. The institutes and labs will remain unnamed, but the identical problem occurred where two laboratories were

getting different answers, always by a factor of two, on the same "sample." It was only when that situation was submitted to a side-by-side comparison of all of the 26 variables that were listed at that time that could be involved in giving rise to the difference was it realized that the two laboratories were doing everything identical, including the catalog number and lot number of the reagent, but they were using different lysing procedures.

Question, cell count -- would a standard control that could be purchased, a standardized could blood sample, a 1 ml sample that would cost, say, $33 to maybe $55 a sample -- would that be useful in establishing inter-laboratory variation for just doing counts? This is a product that could be available. It is being investigated for CD34 determinations in the allogeneic adult world. In the cord blood world, would such a product, a standard control, a positive control be useful?

DR. WAGNER: This is only a comment for the banks. Remember that at least for the Duke-Minnesota data set, first off, I never showed you the Duke CD34 analysis. So, that you haven't seen.

Number two, you know, when we talk about nucleated cell dose, 90 percent roughly came from the New York Blood Center. So, they are all from one bank. But really when there are different banks involved, you know,

we are talking about pre-cryopreserved cell dose and that is a banking issue. There is only a finite number of banks so they will have to address that point, unless we start looking at post-thaw nucleated cell counts in which case every transplant center in the world would have to be able to, you know, do the same type of system that you are proposing.

DR. KURTZBERG: I am having trouble envisioning what this product would really be, and if you think about how the CD34 analysis is done, there are a lot of decisions that are made at various points along the way about how you come up with your final number, and different groups make different decisions about how to do those calculations. I don't think any of us know what is right or wrong. I just think there has to be some uniformity in terms of the approach.

We do a method that used beads and gives us the count per ml but a count per ml doesn't correlate with the count per ml that we get off the automated counter or the hemocytometer when we actually buy that product and look at the product. So, what we decided to do was to use the count per ml from the automated counter of the product and use the percent from the actual CD34 counter and then come up with an absolute number. Whether that is right or wrong, that is how we made the decision.

DR. MARTI: My question for you would be what is your positive control for that count off the machine? And, it sounds like you don't have one or you don't use one. Maybe the laboratory that is setting up the automated counter uses one but you don't.

DR. KURTZBERG: Well, it is the same laboratory but they use just the standard controls for blood.

DR. MARTI: I made the comment yesterday that I don't think the automated cell counters that were licensed were licensed on the basis of databases that included cord blood.

DR. KURTZBERG: No, you are absolutely right, but we do internal correlations with hemocytometers to show that what we are counting is counting nucleated cells, period.

DR. MARTI: My thought is that if you had a stabilized cord blood sample that had a shelf-life of, say, 30-90 days what is the variation between two laboratories on that, and if that variation is acceptable could you use that as a positive control between laboratories, and would it be useful?

DR. KURTZBERG: Yes. That is my personal answer.

DR. MARTI: Thank you.

DR. VISSEK: I am Jan Vissek, New York Blood Center. A lot of the CD34 counts are done, and I think it

would be a very useful to have a product now to mobilize peripheral bloods or just peripheral blood as daily controls. So, just for an internal control and for educational purposes it would be nice to have a product that was stable. They are available through other companies but not for cord blood, and most of the products that companies sell are to mobilize peripheral blood. So, if there is something more specific for cord blood we would use it.

DR. MARTI: Recently there was a collaborative meeting between the FDA in England and the FDA here, and one of the issues was standard cell controls in the area of flow cytometry. The English are particularly interested in developing cellular controls for leukocyte depletion. They want a positive control for a little depletion, a lot of depletion, moderate depletion, and this is where I got that idea that it would probably be good to have a cord blood sample. So, I will pursue that.

DR. HOROWITZ: I think we sort of got interrupted in our move down the panel here in terms of acceptable units, appropriate patients.

DR. WALL: Right, in our subset analysis what is coming up with us is a 3 X 10^7/kg cell count of the post-processing cell count -- not the thawed cell count but the post-processing cell count -- reliably results in

engraftment. So, if that is a target cell dose, to be above that cell dose. Then, it is pretty much any man's game as to what is the acceptable cell dose versus a non-acceptable cell dose.

I would like to echo Joanne, if I may. In pediatrics I think the shift towards cord blood as an unrelated donor source really needs to be looked at from a number of directions. In addition to the points that she had made, I would like to add that the cord bloods are EBV negative and tend to be CMV negative and our population tend to be EBV and CMV negative so this is a major plus. So, at our institution, which is more of a regional transplant program for children pretty much under the age of five, we will move to cord blood as our preferred stem cell source in the unrelated setting.

DR. SHPALL: E.J. Shpall, from Denver. I just wanted to echo Ian's comments about being specific in terms of what patients we are talking about, and that a cell dose of 3 X 10^7/kg would not be doable. I have never had an adult patient who had that cell dose. So, I think it looks like 10^7/kilo for adults would be a somewhat reasonable target, although 50 percent of the patients treated at our center wouldn't have a graft if that were required.

DR. HOROWITZ: I was just about to make that point, and I think we have data on this from the COBLT

study and maybe Shelly can fill in some numbers in terms of what percent of adult transplant candidates would be precluded from having a cord blood transplant using a level of 1 X 10^7, 2 X 10^7 nucleated cells. I know we have some information on that.

DR. CARTER: The data we have is pretty old but I think it was an 80 kg person. If you looked at the COBLT registry at that time, it was 5 percent of them that would have an acceptable graft.

DR. HOROWITZ: So, 5 percent would have 1 X 10^7 --

DR. KURTZBERG: No, 5 percent would have 3 X 10^7.

DR. HOROWITZ: Okay, only 5 percent would have 3.

DR. KURTZBERG: I will show that slide this afternoon.

DR. WAGNER: Similarly, sort of not taking a single, you know, weight cut-off, what we did was we looked at the University of Minnesota for all the adult patients who were referred for unrelated donor transplant and we found that almost 90 percent would have had an acceptable graft on the basis of HLA typing because we allowed the 2-antigen mismatched graft. However, despite that fact we found that only 20 percent would have met the cut-off of 1 X 10^7 nuclear cells/kg. So, clearly, there is a substantial

reduction within our patient referral pattern at the University of Minnesota.

DR. HOROWITZ: So, even if we don't look at transplant outcomes since most centers are using some minimum cut-off for cell dose, in order for cord blood transplantation to be a practical option for treating the majority of people with these diseases who are adults, then the issue of cell dose is the prime one that would need to be addressed.

NY BLOOD CENTER PARTICIPANT: I just wanted to add a comment about the appropriate patients for cord blood. The data we presented from the blood center looks at all patients together. We haven't shown you any very specific analysis, but we are in the process of analyzing specific diseases and looking at outcome, and we have identified some groups with the process of saying who would be the best or the worst candidates for cord blood and, of course, setting up some comparison studies with bone marrow. So, I just wanted to share with you information that we have identified that infants with leukemia do seem to have a much better outcome with cord blood transplants than at least what has been reported with chemotherapy, and we are very much interested in looking for the comparison study with bone marrow.

DR. WAGNER: Well, I think you have to be very careful. The data is very encouraging, on the other hand, it is not really a comparative trial. So, therefore, yes, compared to the historical literature it may seem encouraging but we have to be careful about that statement, you know, is it better or is it equal to -- you know, all we can say is that it is encouraging at this point until larger studies are done where they are actually doing a comparative analysis between bone marrow versus cord blood versus chemotherapy.

NY BLOOD CENTER PARTICIPANT: Exactly, and that is what I was trying to stress, that from our side we can look at the data only of cord blood but it would be very important to set up comparative studies.

DR. RUBINSTEIN I would like to say that while I agree with the comments just made, the same objection applies to anything we do at the moment. There have not been any comparative trials, and all the analysis is retrospective at this point. All we are trying to establish is some idea about how this whole thing shakes up. But, of course, definitive evidence can only come from classically organized trials and we are hoping that those will become possible in the future, but I am not entirely sure that they will be in all cases.

DR. HOROWITZ: I think one other comment I wanted to bring up is that the results that were shown both in the unrelated and the related donor transplants show survivals that are -- well, in the related donor study identical and in the unrelated donor study appear to be similar, although there is not a direct comparison to those reported with bone marrow transplants. Intriguing to me is the consistent report of very low rates of chronic graft versus host disease, something that we are only beginning to appreciate now as we have longer follow up on many of these patients.

In analysis of late deaths after bone marrow transplants, chronic GVHD accounts for about half of them and is a significant cause of morbidity among transplant survivors. To me, one of the things about cord blood transplants that is appealing is the potential for decreased morbidity because of a lower incidence of chronic GVHD, and I wonder if any of the panelists would want to comment on that.

DR. LAUGHLIN: We have not done a formal comparison with adult recipients receiving matched unrelated donor grafting versus partially mismatched unrelated cord blood grafting, however, it has been our impression with a review of the literature -- obviously, all fraught with problems of using historical controls in

comparison of a new stem cell source, but there is a hint that both the incidence of severe acute graft versus host disease as well as incidence of severity of chronic graft versus host disease is reduced in adult recipients of unrelated umbilical cord blood transplant.

If we take a step back not a long time, the initial trials in using this new stem cell source which were primarily reported in pediatric recipients, the comments, not necessary formally made at conferences, were that the observed reduced acute and chronic graft versus host disease was due to the fact that the patients were pediatric recipients. Certainly, the numbers of adults that have been transplanted is very small and we don't want to overstate the preliminary observations, but these preliminary observations do point to a trend towards reduced GVHD.

DR. KURTZBERG: As I said before, I think in children it is pretty evident, and it is important, and it is a reason to be using this as an unrelated stem cell source over bone marrow because of the long-term potential for survival that children have.

But I would also like to say that I think that there is a syndrome that many of these patients have that may relate to mismatching that looks to me very similar to heavily T-depleted patients, and I think it is more

prominent in the adults where the first year to 18 months these patients -- they are not thriving. They may not have rash and diarrhea but they don't gain weight well. They often lose weight. They are okay but they are not really well. And, some people call that chronic GVHD and some people don't call that chronic GVHD, and it goes away when the immune system begins functioning again. Once you have been working with these patients you don't have to do immune function tests. You can just see them walk through the door and you can tell when that happens.

I think we can get caught up in semantics about what you call that, but what is impressive to me is that in cord blood it goes away, but it doesn't go away in two months; it goes away in 12-18 months. I think when you transplant patients they need to know that, and when you prepare yourselves to take care of patients you need to know that and, again, I think in the adult setting it is more prominent than in the pediatric setting.

DR. WAGNER: Just to make one further comment about the comparisons between bone marrow versus cord blood, I mean, clearly an updated analysis needs to be done. It doesn't exist right now. What we have done at the University of Minnesota is we recently performed a matched paired analysis where we matched with age, disease, disease status and treatment, and we compared the results

of cord blood to those who received unrelated marrow. Clearly, they were primarily pediatric in age, but it gives us a clue as to what the real differences and similarities are between bone marrow versus cord blood. Interestingly, in this analysis what we found is that the results we umbilical cord blood transplant, being primarily mismatched, were virtually identical with HLA matched bone marrow in terms of engraftment, survival and graft versus host disease. That included neutrophil and platelet recovery, acute and chronic GVHD -- everything was virtually identical.

So, it appears that mismatched cord blood, though the results are still preliminary, may be equivalent to matched bone marrow. But, fortunately, the real benefit of cord blood is that it allows us to achieve that level of success despite greater degrees of HLA disparity. When you compare the results to mismatched bone marrow, you find that the results with cord blood were significantly better in terms of graft versus host disease and survival. So, therefore, you know, when we can only find matched donors for only a proportion of our patients this may be the option, or the better option for those who can't find matched marrow donors. That remains to be proven.

DR. LAUGHLIN: I wanted to add a couple of comments on phenomenology of chronic graft versus host

disease, which I think is very difficult to code for statistical analysis in assessing morbidity in patients undergoing unrelated grafting. Certainly, the clinical syndrome that Dr. Kurtzberg described is common in the adult recipients but contrast favorably with the sclera dermatitis type progressive graft versus host disease that we see in our matched unrelated donor recipients.

DR. KURTZBERG: I think we should begin to close and it is time for lunch, so that we can stay on schedule.

DR. HOROWITZ: Thank you very much.

[Applause]

[Whereupon, a luncheon recess was taken to reconvene at 1:00 p.m.]

AFTERNOON PROCEEDINGS

[1:09 p.m.]

Session 4: Ex Vivo Expansion

DR. GEE: Good afternoon. I would like to get the afternoon session on track, if we could, because I know several of you have planes to catch.

You heard in this morning's session that in cord blood transplantation we are essentially trying to combat what apparently are opposing forces, one of which being that cord blood is a one-time collection of limited volume and limited numbers of cells, and the second being, as you have heard from virtually every speaker this morning, that the more cells we can infuse, generally the better the time to engraftment and, in some cases, the better the outcome of the transplant.

One way that we can potentially think about resolving that apparent dichotomy is to use ex vivo expansion for cord blood cells. Just to kind of get this session into some kind of focus, what I wanted to do very, very briefly was to outline the areas that perhaps we ought to be considering when we listen to these speakers and to raise for the discussion.

One is the condition for expansions: What kind of media and sera; what kind of growth factors; what kind

of antibiotics, if any, we should be using; whether there are other supplements and how we deal with those, or even other cell types that we ought to co-culture with cord blood cells; what kind of containers we should be using, should we be using bags or bioreactors, and how long should we be culturing the cells?

What kinds of methods shall we use to talk about levels of expansion? Is fold-expansion a useful term for most of us or should we be talking in some other quantitative unit? What fraction of the cord blood unit should we expand, and what fraction should we retain?

How do we characterize what we have expanded? We heard a little bit about that this morning. Should we be using CD34? Is that good enough alone, or should we be using it as part of a more comprehensive panel? When should we use colony assays? Should we have criteria for releasing the product after expansion? What kind of testing should be done? Is it good enough to do sterility? Should we be doing mycoplasmas, endotoxins, etc.?

In terms of clinical studies, is it sufficient to look at engraftment times of neutrophils and platelets? How should we monitor adverse events, and how should we consider an adverse event in the context of a cord blood transplant? And, what has been the eventual clinical outcome of using expanded cell populations?

What I have done is kind of summarized those as both the technical and potentially the clinical issues, but I think also perhaps in the discussion we can address issues related to the regulatory side of cord blood. We are talking about expanding cells when, in general, we have very few media, or reagents, or pieces of equipment that are approved to do such procedures, and also the financial issues.

Speakers this afternoon are Ian McNeice, from Denver, talking about expansion in bags; Joanne Kurtzberg, from Duke, talking about expansion in bioreactors; and Catherine Verfaille, from Minnesota, talking about stromal factors.

So without further ado, let me introduce the first speaker, Ian McNeice, who is going to talk about expansion in bags.

Expansion in Bags

DR. MCNEICE: I would like to thank the organizers for the opportunity to present our data today. What I would like to try to do is discuss some of the issues associated with expansion and then present some of our data on the clinical application of these expanded cells.

This first slide partly addresses one of the questions raised in the last discussion. This is taken

from units in our bank in Denver, where we have over 5500 units. Looking at the median cell number of 1 X 10^9 nucleated cells and then looking at different body weights, you can see within the bank as you get the larger patients, there become fewer products that are available -- which are applicable if you are after a minimum target of 1 X 10^7 cells/kg. So, if a patient is around 60-80 kg you are looking at less than half of these patients who would have a product available. Half of these cords would be appropriate for these patients. So, this certainly is one reason that we want to look at expansion. If we can increase the cell numbers, then potentially we can improve the outcome of these patients.

I think there are a number of different approaches people have proposed and are taking in terms of trying to do expansion. If we think about the types of cells involved in grafts and the application of those cells -- stem cells, the long-term engraftment would be targeted for gene therapy. In the context of cord blood, I think the data we have seen earlier today demonstrated that there are no late graft failures in any of these patients. So, there doesn't appear to be any issue with cord bloods containing enough stem cells to provide durable engraftment.

I think there is some question about whether stem cells actually contribute to short-term engraftment. My evaluation of the data is that there is no hard and fast data that convinces me that stem cells have any contribution to early engraftment. Maybe that is something we can discuss in the panel later.

But I think the focus we have taken is to look at progenitor cells and mature cells. Progenitor cells, theoretically, are going to give rise to the intermediate and short-term engraftment and mature cells are going to drive the short-term engraftment which, in my opinion, is certainly the issue we are trying to achieve with cord blood.

