UNITED STATES OF AMERICA

+ + + + +

DEPARTMENT OF HEALTH AND HUMAN SERVICES

+ + + + +

PUBLIC HEALTH SERVICE

+ + + + +

FOOD AND DRUG ADMINISTRATION

+ + + + +

CENTER FOR BIOLOGICS EVALUATION
RESEARCH

+ + + + +

BIOLOGICAL RESPONSE MODIFIERS
ADVISORY COMMITTEE

MEETING

+ + + + +

TUESDAY
MARCH 24, 1998

The meeting took place in Plaza II and III, Doubletree Hotel, Rockville, MD at 8:30 a.m., Julie M. Vose, M.D., Chair, presiding.

PRESENT:

JULIE M. VOSE, M.D., Chair
GAIL DAPOLITO, Executive Secretary
W. FRENCH ANDERSON, M.D., Member
HUGH AUCHINCLOSS, JR., M.D., Member
ELLIN R. BERMAN, M.D., Member
VIRGINIA C. BROUDY, M.D., Member
PAMELA M. HARTIGAN, Ph.D., Member

RICHARD HONG, M.D., Member
MICHAEL S. KATZ, Patient Representative
EUGENIE S. KLEINERMAN, M.D., Member
KATHERINE E. KNOWLES, Consumer Representative
ABBEY S. MEYERS, Consumer Representative (by phone)

PRESENT (cont'd)

WILLIAM M. O'FALLON, Ph.D., Member
JONATHAN SILVER, M.D., Member
ALTON FLOYD, Ph.D., Temporary non-voting Member
PATRICIA KEEGAN, M.D., FDA Representative
CAROLE B. MILLER, M.D., FDA Representative
JAY P. SIEGEL, M.D., FDA Representative
KAREN WEISS, M.D., FDA Representative

EDA BLOOM, Ph.D., FDA Speaker
JUDITH KASSIS, Ph.D., FDA Speaker
STEPHEN LITWIN, M.D., FDA Speaker
EDWARD MAX, M.D., FDA Speaker
PHILIP NOGUCHI, M.D., FDA Speaker
JAMES BERENSON, M.D., Sponsor Represenative
CINDY JACOBS, Ph.D., M.D., Sponsor Representative

MONIKA KRIEGER, Ph.D., Sponsor Representative

ALSO PRESENT:

KEN ANDERSON, M.D.
KEITH STEWART, M.D.
JOE TARNOWSKI, Ph.D.

BOB VESCIO, M.D.
MIKE WHITE, Ph.D.

A-G-E-N-D-A

Overview of Research Programs Laboratory of Cellular

Immunology Laboratory of Developmental Biology

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

(8:38 a.m.)

MS. DAPOLITO: Good morning. I would like to welcome Dr. Vose to the 22nd meeting of the Biological Response Modifiers Advisory Committee. I am Gail Dapolito, and I am the designated federal official for today's proceedings. I would like to begin by introducing the committee members and consultants seated on our panel today. I will begin on my left. Dr. Mike O'Fallon, Mayo Clinic; Dr. Jonathan Silvers is here from the National Institutes of Health; Dr. Virginia Broudy, University of Washington, School of Medicine; our industry representative, Dr. Alton Floyd of Trigon Technologies; Dr. Eugenie Kleinerman, MD Anderson Cancer Center; Dr. Richard Hong, University of Vermont; our Chair, Dr. Julie Vose, University of Nebraska; Dr. Ellin Berman, Memorial Sloan-Kettering

Cancer Center; Dr. Pamela Hartigan, VA Medical Center, Westhaven; Dr. Hugh Auchincloss, Harvard Medical School; Ms. Katherine Knowles, Health Information Network. Ms. Knowles has graciously accommodated our very last minute request to join us as a consumer representative today. We thank her very much. Next is our patient represenative, Mr. Michael Katz,

International Myeloma Foundation, and Dr. French Anderson, University of California.

The Center for Biologics Evaluation and Research is represented today by Dr. Patricia Keegan, Dr. Karen Weiss, and Dr. Jay Siegel, who will join us later.

Dr. Vose, with your permission, I will now read the conflict of interest statement. The following announcement is made part of the pubic record to preclude even the appearance of a conflict of interest at this meeting. Pursuant to the authority granted under the Committee charter, the Commissioner of FDA has appointed Ms. Katherine Knowles as a temporary voting consumer representative, and Dr. Alton Floyd as a temporary non-voting industry representative. In addition, the Director of the Center for Biologics Evaluation and Research has appointed the following individuals as temporary

voting members: Mr. Michael Katz and Dr. Jonathan Silver. Based on the agenda made available, it has been determined that all financial interests in firms regulated by the Center for Biologics Evaluation and Research, which have been reported by the participating members and consultants as of this date present no potential for an appearance of a conflict

of interest at this meeting with the following notations to preclude even the appearance of a conflict of interest.

Mr. Michael Katz, a patient representative, reported that he is a volunteer member of the Executive Board of Directors of the International Myeloma Foundation, a non-profit organization. The International Myeloma Foundation is being reimbursed by CellPro for the production costs of an informational booklet on bone marrow transplantation. Mr. Katz participated in a previous meeting between the IMF and a representative from CellPro regarding bone marrow transplantation. Mr. Katz received no personal remuneration for attending this meeting.

Dr. Carol Miller has recused herself from today's Committee discussions. The following members and consultant have no interests to disclose: Dr.

French Anderson, Dr. Hugh Auchincloss, Dr. Ellin Berman, Dr. Virginia Broudy, Dr. Alton Floyd, Dr. Pamela Hartigan, Dr. Richard Hong, Dr. Eugenie Kleinerman, Ms. Katherine Knowles, Ms. Abbey Meyers, Dr. Michael O'Fallon, Dr. Jonathan Silver, and Dr. Julie Vose. In the event that the discussions involve other products or firms not already on the agenda for

which the FDA participants have a financial interest, the participants are aware of the need to exclude themselves from such involvement and their exclusion will be noted for the public record. I turn it over to you, Dr. Vose.

CHAIRPERSON VOSE: Thank you. I'd like to welcome everyone to the hearings today. And I think that we will go ahead and -- is there anyone today that would like to come forward for the open public comment? Not seeing anyone at this time, we will go ahead with the hearings today. First would be to initiate the discussion on the pre-market approval for Ceprate, CellPro Incorporated. And we will go ahead with the presentation by CellPro.

DR. KRIEGER: Good morning. On behalf of everyone at CellPro, I would like to thank the FDA for inviting us to speak this morning and present the results of the Phase III Study. This study was

designed to expand the label indication for the CellPro Ceprate SC stem cell concentration system to include the use of peripheral blood.

The first slide here shows the instrument, the CellPro instrument system with the disposables attached to it. This system is currently in use at 300 medical centers around the world and more than

6,000 patients have been treated with cells that have been selected using the CellPro system.

Our presentation today will be divided into three parts. Dr. James Berenson, Professor of Medicine at UCLA will present the first part. He will discuss the Phase I/II study, which was the basis for our Phase III trial. He will present the development of the tumor detection assay which was used in our study as well as the clinical trial results from the Phase I/II study.

Then Dr. Cindy Jacobs from CellPro, our Vice President of Clinical Research, will present the results of the Phase III study, and she will also summarize the literature on additional tumor purging results using the Ceprate SC in patients with multiple myeloma, breast cancer, and lymphoma.