I think it is important to make a couple of comments in terms of cells that we are looking at to evaluate and how we go about this. This is a classic developmental tree that we have for hematopoiesis. CD34 cells represent a compartment up here. I don't think they are the cells that are going to give you the rapid recovery. The neutrophils are going to be here, in this area of development. The platelets will probably mature megakaryocytes, and then probably quite distinctly we need something in the lymphoid lineage here, maybe something back here after pre-T cells or something in this range, to try to develop all of these three lineages which I think

are important in terms of long-term outcome in these patients.

So, in terms of what we are trying to do, I think we need to look at the adult situation, and one of the areas that I think is very important to try to think about is what cells are really responsible for all of these different lineages and rapid engraftment.

For PDPC products I still don't think we have a clear definition, even after all the transplants we have done, which of these cell populations truly can give you the rapid engraftment or how many of those populations we have. So, I think this is, even today, a major question that we have very little understanding of, and which certainly needs a lot of attention paid to.

I put this in just to make a point about CD34. This is in breast cancer patients, looking at the dose of CD34 and the correlation or the relationship to time to platelet recovery, and this has been reported by a number of groups where there is this relationship at low numbers where there appears to be a relationship towards the number of 34 cells, and there is a threshold of around about 2-3 million, and even up to 30-40 million CD34 cells infused you get no faster engraftment.

So, although the level of CD34 can give you an understanding of the potential of that product, it

certainly does not predict for how that product will behave. You can see down here, even some products that have less than 1 million CD34 cells/kg give very rapid platelet engraftment. So, although it does give you an indication, it is certainly not a direct correlation and I think we have to be very careful in how we use these types of endpoints in evaluating their potential in a graft.

Adrian went through a number of the questions and issues associated with doing expansion. The approach that we have taken is to try to have a system which uses components which meet GMP quality, including growth factors, media and culture vessels. Our system does not have any animal products. We need to CD34 enrich, which is certainly somewhat of a task associated with cord blood. You are dealing with frozen products. It would be nice if there were selection systems that were available for cord blood, seeing that there are such few cells that the clinical systems are limited, and we do incur significant losses of CD34 cells during the selection of the cords.

Another big problem I think which is becoming even more of an issue is the lack of interest in industry in terms of supporting these studies. So, it is becoming a bigger burden, I think, on the individual institutes to try to run these studies, and this is very important in terms of the FDA in considering a lot of the requirements they

would like to have. A lot of these just become cost prohibitive when we have to support all these studies entirely within an institute.

So, moving on to the expansion and the conditions that we have developed, CD34 cells are selected using the Isolex 300i. The total cells from a selection are cultured in a 1 L bag in 800 ml. So, in general, there are about 1-2 million cells that are recovered after selection. The cocktail we use of growth factors is stem cell factor, G-CSF and MD-DF. These are all at 100 ng/ml. These are steady cultures so there is no washing or refeeding. The cells are cultured for 10 days. The medium we use is a fully defined medium that was developed when I was at Amgen. The culture bags are 1 L Teflon bags which are purchased from American Fluoroseal.

This is a study schema. There are two cohorts that we enroll patients onto, depending how the products are frozen. This is the first cohort where the products are frozen as a single unit. On day zero the product is thawed and 60 percent is given directly to the patient, then the other 40 percent is selected, expanded in the conditions I have described and the expanded cells are given back on day plus-10. G-CSF is given from day 0 through to neutrophil recovery.

We have now progressed into giving back 40 percent unexpanded and 60 percent expanded, and the data is very similar between the two groups so I won't break it up into the different levels.

The second stratum is products which are frozen in aliquots. In this situation we can thaw part of the product on day minus-10. It is put into the expansion conditions. On day 0 the patient receives both the expanded and the unexpanded, and once again G-CSF is given until neutrophil recovery.

We have actually transplanted 37 patients to date. I haven't had a chance to update these slides. But I will present the data for the first 31 and the data is very consistent for the other patients as well. And, 23/31 were adults and 8 pediatric patients; 11 male, 17 female. The median weight was 79 kg, and the range is shown here. The median age was 49 years.

This slide shows the malignancies of the patients, primarily leukemias and three breast cancer patients. These are the sources of the cord units that were expanded -- New York Blood Center, 7 products; St. Louis, 9 products; a couple from Sydney. These are products that were in one fraction as I described in the schema. The two fractions were products from our own bank. All our products are frozen in aliquots so that we have the

option of enrolling under this cohort, and one of the products was frozen from aliquots from Dusseldorf.

This is the HLA matching. One of the patients was 6/6; 18, 5/6; and 4 of the patients had a 4/6 match. Looking at the expansion conditions, this is looking at total nucleated cells. There was a median of 111-fold expansion of total nucleated cells. The range is shown here. The total nucleated cells infused -- the median was 1.8×10^7, and you can see the range here with the number of patients receiving well below 1×10^7/kg.

Looking at CD34 in the expansion conditions, we attained about 4.5-fold expansion in the range shown here. CD34 cells infused were 1.4×10^5/kg. This is just breaking down into the total cells reinfused in terms of the unexpanded and expanded component. Once again, the median total cells infused was 1.8.

This is engraftment data. We have now had 31 patients evaluable for neutrophil engraftment. The median has stayed at 26 days. Of those 31, 30 patients engrafted neutrophils within 60 days. The other patient had extensive disease, progression of disease in the marrow and actually had an autologous product given back, and that failed to engraft also. So, the majority or virtually all of our patients are attaining neutrophil recovery by day 60.

Platelet engraftment, the median was day 59 with a broad range, as reported by others. We looked at chimerism and all patients had dinochimerism that have been evaluated so far, and this was 99 percent on the median.

This is taken from The New England Journal article by Eileen Gluckman. They looked at their patients for neutrophil engraftment at 60 days, and in the larger patients that they reported in this group only 11/23 patients had actually engrafted neutrophils by day 60. This possibly is one application where expanded cells may have a contribution. I think in some of the data being presented here and reported in the literature, there are some patients who fail to engraft neutrophils and perhaps that is one area where we could apply expanded cells to try to have all patients achieving neutrophil engraftment within the 60 days.

This is the current status of the patients in this study. Nine of the patients are now alive without disease, and I think there are now some patients up to three years post-transplant. Four patients died of relapse and eight patients died of non-relapse causes.

This is looking at the non-relapse mortality. Two patients died of GVH. One patient died of acute GVH. The incidence of GVHD -- of 32 evaluable patients, 16 had a score of 0, 8 in the range of 1-2, and 8 patients in the

range of 3-4. So, there was 50 percent of the patients who had acute GVH. In chronic GVH there were also 32 patients evaluable, and 24 of the patients had no chronic GVH; 1 patient had limited GVH; and 7 patients had extensive GVH. So, 22 percent of the patients had extensive, and 1 patient also died of chronic GVH.

To conclude the clinical part of this, data shows that it is feasible to select and expand. We have looked at both 40 percent fractions and 60 percent fractions of cord units. No toxicity was associated with the expanded products. Whether the expanded products actually have an ability to improve engraftment, I think we certainly don't have any convincing data to date that there is a major contribution.

I think there is a need to try to understand what cells we really need to expand, and I would like to tell you about some of our preclinical work where I think we at least have some theories in terms of the types of cells we would like to progress to.

This is just looking at correlations between the number of 34 cells and time to engraftment, and there certainly is not a significant correlation. We don't see a significant correlation either in terms of the total nucleated cells infused to the time to neutrophil recovery and, once again, this is very low, 0.15. So, certainly do

date none of these parameters are measuring in this group of patients exactly what cells are able to provide neutrophil engraftment.

This is looking at the morphology of the cells. I would like to point out that the myeloid cells are somewhat immature. I will show you in a minute a comparison to expanded peripheral blood cells which have much more mature looking neutrophil elements.

This is immunohistochemistry staining for megakaryocytes. We do have extensive numbers of megs and exactly why we are not getting an impact on platelet recovery may be due to the fact that we are expanding these cells in growth factors, reinfusing them, and then they lack growth factors and may not mature as we would like them to. So, one of the things we are interested in exploring is the use of thrombopoietin post-transplant with the cells as well.

Just quickly, I wanted to touch on a PPC expansion study that we have done. The culture conditions were exactly the same. This was CD34 selected cells. In this study, what we are able to show is when we compared to control patients, the median time to neutrophil recovery with these PPC products was around nine days. The expanded products gave us a shift to the left with one patient engrafting on day 4 and significant numbers of patients on

days 5 and 6, which we have never seen in any patients before, suggesting that the expanded PPC products can impact time to neutrophil recovery.

When we have looked at analysis of the cells and what correlates -- this is looking at CD34 cells. There is an r-squared of 0.25. So, CD34 cells don't correlate with time to engraftment. The only parameter we found that correlated with an r-squared of 0.77 is total nucleated cells. So, all patients that received more than 40 million expanded cells had very rapid engraftment.

This is expanded PPC. You can see that there are much more mature looking neutrophil elements. So what we set about to do is to come up with culture conditions that might try to mirror the levels of the cells that we had in the expanded PPC with cord blood. So, we developed this two-step culture system. The selected cells are put into 100 ml Teflon bags; incubated for 7 days; transferred directly without any washing, and this can be done in a sterile way with sterile docking into a 1 l bag for a further 7 days, and now the cells are harvested on day 14.

What we found is that comparing to the 10-day conditions we get about a 4- or 5-fold increase in the total nucleated cells that are expanded. This has been repeated in about 16 clinical scale experiments.

This is looking at the day-10 cells from expansion, and it is consistent with what I showed you earlier. This is now the type of product we get with the 14-day culture. So, we think this is more typical of what we have seen in expanded peripheral blood. So, we feel that this may be able to help hasten the time to neutrophil engraftment.

Just quickly, we get increased expansion of committed progenitors and also of more primitive progenitors, the high proliferative potential CFC compared to the 10-day cultures.

We looked at CD34 numbers where, in these conditions, we are now obtaining a median of 29-fold expansion of CD34 cells, once again, about 4 or 5 times higher than what we are getting in the single step. So, the increased total cell numbers generated in these two steps -- there is increased committed and primitive progenitors, and the cell product is a more mature cell product when it is generated with the 2-step conditions compared to the 10-day single step. We plan to use these conditions in our clinical protocols, and have submitted the proposal to the FDA, and are interested in progressing with this procedure.

So, I would just like to finish off in terms of trying to think about, once again, what we need for

engraftment in these patients. There are clearly three parameters, the early engraftment, the intermediate engraftment and the long-term engraftment. I think these parameters are certainly covered already with unexpanded cord blood, and I think we need to at least generate this population of cells and then provide very rapid and durable long-term engraftment.

I will finish there and I would just like to thank my collaborators. E.J. Shpall heads the clinical group in the cord blood work, people in the clinical lab and Amgen and Excel for supplying reagents. Thank you.

[Applause]

DR. GEE: As you heard, that was expansion in static culture in Teflon bags. We are now going to switch to Dr. Joanne Kurtzberg, from Duke, talking about expansion in bioreactors.

Expansion in a Bioreactor

DR. KURTZBERG: If I can have the first slide, please? I am going to tell you about the results of a Phase I study that we performed in patients receiving partially expanded cord blood, augmenting on manipulated cord blood, but I wanted to go through a few principles before I get to the study results.

As far as the study goes though, the cohort of patients we transplanted were identical to the cohort that

John presented this morning, although not included in that cohort, and it was a mixed group of patients with malignancies and non-malignant conditions. All but two were children, and out of 28 patients there was one related donor and the rest were unrelated donors, with units all coming from the New York Blood Center.

I just want to remind you, and you have seen this many times, but the cell doses that we are giving for cord blood transplants are all a log lower than what we give for bone marrow transplants. This is all CD34 data from Duke and Minnesota. It looks like there may be a threshold effect of CD34 dosing, again, not paying as much attention to the actual number as to the fact that there is a difference between the curves, and that perhaps CD34 dosing may serve as some functional endpoint for targeting expansion in culture and then looking to see if there is any correlation with the clinical response.

This is data put together by EMMES, initially presented at ASBMT and then updated as ISAAGE. But, if we look at cord blood collections and we try to think about what influences the total nucleated cell count, you can see that the two things that influence how many cells we collect are volume and then ethnicity with African-American donors donating per volume less cells. Without going into that in great detail, that may be because of margination of

leukocytes along blood vessels, but it is just an interesting aside.

But even factoring in these things, there is not an easy way that we can increase the number of cells that we are collecting more than 10 or maybe 15 percent, and that may be optimistic as well. So, we are not going to increase cell doses from individual cord blood units without some manipulation.

Again just to remind you, this is data presented at ISAAGE also from COBLT, but you can see that the median number of nucleated cells in a post-process unit is about 800 million. I don't think I put the slide in, but the median number of CD34s is about 2.6 million.

We alluded to this, this morning saying in the COBLT bank how many units would be available to provide a cell dose of X to a patient of weight X. So, if you look at an 80 kg individual, and this is 0-5 X 10^7 cells/kg, you can see that there is a small proportion of units that can provide an 80 kg person that kind of cell dose. If you get to larger patients it is even less yielding.

This is another way to present the same data and it just shows you that patients greater than 80 kg -- that 66 percent are going to find a unit that is delivering less than 1 X 10^7 cells/kg, and only 0.3 can find a unit that would deliver more than 3 X 10^7 cells/kg, again illustrating

that for adults, if we think that we have to achieve cell doses in these ranges, we are not going to do it with individual manipulated units.

Again to remind you, in multiple regression analysis of the Duke-Minnesota data, cell dose expressed as CD34 and age were the most important parameters influencing alternate outcome and often non-relapse mortality.

When we looked at the reasons why people were dying, and I alluded to this, this morning, the biggest reason for death is infection, and this is equally divided between fungal, bacterial and viral infections and we don't know exactly why this is. I want to stress that these infections are not just occurring in people who have delayed engraftment. These are infections that are occurring after engraftment when the neutrophil count is over 500.

I think it is important to mention that in children versus adults there are different approaches to supportive care. I am not suggesting this is the entire explanation, but I think that one explanation for the improved outcomes in children may be that the supportive care is more aggressive. There is a little bit less pressure to get kids out of the hospital as quickly as adults. Some of the managed care issues are a little bit less intense or can be gotten around a little bit more

easily in children. Children don't take medicines by mouth as willingly as adults do. So, they often stay on IV prophylaxis with various antibiotics or antifungals longer than an adult might.

I know in our own center the children stay in the hospital longer. They stay on IV therapy longer, and their overall level of supportive care is higher. In our center, if a child comes in with a history of a fungal infection they get supported with G-mobilized irradiated granulocytes that are harvested from their parents through their entire aplasia and until their ANC is 10,000. That is not something that adults are doing, and it wouldn't even be as easy to do in an adult because a parent can donate a granulocyte collection after a single dose of G 12 hours earlier, and that will keep a young child's white count above -- somewhere in the 0,5 to 0.8 range for 3 days. We split if over 3 days. Whereas, that same number of granulocytes in an adult body won't last as long and the numbers really may or may not reflect protection but that is a difference.

This is data that Nelson Chow put together looking at immune reconstitution. It is a little bit of a complicated slide but I want to take you through it. When we look at immune reconstitution, and this is looking at growth of CD45RA cells versus RO cells, and these curves

are parallelled with CD4 recovery, PHA recovery, other mitogenic responses and T-cell responses to antigens. What you see in children -- this is 12 months, 24 months and 36 months, is that somewhere between 9 and 12 months all of these things correct and this is also the time that GVH prophylaxis is being weaned so that the average child is coming off of cyclosporine and all steroids by that time. But, at 12 months they generally have a healthy number of lymphocytes, a healthy number of CD4 cells, good PHA responses and a high number of CD45RA cells, which we don't have a complete explanation for but which persist as many years as we have been able to follow it.

Likewise, in the pediatric population when you look at TREC formation, these are a reflection of thymically educated cells that have recently emigrated from the thymus, you can see high levels and normal levels of these cells and, in fact, we see them coming up between 9 and 12 months.

In contrast, when you look at the adults -- and again this is the same time frame so 12 months, 24 months, 36 months -- you see that the immune responses remain flat for up to two years. This, again, is parallelled by low lymphocyte counts, low CD4 counts, low PHA responses, poor T-cell proliferation to antigens, and low numbers of TRECs.

I think that this may impact the non-relapse mortality that we are seeing in the first 100 days, as well as neutrophil count and that we shouldn't forget about this because the strategies to support the immune system may be very different than the strategies we would use to grow neutrophils or megakaryocytes or platelet and myeloid precursors, and there may be some cytokines that can be helpful here that are produced by a pediatric thymus and not produced by an adult thymus.

So, I just raise this as another avenue to pursue. We may have ex vivo expansion strategies that are actually compartmentalized, where one fraction of cells goes to make platelet precursors, another fraction may go to make myeloid precursors, and a third fraction may go to grow lymphoid precursors. We haven't had a chance to look yet but we wonder if this is impacted by stem cell dosing, but we don't know.

In my talk before I presented the same thing. The only point that I want to make is that I think we don't know how to define stem cells and we would use different strategies to expand stem cells compared to progenitor cells. The focus of the ex vivo trial that we did was to expand progenitor cells, hoping to decrease engraftment time which would have a practical impact in that it would

decrease resource utilization and it would also, hopefully, decrease non-relapse mortality.

We worked on a collaborative study with Aastrom which had done some preclinical work using their bioreactor to look at a cocktail that included flit-3 ligand, low dose erythropoietin and pixy in media that contained fetal calf and horse serum to expand cord blood cells. In their average conditions they were getting about a 3-fold expansion of total nucleated cells, 150-200-fold expansion of CFU-GMs, no expansion of CD34 lineage negative cells -- just kind of an equal output, a 4-fold expansion of myeloid cells and no expansion of lymphoid cells.

This is just a picture of the bioreactor or the cassette that goes into the bioreactor. It is sterile and there is an individual cassette used for each patient on the trial. No one has had to pay for these cassettes but they are going to be expensive, I am sure. Media goes here and perfuses through a control pump over the cells which are laid out on this membrane, and there is also gas exchange which is controlled, however you set it, by a computer system. The average expansions have been 12 days, but the bioreactor could be programmed at any gas concentration, any temperature and any perfusion rate.

This is just a picture of what the system looks like. The cassettes, now filled with media, go into this

chamber that kind of looks like a microwave, and there is a cold side and a warm side, and the gases are in back, and the computer controls the whole thing. So, it is a closed system. The techs don't mind using it. You kind of load it and it does its own monitoring. There are ports to take cultures out and things like that, but it does its own thing for 12 days and then you harvest.

The trial that we did -- and we really conducted this from 1998 to 1999, was that we took cord blood recipients and in the adult population they had to have a unit that delivered more than 1 X 10^7 cells/kg and in the pediatric population more than 3 X 10^7 cells/kg, and all, but the one patient who received the directed donor unit which was harvested and frozen at our center, all the other patients had older units from New York Blood Center which were frozen in single bags. So, we did not have the option to do pre-transplant expansion. On day 0, which was the day of transplant, after a standard preparative regimen which John talked about this morning, the unit was harvested, washed in dextrase albumin which is the method that Dr. Rubinstein developed and that we use routinely, and the roughly 1-2 X 10^7 cells/kg was given as an unmanipulated standard graft and the remaining cells were put into the chamber and expanded. The number of chambers that were used depended on the number of cells that were

available. So, in a few very small infants on the trial we were able to do two chambers. We put up to 400 million cells per chamber. But in most patients it was one chamber. We targeted 150 million cells per chamber but we had 3 patients who were larger and we didn't have that many cells and we actually put as few as 40-80 million cells in the chamber.

The patient got transplanted in the standard way, got the standard post-transplant care and GVH prophylaxis. Then, on day plus-12 the cells were harvested from the bioreactor. Whatever was available was infused into the central catheter of the recipient without any other change in the transplant regimen. Then we looked at the usual endpoints of recovery with platelet and ANC recovery, number of infections and overall event-free survival.

This just shows you the expansion numbers. The median cell dose was 2×10^7 cells/kg. We gave an additional median cell dose of 2×10^7 expanded cells for a total of 4. The median CFU dose was 0.35×10^4 from the unexpanded. We increased that significantly so that the total dose was 97×10^4 and the median CD34 dose did increase a little bit with the expanded cells.

When we looked at recovery we saw no differences between recovery without expansion and with expansion. These just give you the days to recovery, which are really

very similar to what we saw in patients receiving conventional grafts.

These are Kaplan-Meier plots looking at probability. This is to ANC 500, and one curve is a 73-patient cohort who got 2 or less X 10^7 cells/kg of traditional graft, and they were historical look and prospective controls, and then the study patients, and the curves overlap.

This is platelet recovery and you can see that that overlaps too. So, we found actually no effect on recovery of a cell number.

But we did see effect on overall event-free survival, as well as 100-day survival, and I can't explain it. It has us intrigued but it makes me think that there may be something to doing this and that we ought to pursue it and refine the conditions that we expand in.

Now, this just basically says that by giving a higher dose of CFU-GM maybe we have had an impact on overall event-free survival but we don't know and it should be tested. We had a randomized trial, approved by the FDA, but then there were financial issues and it could not be initiated. We are about to go back and initiate another trial.

Let me just end by showing you some of the in vitro work that we are doing now to look at optimizing

conditions. Again, the obstacles here have not been the background work or the knowledge of different cytokines to use, but the obstacles here have been that we can't get the different pharmaceutical companies to cooperate with each other because of protective interests in developing products. It would be very nice, from the point of view of us, if the FDA could do something to have sort of a limbo period where things could be tested and piloted, and then if they had promise they could be taken forward before anybody made a major commitment to developing a product.

This just shows you, in red, the Aastrom control media, and this is expansion of cord blood cells by fold-increase and just total nucleated cells, but CD34 and CFU-C as well as LTC-IC all parallel. You can see in control conditions what we get, and then this is if we add stem cell factor but no stroma to the Aastrom cocktail. This is if we add placental adherent cells, and these are placental donor cells from the mother of the cord blood donor. So, this is the Aastrom condition with placenta; Aastrom condition with placenta and stem cell factor. This is the same thing with two different densities. Bone marrow shows that we get improved expansion just with stem cell factor, but even more when we add stroma and stem cell factor.

One strategy we are also considering is to take the patient's own marrow, expand it in the bioreactor

before we actually put the cord blood cells in, irradiate it at day 0 and then add the cord blood cells with whatever growth factors we can get our hands on and then look and see if we have improved expansion as a result of that.

I know my time is up, so let me just switch to my acknowledgment slide. This is our longest survivor of unrelated cord blood transplant. He was called a 4/6 but he actually received a 3/6 graft and he is 7 years out now, going into third grade and doing well.

Then, I want to acknowledge many of the people who impacted this work -- our laboratory staff who worked with Aastrom to do the expansions, the group at the placental blood program in New York who provided all the units, and then the group at Aastrom and MedSep who provided the bags, etc. I will stop there.

[Applause]

DR. GEE: I wanted to thank Joanne for that nice segway in her final slides into stromal factors. This is a whole area that we are becoming a little bit more familiar with about unknown factors and unknown cell types that can influence outcome, and here to talk about stromal factors, from the University of Minnesota, is Dr. Catherine Verfaille.

Stromal Factors

DR. VERFAILLE: Thank you. At the University of Minnesota we have been interested in stromal factors and how they may influence expansion of cord blood. We have focused on two questions, not the middle one here; that is an old slide. But, we have essentially asked the question whether we could expand hemopoietic stem cells as well as progenitor cells, but focusing much more on stem cells rather than progenitor cells, as well as can we then at the same time also genetically alter these cells for two reasons: One of them is in the allogeneic transplant setting to use this approach to just mark the cells and actually ask the question whether expansion truly helps long-term as well as short-term engraftment, or, in the autologous transplant setting, to genetically correct cord blood cells from patients with inborn errors of disease.

So, in contrast to the previous two speakers, we have really focused on trying to get at a maintenance or expansion of stem cells. I know that in the later sessions the definition of stem cells will be approached. We have used a number of different assays to try to address that, and I will get back to that in just a minute.

The stromal feeder that we have used -- we used to use adult bone marrow stroma but because of the high variability between different stromal feeders we have really focused essentially on the stromal feeder line made

by Ijo Lemichka, called AFT024, and you may well be able to use other feeders like MS5 or even S17 which may have the same effect as what we showed with AFT024, though we haven't rigorously tested that.

This feeder layer is derived from murine fetal liver, from mice that are SV40 transformed, and which is temperature sensitive. So, the cell line essentially goes indefinitely but there is obviously a problem with using this in a clinical setting since it is a transformed cell line.

Terry Moor and his group have shown that this AFT024 supports murine hemopoietic stem cells tested in a competitive repopulation assay for at least 6 weeks ex vivo, and they are in the process of trying to define which factors are important.

Studies from the group in Minnesota, including Jeff Miller and myself, as well as Gay Cook, have shown that this AFT024 fetus supports human cord blood, bone marrow and peripheral blood long-term culture initiating cells as one measure of primitive myeloid cells, natural killer initiating cells, which is actually missing from this list, as a measure for lymphoid progenitor cells capable of differentiating into B cells and NK cells. I will show you just a brief little bit of data on myeloid lymphoid initiating cells defined in our laboratory, and

Gay Cook showed that extended long-term culture initiating cells are also very well supported by this feeder. And, I will also show you data that this feeder supports SCID repopulating cells as well as sheep repopulating cells.

We have been able to show that this can be done in contact and in non-contact, which gets closer to a potentially clinically applicable system since the feeder layer would not be in the same compartment as the hemopoietic cells.

AFT024 makes a number of cytokines, although not that much. It makes quite a bit of IL-6, G-CSF, three chemokines, stem cell factor and a very large amount of vascular endothelial growth factor. It also makes 6 O-sulfated glycoproteins, aminoglycans, and our group has actually shown that this is very important in supporting primitive hemopoietic cells LTC-IC. So we have actually tried to combine all these factors and tried to replace stroma with these factors, and I will show you data on that.

In addition, there are a number of factors made by this feeder layer in a context that would be available to the cells when they are cultured in contact. Whether these are important isn't clear to me since we can actually do most of our studies and have the same results when we

culture cells in a non-contact system, meaning in a transwell above AFT024.

So, to just show you two little pieces of non-preclinical data, this is essentially that we asked whether the AFT024 culture system, in a non-contact system, would allow divisions of primitive cells like MO-IC which would be acquired for gene transfer. Essentially, we plate 34-plus/minus cells in a transwell above AFT024 with cocktails of cytokine, FCF and IL-7 with and without GCF. Then we sort out 34-plus/minus cells that have divided, and we can say so because we label these with PKH26 or CF8C, and ask the question how much primitive myeloid lymphoid initiating cells are still present in here.

You can see that we essentially maintain the cells, but these cells have undergone at least three or four cell divisions, which is one of the things that we wanted to show to demonstrate that we can then use this system to genetically modify the cells using murine retroviral vectors.

We have actually shown that plating CD34 positive cord blood cells in an AFT024 non-contact culture system supports SCID repopulating cells. The way we did this, we did limiting dilutions of uncultured cells and cultured cells into NOD-SCID mice and actually measured the frequency of SCID repopulating cells.

You can see here, in blue, the unmanipulated cells; in orange, the 7-day expanded cells; and in green, the 14-day expanded cells and you can see that we can maintain SCID repopulating cells in this assay system. Ian then took the cells from primary mice and transplanted them to secondary mice to measure longer term repopulating cells. As is shown here, you can see again that we can transfer the cells whether they are from uncultured cord blood CD34 positive cells or CD34 positive cells cultured for 7 days and 14 days. In the mouse model we were not able to transfer them into tertiary recipients.

In collaboration, we have also tested this in a fetal sheep model, and shown here is unmanipulated cells, in blue, and 7-day cultured cells, in orange, and you can see that if we analyze the primary fetal sheep at 2 months or 6 months after transplantation we essentially maintain cells that can be populated in fetal sheep. This was then taken from the primary recipients and given to a secondary recipient, and even a tertiary recipient, and you can see that 7-day expanded cells persist and can actually engraft in the third fetal sheep, suggesting very strongly that long-term repopulating cells can be maintained in an AFT contact culture system.

So, for the next set of studies what we have actually focused on was not so much cytokines, as I

mentioned, that would expand CFC and 34 positive cells but really cytokines that would expand LTC-IC, NK-IC, and in certain instances we also have data for SCID repopulating cells. This is the non-contact system I described before, AFT024, using media with fetal calf serum and in general four cytokines, FCS, IL7 and thrombopoietin of MGDF. We then tried to come up with a system that would be clinically suitable. So, one of the things we did, we made AFT condition medium, and to do that, we took AFT fetus that were irradiated and conditioned them in the presence of horse serum and these cytokines. We collected the media and then added it three times per week to cultures with CD34 positive cord blood cells. We never washed the cells. We just kept adding fresh media, and actually doubled the media every two to three days, which would be clinically applicable although we haven't really tested this on a large scale.

The other culture we made is the stroma-free system in which we tried to replace this. Essentially, we added the 6-O-sulfated heparin which is a chemically defined agent that can be added in, and again this same mixture of cytokines. This media was frozen and then again added three times per week to the CD34 positive cell cultures.

The readouts were colony-forming cells, NK-IC or cells that give rise over a long period of time to natural killer cells, B cells, CD15 positive myeloid cells and also CD1A positives dendritic cells and SCID repopulating cells with secondary transfer. We actually didn't look very much at ML-IC.

These are all the same slides and I will just go through this first one in detail. Essentially what we tested is what the effect was on colony-forming cell expansion. The first three rows here are artificial condition medium without AFT024 conditioning, and four and five are medium conditioned by the AFT024 feeder. The different cytokines added are on the bottom here. Then, these are the cytokines that were added.

If you just focus on the first three versus the last two columns, at one week we see slightly improved expansion which is much more pronounced at week two. So, we get about 20-30-fold expansion of colony-forming cells in the absence of stroma-conditioned media, and it is actually significantly worse when stroma-conditioned media is added.

The same thing is true for LTC-IC and it is actually probably even more pronounced. If you look here, at one week, we get a two-fold expansion of LTC-IC but we get close to a six-fold expansion of LTC-IC in a stroma-

free system supplemented with all these growth factors. Thrombopoietin seems to be important and stem cell factors also seem to be important since there is a significant difference between column one and two, and two and three. Again, AFT02- conditioned medium did not perform nearly as well.

The same thing is true for NK-IC. Again, at week 1 we have about a 10-fold expansion of NK-IC which goes up to about a 20-fold expansion at week 2 and here again thrombopoietin seems to be important. FCS seems to be slightly less important but, again, media conditioned by AFT024 performed less well than the artificial media that we generated using 6-O-sulfated heparin.

We then tested whether these cells could be transplanted in the fetal sheep model, and this is the FT culture condition. These are what I showed you before, unmanipulated FS7 culture and FT culture, and you can see that we have maintenance of the CD34 positive cells that can engraft in NOD-SCID mice. Not shown here is that these can also be transferred to secondary NOD-SCID mice. We haven't tested this yet in the fetal sheep model.

The second question that we asked is whether this now would also allow us to retrovirally transfuse the cells, and in the AFT024 non-contact system CD34 positive cells are placed in a transwell, coated with fibronectin

and the retrovirus is essentially filtered through the transwell to allow increased contact between virus and the cells on days 3 and 4, each time for 6 hours, and for the other 18 hours the media is replaced by regular culture media.

Aside from this system, we also tried to use a more clinically feasible system, again either using AFT024-conditioned media or a stroma-free system, together with all these cytokines. In this particular instance we plated the cells in the bottom of wells so we do not have the added effect of filtering the retrovirus through the transwell. Again, we transduced the cells on days 3 and 4 of culture again for 6 hours. Here, we actually assessed the cells immediately following transduction and here we expanded the cells another 3 days to measure both expansion as well as transduction of committed and primitive progenitor cells.

This is not preclinical data. These are highly purified CD34 positive cells, CD33 and CD38 negative that we transduced into AFT024 non-contact culture system, and you can see a very high degree of retroviral transduction using GFP and AFT024 non-contact culture system.

If you measured the transduction frequency in myeloid lymphoid initiating cells, again we have a very, very high percent transduction, 74 percent of ML-IC

transfused with the GFP positive vector. If we test this in a mouse model and actually transplant these mice you can see that almost all the human cells in this particular mouse that was transplanted with GFP transduced cells were GFP positive. We are in the process of testing currently whether these cells can be transferred to secondary recipients and we don't really have data yet.

When we started looking at a more clinical situation we found some very interesting and yet to be explained results. Each time here for CFC, LTC-IC and NK-IC we show a fold-expansion of the progenitor population, percent transduction of the progenitor population and the yield of transduced progenitors by multiplying these two with one another.

As we have shown before, colony-forming cell expansion after seven days is relatively low in the AFT024 non-contact culture systems and is higher if you do it in a defined culture condition and, again, lower if you use AFT024 condition medium, shown here.