After the presentation, if there are questions, we have a number of people with us prepared

to answer questions. We have four clinical investigators with us who were part of the Phase III trail; Dr. Berenson, Dr. Vescio, Dr. Stewart, and Dr. Anderson. We also have two statisticians with us who can answer questions; Dr. Mike White of CellPro and Dr. Brent Blumenstein of the Fred Hutchinson. In terms of questions on the PCR assay, we have some

additional people; Dr. Jerry Radich from Fred Hutchinson, Dr. Amy Ross from MRD Diagnostics. And then if there are questions on the clinical trials, there are, of course, those of us from CellPro, including Dr. Jacobs, Dr. Sing, and myself.

Okay, I would like to now turn the meeting over to Dr. Berenson.

DR. BERENSON: Good morning. Multiple myeloma is a bone marrow-based malignancy characterized by monoclonal plasma cells, these fried egg-appearing cells in this patient's bone marrow. This is the second most common hematological malignancy with 15,000 new cases per year. Although a variety of different chemotherapeutic and biologics have been tried with varying response rates. none of these have translated to cure with a median survival of about 30 months. The use of high-dose therapy followed by either allogeneic or autologous bone

marrow support has a higher response rate than the conventional regimens. Whether it leads to an improvement in survival was not clear until the publication of a recent randomized study from the French Intergroup, in which 200 patients were randomized to either receive conventional therapy or conventional therapy followed by high-dose with

Melphalan and TBI followed by in this case autologous bone marrow transplant. In this study published in the New England Journal of Medicine, there was an improvement in overall survival as shown here in the high-dose arm compared to the conventional arm, and a recent update of this study presented to the ASH Meeting in San Diego in December continues to show the improvement in overall survival in the arm that underwent the high-dose followed by bone marrow support.

Although one thinks of myeloma as a bone marrow-based disease, there is evidence for peripheral blood involvement in most if not all patients, the most specific of which is based on molecular techniques developed in our laboratory and others. So that, therefore, it becomes not only of biologic, but possibly clinical importance to determine where in B cell differentiation this tumor may begin. In fact,

we know that CD34 is expressed on the pluripotent stem cell, and cells selected for this in the peripheral blood are capable of engrafting patients following myeloablative chemotherapy. However, we know that this antigen is also expressed on early B cells in this cascade. So, therefore, it became important to determine if any CD34 expressing cells were also part

of the malignant clone.

Now we are fortunate in multiple myelomas to have an excellent marker for the tumor, and that is based on the antibody that is produced by those monoclonal plasma cells and the specificity resides in the variable region, the amino terminal portion of the molecule which gives rise to its antigen recognition specificity. Three parts of that portion of the molecule called hyper-variable regions are the areas of the molecule with directly contact antigen. These have also been called complimentarity determining regions.

Thus, a molecular marker is possible in myeloma to determine malignancy based on the sequence at the gene level which gives rise to that portion of the molecule. Now the variable region is made up of 50 different genes which have been divided into 7 different families. Thus, one can develop a set of

primers here in the 5 prime region in the leader part,

and then in the 3 prime region, one can develop a set of primers based on C-gamma for IgG-producing myeloma or C-alpha for IgA producing myeloma. And using PCR technique on RNA turned into DNA by reverse transcriptase, one can obtain the sequence of this portion of the molecule.

Now importantly, those CDR's are again very specific in terms of determining malignancy, and that is because they are the portions of the molecule which bind to antigen. The CDR3 region is ont only made of a short stretch of nucleotides called diversity segment, but also is accompanied by the addition of non-germ line nucleotides called end-region addition. So it has additional specificity.

Thus, by obtaining that sequence that is expressing that part of the molecule, one has an excellent molecular marker.

Here is an example of one of the sequences that we obtained. In fact, this was one of the first ones many years ago, and this is what we called WAD#1, the patient sequence, and this is the most homologous germ line sequence called V1DP5. You can see in most parts of the molecule, there is identity between the patient sequence and the germ line sequence indicated

by the dots. But in those regions that bind to antigen by that process of somatic mutation, so the final antibody produced can more avidly bind to antigen, there are differences, again called somatic mutations. So this becomes an excellent specific tumor marker that we can develop primers for to detect malignant cells in the CDR1 and an accompanied 3 prime

primer encompassing that CDR3, and if you recall, that consists of that D segment plus the addition of non-germ line nucleotides. Thus, the combination of the CDR1 and the CDR3, pairs of those primers, becomes very specific at detecting only malignant cells in mixed population.

So in order to first answer the question of whether CD34 was expressed onto any malignant cells, CD34 cells were first enriched using the Ceprate device followed by flow sorting using a Ceprate antibody against CD34. And using patient-specific primers as I showed were generated in a similar manner to the previous slide, we could not find any amplified PCR product in the CD34 purified population, and yet the bone marrow from that same patient containing the tumor cells diluted out even 10,000-fold, we were able to find amplified product, suggesting indeed that CD34 was not expressed on the

malignant clone.

So this then provided the rationale for our Phase II study in which patients with advanced myeloma underwent high-dose chemotherapy followed by the use of autologous CD34 selected peripheral blood stem cells. The rationale, of course, the bone marrow has a lot of tumor and the blood has a little tumor,

and theoretically selecting for CD34 will reduced the tumor burden that will be given back to the patient following the myeloablative chemotherapy.

The regimen used is outlined here. We mobilized stem cells using intravenous cyclophosphamide, four days of oral prednisone, and daily injections of G-CSF. The stem cells were collected and then selected using the Ceprate device and stored away. Patients were then given high doses of Busulfan at 15/kg and cyclophosphamide at 120/kg. They then received back the stem cells, which again were CD34 selected. GM-CSF was used to enhance recovery. And in this particular Phase II trial, we used interferon and dexamethasone for one year post-transplant as maintenance treatment.

Now the tumor detection assay developed by Bob Vescio in our group is outlined in the next few slides. Again, primers were made that were

complimentary to the unique regions in the CDRs. We employed a 60-cycle PCR using our sample DNA, in this case either CD34 selected or unselected autograft material, which was serially diluted in normal, in this case, placental DNA to a final concentration containing the worth of 100,000 cells of DNA, which is .6 micrograms. And then the products were

electrophoresed and stained appropriately.

Now the quantitative nature of the assay was because we actually did serial replicates at each of these dilutions. I will outline this briefly for you. This is in cartoon form, of course. In this case, LK for leukophoresis DNA in the undiluted sample, there is only leukophoresis DNA. With serial dilution in placental DNA, we have, of course, less and less of our sample DNA and more and more of our normal DNA. And as you see out here, very little sample DNA and much normal DNA. So theoretically, a tumor containing sample, all of these reactions would be possible. All of these may be positive. And slowly this number would become zero out of 5. And then one can convert this to a percentage contamination in the undiluted sample using Poisson distribution analysis. And this is based on the fact that we can detect one copy of the target DNA, in this case that tumor-

specific immunoglobulin gene primer pairs, in our PCR tube. Again, as I showed you, five replicates done at each serial dilution. And then we can use the old computer using Poisson distribution analysis to determine the percentage of tumor in the undiluted sample.

And here is an example of one of the

patients actually from the Phase III trial, and here we are looking at post mobilization chemotherapy peripheral blood mononuclear cells. In part A here is the undiluted blood, and we serially dilute this out. So you can see the reactions using patient-specific primers. All five of these are positive, five of these, et cetera. And as we move out here slowly, these become negative. And again one then can convert using Poisson distribution analysis to determine the percentage of tumor in the undiluted sample and multiply that by the amount of leukophoresis cells obtained and obtain a number of tumor cells per kilogram in the leukophoresis product and then in the CD34 selected absorbed product.

These are the results from the clinical samples from the Phase II trial in which 18 patients were analyzed. 11 out of the 18 contain tumor in the unselected fraction as you see here, ranging from

about 4,000/kg to a high of 2 million. Following CD34 selection in the absorbed fraction, the tumor cell numbers were reduced below the level of sensitivity of this assay, which is 1:700,000 normal cells in the 8 cases you see. In the three in which we could obtain numbers, you can see they range from 83 to a high of 2,000, with a log reduction -- in most cases, we

couldn't give a quantitative number. It was a greater than because we couldn't obtain any tumor cells in the CD34 selected fraction. In the three cases that we could, there was approximately a 3 log reduction in tumor burden following CD34 selection.

Now despite that excellent reduction in tumor burden, there was very quick engraftment in that neutrophil recovery by a median of 12-days occurs, and platelets to 20,000 and 50,000 occurred at 12 and 13 days respectively. Importantly in this Phase II study, we also establish a threshold dose of CD34 required, and that was 2 million cells per kilogram.

In patients who received less than that threshold dose, there was a delay in neutrophil recovery and the patients had a delay in platelet recovery as well and required, as you see here, more red cell transfusions and platelet transfusions. This established our goal for a threshold dose of CD34 for the Phase III trial,

which will now be presented by Dr. Cindy Jacobs.

Thank you.

DR. JACOBS: For the Phase III study, I will first present the study design and then the patient characteristics and processing results, next the primary and secondary endpoints, and the long-term follow-up results at one year post-transplant.

This is a controlled Phase III study randomizing patients to either have their stem cells CD34 selected using the Ceprate system or unselected. The 15 participating sites are listed here in order of accrual, with UCLA, Toronto Hospital, Dana Farber Cancer Institute, Washington University, and the University of South Florida occurring the majority of the patients.

This shows the scheme of treatment. Patients were registered if they had a diagnosis of multiple myeloma and stable or responsive disease after 3 cycles of chemotherapy. They were excluded if they had progressive disease or greater than 3 months of ablative therapy or a total of greater than six months of chemotherapy. All patients were mobilized using cytoxin, prednisone, and G-CSF. The patients were randomized just prior to starting leukophoresis and PBPC collection to have their stem cells either

Ceprate selected or unselected using standard procedures. All patients received high-dose chemotherapy consisting of Busulfan and cytoxin, and then either received their CD34 selected stem cells or their unselected stem cells. All patients received GM-CSF post-transplant. The primary study period was six months, and we have annual follow-up that is

ongoing.

There were two primary study objectives, one for safety and one for efficacy. The safety objective was to demonstrate equivalent neutrophil engraftment for both arms. The efficacy objective was to demonstrate a reduction in tumor cells in the Ceprate selected arm.

There were four main secondary safety endpoints; time to neutrophil engraftment, time to platelet engraftment, percent of patients with infections, and overall survival at 6 months. There were a number of other secondary endpoints. The first six listed here were additional secondary safety endpoints. The next three listed are additional secondary efficacy endpoints. And the last two were long-term follow-up endpoints.

I would like to now show the patient characteristics and processing results. There were

134 patients that were registered on the study. Three of those patients were not randomized to the study. So of the 131 patients registered and randomized onto the study, 67 were in the CD34 selected arm and 64 were in the unselected arm.

Of the 131 patients randomized, all were infused except for one patient. This patient had

inadequate mobilization of stem cells. This patient ended up withdrawing consent for transplant on this study. So there were 130 patients that were infused.

This is a summary of the demographic characteristics. The arms were comparable for the demographic characteristics except for one, gender. There were more females in the CD34 selected arm than in the unselected arm. For the other parameters -- race, age, weight, and years since first diagnosis -- the arms were comparable. Although not shown here, the arms were also comparable for other disease characteristics as well as prior cancer-related therapy.

Of the 40 various laboratory parameters that we measured at baseline and at randomization, all were comparable between the arms except for four, and they are listed here. For the white blood cell count and platelet count, the CD34 selected arm had

significantly lower counts in the unselected arm. For the median white blood cell count, the CD34 selected arm had approximately 16,000 compared to 26,000 with a P value of .04. The median platelet count for the CD34 selected arm was 110,000 versus 152,000, with a P value of less than .01.

The CD4/CD8 ratio was also significantly

lower in the CD34 selected arm compared to the unselected arm with a P value of .03. And the number of patients that had reactivity to CMV were higher in the CD34 selected arm than in the unselected arm with a P value of .01.

This is intended to keep you awake. For the first two parameters, the clinical relevance of this will be discussed later. For the latter two parameters, the clinical relevance is unknown at this point. However, the overall trend for these four parameters was in favor of the unselected arm.

Before going over the processing results, I would like to briefly go over each step in each of the arms. For the CD34 selected arm, the product to start is incubated with the CD34 antibody. Then the cells are processed using the Ceprate system yielding a CD34-enriched product and a CD34-depleted product.

For the CD34-enriched product, it is cryo-

preserved until transplant was thawed just prior to infusion. For the unselected arm, the product to start was cryopreserved until transplant and then thawed just prior to infusion.

Let's first look at the number of leukophoresis. The majority of the patients required two leukophoreses. However, if you look at the

overall general distribution, you can see there were a few less patients in the CD34 selected arm requiring two and a few more requiring three and four when compared to the unselected arm. This overall distribution was statistically significant.

So we did additional analyses to look at factors influencing the number of leukophoresis and there were two important factors. First of all, this study required that a minimum of two leukophoreses were obtained, and leukophoresing continued until all patients had 5 x 108 nucleated cells per kilogram. For the CD34 selected arm, there was an additional criterion for post-processing to have at least 4 x 106 nucleated cells per kilogram. This is really based on achieving a minimum of 50 percent CD34 cells, so the minimum target of 2 x 106 CD34 cells per kilogram would be achieved. 18 of the 67 patients or 27 percent of the patients required an additional leukophoresis

because of this criterion. However, this is where, again, the baseline differences between the arms also appeared. As with the WBC or the hematologic values at randomization, which occurred just prior to leukophoresing, the trend toward -- there was a trend toward the CD34 selected arm requiring an additional leukophoresis than the unselected arm to at least

achieve the first criterion of 5 x 108 nucleated cells per kilogram in the peripheral blood product. So both of these played a factor.

This slide summarizes the processing results so you can see the median of 2 leukophoreses in both arms with overlapping ranges. The number of CD34 cells at the various processing steps are here. Both arms were comparable for the number of CD34 cells in the initial product with the median of 10 x 106 CD34 cells per kilogram in the CD34 selected arm and 8.7 in the unselected arm. The overall yield in this trial was 60 percent. So in the enriched product, the median CD34 cells infused was 5.4 x 106 per kilogram compared to the 8.7 x 106 for the unselected arm.

Let's now review the primary safety and efficacy endpoints. The primary safety endpoint was successful neutrophil engraftment on or by day 14. For the CD34 selected arm, 94 percent of the patients

achieved this and 100 percent in the unselected arm.

Of the four patients who did not achieve neutrophil engraftment by day 14, they are listed here. Three of those patients engrafted on day 15. One of the patients was not infused, and this is the patient you saw previously that had inadequate mobilization and withdrew consent for transplant on the study. Since

this was an intent to treat analysis, this patient was considered a failure to engraft. The intent to treat analysis for the study did show that the arms were equivalent in successful neutrophil engraftment.