What is somewhat puzzling to us is the fact that the transduction frequency in this condition, where we get significant cell expansion, is extremely low, which is not true here where the expansion is actually not quite as high but the transduction frequency is better. That then leads actually to an equal yield of transduced cells. This is

true for all the cell populations we looked at. So, the LTC-IC fold-expansion here is similar between AFT024 non-contact and defined medium but, as the transduction efficiency again falls off it is better in AFT024 conditioned medium even though the expansion is poorer, which actually yields more transduced cells in AFT024 conditioned medium than in the defined culture conditioned medium.

The same is true for NK-IC -- again, better expansion, poorer transduction, equivalent yield of transduced progenitors.

Now, where does this leave us? And, the question is do we actually need stroma? What we have shown is that SCID repopulating cells are preserved equally well in AFT024 non-contact cultures as in stroma-free cultures if we add the 6-O-sulfated heparin and the cytokines I showed you. Whether we need every single cytokine on that list we don't really know at this point in time and we will go back and test that.

Likewise, CFC, LTC-IC and NI-IC are expanded equally well in non-contact cultures as in stroma-free cultures with 6-O-sulfated heparin and cytokines. But, interestingly enough, when we used AFT024 conditioned medium the expansion of all these progenitors is actually significantly poorer.

As far as transduction, we have shown that transduction AFT024 non-contact cultures yield significantly higher numbers of transduced CFC, LTC-IC and NK-IC than in AFT024 conditioned medium and, for sure, if we do it in stroma-free cultures. What I pointed out is that for the AFT024 non-contact culture we transduce in transwells since we actually increase the concentration of the retroviruses by filtering, and so we are going back and asking that question and the preliminary studies indicate that we can improve transduction by using transwells.

Using this stroma-free system, we added very high concentrations of proteoglycans and they may interfere with the interaction between the retrovirus and the hemopoietic cells, for sure, if it is based on a GLV pseudotype vector as we have been using. So, we are going to test VSGV pseudotyping and we are also going to titrate down the concentration of the glycans to see if that will improve transduction without losing maintenance of the progenitor cell populations.

So, I would say that at this point in time we may be able to replace stroma conditioned medium by a number of ingredients which may not all GMP at this point in time, and so we are working on trying to figure out if this can be done in a clinical setting.

What we are hoping to do is two sets of trials, and actually probably the first one that we will do will be on the adult side where we use two different cord bloods to test whether two cord bloods might help engraftment in patients for whom we don't have a sufficiently large first cord blood. Because these cells are spontaneously marked so there could be p differences between the two cords, we hope to be able to the following experiment: Use one cord blood unexpanded; expand the second cord blood and infuse them at the same time. This should allow us to measure early, as well as intermediate and late engraftment by the unmanipulated and manipulated cord in the same patient.

In children, if we can figure out how to improve retroviral transduction, what we hope to do is to take cord bloods and take 60 percent of the cord blood and transplant this in an unexpanded state and take one-third of the cord blood, expand it and retrovirally mark it, again with the question whether the expanded population will contribute to early as well as late engraftment in children. I will stop there Thanks.

[Applause]

Discussion: What Do We Need to Know?

DR. GEE: Could I ask Ian and John to join us? Do we have questions from the audience? Will you please identify yourself?

DR. KEANE-MOORE: I am Michele Keane-Moore, from the FDA. The question I had was for those of you who have used units of cord blood from more than one blood bank, could you tell me if you have seen any difference in the expansion capability of the cells or the resulting populations or, in the absence of that, would you speculate as to what quality of the unit you need in order to expand the relevant cell populations?

DR. MCNEICE: I don't think that we have identified any difference in the products. Obviously, for us the big question is if we can get them in aliquots that is certainly an advantage to be able to give the expanded cells on day 0.

I think in terms of the questions you are asking, like my comments in the presentation, if I knew what cell I really needed to expand then I could address that question much easier. I think that is one of the things that still needs quite a bit of work, to try to identify the target cell that is really going to provide rapid neutrophil recovery. I predict it is a different cell type that will give us rapid platelet recovery, and then a third population will give rapid immune recovery. But, until we identify those cells I think we are all guessing. We are trying to use what we know and I think making the best

guesses we can to date, but I don't think we have the answers.

DR. GEE: But in larger recipients, aren't you going to have to think about expanding the stem cell population?

DR. MCNEICE: In the peripheral blood expansion work we have given back just expanded PPC cells and, you know, they may have endogenous recovery but we have done some experiments NOD-SCID. The data shown on the two-step actually shows we are expanding primitive HPPC --

DR. GEE: So, you think in a single culture system for a large recipient you could achieve both progenitors and true stem cell expansion?

DR. MCNEICE: I don't think we are getting expansion of true stem cells, but once again I would come back to our clinical data that was presented earlier, suggesting that there is no issue with the stem cell number. So, I don't believe that we need to increase stem cell numbers. The products we are giving back today provide durable engraftment. Maybe I could put it back to the floor, does anyone know of any unmanipulated products that have resulted in late graft failure?

DR. VERFAILLE: I agree that right now the earlier graft is a problem and the patients that don't engraft early don't engraft at all. So, it is an

impossible currently to try to determine whether even if there is sufficient progenitor cells for early engraftment, whether there may be a small number of stem cells within the same cord that has a low number of early engraftment cells. So, I don't think we can actually answer the question. You know, in Minnesota we hope that we can answer the question by using marked cells whether it is in a double cord transplant or by using a single cord in which part of it is marked, which would allow us to tell whether the expanded cells contribute to early engraftment as well as to late engraftment, which currently is hard to determine.

DR. MCNEICE: Well, I think that partly comes back to our disagreement. I still don't believe that there is any data that suggests stem cells contribute to short-term engraftment. I would also say that in some of our patients who received well less than 1 X 10^7/kg, they are out almost 3 years now with no issues of losing their graft. So, at least to date, what we have evaluated what we are giving back is providing long-term engraftment.

DR. GEE: So, then is there a lower limit ?

DR. MCNEICE: I don't think we understand the quality of the cells to put a lower limit on anything until we can determine which cells are important for what, how do we quantitate how many of those we need?

DR. GEE: Yes. Joanne, did you have a comment?

DR. KURTZBERG: No, other than I agree with Ian. We are not seeing any late graft failures, although in the low cell dose group we have very few long-term survivors so that I don't know if we have adequately had a chance to look at that population from that perspective.

DR. RUBINSTEIN: That was really what I wanted to ask Ian. If we don't have late failures, could that be because only the ones that have enough stem cells have engrafted? I don't think the absence of late failures assures the lack of evidence that we don't have enough stem cells. It is kind of a circular reasoning. It is only true if you believe that you can engraft somebody without them.

DR. MCNEICE: Well, I think if you look at some of the data in the literature -- I know when we did some studies with SCF-mobilized VOPC we actually engrafted the patients early and then they lost the graft, and I think that has been described in other areas of transplant where you can have a transient early engraftment without durable engraftment and that has been shown in many mouse studies. So, my reading of that is that there are different populations that derive the very rapid, the intermediate and the long-term engraftment. So, I think if it was just a stem cell issue -- if we had a lack of stem cells, I

would expect that on some occasions we would see an early engraftment and then the graft would be lost, and my take on the data is that we are not seeing that.

DR. VERFAILLE: I agree with that but I think if you can't assess early engraftment it is also impossible to actually definitively say that there will be enough long-term repopulating cells. It may be both. There may be short-term repopulating cells as well as long-term repopulating cells, and giving only ex vivo expanded short-term repopulating cells may ultimately lead to loss of graft in the long-term.

DR. MCNEICE: Right, and that would be the only setting we can evaluate --

DR. VERFAILLE: Right.

DR. LASKY: Larry Lasky, from Columbus, Ohio. Given that we don't know what the best expansion technique is, some of us are storing cord bloods in 2 aliquots; some of us are storing them in the MedSep bags which are divided 20/80; and some of us are just storing them in one big lump. Is there a consensus among you about what we should be doing?

DR. KURTZBERG: Well, I will tell you our first choice was to store in two MedSep bags because it gave us almost every combination of percentages, but that proved to be too expensive because we would have needed double the

freezers and double the bags and we couldn't finance that. But that was our first choice. I think if we can expand, probably the 20/80 mix will be sufficient but it is a guess.

DR. MCNEICE: We freeze in 40 percent and 60 percent aliquots. So, we have had the option of doing 40/60.

DR. GEE: Tom?

DR. LANE: Lane, San Diego. I was fascinated with Joanne's expansion trial and particularly perhaps apparent difference in overall survival. I have just a couple of questions about that. One was, you mentioned that you weren't quite sure why there was this possible difference, but could you expand on that? Have you actually looked to see whether there might have been diminished infections?

The specific question I had is do you think immune recovery was affected by the expansion? The second question is, it looked to me like the control group actually had diminished survival compared to the other data you showed, but was that because there was low dose?

DR. KURTZBERG: Yes, I don't know if I can remember all those questions.

DR. LANE: Okay, I will ask them again.

DR. KURTZBERG: The answer to the third question is, yes, the control group is patients who got less than 2 X 10^7 cells/kg unmanipulated, the majority of which were done before that trial, but then we had a year follow-up because of the funding issues and we added on patients who were done in the subsequent year. At first I thought maybe it was just a learning curve and that we, as a center, were improving. Our survival had improved year by year and I thought maybe it was just an artifact of that. But then when we looked retrospectively at sort of 1999 compared to '97, '96, '95 we had the same data for that group of patients and the advantage to the people who received the augmented cells was still there. So, that made me believe that it might be more real. But I don't know why.

We had not, at that point, studied immune reconstitution in the first 100 days. We do at day 100 time point. We are now doing that and we are seeing differences. We have some flow-base techniques that allow us to look at lymphocyte counts and proliferation with very small numbers of cells. So, we are seeing differences between individual patients at day 60. But we didn't look at this cohort of patients so I can't say anything about their function before day 100.

We looked at the survival endpoint because we were hoping to find something that gave us some motivation

to continue, and we hoped that maybe we would see a survival advantage, but when we looked at numbers of infections or types of infections, they were not different. So, you know, I can't explain it on the basis of any of the routine things.

DR. LANE: Nor relapse?

DR. KURTZBERG: No, GVH rates, relapse rates, etc. were all the same. Two comments in that regard though, one, because we boost on day-plus-12, we weren't sure that we weren't overlapping the engraftment of the expanded cells with the engraftment of the unmanipulated cells. So, we couldn't really address that question in this particular study.

Second, we did have one adult who did very well early on and engrafted and was out about a year and nine months and then developed myelodysplastic syndrome with mono-77. He never came back to Duke so we don't really have validation that this was in donor cells. But there was one study done that said that this myelodysplasia was in donor cells, not in host cells that had relapsed. I think that raises a point of concern in terms of if we are stimulating all these cells and could we be inducing transformation.

DR. REEMS: Joanna Reems, Seattle, Washington. Catherine, could you tell me if you extended your culture

period and looked at SRC values with extended culture periods, instead of 2 weeks going out to 4 and 8 weeks?

DR. VERFAILLE: No, for the SRCs we only looked at the 2-week endpoint. For all the other data points we looked at longer time points and actually, you know, if you wait 5 weeks the expansion ability of CICs increases but we haven't really retested that for SRC except in a few animals where we looked at 4 weeks of expansion using the non-contact culture system and we still had SRC present, but we didn't extensively evaluate that by multiple limiting elutions and things like that.

DR. REEMS: Okay, thank you.

DR. LAUGHLIN: May Laughlin, Case Western. I had a question for Joanne Kurtzberg and Ian McNeice. Did you incorporate assessment of bone marrow somewhere around day 30 and, if so, did you see any differences histologically in patients receiving expanded grafts versus non-manipulated grafts, presence of megakaryocytes, differences in myeloid or erythroid engraftment?

DR. KURTZBERG: Yes, we did look at that and we did not see any differences, and we looked at CFU generation as well and didn't see any differences.

DR. MCNEICE: I need to defer to E.J.

DR. SHPALL: We don't see any real difference although we don't have a lot of [not at microphone; inaudible].

DR. GEE: One last question on this, is the practicality of doing this in a routine processing lab, I mean, is the proposal that one will keep these cells in culture? How are we going to do it for a specific time and get them into a recipient at the appropriate time? And, if we are not going to be able to do that kind of in real time, has anybody looked at the effect of cryopreservation on these cell types, particularly the more mature myeloid elements in terms of their survivability of the cryopreservation procedure?

DR. MCNEICE: We have looked preclinically at this issue and, clearly, if you refreeze you lose some mature neutrophil elements. They are going to fall apart. So, it is sort of defeating yourself if you want to take the approach that we are taking of trying to really drive the mature neutrophils, to refreeze those products you are basically defeating yourself.

DR. GEE: Do you have enough reproducibility in the culture conditions that you can say I need this number of cells on day 14 or day 12, and know that for the majority of recipients you are going to have something in that range?

DR. MCNEICE: I would come back again to the issue that we don't know what number we need. The products vary a lot. The expansions vary a lot. This is not a consistent thing where you can predict the number of cells you are going to have at the end because you get variable purities off the selection, you get various losses during the selection, and then in our hands we have found the purity of the selected population influences the amount of expansion we get. So, there is a whole lot of variables that are going to impact your output, and I don't think we are at the stage where we can control -- I am not sure we want to yet when we don't know what we want to do necessarily.

DR. KURTZBERG: I would echo that. We had a lot of variability between the level of expansion, and then when you factored in the size of the patient and the size of the unit and you wanted to aim for a certain cell dose per kilo, it was all over the place. And, I think in the clinical trial design of ex vivo expanded products that becomes a big difficulty unless you are expanding from a very small number of cells.

DR. GEE: So, probably the take-home message, or at least one of several of the take-home messages for this session is that we need more funding to do these kinds of studies --

[Laughter]

-- and we probably need some help with regulatory issues, as was referred to by all speakers, because of the complexity of getting these kinds of conditions together from different companies, from different groups in order to do these kinds of studies. Thank you.

[Applause]

[Brief recess]

Session 5: What is a Stem Cell?

Introduction and Overview

DR. BROXMEYER: If we can get started again, please? You can feel free to talk during my presentation but, please, don't talk during the rest of the presentations.

Basic laboratory research has gotten us pretty far. It was the basis for the first cord blood transplants and, hopefully, continued research in the laboratory will help us in the future to answer some of the questions that have been coming up over the past two days.

So, what we know is that you have a stem cell but you don't have a stem cell. What you have is a whole hierarchy of cells, from the earliest subsets of these cells with long-term marrow repopulating capability to cells which could still have some stem-ness but are not necessarily long-term marrow repopulating cells. And, we

know that these give rise to a whole bunch of progenitor cells.

So, what is a stem cell? A stem cell is not a CD34 positive cell. Okay? I just want to get that clear. That is only a marker which picks up a lot of different cells. So, what is a stem cell? It is the composite of all of its functions, and that is what we need to concentrate on. And, this is what a stem cell can do -- it can proliferate, differentiate; it can self-renew. But that is not enough because it has to move; it has to home to where it needs to be to get to the right environment to do what it has to do. That includes migration and probably chemotaxis directed movement. And, if the cell doesn't survive, it can't get anywhere and it can't proliferate. So, that is what we need to think about.

What I am going to do is focus on two of the areas that not many people talk about, and that is the movement -- migration, chemotaxis and the survival, and just give you some ideas of where I am coming from.

So, we know almost nothing about what allows a cell to get to the right environment and, in fact, most of the cells that one infuses probably don't get to the environment they need to get to in order to do what they are supposed to do, or what they can do under the right conditions.

So, think about this, if you could figure out how to take a stem cell, or stem cells, or progenitor cells and get them to more efficiently go to the correct microenvironment, you probably could get away with a lot less cells than you are infusing right now. I don't want to say that we don't have to ex vivo expand cells, but maybe we wouldn't if we knew enough about it. So, we certainly need work in the area of cell movement.

I also worry about the fact when we ex vivo expand cells that we are changing the homing characteristics, and I don't think anybody would disagree with what I have just said. We just don't know where we are going with this yet.

So, there is a group of molecules, and I am limiting myself to these molecules but it is not only these molecules that may be involved, called chemokines -- cehmo-attractant cytokines. There are now well over 60 of these that have been identified and they fall into four different categories based on their cysteine motifs. Chemokines have been known for a long time to chemo-attract leukocytes, to chemotax leukocytes, and these are some of the abbreviations for a number of chemokines that are known to chemo-attract different subsets of lymphocytes and for different inflammatory cells, including neutrophils, monocytes and NK cells.