For the primary efficacy endpoint, an attempt was made for all patients to obtain a clonal immunoglobulin sequence and then to assess the number of tumor cells in the initial PBPC product. To that end, 42 percent of the patients in the CD34 selected arm and 30 percent in the unselected arm did have their clonal immunoglobulin sequence obtained. Thus, for those patients, we could look at the number of tumor cells in the initial PBPC product. The median number of tumor cells in the CD34 selected arm was 2.6 million. For the unselected arm, it was 2.3 million. Both of these parameters were comparable between the arms prior to processing.

The reasons that a clonal immunoglobulin

sequence were not obtained are listed here. Although the reasons varied, the arms were comparable for the various reasons that a clonal immunoglobulin sequence was not obtained.

In looking at the Ceprate arm then prior to and after processing, in the initial PBPC product, the median, as you saw before, of tumor cells was 2.6

million with a range of zero to 363 million tumor cells. In the CD34 enriched product, the median was zero. This is mainly because the majority of the patients had no detectable tumor cells. The range still showed zero to 1.2 million tumor cells. In the CD34 depleted product, the median number of tumor cells was approximately 9 million with the range of zero to 375 million similar to the PBPC product.

If you now take these numbers for each individual patient and calculate the log depletion of tumor cells for before to after processing, the median log of tumor cells was 3.1. The range was 1.6 to 6 logs of tumor cell depletion. For those patients that had no detectable tumor cells in their enriched product, we calculated for each patient the lower limit of detection and considered there was residual tumor cell and used that value to calculate the log depletion. So the log depletion is underestimated for

those patients.

We also, of the 28 patients -- there were four patients who had no detectable tumor cells in the initial PBPC product and no detectable tumor cells after processing, and those four patients are not in this analysis.

We did a couple of analyses looking at

mass balance of tumor cells. This figure represents one of them. We looked at linear regression, taking the number of tumor cells in the enriched product plus the number of tumor cells in the depleted product and compared it to the number of tumor cells in the initial PBPC product. This shows the linear regression line. One would expect the intercept of 0 and a slope of 1. The slope of this line was 1.02 and an r coefficient of .98.

I would like to now review the main secondary safety results. Based on neutrophil engraftment, the median day was 12 in both arms with overlapping ranges. Based on platelet engraftment, the median was day 11 in the CD34 selected arm versus day 9 in the unselected arm. This two-day difference was statistically significant. I think you can better see it in the Kaplan Meier curve, where you are looking at the CD34 selected arm in the solid line and

the unselected arm in the dash line. You can see there is a slight delay here.

In this protocol, we had prospectively planned to do an analysis looking for factors influencing engraftment just as we had done in the previous Phase III trial. And as we had done in the previous Phase III trial, we looked at various

clinical variables and processing variables and did a multi-variate analysis looking for which factors were the most significant in influencing platelet engraftment and platelet recovery. Platelet engraftment is platelet transfusion independence. Platelet recovery is platelet transfusion independence and a platelet count of greater than 20,000. The analysis for both of these is the same, so they will be presented together.

The multivariate analysis showed two significant factors, CD34 cells per kilogram in the infused product and the platelet count at randomization. Since we had already put in the trial to look at all engraftment, we did the same analysis for neutrophil engraftment as well. Only one factor came out in neutrophil engraftment and that was CD34 cells per kilogram in the infused product.

Now we were already sensitive to the

number of CD34 cells because in the previous Phase I/II trial, we already knew that the target should be 2 x 106 CD34 cells per kilogram. So we did look at the population of patients, regardless of arm in this study, evaluating that. For the 130 patients, five patients we did not have CD34 values. So 108 patients here received greater than 2 x 106 CD34 cells. The

remaining 17 patients had less than 2 x 106 CD34 cells. And you can see here the significant delay in platelet engraftment. Of the 17 patients that received less than 2 x 106, there were 10 in the Ceprate arm and 7 in the unselected arm.

We then looked at comparing the arms for the 108 patients with greater than 2 x 106, and there was no significant difference in time to platelet engraftment.

We also looked at adjusting for CD34 cells per kilogram and platelet count at randomization. If you also adjust for those factors, there is no significant difference between the arms.

Incidence of infections was a main secondary safety endpoint. As you can see here, the number of patients with at least one infection from day zero to day 100 was comparable between the arms. Also out to day 6 months was comparable. Severe or

life-threatening infections from day zero to day 100 were comparable and out to 6 months were also comparable.

The main secondary endpoint of survival is actually shown here as the number of deaths. There were only 7 deaths that occurred six months post-transplant. Two in the CD34 selected arm, 5 in the

unselected arm. The two in the CD34 selected arm died of underlying malignancy. Of the five in the unselected, one died of infection, one of VOD, and the other three of underlying malignancy. So the arms were comparable.

Let's now look at the other secondary safety results. Number of transfusions were evaluated to day 100. Platelet transfusion events, the median was 3 in the CD34 selected arm versus 2. This was statistically significant. Since platelet transfusion independence was a criteria for platelet engraftment and recovery, we looked at the number of platelet transfusion events for those patients greater than 2 x 106, and there was no significant difference between the arms. We also adjusted for platelet count at randomization. And if you adjust for platelet count at randomization, again there is no significant difference between the arms for platelet transfusion

events. There was no difference between the arms as far as red blood cell units given.

The incidence of bleeding events were comparable between the arms. There were no new bleeding events reported after day 100.

The immunity reconstitution was evaluated in this study for all patients looking at

immunophenotyping and serum quantitative immunoglobulin levels. We also looked at lymphocyte proliferation and viral serologies in a subgroup of patients. For cell-mediated immunity, there were no differences between the arms at baseline, day 100 or 6 months visits for total lymphocytes, CD3 cells, CD8, and CD56 cells. There was also no difference between the arms at baseline day 100 and 6 months for PHA ConA and tetanus for the lymphocyte proliferation assays. There was a significant difference in CD4 counts at day 100 and 6 months. For example, at day 100, the median CD34 count in the selected arm was 211 compared to 298 in the unselected arm. The relevance of this significance is unknown.

For humoral immunities, there were no differences between the arms for the quantitative immunoglobulin levels assessed or reestablishment of antibody reactivity to various viruses. If you look

at late infections, there were no differences between the arms in incidence of infections after day 100 to the 6-month visit for the arms, and there were no differences between the arms in the type of late infections either.

Let's look at the secondary efficacy results. We looked at the number of tumor cells

infused not only for the Ceprate selected arm but the unselected arm for those patients that we obtained a clonal immunoglobulin sequence. You have seen these numbers before with the median of zero. The unselected arm, the median was 2.3 million, and this was significant.

Here we see the number of patients that had no detectable tumor cells in the initial leukophoresis prior to processing or in the initial product. There were four patients in the selected arm that had no detectable tumor cells or 14 percent, and also 4 in the unselected arm. There was no significant difference between the arms prior to processing. After processing, 54 percent of the patients had no detectable tumor cells and this was significantly different.

Incidence of infusional toxicities were also evaluated. The incidence of hypertension was

significantly lower in the CD34 selected arm compared to the unselected arm. The incidence of bradycardia was also lower in the CD34 selected arm compared to the unselected and approached significance. There was no difference in heart block or atrial arrhythmias. So the reduction in hypertension and bradycardia is similar to our previous approval for bone marrow.

Let's look a the long-term follow-up results. We looked at hematologic values at 6 months for ANC, platelet counts of less than 50,000, hemoglobin of less than 10 grams, and there was no difference. The arms were comparable. We had one patient in the CD34 selected arm that received the back-up depleted product at day 119 for platelet count of less than 20,000. This patient was platelet transfusion independent. And even after infusion of the back-up, platelet counts remained below 20,000.

Although the curve is immature, this is a Kaplan Meier for overall survival, and there is no difference between the arms. And again, although the curve is immature, this is the progression pre-survival for the selected arm and the unselected arm and there is no difference between the arms at this point.

So in summary, this study successfully met

the primary safety and efficacy endpoints, the safety being successful neutrophil engraftment by day 14 and the efficacy for a significant reduction of tumor cells in the Ceprate arm. There were no statistically significant differences between the arms for time to neutrophil engraftment, incidence of infections, and overall survival. However, there was a significant

difference in median time to platelet engraftment, which was 11 in the Ceprate arm and 9 days in the unselected arm. When evaluating this further, the time to platelet engraftment and the number of platelet transfusion events were influenced by the number of CD34 cells infused and the platelet count at randomization. There were no significant treatment arm effects after adjusting for these factors and there were also no significant differences if you evaluate patients who received at least 2 x 106 CD34 cells per kilogram.

There were no statistical significant differences between the arms in the number of other secondary safety endpoints. Some of them I have not presented like days of hospitalization and incidence of adverse events.

The secondary efficacy endpoints, there was a reduction in the number of tumor cells infused.

I didn't present this, but there was a median 74-fold enrichment of CD34 cells and a reduced proportion of patients with hypertension post-infusion.

I would now like to just briefly summarize the additional tumor purging results from other investigative studies that are published using the Ceprate system. In your briefing document, we have

presented results from the Phase I/II multiple myeloma study that Dr. Berenson showed you. We also went back and looked at the breast cancer purging in the previous Phase I/II study from the prior approval. Since these data are presented in the publications, I am not going to present it separately.

Let's just first go to the publications looking at tumor purging for multiple myeloma patients. Of the studies, three of them looked at log tumor depletion. The Schiller publication does contain some of the Phase I/II data. This showed a range of 2.5 to greater than 4.5 log tumor depletion.

Looking at the number of contaminated products purged below detection after the Ceprate system, it was 63 percent, 140, or 100 percent depending on the samples.

Of the publications looking at tumor purging for patients with breast cancer, both peripheral blood and bone marrow have been evaluated.

The log depletion, the median was 2 with a range of 1 to greater than 4 for both bone marrow and peripheral blood. For the number of contaminated products purged below detection, it was 18 for bone marrow and 67 percent here for peripheral blood and 100 percent here in the Vogel article.

This slide shows tumor purging for

patients with non-Hodgkin's lymphoma, again evaluating both peripheral blood and bone marrow. There were two publications that actually quantitated the log tumor depletion showing a range from 1 to 3 logs of tumor depletion. For the number of contaminated products purged below detection, there was 89 percent, 60, 80, and 75 percent.

Thus, based on the Phase I/II study, the Phase III study, and the additional investigative studies in the literature, CellPro would like to expand our indication to include peripheral blood progenitor cells in addition to the autologous bone marrow. We would like to include a selection of peripheral blood results in greater than 100-fold or two log reduction in the number of tumor cells present in the autograft. And we will also recommend for peripheral blood that at least 2 x 106 CD34 cells per kilogram be collected after selection. That is it.

CHAIRPERSON VOSE: Thank you, Dr. Jacobs.

I would just like to announce that Abbey Meyers, the consumer representative, is joining us by phone before we start into questions. And I would like to open it up for questions of the sponsor from the committee. I guess we can't turn up the lights apparently, so we are in the dark. They are permanently dim. Dr.

Auchincloss?

DR. AUCHINCLOSS: Can I come back to Dr. Jacobs, and in particular to your comparison between the number of tumor cells before and after processing. The slope of the curve that you showed us was 1 or essentially 1, but there are individual points, obviously, that are well outside of the line. And in particular, you have at least two that appear to have 105 cells prior to processing and then 108 cells after. What does that say about the accuracy of the assay?

DR. JACOBS: You mean at -- there were no patients that actually had more tumor cells after processing. There were three outliers there, but none of the patients had a total of more tumor cells after processing.

DR. AUCHINCLOSS: No. I am not saying in the product that you infused, but when you calculate

the number in the enriched plus depleted, there were 108 cells calculated, whereas there were 105 cells in the original product. How did you gain three logs worth of cells?

DR. JACOBS: Mike, do you know? I am not quite sure I am understanding. Heather, can you put the slide with the linear regression line? If you

would just put it in the middle of the carousel, I can get it to that slide. I am not quite sure -- keep going forward just a little bit. There you go.

DR. AUCHINCLOSS: So if you just focus on those two points in the upper left-hand area, you originally start with a calculation that says that you have 105 tumor cells. And that is -- at the end, it measures out to 108 tumor cells in your combined enriched and depleted product. And my concern is what does this say about the accuracy of the individual numbers that you have if you can go from 105 to 108 cells in one preparation?

DR. VESCIO: Well, my name is Dr. Vescio, and I am one -- I guess the person that oversaw the tumor quantitation part of the trial. And I think as with any assay that one develops, there is going to be some variability. And we did -- as was submitted to the Board, we did an initial set of experiments to

verify the accuracy of the assay and we found that in general the assay was accurate within a half a log or actually in the majority of cases, less than half a log, 0.2 logs by the standard deviation. Obviously these points here, there was more variability than one would have -- than that would have suggested. But again, it is just a matter of the variability between

the assays. These are all done by hand and there is going to be, as expected, some variability. But if you look at overall, the results were very comparable and what one would expect comparing tumor burden in the combined enriched and depleted product versus the leukophoresis product itself. So I think obviously one did not create tumor cells out of air. But I think it just shows that at least just for these two cases, there was a little bit of variability in the assay.

DR. AUCHINCLOSS: Well, as I understand it, the central issue here is the degree of accuracy in the assay.

DR. VESCIO: Sure.

DR. AUCHINCLOSS: And for you to suggest that it is accurate to within half a log I guess doesn't fit with a picture that says -- there are two patients up there at least, and actually there are

several others -- but there are two at least there where it is not accurate within 103.

DR. JACOBS: Actually, with this analysis, if you are adding enriched and depleted, that half log variability for both would probably be a full log of variation.

DR. VESCIO: And I think that if -- you

know, again, there is always possibility for some error. We could only run the samples once. And I think that if one looked at, again, when the assay was verified, all the results were done there and those were done in quadruplicate. And in those particular cases, the assay variability was less than a half a log. We also did some controls looking at beta-actin quantitatively and also in all of those cases, the variability was less than a half a log. So I think although I agree in these particular two examples out of the numerous cases that were done in assay, those fell outside of the variability that one would have seen. But I think the majority of the patients followed what one would have expected. And for this very complicated assay, I think that in general it -- one would expect the variability to be balanced on both ends, and I am not sure that it really would have influenced the results.

CHAIRPERSON VOSE: Dr. Silver?