So, we know very little about movement of hematopoietic stem and progenitor cells. Of the greater than 60 chemokines that have been identified, to my knowledge there are only 3 that so far have been shown to chemotax hematopoietic stem and/or progenitor cells. One of them is SDF1. It has a single receptor which is called CXCR4. You may know that receptor because it is a co-receptor for HIV. Also, CK-beta-11, which comes under a bunch of different names and now has been classified as CCL19, and another chemokine, SCL-CCL21. These two bind the same receptor, CCR7.

So, if you look at the migration of either bone marrow or cord blood CD34 positive cells and you use a chamber system which allows you to differentiate chemotaxis, directed movement, from chemokinesis, which is more random movement, you can show that SDF1, stromal-derived factor 1, has the capability of chemotaxing a high percentage of erythroid granulocyte macrophage multi-potential progenitors. Others have shown that chemotaxis LTC-IC -- and there is some evidence that it may be involved in the movement of the SCID repopulating cells, the human cells that will repopulate SCID mice.

So, this was done by a group before we got into the area, and the work I am showing you here is of a former graduate student of mine who is now doing his postdoc at

Stanford. In addition to following up on this work, he noticed that CK-beta-11 also chemotaxed CD34 positive cells but that a lower percent chemotaxed. It turned out that CD-beta-11 was mainly chemotaxing the granulocyte macrophage progenitors, and it became even more specific than that in that it was chemotaxing the macrophage colony-stimulating factor responsive cells. If you looked at the colonies that formed from the cells that chemotaxed the CK-beta-11, they were essentially all macrophages.

So, right now we have SDF1 which chemotaxes a multiple of early subsets of cells. We don't know yet if it really chemotaxes the long-term marrow repopulating cell but I think there is probably a reasonable chance that it does, and now we have two chemokines, CK-beta-11 and SLC, which bind the same receptor which chemotax pretty specifically macrophage progenitors. And, there may be other chemokines, not yet identified, that will chemotax other types of cells.

So, in order to deal with the biology of this, before Chang Kim left his graduate training because I wouldn't let him out unless he prepared the vectors for a number of transgenic mice, he prepared CK-beta-11 transgenics which were under an LCK promoter which then localized the expression of CK-beta-11 to lymphoid tissues, and that is shown here. This is expression of CK-beta-11

in the thymus and in spleen cells, not merely in bone marrow and not in liver.

What we looked at is the absolute numbers of progenitor cells in the transgenic animals, in orange, versus the control, in blue. What we see is that if you look at cells that are stimulated in vitro, after you take them out of the animal, with multiple cytokines which essentially is getting you the earlier, more immature subsets of the granulocyte macrophage, erythroid or multi-potential progenitor cells you have a decrease in absolute numbers in the transgenic animals of CFG-UM and an increase here that doesn't look too impressive, but when you break it down and look at the CFG-UM as the M-CSF or macrophage CSF responsive cells, the increase in the spleen is greater and you are not really seeing much of a change with the GMC-SF responsive cells. So, it appears to be macrophage colony-stimulating factor responsive.

Interestingly, if you look at the cycling of those progenitors, you will notice in the bone marrow the cells are essentially out of cycle, a slow and uncycling state -- these are the macrophage progenitors. In the spleen they are cycling very rapidly. Now, in the spleen of normal mice you don't see usually any cycling of progenitor cells of any type.

So, based on this information, and there may be other interpretations but right now this tissue specific expression of CK-beta-11 is coincident with enhanced levels of cycling macrophage progenitor cells in the spleen. So, we have tentatively concluded that this may be due to preferential attraction of cycling CFU-GM to the spleen, which would be very consistent with the in vitro data that we have shown you.

Now, we were also very interested in SDF1, not necessarily for its ability to chemotax cells but because we had some suggestions that it might act as a survival factor. So, in order to carry through on some studies we did where we preliminarily showed that we can take soluble SDF1 and prolong the survival of a lot of different types of progenitor cells, including the most immature cells or subsets within that category.

So, Chang made the vectors in order to produce transgenic mice which expressed SDF1 in pretty much all of the organs. It was under an RSV promoter. And, the first thing that we saw was that there was enhanced proliferation -- the blue is the transgenics -- of all the different types of progenitors in the bone marrow and in the spleen, but we have never seen SDF1 in vitro act as a stimulating molecule, as a synergistic co-stimulating molecule, as a

suppressor molecule. In fact, the only thing we have ever seen it do is chemotax progenitor cells.

So, what could explain this? We felt maybe it could be explained by the fact that SDF1 was acting as a survival factor for the cells. So, we set up an assessment of myeloid progenitor cell survival by plating cells in a semi-solid medium at time 0 in the absence or presence of SDF1, and then we added growth factors, a whole bunch of them, at either time 0, or we delayed the addition -- now, hematopoietic progenitor cells and probably stem cells die by apoptotic death if they don't have growth factors around. So, the survival is an indication of anti-apoptotic effects.

This shows you the CFU-GM using unseparated marrow of the SDF1 transgenics, in blue, surviving better than the control progenitor cells. This is after delayed growth factor addition either 24, 48 or 72 hours later.

This shows you after 24 hours an enhanced survival also of the erythroid and multi-potential progenitor cells when they come from the SDF transgenic mice.

This shows you that SDF1 itself can prolong survival of human progenitor cells. We have done these with unseparated cells. We have done them with very highly purified cells. So, SDF1 can act as a survival factor.

So, where do we stand? We have cells, hematopoietic stem cells that really must be defined by their function, and the better we know their function the better we can design more rational clinical trials, especially in my case the interest is in cord blood transplantation.

I would like to suggest that SDF1 and/or other chemokines potentially alone or in combination with other cytokines -- and we have lots of evidence for that which I haven't shown you -- may be the attractants that get the cells to home, when you put them in intravenously, to where they have to be, but that now has to be dissected experimentally.

I would also like to say that if you can find a way to enhance or prolong the survival of the stem and progenitors, you will do at least two things. It will probably enhance ex vivo expansion and probably enhance the ability to do gene transfer and potentially gene therapy. Thank you very much.

[Applause]

We are going to wait until the end for discussion, and our next speaker is going to be Esmail Zanjani, and he will be comparing umbilical cord blood with bone marrow stem cells, or whatever else he wants to talk about.

Comparing UCB and BM Stem Cells

DR. ZANJANI: Thank you very much. It is quite clear that what we need is an assay system that could predict what happens to human stem cells in a clinical setting.

We have been thinking that hematopoietic stem cells should provide a life-long supply of lymphohematopoeitic cells at adequate levels, under normal and occasional stress conditions in vivo, and basing our definition on this, we developed an assay system for human hematopoietic stem progenitor cells in the sheep.

What we do, we inject the cells of interest into sheep at about 60 days of gestation. The animals are pre-immune at the time. Three months later these animals are born and then we evaluate these animals after the injection at intervals for evidence of human cell activity. Please note that this is a large animal and, therefore, you can do long-term observations at multiple times over several years.

We also thought that the most important element in such an assay would be that the assay be able to distinguish between different classes of human hematopoietic stem cells, principally between those that have short-term activity -- these are the committed

progenitors -- versus those that provide life-long engraftment and production of hematopoietic stem cells.

So, the first thing we did was establish that our model, in fact, does distinguish between these different classes of stem cells. It is very important that this actually be achieved because there is a lot of evidence to suggest that committed progenitors could last a long time in vivo and in different animal models. For example, Weissman has shown, and others too, that in mice committed progenitors could survival for up to four months. In cats, a group in Seattle has shown they could last almost one to four years. If you were to relate it by the number of years these animals survive, as well as the size, how long would then human hematopoietic progenitors that are already committed -- how long would they survive? We have evidence that in the sheep the committed progenitors could last as long as one year.

And, how did we demonstrate that? We separated cells on the basis of CD34 expression which, as Hal said, isn't really a sufficient indication of whether this is a stem cell or not -- one has to look at it by function, and these were then separated on the basis of expression of CD38 or lack thereof.

When we transplanted these cells into the fetal sheep both produced multilineage hematopoietic cells of

human lineages, but when we looked at these animals over a long-term period we saw there was a significant difference in the engraftment of these two populations of cells. So, the cells that received 38 positive began to peter out at about one year after the transplant, whereas those animals that were transplanted 38 negative population continued to express hematopoietic cells over time. Actually, we followed those for years and they are still there.

Now, one can't really wait for all that time in order to evaluation whether a human stem cell candidate is a long-term or a short-term acting cell. One can take advantage of the fact that animal studies have shown another stem cell characteristic, its ability to engraft secondary recipients. So, one can use that to distinguish between the different populations. In this case, we again looked at the 38 positive versus negative ones and, as you can see, in the secondary recipients there is no engraftment of these 38 positive cells that were taken from primary animals transplanted with this cell population. So, committed progenitors can engraft primary recipients, usually for a shorter time period, and they do not transfer out into secondary recipients.

So, we have been trying to define the long-term human hematopoietic stem cells on the basis of their ability to engraft in primary recipients long-term, and

also be able to engraft secondary recipients, and we are now looking at tertiary animals as well. In all cases they should allow the production of all elements of blood, lymphoid and myeloid elements.

So, we have been using this now to evaluation hematopoietic stem cell candidates from a variety of sources and we have been doing this on a cell per cell basis. Here, for example, are results of 10,000 cells from CD34 positive, 38 negative cells from these different human sources -- fetal liver, cord blood, bone marrow and peripheral blood, and, as you can see, we followed them for a year and they all engraft. Actually, if we follow these even longer they would show the same level of engraftment over time, although the peripheral blood cells will begin to decline as you will see presently.

The difference here is that while we could transfer into secondary animals cells taken from primary sheep that were transplanted with fetal liver, cord blood, or human bone marrow, we could not do so with peripheral blood. Now, remember, this is on a cell per cell basis. We are transplanting limited numbers of cells into these animals.

This is interesting because we were able to confirm this not only for the 38 negative cell population, but in collaboration with Catherine Verfaille, with other

populations of CD34 positive and negative cells that we took from normal individuals who had been mobilized with DCSF, and actually compared also with the CD34 negative population. One of the things that was rather pleasing that we observed in the primary animals was that CD34 cells for mobilized peripheral blood, in fact, produced a higher level of differentiated products early after the transplant, but later it became basically equalized with the other cell populations that we had put there.

What was interesting was that neither the CD34 negative population nor the CD34 positive population from mobilized peripheral blood of normal individuals could engraft secondary recipients, and this, on a cell per cell basis, tells us there is a difference between these candidate stem cells with those that are present in the bone marrow.

We have been comparing cord blood with bone marrow. First I would like to show you some data in terms of limiting dilution studies. One we transplanted 100 CD34 positive/38 negative cells from normal human bone marrow, none of our animals engrafted. At about 200 cells, as you can see, about 25 percent or so of the animals show engraftment. Her is the data for the rest of it.

By contrast, even as few as 50 cells, with a phenotype of 34 positive, 38 negative cells, engraft a

significant number of the animals we transplant in. With 75 cells per fetus we can get almost 35 percent of the animals to show engraftment. In all cases the engraftments are multilinear. So, there is a significant difference between cord blood in terms of engraftment ability -- perhaps it has something to do with the chemokines, their ability to respond to chemokines, that Hal was talking about -- when you compare them to normal human bone marrow.

So, we did two series of cord blood studies. We studied single-unit collections, and these were done primarily in collaboration with Hal Broxmeyer, versus those that had been pooled.

Here are three animals transplanted with, again, limiting numbers. I believe these are 34,000 CD34 positive cells per animal. The three animals were followed over a three-year period, shown here -- actually, it is almost four. You can see that they maintain their engraftment over this period and there is this bump in the human cell activity in these animals after about one year of engraftment.

We did two series of studies with pooled collections. In the first one we pooled 11 separate collections together, isolated the 34 positive cells and injected about 80,000 cells per fetus. Here are the

results from 10 of the animals given these, and you can see they maintain them over the 3-year period.

When you compare the pooled units versus the single units, however, you see that the activity in these animals that were transplanted with single-unit cells, basically the same number of cells, were much higher than those that were given the pooled unit.

We did secondary transplants. In both cases, whether the secondary transplants were done after the primary animals had been engrafted for 12 months or 24 months, one can get transfer into the secondary recipients. This is the pooled unit, for example, after 24 months in the primary animals and, as you can see, this is a single unit. They show generally a little higher level of activity but it is not significantly different.

In the second study which gave us a little more information, we took a male and a female cord blood donor, with the number of CD34 positive and negative cells as shown, and we injected 10,000 cells from each of these that were combined into the same fetus. The results in the primary animals show -- and here we followed them for 18 months and we have subsequently looked at them even longer -- that there is significant activity that has been maintained over this period of time.

When we began to examine where this activity comes from, which donor is predominating or are both being represented over this 18-month period, the result was rather interesting. It turns out that nearly 100 percent of the activity at 18 months can be attributed to donor A, donor B having been basically lost, as you can see, over this time period.

We did secondary transplants at both 4 months after the cells being present in the primary animals and at 12 months later to see actually whether we can confirm what we are seeing in the primary animals, that is, secondary animals taken at 12 months should basically have only donor A type cells in them. This is, in fact, what shows up in both cases. So, only one of the two has persisted in the system.

Now, there is also a possibility that if one uses pooled preparations of cord blood cells that maybe one can induce tolerance in the recipient and the remaining material from the pool could be used to boost the system. We have been looking at that, especially in our in utero setting. As you can see, a significant number of the animals, about 51 percent or so, are tolerant of the original donor pool. There is a boost in the level of donor cell activity, as shown in these four animals, but ultimately the levels in some of them come down.

We have looked, again with Catherine Verfaille, at expanded cell populations. She showed you the data for day 7. We find that they do, in fact, persist in primary animals. We have never been able to show activity at day 14 that has persisted, actually with her material or anybody else's. At day 7 we find there is persistence of hematopoietic stem cells but not afterwards.

I am going to just end -- a couple of minutes are left to me -- by showing the ability of cord blood cells to produce human hepatocytes in the system much more than normal human bone marrow would do.

This is a normal human with this specific antibody to human hepatocytes. In close-up you can see the hepatocytes showing up, reacting with this antibody.

Here is a normal sheep liver, and you can see there is no interaction.

Here it is at a higher level. The antibody is highly specific for human hepatocytes in this system.

When you transplant human hematopoietic cells either from bone marrow or cord blood into these animals, there are these foci of human hepatocyte activity that you can see in a close-up here. These cells are clearly of human origin.

When you compare this to what happens in animals that have been transplanted with cord blood the result is

really impressive. Here is a low power shot of a fetal sheep liver that shows huge numbers of human hepatocytes, and these were transplanted with as few as 5000 to 10,000 cord blood CD34 positive lin-negative cells.

Here it is in close-up. This is the portal vein. You can see the area is just covered with these hepatic cells that are of human origin. Thank you.

[Applause]

DR. BROXMEYER: Our next speaker is Clay Smith, and Clay is going to talk about potential new markers, ADHase.

Potential New Markers: ADHase

DR. SMITH: It is going to be a little less exciting than seeing a liver developed from cord blood, but we have been looking for other markers for hematopoietic stem cells, other than CD34. A number of people have alluded to some of the problems with using CD34 and we have been looking for markers that may reflect some activities that are really intrinsic to stem cells, and one of these activities is aldehyde dehydrogenase. Aldehyde dehydrogenase is known to play a very important role in retinoid acid metabolism in many developing tissues, presumably including the hematopoietic system.

There have been a number of immunohistochemical and other studies that have shown that ALDH is expressed at

high levels in stem cells and various species and in various tissues, and we know from clinical studies that human hematopoietic stem cells have lots of ALDH because that is the enzyme that renders them resistant to purging with 4HC. Finally, Rich Jones, a few years ago had made a fluorescence substrate for ALDH which he showed could enrich somewhat murine long-term repopulating cells.

We were looking for a reagent that had spectral properties that would allow it to be used with other markers and would be suitable for human cells. So, we got together with Mike Colden at Duke, and he synthesized this compound which, coincidentally following Dr. Zanjani's talk, actually sounds like what a sheep sounds like, BAA, which is BODIPY amino acid aldehyde. What this is, is a BODIPY moiety which is a fluorescent compound, and it has been conjugated to acid aldehyde which is a substrate for aldehyde dehydrogenase. This is a non-polar compound so when you incubate this compound with cells that apparently freely diffuse into the cytoplasm, it is acted upon by ALDH and converted to a carboxylic acid which then, because of its negative charge, causes it to be trapped within the cytoplasm. So, the more ALDH activity you have, the more of this dye is accumulated.