DR. SILVER: I'm not sure I understand fully the details of the collection process, but the essence of my question is the enriched product contains maybe 50 percent or 20 percent or some percentage of CD34 negative cells which are not selected against in this process -- contaminating the

enriched population. Is there any reason that you would not expect the number of tumor cells in that half of the cells which are not CD34 positive to be depleted if half of the enriched product is basically a random sampling of the non-CD34 cells, and that includes the tumor cells, then wouldn't you expect about half of the product to contain the original number of tumor cells?

DR. VESCIO: That is a very good point and in fact that is basically what is seen. Really there was some additional elimination of tumor cells. But if you actually look at the percent contamination within the product, it is not that dramatic. It is maybe a half log or maybe a log less in tumor cells within the 34-enriched product. But the effectiveness of the separation procedure is primarily by reducing the total quantity of cells that one gives back to the patient, since one gives back to the patient two logs

less of autograft cells with the CD34 selection. So I think when we measure actually tumor within the product, the percent contamination of the enriched product is only about as one would have expected, about a half to one log less by that procedure.

DR. JACOBS: If you look at the Ceprate procedure, what we look at is probably about a two log

depletion due to the loss of the contaminating cells while you are purifying or you are selecting the CD34 cells. And a log of 3 for the tumor purging -- I mean, there may be some additional tumor purging depending on the type of tumor cell going through actually easier in the wash in the depleted. I don't know, Jim, if you have anything more to add from the Phase I/II study and what you have seen in tumor purging.

DR. SILVER: Could you clarify, do you wash the column before you elute or do you just collect the cells that are CD34 positive and then elute them?

DR. JACOBS: You wash or you elute the column as far as the depleted cells. And then to get the CD34 selected cells off, there is a little mechanical stir bar that gently washes the cells off when you put them, RPMI in this case, to the column.

DR. BERENSON: Well, I think the only

comment I would make is that the sensitivity of the assay is about where the tumor contamination is. You saw in several cases that the unselected product, approximately 20 percent in both the Phase II and Phase III, did not contain tumor and that is why we had to put greater than signs in those patients that went completely to negative. But obviously we can't

tell you the exact number. But again, your point is well taken that we are going from approximately 1 percent positive to 80 percent positive or 99 percent negative to approximately 20 percent negative, which is less than a log per se. But again, we have more than two logs simply by cell number. The additional close to approaching the log approximates the three logs that Dr. Vescio found, very close to what we would expect. But I would not argue against that the column itself could add additional stickiness in terms of possibly the cell sticking on. We don't know. I mean, that really has not been looked into. But three logs is pretty much what we would expect and that is what we got.

DR. SILVER: I thought two logs is what you would expect from your selecting 1 percent of the cells?

DR. BERENSON: No, that is incorrect. As

you know -- as Dr. Jacobs presented, we do actually give back less than 1 percent of the cells. And in addition, we go from 99 percent, if you will, of CD34 negative to approximately 30 percent CD34 negative.

So adding that additional approximated log, we would expect about 2.2 logs, 2.3 plus .7, about 3 logs, which is what we got.

DR. O'FALLON: Well, just let me comment on this figure. I find it hard to believe that the correlation coefficient is as high as you are quoting. It seems unlikely to be .98 with those two points that we have already attended to being that far away from the line.

DR. WHITE: I am Mike White from CellPro. I don't know what to say, Dr. O'Fallon. We ran the analysis in SAS. This analysis is not meant to be predictive. This is meant to be a descriptive analysis of what is going on. We aren't trying to suggest that there is any sort of prediction attached to the regression. It was a zero intercept model where we are only estimating the slope of the regression. And as Dr. Jacobs presented, the slope was essentially one. We have also done other analyses look at the ratio of the number of tumor cells in the PBPC product to the sum of the number in the depleted

and enriched product. Again, in this case when you look at the log ratio, you would expect it to be zero. We tested it to see if it was significantly different from zero. It was not significantly different from zero. That is not to say that it is equal to zero, but simply that we did not see any trend in the data for loss of tumor cells in the depleted plus the

enriched product. And that is really all we were trying to show there. Not so much is the slope 1, but is there any trend toward loss of cells. And that is what we were trying to get at in these analyses.

CHAIRPERSON VOSE: Dr. Berman?

DR. BERMAN: Is the claim that there is reduced toxicity from the separated product, that is just based on the fact that there is less DMSO? It is a 7.5 percent versus 10 percent, right?

DR. JACOBS: Also there was a reduction in the volume of infusate also that made a difference as well.

CHAIRPERSON VOSE: Mr. Katz?

MR. KATZ: The four patients that had a higher percentage of tumor cells in the purified product, does it follow that it is still better for them to have that product infused versus the original?

DR. JACOBS: If you take the percent and

then you multiply it by the number of nucleated cells in the infused product, two of those four patients had the 1.6 log depletion, the other one was close to 2 logs, and the other two were greater than 2 logs. So even what you give back is still going to be roughly 1.5 to 2.5 logs even for those patients.

MR. KATZ: Thank you.

CHAIRPERSON VOSE: Just to get back to the MRD assay for a minute. The CDR3 assay is such a difficult assay to perform it so individualized for patients, and there is a wide variety, as you have shown, for patients that couldn't be amplified or other reasons that in the end you end up with a fairly small number of patients that could actually be used for that. So do you want to comment on that relative to the small number of patients that could actually be used for your analysis?

DR. JACOBS: Actually, one of the problems that we had was the criteria -- the inclusion criteria. The patients had to have responsive or stable disease after a minimum of three cycles of chemotherapy. The bone marrow sample for which the sequence was attempted to be obtained was right at baseline just prior to going on to the study. So in many of the patients, obviously, with responsive

disease, it was more difficult to obtain the clonal sequence in those patients. I think we would have gotten a greater percentage of patients if we had had like de novo patients, where on their initial diagnosis we were able to obtain a bone marrow sample. So we were at a little bit of a disadvantage in this study.

DR. BERENSON: Julie, in the Phase II trial, we actually obtained bone marrow earlier on. So in that trial in the patients we attempted, we could actually obtain sequences with primers that were usable in about 70 percent of the cases. And you saw the data was quite consistent with Phase III. Obviously that wasn't a blinded kind of trial because it didn't come under FDA jurisdiction.

DR. JACOBS: Actually, that was a heated discussion with the investigators as well because they wanted to use -- if they had a bone marrow sample from diagnosis, to use it. But we were very conservative and we said no because then we could have injected bias into the number of patients from arm versus the other that could have had their clonal sequence. So we were very strict on it had to be the bone marrow sample at the time that the patient was registered and had informed consent.

CHAIRPERSON VOSE: Well, I think that is the correct thing to do. It is just a little concerning that in the end we only ended up with a fairly small number of patients that could be analyzed, even though that was the crux of it.

DR. JACOBS: Right.

CHAIRPERSON VOSE: Additional questions

for the sponsor? Dr. Auchincloss?

DR. AUCHINCLOSS: The FDA document that we are going to be looking at a little bit later on talks about three patients in whom -- these are 008, 009, and 0013 -- in whom the reduction in total nucleated cells was approximately equal to the reduction in tumor cells. I just want your comment on those three patients that we will be hearing about. Does that imply that tumor cells were, in fact, sticking to the column there?

DR. JACOBS: Well, we don't have any evidence for that. I mean, we haven't looked at that.

What was the site number? The first two numbers on the patient --

DR. AUCHINCLOSS: They are all from 2600 or 260.

DR. JACOBS: Okay. Actually, those patients were from a Dana Farber, and there was

nothing that was -- I mean, Ken, if you would like to comment as far as processing results for those patients abnormal. I think those patients were just -- two of them were the ones that were on the lower end of tumor purging, 1.5 to 2.5 logs.

DR. AUCHINCLOSS: And again, I am just asking because I assume we will hear about these

later. I wanted to hear any comments. But again, does it suggest to you that there is some variability in the assay?

DR. JACOBS: Well, I mean, again I think we realize the variability of the assay is at least half a log.

DR. AUCHINCLOSS: And one other question for you. That is in your progression free survival curve, have you made any effort to determine whether those who have not shown progression free -- sorry -- those who have shown progression free survival came from the group that got zero tumor in the --

DR. JACOBS: No. Right now there is no correlation although the numbers are small. We will be looking at that, but there is none at this point.

DR. AUCHINCLOSS: Is it fair to say, therefore, that you have no evidence that this procedure of tumor cell depletion does a patient any

good?

DR. JACOBS: At this point, no, this study wasn't designed to show that obviously with the numbers.

DR. AUCHINCLOSS: I understand that.

DR. JACOBS: But obviously the principle is giving back tumor cells is not good. And we do

have plans to follow this study for long-term, although we may or may not see a significant difference with the number of patients that we have in this study.

DR. AUCHINCLOSS: Well, there is -- yes, I agree with you. There is sort of a general sense that it is probably not good to give back tumor cells. That seems like a reasonable supposition. On the other hand, but putting tumor depletion in your labeling, there is an implication, I think, that you are doing somebody some good. Is that your feeling?

DR. JACOBS: Well, I mean when this was originally discussed as a primary efficacy endpoint, it came from the BRMAC meetings that happened in 1994 that at least if there were no safety issues that depleting tumor cells would be an endpoint for a pivotal study.

DR. AUCHINCLOSS: I am familiar with the

meeting that you are referring to. Also, that meeting suggested that it would be tumor specific. Do you agree with that point also made by the committee at that point?

DR. JACOBS: Right now our Phase I/II and Phase III, that is why we chose multiple myelomas because of the specificity of the assay, so we could

show proof of principle. The only other publications that we have were with breast cancer and non-Hodgkin's lymphoma.

DR. AUCHINCLOSS: My only point being that if one simply took the word of the committee in 1994, you wouldn't label, based on the current trial, for more than multiple myeloma.

CHAIRPERSON VOSE: Dr. Silver.

DR. SILVER: One other question about the assay, the PCR assay. Probably the major concern is that you might be missing some cells -- that the assay might not be sensitive to a single positive cell. Was the assay tested for its ability to detect a single copy of DNA on more than just one of the primer pairs because all of the primer pairs are different for each patient?

DR. VESCIO: I think because of the -- there is no gold standard for getting a sample of say

myeloma bone marrow in these patients, particularly patients that have been treated for three or four months. There is no way of having a standard that one could insure and be absolutely positive that one could detect just one copy of tumor cell DNA within a tube. However, in the majority of cases -- actually, in the case when we compared tumor contamination within the

bone marrow, we initially looked at tumor contamination in that original bone marrow specimen, and it was consistent with the amount of contamination that one found on the cytospins of those same bone marrow specimens. So one could then assume that, indeed, we were able to detect one copy of the target gene. We have also subsequently quantified the presence of the beta-actin gene within cells from various patients and did indeed show in those three patients from the leukophoresis and the 34-enriched specimens, that we were able to in all of those cases detect one copy of the beta-actin gene by the assay itself. So I think there is no way of absolutely proving in an individual patient that one could detect one copy per gene. In this particular analysis, it is a relative comparison anyway. And so if by some chance there are some patients where the detection is less than one or we need more than one copy of the

gene per cell, it should not influence the results, because again it is a relative comparison. The absolute numbers may be a little bit off in those cases if that occurs. But the percent of tumor log depletion would not have been changed by the sensitivity of the assay.

DR. SILVER: Since you cloned the gene for

each patient and sequenced it, you could have done a reconstruction experiment where you dope in a certain number of molecules of the clone target into a pool of .6 micrograms of genomic DNA or placental DNA and see what the sensitivity of the assay is. Was that done on --

DR. VESCIO: We did not do that. You can -- that can be done. The problem with even that particular technique is that it is different to dope genomic DNA with plasma DNA, which may be more easily amplifiable than the actual genomic DNA. I think genomic DNA is more difficult to amplify in the first place. So some groups have now subsequently looked at that, but again I think the results looking at the original tumor contamination within that primary bone marrow specimen was pretty convincing that we were, indeed, able to detect one copy of the target cell within a PCR tube.

DR. BERENSON: One thing to reemphasize is that we, as part of the validation, looked at, as you know, multiple bone marrows as well as cell lines. And certainly we were accurate using three different techs who were blinded within this .5. In most cases, it was within .2 or .3. So to somehow argue that this assay is more off than that would be, I think,

misleading, given the fact that we have taken fresh bone marrows from myeloma patients where we knew the percent plasma cells as well as tumor cell lines. Somehow you would have to argue not only is the assay inaccurate, but that it is biased somehow toward the CD34 arm, which I think that is a lot to fathom.

DR. SILVER: One last relating question. When you see a significant or a major drop in the percent of tumor cells in the enriched product, one concern is that you are getting a false -- and you don't see any tumor cells -- the concern is that you are getting a false negative to say something inhibiting the PCR or the sensitivity of the PCR down to one molecule in those assays. Did you do any mixing experiments where you mixed a sample from the enriched cells in which you couldn't detect tumor with DNA from cells in which you knew there was tumor present or tumor DNA present to see if you inhibited

the ability to detect a small number of copies.

DR. BERENSON: Well, I think the beta-actin really is against what you say. That Dr. Vescio has run beta-actin primers on several different patients and indeed has shown an accuracy of the assay for detecting DNA to within .5, in fact in most cases it was within .2 to .3 logs. I don't know, Bob, if

you have another comment.

DR. VESCIO: Yes. I think one of the things to insure that indeed these results were reproducible, one of the additional mechanisms and procedures that we did to insure that the results were reproducible is that we always processed the 34-enriched specimen and the leukophoresis specimen and some bone marrow specimens that were diluted to a point where the bone marrow became at the limits of detectability, and those were all processed at the same time in the same PCR machine. So, again, we showed in those cases the results -- the bone marrow controls were consistent to what one would have expected. And again, the 34-enriched and the leukophoresis products were processed at the same time in the same machine, and we had this beta-actin control that was always positive in the enriched specimen and also in the leukophoresis specimen to

show that the DNA was, in fact, qualitatively sufficient for the procedure itself. So I think with all those controls, we feel very confident of the results that were obtained.

CHAIRPERSON VOSE: Dr. O'Fallon?

DR. O'FALLON: Three questions. Was randomization of the patients stratified in any way?

I couldn't detect that from reading your description.

DR. JACOBS: The patients were stratified for age greater than 55 and less than 55.

DR. O'FALLON: Okay.

DR. JACOBS: That was the only stratification that we used.

DR. O'FALLON: Were they stratified within the individual centers, then, separately?

DR. JACOBS: Yes.

DR. O'FALLON: Okay. Several of the centers had very small numbers of participants. Did any of the analyses change if you analyzed only those centers which had more than --

DR. JACOBS: No. There was no variation or no site effect that we could see when we did those analyses.