One of the things we found out after some months of not quite understanding why this wasn't working quite as

well as we thought it was is that it turns out that these compounds, and many of the fluorescent dyes that we have used since then, get pumped out by MDR and other ABC type pumps. So, it turns out if you block this pump with verapamil or other inhibitors you can get a much better accumulation of this because many of these pumps are also expressed at very high levels in hematopoietic stem cells. So, while the ADLH is busy making this compound the MDR is just as busy pumping it out.

But if you block this and add this substrate and you stain cord blood -- this is an entire cord blood that was treated -- I believe this one has only been through hetastarch and nothing else, and it has been stained for 30 minutes in the presence of the BODIPY dye and verapamil, and if you look within this R1 gate, which are the small cells -- this is size on the Y axis and intensity of BODIPY staining on the X axis, and I am sorry, I didn't show the control here -- if you add an inhibitor of ALDH, called DAB, it abolishes this window. If you don't add the verapamil you don't see this very well. But if you sort this population to purity you find that it is anywhere from 70-90 percent enriched for CD34, bright; CD38, dim; lin-minus cells. It does have some 34-minus cells and I will come back to that in a few minutes.

Conversely, if you stain a cord blood the standard way with 34 and 38, and you sort those to purity and you stain them with a BODIPY dye you find that virtually all of the 34 positive/38 negative stain very brightly and are in this small window.

So, we have gone on to characterize this population pretty extensively, and I will just show you some of that data. One of the things that we noticed was that the brighter the cell stained with ALDH, the dimmer they are for CD38 and other activation markers like CD71, and sometimes the brighter they are on ALDH the brighter they are in CD34.

The other thing is that this ALDH bright population is very highly enriched for colony-forming units, for 5-week long-term culture activity units. Again, each of the bars represents the ALDH bright population that I just showed you, and they are quite enriched, particularly if you sort CD34 positive cells to purity for extended long-term culture activity, and this is thought to be a better assay of more primitive cells than the 5-week long-term culture assay.

In addition, these ALDH bright cells in cultures that are very similar to what Catherine Verfaille described earlier generate NK cells and other lymphoid cells that are very high frequency. This is just showing NK cells

generating from a very small number of ALDH bright cells and this is just a summary of some experiments. Occasionally we will see some B cells in these cultures as well.

They also engraft the small animal stem cell model du jour, the NOD-SCID mice very nicely. These are human cells. Like most of the time in the NOD-SCIDs, they have B cell markers but you can also see some islet markers -- again, very small numbers of these cells do very well in this assay.

So, it looks like BAA is a good substrate for ALDH. One of the advantages of this substrate is that because it is pumped out you can get rid of it from the cells very quickly by simply washing them. In 30 minutes to an hour, you no longer see any fluorescence. And, we haven't seen any toxicity with this dye, whatsoever, although certainly more studies need to be done. It very nicely enriches for 34 positive, 34-plus, 34-minus stem cells, lymphoid, progenitors, NON-SCID repopulating units, etc.

We have also got some very preliminary data that it lights up baboon bone marrow and that, hopefully, will be a nice model to test whether these are truly engrafting cells before we go on to see whether humans could be engrafted with these cells.

I would like to close with a couple of slides on the issue of are there some CD34-minus cells in cord blood that might be important to be enumerating and looking at for expansion as well. I think Dr. Zanjani just showed a little bit of data showing that some 34-minus cells can engraft in the SCID mice.

This slide just shows that these bright cells are heterogeneous with a large number of markers including Kit. Here you see some cells are Kit positive; some are Kit negative. The IL7 receptors, some express and some don't. Again, this is CD34 so you can see here, again, that there is a population of cells here that routinely show up that are negative for CD34 and most of those cells actually don't stain for other lineage commitment markers as well, and they do stain for this IL7 receptor which has been shown to be, at least in mice, present on a common lymphoid progenitor.

Because of this and other observations, we have been trying to characterize whether this population has progenitor-like activity. I don't have any data on that today but I would like to show you, just very quickly, some data on what looks like some lymphoid progenitor activity in another CD34-minus population which looks like it may end up being the same cells, but we are not quite done with those experiments yet.

These CD34-minus cells were got at by using another dye staining technique that was developed by Peggy Gidell, which is called the side population identification using staining with a Hoechst dye, 33342, and you look for emission in both blue and red and what Peggy showed is that in mice you get this nice, little small population sticking out here that she called the side population. When she sorted those to purity, all of the long-term reconstituting activity of the mouse bone marrow was localized here and very little of it was out in this other population.

So, we collaborated with them and looked at cord blood, and you can see a little bit of a side population. It is not quite as dramatic as the mouse. But we went on and looked very extensively within that side population and initially found that a lot of the cells that were mature B-cells, T-cells and then K-cells. But if you lineage-deplete those you end up with lin-minus cord blood. You get about a 20-fold enrichment for the SP population, and if you look specifically within that you will find that there is a kind of classic looking 34 positive/38 negative population, and like with the ALDH, you see this CD34 negative/CD38 negative population. This stains for virtually nothing between CD1 and 150, other than a number of different adhesion markers, except for CD7, for the most

part. There is a CD7 negative/34 minus population here as well.

Because CD7 was discovered at Duke, in large part by Dr. Kurtzberg, we took this as a sign that we should be looking at this population. In addition, my department chairman was one of the lead investigators on that. So, that was an additional sign. So, we spent a lot of our time looking at this population. To make a long story short, it has lymphoid progenitor activity in just about every assay that we have looked at.

This is just one example of that. These were these 34 minus lin, very extensively lineage-depleted CD7 positive cells engrafting in SCID/hu-5 mice, done in collaboration with David Camarini at UVA. These are discriminated based on their HLA type for donor versus the recipient thymus, and you can see nice single and in some cases double positive T-cells growing out in many, many thousand-fold expansion over a few weeks in these mice.

I don't have time to show you the rest of the data but just to summarize it, this 34 minus population, again, expresses no lineage commitment markers. It is virtually all in G0. We haven't been able to grow them in suspension cultures at all. They require stroma and it takes them a long time to get going, but when they get going they expand quite dramatically. They stain with a

Hoechst dye identically to murine long-term reconstituting cells. Joanne has isolated some leukemia that would become both myeloid and lymphoid that has exactly the same phenotype, interestingly enough.

Finally, I should have quotations around this, the myeloid capacity of this population is pretty minimal and sporadic but it very nicely differentiates into NK-B and T-cells in a variety of different assays.

So, I think it raises the question about whether we should be looking at expanding this population or other similar populations to try to enhance immune reconstitution post-transplant. If we start to process these cells, we might want to be careful not to throw some of these 34 minus populations away in the trash as we start to do that.

Let me finish up just by acknowledging that the majority of this work was done by Bob Sterns, who is now on the faculty at Duke, who I am sure would be happy to discuss this further with anybody. Thanks.

[Applause]

DR. BROXMEYER: So, our next speaker is Jeff Moore, and he is going to talk about potential new markers, and since I don't know what FRIL stands for I won't tell you.

Potential New Markers: FRIL

DR. MOORE: He needs to read the papers I sent to him!

[Laughter]

Thank you, Hal. This afternoon, I would like to tell you about an interesting molecule that I think may have some applications in cord blood banking and transplantation. The molecule is a legume lectin that we call Flt3 receptor-interacting lectin, or FRIL, and I will describe how that name evolved in a minute.

It has two very interesting properties. One is that when the lectin FRIL by itself preserves cord blood SCID repopulating cells and progenitors in a dormant state up till mounting suspension culture without medium changes. The second property is that when attached to magnetic beads, either through direct or indirect magnetic bead separation, isolates a rare population of Flt3 cord blood cells.

So, just to orient you, there are a couple of things about the lectin that, you know, many of us have worked with over the years that are important to think about. One is that in this capacity it acts more like a cytokine but, unlike cytokines that we have worked with, we only need to add it at the beginning of culture and it is very stable.

The second is it kind of has an antibody type property wherein it actually will capture and pull out cells. But what differs from an antibody is that you cannot use an antibody, say, against Flt3 because of the low density, but the apparent specificity of this for Flt3 is binding to a carbohydrate, and there are two binding sites on the plant lectin.

So, I would like to give you just a brief history of FRIL. Several years ago we were looking for the Flt3 ligand and we developed an assay using Flt3 transvected 323 cells, and since these are a vector independent cell line we developed a rescue assay and when to our favorite source for hematopoietic stimulators, PHA-conditioned media. It turns out that PHA-conditioned media has very high levels of the Flt3 ligands, probably the best T-cell source, but during a biochemical purification we actually came up with a different protein, and that protein was a second legume lectin that was in actually the red kidney bean extract used to make PHA-conditioned media. So, PHA that people buy is actually nothing but a red kidney bean extract.

This lectin, in contrast to PHA which has a complex sugar specificity, has specificity for mannose and glucose and, in fact, its tightest binding is to a trimannoseal core. This just turned out to have a huge advantage for us because we can purify this protein in the

gram level and it has really accelerated a lot of our characterization both in vitro and in vivo.

So, understanding how FRIL interacts with the Flt3 receptors or differs -- acts in a different way than Flt3. So, here is Flt3; binds to receptor; induces proliferation. FRIL interacts with a mannose carbohydrate on Flt3 and actually induces this quiescence effect that I have described.

This is shown here by Kollet, and published last month. The next series of slides will be from this experimental hematology paper. They took cord blood CD34 cells and cultured them in FRIL or cytokines for 3, 6 or 10 days. At each of these time points they looked at the number of cells and cycles. They started with about 1500 cells and what they are measuring in the table are the S, SG2 cells here. In FRIL the number of cells remain fairly constant over the first 3-6 days, and then at day 10 go down to about half of what they were at 3.2. And in these 2 different cytokine cocktails you get the expected large expansion over those 3-10 days, and it ends up being anywhere from 15-150-fold number of cells.

Well, there are a lot of reasons why you can end up with no cells -- no cytokines at all, you end up with no cells, but these cells have some interesting properties. This is a busy slide but it really makes the point well.

These were cultures in either calf serum or serum defined medium, and this is looking at the total number of viable cells, in green. This is with FRIL alone. So, you start out with this number of cells -- with FRIL it is about 40 ng/ml but it is in concentration that you would use for a cytokine, and over time, out to 20 days, the number of cells in the culture decrease fairly dramatically, which is consistent with what we showed in the previous slide. Similarly, the number of progenitors kind of decreases over time. We didn't test at day 0, here.

What is interesting is that if the cells are harvested at day 6 and split in two and either put back into FRIL or put into one of those cytokine cocktails I described in the previous slide, you see this large increase in the total number of viable cells, shown here in red. Similarly, this 10 plus 3 more days, or going out to 13-plus 7 day sin cytokines increases the number of viable cells dramatically. The similar pattern is seen with progenitors. Again, this 6-plus 4, here back in FRIL. Here is what happens when they are in cytokines, and then down again, day 13 when these are put back in cytokines there is a large expansion.

So, our concept of how FRIL was working at this point was that it was somehow acting on these primitive cells, holding them in a quiescent state, but it was

reversible. From a practical point of view, what is really exciting about this molecule is that you can see the number of cells that go down over time and, yet, when you take them out you can expand, as opposed to cytokines where you are just kind of expanding the whole thing as you are going. This allows basically a synchronization in holding those cells and then being able to expand them.

Actually in these same cultures, we also harvested some of these and looked at their ability to engraft in the NOD-SCID mice. Again, this work was done by Kollet. On the right are the human mouse DNA mixed ratios, and here is the bone marrow probe for human DNA by Southern Blot analysis for cells that were cultured for 6 days in FRIL, 10 days in FRIL, in the middle, and then that idea of 6-plus 4, again looking for an expansion of these SCID repopulating cells. In this case there is some evidence for that. Their experience is shown here with 10 mice here and 30 mice, and there is some evidence that there is an increase.

Now, they also looked at these cells by flow cytometry, and there is myeloid, lymphoid and K-cell engraftment here. And, they took the bone marrow from these mice and did a serial transplantation and the secondary recipients got engraftment, again myeloid, lymphoid engraftment.

So, what are the possible applications for a FRIL in cord blood banking and transplantation? Maybe one just has to work with the molecule to get this impression, but this ability to functionally select primitive cells, again, reduces probably the loss of primitive cells resulting from cytokine stimulation. So, again, even with normal cocktails of cytokines where you can maintain SRC, you get this huge expansion of other cells in the culture. And here, with FRIL, I have shown you that there are very few cells in those cultures and that gives you a lot more possibility.

I think one of the things that would be interesting with those cells is how they home and whether chemokines -- whether they need a little dose of chemokines at this point to try to up-regulate their homing molecules.

Importantly, subsequent exposure from of FRIL preserved cells to cytokines expands the number of progenitors and probably SRC.

The question here is, is this ability to functionally select in in vitro, and I will also show some flow cytometry data, cell selection data in a minute -- is it possible that FRIL, since it is binding to a relevant stem cell receptor, Flt3, can provide really a more uniform product for ex vivo expansion and transplantation?

The other thing with those few primitive cells that are held in culture is that FRIL synchronizes these cells, and would this result in higher viability during cryopreservation? Also, this cell population, since this is a small number of cells in a dormant state, would this be a better target population for gene therapy?

Our thought along these lines is could you take these sleeping cells, wash them free of FRIL, add cytokines and expand them bringing into cycle, use the retroviral vectors and then put the cells back in a quiescent state so that you can spend some time analyzing them, and we are eager to collaborate in this area.

I am going to switch now to the last part to talk about using FRIL attached to magnetic beads to capture cells. Many people have used lectins over the years, wheat germ, agglutinin and other lectins that are either FITSY or PE labeled to identify cells. I am not a kind of patient cell sorter so I like to use the magnetic beads because it is fast. In fact, when we use the beads we get a 0.3 percent recovery from cord blood mononuclear cells, and then we have analyzed these by flow cytometry, PCR, some colony assays and are looking forward to starting some collaborations with the NOD-SCID mice.

So, here is a representative experiment from taking FRIL-selected cells, again about 0.3 percent cord

blood mononuclear cells. We gated on this population, forward versus side scatter -- I guess this kind of got bleached out in the back, but of those cells, 50 percent of them were Flt3 positive cells. Now, theoretically they should all be Flt3, and we actually spent most of the summer how to get rid of all those cells and also to just gate this whole population, and I think there are some technical issues that we should be able to get a more purified cell population.

What is the activation status of these? Looking at Flt3 on the ordinate and CD38, about two-thirds of this particular cord blood sample was Flt3 positive/CD38 negative, and we need to look at it more to get a better impression of this.

Now, one thing that has been interesting about this work is that the cell selection we started was probably in about 1994 or 1995 and we were very troubled by the fact that the cells didn't express C34. We sat and kind of mulled over this and said that the C34 negative story has been very interesting and, in fact, the cells that I have shown you are C34 negative and we need to do more extensive analysis to characterize those cells.

We have also looked for other tyrosine kinase receptors that are expressed on very primitive cells, and they include Flt3, Kit, Flk1/KDR, Flt4 and Flt1. This

shows that probably Flt1 illustrates it best -- lane 1 is cord blood mononuclear cells and there is an enrichment in the FRIL-selected population and 34 population. A similar pattern is seen with Flt3 and also these other ones.

In fact, what has been really interesting about these studies is that if you look only by PCR at the receptor expression of these, they look identical to 34 negative cells and, yet, there are two distinct populations. We can first isolate FRIL-selected cells and isolate CD34 and vice versa. So, it is a distinct cell population that we are able to capture.

This is just preliminary data taking cord blood mononuclear cells and culturing them either in VEGF or hematopoietic colony assays and just showing that we get some enrichment there.

So, I would like to leave with this question, can FRIL selection, either by a bead selection or an in vitro, ex vivo selection provide a better and more uniform product for ex vivo expansion and transplantation? Thanks very much.

[Applause]

DR. BROXMEYER: Our next is going to be John Adamson. John is going to be talking about the importance of CFU-Meg assay, and he brings his own computer.

DR. ADAMSON: I would like to begin by thanking the organizers for inviting me to participate. I am here really representing the joint effort of several laboratories that have contributed to the New York Blood Center's Placental Blood Program.

Unlike both the written program and Hal's introduction, that title is really not only a bit off, it is totally off. What I am really going to talk about is the relationship of the analysis, if you will, of colony-forming cell content of cord blood samples with clinical outcomes. Some of the data, toward the end of the presentation, was published in May or June in Bone Marrow Transplantation and most of the data at the beginning of the presentation is actually in press in Blood.

I want to start by going back in time to the beginning of the cord blood program in New York when the decision was made, for a variety of reasons including some that you have heard mentioned here yesterday, that rather than rely on CD34 counts, because of the uncertainty of what CD34 represented in cord blood samples compared to cytokine mobilized peripheral blood or bone marrow, we decided that we wanted a functional assay for the cord blood samples that were being collected in the early days of the program.

So, the decision was made to culture each cord blood sample as it came in to Dr. Rubinstein's laboratory in the blood center for its content of colony-forming units. In total, we have data on over 4000 placental cord blood stem cell preparations that are in storage or have been used. The reason that that number is not the 11,000 which currently is the number that has been processed and either exists in storage or is a combination of used and stored units is that we focused, after the initial period of time, on those samples between 40-60 ml in volume.

Second, beginning later in the life of the program or the project, we added a quantitative assay for megakaryocytic colony-forming cells. However, because the number of these cells or the number of those units that have gone on to transplantation has been relatively few, we analyzed the relationship of meg colony-forming cell numbers to total colony-forming cell numbers in a fair number of cord blood units with the intent to begin to assess the possible relationship of their numbers to time for platelet engraftment which, as all of you know who have been involved in cord blood transplantation, is something which has been somewhat vexing.

So, the studies that I want to report on today, which are clinical correlation studies or laboratory and clinical correlation studies, is to present data on the

correlation of the number of colony-forming cells with total nucleated cells in a large number of cord blood units, and to compare the relative contribution of colony-forming cell counts to total nucleated cell counts to transplant outcomes. Then, as I mentioned, to initially assess the possible added value of routinely performing meg colony-forming cell assays on all cord blood preparations.

The first study was performed on 204 out of 562 patients whose transplant outcomes have been reported previously, specifically in the November issue of 1998 of The New England Journal of Medicine by the Cord Blood Program at the New York Blood Center. In the first analysis, recognizing that the data that you will see on outcomes is a subset of the 526 patients who were published about two years ago, almost two years ago, was an analysis of the total nucleated cell count against the total number of colony-forming cells per unit. As you can see, there was a reasonable correlation coefficient with a highly significant p value given the fact that we were dealing with 192 samples.

We went on then to take these same data sets, total nucleated cell counts and colony-forming cells of all kinds other than meg CFC, and look at clinical outcomes.

This slide is very similar to the figures shown in the publication in the New England Journal in November

of 1998, and it groups patients on the basis of the total nucleated cell count which they received in their graft. Recognize that these numbers, whether colony-forming cell counts or nucleated cell counts, are pre-cryopreservation. There are 4 groups and with increasing nucleated cell dose, and this is times 10^6 so the group that got the highest dose of nucleated cells received 100 X 10^6/kg or 10^8/kg or more, and the group that received the lowest cell dose received 25 million nucleated cells per kg or less. As you can see, there was a cell dose related effect in terms of the time to neutrophil engraftment, which was defined as reaching and maintaining a neutrophil count of 500/microliter. The median days to achieve myeloid recovery is shown in the far right column.

The two features that were true in the larger data set were true in the smaller data set, and that is, the greater the cell dose the more rapid was myeloid recover, and at the lowest cell dose not only was there slower myeloid recovery but there was a higher degree of failure to engraft.

These data were then compared to the colony-forming cell dose that these patients received and, again, there was stratification and, in this case, a little bit better stratification than was seen with the nucleated cell dose but in both cases at the highest cell dose, whether

nucleated cells or colony-forming cells, were associated with the shortest time to myeloid engraftment. And, the group that appeared most at risk, and even more gravely at risk perhaps than based on total nucleated cell counts, were those who received less than 50,000 colony-forming cells per kilogram recipient body weight. Again, the relationships where highly statistically significant.

What of platelet engraftment? Again this was total nucleated cell dose and here, in fact, there was a reasonable compression around the 50 percent point in terms of the cell dose, with separation coming perhaps further out in the days after transplantation and, very similarly to the report of the larger patient group in November of 1998, at the two highest nucleated cell doses, in fact, there didn't seem to be advantage of the higher cell dose over a lower cell dose. But, again, the difference across the groups was highly statistically significant.

When one converted to colony-forming cells, however, the differences were even more magnified. At the lowest colony-forming cell dose the patients were at considerable risk for delayed platelet engraftment, in this case with a median time to engraftment of 140 days but, again, even with the highest colony-forming cell doses there wasn't a distinction between the two highest dose levels when colony-forming cells were used compared to

nucleated cells. This cell dose effect was even more greatly statistically significant.

So, the results show that the colony-forming cell dose correlated significantly with total nucleated cell dose, and the colony-forming cell association with neutrophil engraftment was greater than total nucleated cells with RF 0.46 versus RF 0.41 respectively.

What of event-free survival, the length of time alive until there was a transplant-related event? Transplant-related events were death, autologous reconstitution or the need for repeat transplant, the same criteria that were used in the original publication in 1998.

This shows the effect of total nucleated cell dose, and here the higher three dose groups appeared to be relatively similar compared to the lowest cell dose group and the difference was statistically significant.

Very similar results were obtained when colony-forming cells were looked at but, again, the patients who received the lowest colony-forming cell dose, which was less than 50,000 per kilogram recipient body weight, were clearly at much greater risk than the patients that received the three dose levels above, from 50 all the way up to 200 10^3 colony-forming cells per kilogram recipient

body weight. So, this group of patients would appear to be at particular risk.

An important result with the transplant-related events is that when the colony-forming cells or total nucleated cell count is included as a continuous variable, the differences are not statistically significant, whereas, in multivariate tests of the speed of platelet or neutrophil engraftment or the probability of transplant events, the inclusion of colony-forming cell dose in the model displaced the significance of the higher relative risk associated with total nucleated cell dose. So, in fact, in this analysis the colony-forming cell dose was more predictive of neutrophil engraftment, more predictive of platelet engraftment and trended towards association with better or fewer transplant-related events.

In the second study, because relatively few patients had been transplanted with units on which we had done individual meg CFC assays, instead of looking at transplant outcomes we compared the meg CFC numbers to the total number of colony-forming cells in 134 cord blood units. We also looked at 21 bone marrow and 52 cytokine mobilized peripheral blood stem cell preparations.

The important thing here is to note that the relatively high proportion among all colony-forming cells, of the number of colony-forming cells that were identified

as meg colony-forming cells, fully 20 percent of all progenitor cells that grew out in colony assays of one kind or another grew out as megakaryocyte colonies. In mobilized peripheral blood because the numbers were smaller there was obviously a much greater standard error, as there was with bone marrow but the source of stem cells with the lowest proportion of megakaryocytic colony-forming cells was, in fact, the bone marrow.

If we looked at the correlation between met colony-forming cells and total colony-forming cells per milliliter of cord blood, there was a very strong correlation which for all samples was 0.84, with a p value of 1 to the 10^{-4}. This kind of correlation was inferred but not reached statistically with cytokine mobilized peripheral blood, and was inferred but clearly not reached with bone marrow stem cell preparations. With cytokine mobilized peripheral blood and bone marrow the denominator was CD34 positive cells, and no selection was done on the cord blood samples that were used for analysis.

So, our preliminary conclusion, based on the extremely close correlation between colony-forming cells of all types and the number of meg colony-forming cells, strongly suggested to us that the routine measurement or determination of meg colony-forming cell content was probably not warranted in banks or within laboratories that

were studying cord blood cell preparations and their biology for clinical outcomes. Of course, we have a large number of cord blood stem cell preparations in New York -- no longer "we," I apologize -- but the blood program in New York has a large number of units in storage for which meg CFC data will be available at the time the transplants are carried out. So, eventually I think there will be an opportunity to directly correlate meg colony-forming cells numbers with time to platelet recovery but at first blush this does not appear to be a useful routine assay to add to the screening, if you will, or biological characterization of cord blood units. Thank you very much for your attention.

[Applause]

DR. BROXMEYER: Jan Visser is going to talk about the correlation of CD34 positive cells with CFU, unless he has changed his talk.

Correlation of CD34 Positive Cells with CFU

DR. VISSER: I thought I would save some time by not using my computer --

[Laughter]

-- my slides came out very faint so I will rely on the backup system, not the blackboard but the overhead projector. My title didn't change. It is about CD34 CFC counts in placental cord bloods. It is about a series of

experiments that I did, together with the laboratory of Pablo Rubinstein and Cladd Stevens, and it is a continuation of what John Adamson just told you.

It is a good thing to know the CFU counts in cord bloods. They are predictive of the clinical outcome. But when you let your cord blood bank explode from 10,000 to 50,000 units it is hard to imagine doing all the CFC counts, and it is nice to have an alternative that is maybe a little bit easier. So, the CFC counts that we did were similar to the ones described by John just now. So, they are done by the same technique and actually the same growth factors, and you compare those numbers with CD34 counts. After some preliminary experiments we selected the ProCOUNT kit from Beck and Dickerson, which is not really sold to be used for cord blood. They say it is good for mobilized peripheral blood, but with a couple of modifications, as I will show you, and especially with regard to CD34 very bright cells, it is a nice test, except for the expenses.

It uses about 100 microliter of cord blood to begin with, and you add known numbers of beads to each tube so you get an absolute number of CD34 counts, and that is also a very nice feature of it.

The difficulty comes when you look at the number of cord bloods and it turns out that there are differences with respect to the number of CD34 very bright cells in the

population. CD34 very bright cells have been described in the past in the literature as being the ones that are containing the stem cells, and I think the literature goes back to 1992-1995 and maybe the reagents in those days were not so strong and actually the population that I am describing now is the population in these graphs that is very bright and easily distinguishable from the population that Beck and Dickerson says is the stem cell candidate, but it is a surrogate assay. It is like trying to estimate the number of parents and grandparents by counting grandchildren and great-grandchildren.

[Laughter]

It is a way to get an idea about the quality of the graft. So, we looked into these very bright cells and intermediate cells, which I will call regular CD34s. Actually, when you do many of the analyses in the ProCOUNT assay you can just draw a line around the cells that are CD34 and ignore the other ones, and you can get a very nice estimate of the cells that form a nice cluster also in CD45 nucleic acid content and side scatter. The actual numbers obtained this way correlate well with CFU numbers.

This is the distribution. They average about 40 microliters and there are a good number of units with higher numbers of CD34 positive cells, but the bulk is between 10-50 per microliter.

When we look now at the CD34 very bright cells as a percentage for the CD34 positive cells, it turns out that a good number of them, 20-30 percent of all the units, more than 40 percent of the CD34 positive cells are the bright cells. So, if as we talked earlier about people having differences of a factor of 2 between certain units and CD34 numbers, it is in that order of magnitude that you get differences between units.

The occurrence of these CD34 brights is predominantly in the units with lower numbers of CD34 per microliter. This is the correlation between CFC, done with the same method that John Adamson just described, and CD34 cells. There is a good correlation, with a correlation coefficient of 0.89. So, if the CFC correlates well with clinical outcome, it is very likely that the CD34 numbers do as well.

Response is the correlation between the CFC and the CD34 brights, and there is absolutely no correlation between the CD34 brights and the CFC numbers. So, they are not a good measure of clinical outcome.

This is the same thing in a table. If you have about 20 CFC total per microliter as opposed to CD34 positive of about 39, 40, the CD34 intermediates are 32 per microliter, and the brights are 6.8 but, as I said, some units -- about 20-30 percent of the units it is more than

40, 50 percent of the CD34 cells are these brights. The correlation between CFC total and CD34 brights is absent.

When you characterize them in more detail with double labeling, they turn out to be AC133 negative, DR predominantly negative. They were KDR positive, CXCR4 positive, whereas the regulars were mostly AC133 positive, KDR negative. This indicates that this is probably an endothelial cell type and not immature because they would be AC133 positive, but the mature endothelial cell.

Electron microscopy showed that many of them are apoptotic or closed to apoptotic with holes in the nuclear membrane, as opposed to the regular CD34 positive cells where very few of them were apoptotic by electron microscopy.

When we look at apoptosis using an Annexin-V labeling, double labeling, in 3 different units almost 90 percent of the CD34 very brights were Annexin-V positive, and the Annexin-V FITC fluorescence could be blocked or competed away with non-labeled Annexin-V, indicating that this is truly an apoptotic process in most of these cells.

In conclusion, the CFC and CD34 positive cell counts in the placental blood correlate well if we exclude the CD34 positive bright cells. The modifications in ProCOUNT are recommended to facilitate the rapid counting of the progenitors in cord blood, and I think we have data

that show conclusively that most of the CD34 positive bright cells are endothelial cells, apoptotic or in a dying process. Thank you for your attention.

[Applause]

Discussion: Correlation with Outcome

DR. BROXMEYER: We have a majority here. So, we would be happy to answer any questions from any of the people who have gone. Just don't let them know what answers we gave you. Yes, Liana?

DR. HARVATH: Liana Harvath, Bethesda. I have two questions, the first one for John Adamson and it is involving how you quantify the CFU meg, if you could give us some advice. When you were doing your assays did you distinguish between the large colonies and the smaller colonies, or did you just count the total CFU meg? I wondered if you would comment on that.

DR. ADAMSON: We did both. The data that I showed for total meg colony-forming cells, but we arbitrarily looked at large colonies and smaller colonies -- and this is published in BMT -- on the basis of the numbers of cells greater than 50 or less than 50, and there was a much higher proportion, in fact, virtually 100 percent of cord blood samples had cells in them that, when cultured under conditions that we used, formed large colonies as opposed to bone marrow in which the vast

majority of the CD34 positive cells that we cultured, in fact, had no large colonies when the progenitor cells grew out. The peripheral blood cells, as I recall, were somewhere in between but tended toward the low side. So, not only were the numbers of progenitor cells that gave rise to megakaryocytes much greater proportionally in cord blood but they also gave rise to larger colonies on average.

DR. HARVATH: And, I am wondering if you think it would be worthwhile -- since there is this delay time to platelet engraftment we have often wondered if that is related to the information that you could glean from in vitro studies of the sizes of these colonies, and if there is any way that that could be explored further in an assay that may be more predictive of time to platelet engraftment.

DR. ADAMSON: Two things, first, we don't have the direct correlation, for reasons that I explained. Second, I think you can make a strong case, based on the correlations that you saw, that you are not going to learn much from routine assays of meg colony-forming cells. I say that because of the very strong correlation between meg colony-forming cells and total numbers of colony-forming cells that I showed with a coefficient of 0.84. Then I would take you back to the clinical outcome slide that

showed that once you exceeded or once you achieved a certain number of colony-forming cells, you didn't shorten platelet engraftment any more. So, unlike myeloid engraftment, there was not a continuous shortening of the time to engraftment as either the total nucleated cell dose went up or the colony-forming cell dose went up. So, I would argue, particularly when you achieve total colony-forming cells doses of a certain number, say, greater than 100 X 10^3/kg, greater cell doses which would imply greater meg colony-forming cell doses are not going to have a difference.

DR. BROXMEYER: I would like to follow up on Liana's question. Your meg assays -- what are the ingredients that are thrown into the culture? Is this a combination of growth factors?

DR. ADAMSON: Flt3, thrombopoeitic stem cell factor.

DR. BROXMEYER: So, there is a general consensus in the field that if you use a single cytokine or maybe two you are probably going to pick up smaller colonies that derive from more mature subsets within that series. Taking it from there, if you put in a lot of growth factors you are going to get larger colonies, probably derived from the more immature subsets. So, if we are looking for platelet engraftment and to see if meg colony assays could

potentially predict for time to platelet engraftment, maybe we really should be looking at the meg colonies that are coming from the mature subsets of cells. Pretty much what you guys have done is look at probably the earliest subsets of those meg colonies. This is just a thought.

DR. ADAMSON: Again, I don't think that the logic train holds up because regardless of what you call it, you are putting in a larger number of colony-forming cells, and only if you use the argument or the speculation that there is an inverse relationship between the kinds of cells that you are talking about and the more primitive progenitors that would, therefore, favor platelet engraftment when you gave smaller numbers of more primitive progenitors -- you know, you are talking about an in vivo read out which is time to platelet engraftment versus an in vitro readout which is colony-forming cells. All we are saying is that the correlation is very strong, and you achieve a certain level, and you can't get faster, or we didn't see, in the patient outcomes reported to the New York Placental Blood Program, faster platelet engraftment. I think that is probably the more relevant finding.

DR. BROXMEYER: Anybody else want to contradict John?

[Laughter]

DR. LAZARUS: Well, I am not going to contradict any statement that was just made, I just have another comment and question. I am Ellen Lazarus from Bethesda. One question for John Adamson, could you please describe the technique you used to identify the megakaryocyte CFUs in your cultures, whether you used an immunostain or some other technique.

DR. ADAMSON: It was fluorescence.

DR. LAZARUS: And, do you have any comment on the possible application of this assay to post-thaw products and whether there might be some additional benefit to looking at that?

DR. ADAMSON: There could very well be. I think one of the reasons some of the correlation that we have been able to show is possible is because we are doing things in a very routine way and, you know, highly reproducible way in several laboratories at the New York Blood Center, and goodness knows what happens post-thaw.

DR. LAZARUS: Thank you.

DR. ADAMSON: But I think your point is well taken. Again, unlike myeloid recovery, platelet recovery above a certain nucleated cell dose or colony-forming cell dose doesn't seem to budge, and I think there must be an important biological message in that.

DR. HARVATH: I have one question for Dr. Visser. I thought your data are really very, very interesting, and just a comment and a question, I was wondering if you have spoken to the people at BD to tell them about this improvement on the ProCOUNT because when the data were evaluated for clearance of that assay, as you probably know, it was not initially performed with cord blood samples and I think that the information you have provide very important information for the use of this assay now for cord blood rather than for peripheral blood.

DR. VISSER: Yes, we have been talking with them, particularly the designer of the ProCOUNT analysis software, and they are interested in getting an improved version but it will take time.

DR. MARTI: Gerald Marti, Bethesda. I also want to follow up with what Liana said. First of all, you indicated that there were two corrections that needed to be made, perhaps a couple that needed to be made in order to kind of modify the ProCOUNT. One is obviously the location of the bright CD34 apoptotic endothelial cell. Is there anything else?

DR. VISSER: There is one in the nucleated cell count area. It may not be really important but in the application of cord blood the nucleated cell count number is used and the CD34 count is used. For the nucleated cell

count there is a problem of setting the direct gate for cells that are dying, like granulocytes that are falling apart. It is the same in the other nucleated cell count. They all treat it a little bit differently.

DR. MARTI: But even allowing for the difference between the various hematology cell counting devices, with that particular single platform you could get a handle on total nucleated cells, and if you would accept the definition of WBC on the basis of total CD45 you might be able to get a WBC and nucleated -- those two counts.

The other thing that I was wondering was in that assay they use, quote/unquote, an isotype control. Does the isotype control, which I assume is also PE conjugated, does that bind to the CD34 bright cells?

DR. VISSER: No, it doesn't.

DR. MARTI: It doesn't? I was thinking, you know, early on you were always taught that dead cells bind antibodies better than live cells --

DR. VISSER: But these cells don't.

DR. MARTI: They don't. So, that is another caveat that needs to be pointed out. Thank you.

DR. BROXMEYER: I have a question for Jeff. When you get binding of FRIL to the Flt3 receptor, you are talking about a lectin. Does it ever come off? I couldn't

tell from the data you had, does it interfere at all with the Flt3 ligand?

DR. MOORE: Yes, two things, one is that in our early studies it was difficult to get the beads off. So, we can inhibit binding of FRIL to the cells so we don't get any binding. The other thing is using CHO IL-25 labeled Flt3 [not at microphone; inaudible].

DR. BROXMEYER: But it still could change the confirmation of the receptor.

DR. MOORE: It doesn't appear to.

DR. BROXMEYER: Then, I was interested in your SCID repopulating cell studies that you did with the people from Israel, and what I saw was that you got some survival with the FRIL so that you could do the repopulation of the SCID mouse, and then you got an enhanced repopulation when the cells were in the presence of a bunch of cytokines.

DR. MOORE: They were in FRIL for 6 days, for example, were washed and then cultured with cytokines for 4 additional days, and it is that second period where we saw some expansion in the number of total cells of progenitors.

DR. BROXMEYER: I know the paper is accepted already but what I saw missing was the control.

DR. MOORE: I didn't show the control --

DR. BROXMEYER: Okay, and what happened with the cytokine control?

DR. MOORE: The cytokine control at that lab, they didn't get any engraftment in their lab with 6 days of cytokines only or 10 days of cytokines only, the cytokines they used.

DR. BROXMEYER: Great! Ian?

DR. MCNEICE: Ian McNeice, from Denver. Jan, I had a question for you on the bright cells. The method for 34 analysis, as I remember they exclude a bright 45 population that is 34 positive, and they claim that they are lymphocytes. It looked like yours is also high 45. Is it the same cell population? I think that was designed more on peripheral blood from their analysis, but do you know if those two populations are the same?

DR. VISSER: I am not sure. They are not CD45 really bright, they are kind of intermediate, just like the CD34s, the regular CD34s. There is one other device that is descried now in Stem Cells, the last issue, from an Italian group where they find enormous numbers of CD34 bright cells coming from a placenta if you squeeze it --

[Laughter]

-- somehow they have found that you can get a higher volume of cord blood from a placenta by squeezing it, but also that the number of CD34 positive, especially CD34 brights is really high.

DR. RUBINSTEIN: A very short question for Jeff. You were surprised that after isolating your positive cells, when you ran them in the cell sorter you found that only about half of them were positive for the Flt3 marker. Is it possible that this lectin is binding to another population of cells which is not Flt3 positive and which might be a confounding factor in your assays?

DR. MOORE: Yes, that is entirely possible, and we actually spent most of the summer trying to get rid of contaminating cells, and actually the early phases with the mittenyi columns were just to make sure all the cells were running to the column. So, we are still working on that. Also, it may be concentration dependent. So, if we lower the concentration slightly we may get rid of those, but it was surprising and I guess pleasing to us that we could get, you know, 50 percent of the population. We just need to go the rest of the way and see if we can get uniform Flt3 positive cells.

DR. BROXMEYER: LeeAnn?

DR. JENSEN: LeeAnn Jensen, Bethesda. I was hoping somebody else was going to ask question but nobody seems to. There has been some discussion about whether CD34 is what we should be measuring or not, and I am sorry that Dr. Smith isn't here to talk about CD34 positive cells versus CD34 negative cells, and would you like to comment

on whether that is what we should be measuring or whether we should be measuring something else?

DR. BROXMEYER: I don't think I would touch that one.

[Laughter]

But whatever you do, make sure you know that they are lin-negative otherwise you run into a lot of problems because you really can't get much of the CD34 negative if there are other lineage markers there, and it is the same problem with the CD34, you have to have lineage negative otherwise you run into real problems.

DR. VISSER: I agree. We have been looking at 60 cord bloods for the side population cells. They contain all kinds of lineage markers. We did not do what was shown just now, take lineage depleted and then go on with SP. But it is clearly a field where there is a lot of work to be done where we compare the purity of mouse stem cells with those of human stem cells and there is a difference in purity unless the KDR story is true. The KDR positive cells would be in the ball park of purity of what we find in mice. Then what Ian stressed during this meeting, that maybe long-term repopulating cells -- there are plenty of them in cord blood because there is long-term repopulation all the time. Maybe we have to look at the cell type that does this and I think CD34 has something to do with that.

DR. BROXMEYER: Does everybody know what KDR is? It is a receptor for vascular endothelial cell growth factor and there is some suggestion in the literature that maybe the KDR positive cells are picking up an earlier subset of CD34 positive cells but, as Jan has said, there is not real confirmation of that yet and it would be a very small population of cells.

DR. VISSER: Our CD34 brights are always KDR positive, and sometimes it is 34 percent. We use it as an internal control, actually, to look for KDR antibodies.

DR. BROXMEYER: So, one of our tasks was to offer up suggestions for areas of potential funding. So, I am going to put my two cents in and say that about four years ago, Hartman and Blood put out an RFA for homing and the people who got that, like myself -- that is sort of finished now but a lot of the stuff that I did was because of that RFA. So, I would say that I think we need efforts in homing. I think that is really important. We need to get the really good people in the area.

I would also make -- not a plea but a suggestion that, you know, we do more work on cell survival. The field of cell survival is incredibly intense right now. It took me months to learn the literature to play with something that I was doing. There is an incredible amount

of information out there and I think the time is right to start using that information for hematopoietic stem cells.

I think those two areas would be nice areas to look at, and then if there are any other suggestions from other panel members? You guys are very satisfied that you have all the money you need?

[Laughter]

DR. VISSER: I agree with you. Homing is an important aspect. We tried to find heterogeneity. That is what we look for because there is heterogeneity between cord blood units in performance, especially with platelets. So, there must be heterogeneity in those markers, and there are very few markers that we know that show a wide spectrum of differences and we look for those.

DR. BROXMEYER: Great. Any other questions before we let Joanne summarize everything that has gone on in the last two days?

[No response]

Thank you.

[Applause]

Brief Summary and Future Directions

DR. KURTZBERG: I know everybody would like this to be short so it will. I just have three overheads. I think the meeting went well and that there was a lot of

interchange, and that I certainly learned a lot. I think we helped define some directions that we need to take.

One of our tasks was to make some recommendations to the FDA about what kind of cord blood product could be licensed right now versus what we still need more information about. I put this together just to have something to start with. It doesn't mean it is the final document. But, just listening to everybody talking to everybody and putting together some of the comments that have been made during the meeting, this is what I think could be licensed right now: A product that is collected in either a FACT/NETCORD approved lab or whatever the certification body is designated, but the program needs to be approved by one of these product standards organizations. It needs to be collected at a site that is meeting those standards, as well as processed and cryopreserved according to those standards.

The product itself -- again, this is a place to start -- should be sterile, have a minimum volume of 30 malignancy, results of RH and ABO typing, HLA typing and the A, B, and DR-beta-1 loci, and post-processing counts, viability, infectious disease screening, some sort of family history and evidence of maternal consent for donation.

For transplant, and this would be what we would put in quotes as conventional today, not to preclude the other areas of research would be a match that is a minimum of 4/6 antigens or 3/6 alleles, and for pediatric patients -- and this is more size related than age related, so patients who are less than 12 years or less than 50 kg, a cell dose of a minimum of 2 X 10^7 cells/kg, and this is referring to the post-processing count, and for adult patients or patients over 12 or over 50 kg, a minimum cell dose of 1 X 10^7 cells/kg.

So, this is just a place to start to say that this, in my opinion, could be licensed. Comments? Questions?

DR. RUBINSTEIN: A question, when you say minimum volume for the product of 30 ml, do you mean the final product or the collected volume?

DR. KURTZBERG: I mean the collected volume without anticoagulant, and this is really to address situations where very small amounts have been collected for directed donation -- I mean, 5 ml, 7 ml, and we have been led to believe that that is a sufficient amount for a conventional transplant and I think that is inappropriate. Other comments? Cladd?

DR. STEVENS: [Not at microphone; inaudible].

DR. KURTZBERG: Cladd's question was bringing up the point that sterility is sort of a moving target and that we don't really have a test that we would all agree proves that something is sterile. I think my point is that for public banking I don't think we should be banking a product we know is contaminated with bacteria. I don't think that is true in directed donation where there are other reasons why that product might be important and specifically important for a family or a patient. But I think when you have a public bank and you are offering a certain degree of safety using standard culture methodology the product ought to be free of bacteria, would be what I would say but that is just my opinion. Any other comments?

I want to be clear, this doesn't preclude other cell doses, other matches, other kinds of testing. This would just be what could be licensed where you could say to a third-party payer this is FDA approved for this indication and you wouldn't have to go through all the things that we have to go through when it isn't.

Two more overheads -- one, we came up with several ideas to pursue. These would obviously all need funding. One is a voluntary certification program for the banks so that there could be some effort to be able to correlate data from one bank with another and be able to know that cell count A correlates with cell count B, etc.

This might relate just to cell count; it might relate to CD34 assays of CFU-GMs, or whatever is designated as important in the field at the time.

We need more support for studies that will increase cell dosing. The ideas that have been talked about here involve ex vivo expansion or combining units but there may be other strategies that are more innovative that would come to the forefront if there were funding opportunities, and unfortunately it doesn't appear that industry is going to be funding these studies.

One strategy around that might be if there were some regulatory incentive for the industry of pharmaceutical companies to be able to do some pilot studies without having to meet 100 percent of the regulatory requirements that they would have to meet for licensing. Some of the pilot studies could be done as Phase I to at least figure out what needs to be pursued clinically. I think one of the major problems is here because everything we are using as an assay, short of the transplant, is a surrogate and we don't have a good in vitro assay that allows us what is good to expand or not expand, and so we have to do these things right now in the patient and even though in ten years or five years we might have the right assay for stem cells or progenitor cells, we want to be able to make progress right now, and to do that

we need to do short-term pilot phase or Phase I studies in patients. This would be a way to allow that to happen without complicated agreements and complicated regulatory strategies.

We need a DNA-based chip which could be used for ID screening, also for sterility screening, and it would be nice to have a consensus for common definitions for clinical transplant endpoints so that these different strategies could be more easily compared to each other.

Then, there are lots of questions that remain unanswered, but some of the more important ones that I think have been mentioned throughout the two days are, one, that we don't know how to really mark stem and/or progenitor cells and we don't have anything that we can correlated with clinical endpoints yet, and we need to continue to look for those and we need funding to that. Because we don't know the answer to number one we can't answer number two, which is, is there an absolute cell does limit that would really define a unit as definite for engraftment or not.

We need to continue to look clinically as to whether there is a role for ex vivo expansion, and we need to do studies now that measure the contribution of HLA matching at low or high resolution using cord blood as a

source, for instance, observations we have made in bone marrow and then correlating that with cell dose.

We still need to learn the efficacy in adults, and those studies are ongoing. I think we are ready to do comparative studies in a prospective way with bone marrow transplantation in children.

Then, I think it is fascinating to start to consider what the multi-potentiality of cord blood cells is. Certainly, both from Dr. Zanjani's data and also just doing transplants in kids with bone marrows, it really looks that there are multi-potential non-hematopoietic cells in these grafts, and if that is true having a bank of HLA typed units may turn out to have applications that we are not eve thinking of right now, but in the future could be used for generation of cells of other organs that don't involve the hematopoietic system.

Comments? Questions? Liana?

DR. HARVATH: Thank you very much, Joanne, and I wanted to just thank everybody, all of the speakers and all of the participants for sharing such fascinating and interesting data with all of us.

There have been a couple of comments about helping the regulatory arena, and I don't know, are any of my former colleagues from FDA in the audience? Because I no longer officially work there and I don't want to

misrepresent the agency. So, Dr. Solomon, Dr. Marti -- is Dr. Lazarus here? Correct me if I am mis-speaking here but I have heard this recurrent theme of the regulatory agencies fostering the use of our ancillary products. So, they seem to be growth factors, cytokines, etc. And, what we would need to do is to find out how you envision the FDA or any other regulatory body helping with that because the FDA's authority is not really in approaching a particular biotechnology and getting them to work with one another but, rather, to look at the kind of work that has been done on a particular ancillary product.

Just so you know, many Phase I trials get launched with reagents/ancillary products that are not the same grade or the same purity of reagents that would be launched if that was the drug to be studied in a clinical trial. So, there is the ability to launch many Phase I trials, and this is commonly done for somatic cells where there is ex vivo manipulation of them. Usually what is asked for are certificates of analysis and some types of forms from the company to verify what kinds of chemical analyses have been done, and what kinds of microbial cultures have been done. I hope I am not mis-speaking for my colleagues, but when I left a couple of weeks ago that was basically what was looked at.

Is it beyond that that you are asking the agency for guidance on? I think if you could be real specific in what you would think the FDA could do to foster the availability of these and get that back to the agency it would be very helpful and I think it would help them understand the difficulties you are experiencing as investigators in this field. You are probably aware of this but there is an FDA group addressing the entire issue of ancillary products and they are looking for various kinds of input as to what the real problems are. Unless some of my former colleagues would like to comment further, I would encourage all of you to get that information directly back to FDA and, in fact, I would go so far as to give you the name of someone. She is the Deputy Director of the Division of Cellular and Gene Therapy. Her name is Dr. Joyce Frey Vasconcells. I don't have her number memorized, but she is a person who has been heading up the initiative and trying to work through the ancillary products issue. So, I would just like to offer that as a suggestion to all of you who are struggling with that issue. And, thank you very much again.

DR. KURTZBERG: Any other comments? Otherwise, thank you everybody, and we will close the meeting.

[Applause]

[Whereupon, at 4:46 p.m., the proceedings were adjourned]