DR. O'FALLON: Okay, the last question. One of your -- well, your primary safety endpoint is

based on a type of analysis which is somewhat unusual in terms of you are specifying a hypothesis of a 12 percent reduction in responsiveness in the one group. You have four subjects that did not engraft according to schedule, and you quote a P value of .019. Since the numbers were small enough, I thought I could try to replicate that P value, but I was unsuccessful.

Perhaps you could describe to me a little bit what you did there.

DR. WHITE: Mike White from CellPro. The reference for that analysis is the Blackwelder paper on demonstrating equivalents. Rather than specifying your standard null hypothesis, you specify a null hypothesis that a difference exists. The difference we specified was that the proportion of patients with successful neutrophil engraftment in the selected arm was at least 12 percent less than in the unselected arm. When we did the analysis, we used that as our null hypothesis. I used the normal approximation to the binomial. The analysis we reported was the analysis without adjustment for continuity. I also did an analysis adjusting for continuity and also used -- I looked at other alternatives. I wanted to say that I used an exact binomial test, but I did not use an exact binomial test on that one. That would be

pretty tough to do. But the P values were pretty consistent. It is a tough analysis to do because of the non-null hypothesis. You can't go to things like Fisher's exact test, obviously. And I agree the numbers were small. We are in the tail of the binomial. I tried to replicate it with other analyses and came up with a consistent result.

CHAIRPERSON VOSE: Dr. Siegel?

DR. SIEGEL: Yes. Just as a point of information or perhaps a couple on a couple of issues recently, we certainly accept and encourage the idea of a null hypotheses with pre-specified differences.

The primary endpoint, though, as you referred to it is not one that the Agency considers appropriate as a measure of neutrophil engraftment, and that is consistent with advice and discussion of this committee. We prefer not to look primarily and do not look primarily at engraftment by day 14, but rather at time to engraftment analyses, which are more sensitive to across the board shifts of a couple of days, which might be of interest. And also of important secondary interest at percent who have engrafted or failed to engraft at later time points, 30 days or so, as a sign of possible tailing in the distribution, where some therapies may actually increase the number of

engraftment failures. Those issues were discussed with the company. All of those things were measured as secondary endpoints, so we didn't wind up getting into loggerheads specifically on that issue. You have heard all those endpoints presented and we will hear more about them.

I also wanted to say regarding Dr.

Silver's question just to add in that -- since I don't think it was mentioned -- that there is implicit in the limiting dilution analysis an individual test by test assessment of whether in fact the probe has the sensitivity for a single copy of DNA. When you do a limiting dilution analysis and you look at how the percents of positivity drop off from dilution to dilution, if you have sensitivity down to a single cell, you will see a distribution that is a function of the Poisson distribution and of the actual frequency. And if you have a less sensitive test, you may see -- you will see a different pattern of fall-off as you dilute the cell number out. It is not a highly sensitive assessment given the numbers of replications in this, but it is not a highly sensitive assessment of whether there is a single copy sensitivity. But it does provide some evidence -- in this case, some positive evidence that there was that

level of sensitivity.

CHAIRPERSON VOSE: Additional questions?

Mr. Katz?

MR. KATZ: Yes. I have two technical

clarifications I wanted to ask about. One, has there

been any look at the different disease types of the

various patients -- you know, IgA, IgG, light

chain/heavy chain? Is there any reason to expect that that would make a difference in how effective it would be?

DR. JACOBS: Actually, no, that was not done. I don't know if you would expect any differences. I would rather have --

DR. BERENSON: Well, I think the problem, Mike, of course is there is not enough patients to answer that particular question. I don't know the exact number that were IgA and IgG on that CD34 selected. Do you offhand, Bob? It is probably, if I could guess, approximately two-thirds were IgG and one-third are IgA. But we just don't have those numbers. It is too small.

MR. KATZ: Well, I guess the question would be that some other therapies seem to be different, whether it is Kappa or lambda or light chain or God knows what.

DR. BERENSON: Depending on whose trial you read.

MR. KATZ: What?

DR. BERENSON: I don't know, Ken, if you have a comment on this. But there is a lot of variability of results. For example, with Interferon, some trials suggesting differences as you have

suggested with different isotypes. Other trials do not suggest that. Ken, do you want to make a comment on that?

DR. ANDERSON: Yes. I would just echo what Jim has said. I think that this trial was not designed to test the efficacy of high dose therapy versus conventional therapy or subgroup analysis within that. The efficacy endpoint, as you heard, was tumor cell depletion.

MR. KATZ: Well, I guess this is a technical question, though. Is there any reason to believe that the different -- is isotypes the word -- that different types would make any difference in the effectiveness of the selection technology?

DR. ANDERSON: No, there is none. I think to get right to your point, if you look at B cell lineage differentiation or myeloma, the malignant B cell, and you look at CD34 antigen expression, it does

not vary, Mike, with the subtype of myeloma.

MR. KATZ: Okay. One other technical clarification. This has been tested with a trial that uses busulfan and cytoxin as the transplant regimen.

That is, in fact, the minority of transplants that are done in the country, right, or in the world? Most transplants are done with melphalan or possibly with

TBI. Is there any reason to question what this -- how this might behave in patients undergoing those regimens?

DR. BERENSON: Well, that has actually been looked at by the IBMTR, and there is no difference in outcome. Obviously that is not a randomized group, but at least in their registry, the IBMTR has looked at different regimens and shown no difference, whether it is BUCY, TBI-containing, or melphalan. So it does not seem to influence outcome. Obviously that is registry data and fraught with all of those problems, but that is, I guess, about the best we have. We certainly have no randomized data comparing BUCY to melphalan or TBI.

MR. KATZ: Well, I am not asking the question in terms of the effectiveness of the transplant. What I am asking is that presumably this is going to be approved or the proposal is to approve

it for any of these regimens to be used as a purging technique with any of the regimens. So the question is would you have any reason to believe that engraftment might be different or that there might be other issues with people that have different myeloablation?

DR. BERENSON: Myeloablative chemotherapy?

Is that what you are asking, Mike?

MR. KATZ: Yes.

DR. BERENSON: I have no reason to expect that given all the other trials that have been done by CellPro. I don't know if Ken or Keith has any other comments.

DR. ANDERSON: I would just echo those comments. As Jim showed you all, the only randomized trial in myeloma, and we are fortunate to have one that showed a benefit for high-dose therapy versus conventional therapy, is from France. And by chance it had TBI and melphalan as the ablative regimen.

There are four other randomized trials; one in the United States, one in Scandinavia, one in England, and one in Spain which will be using different ablative regimens and in some cases some chemotherapy only, which are asking the high-dose versus conventional therapy question and will be available within the next

several years. But for today, the issue is that in this trial, great care was taken to make sure there were no differences in the conventional chemotherapy and the intensity that was received or the ablative regimen that was given. So that prior to the two-arm experiment, if you will, the prior therapy, including ablation, was not significantly different in the two

groups in order to allow a fair comparison.

CHAIRPERSON VOSE: I think -- I was just going to say that I agree with your comment that I think there is a concern about TBI especially containing regimens and highly selected cell products. There is some data of concern about that area and I think we do have to keep that in consideration in the labeling. So I think that is a concern.

DR. JACOBS: In the final clinical report that we submitted to the FDA, we did a literature search of publications, looking at then the time to neutrophil and time to platelet engraftment, which is very comparable to what we see in the Phase III study. Obviously a variety of regimens were used, not only for multiple myeloma but for breast cancer and lymphoma. So there is a consistent pattern as far as time to neutrophil and platelet recovery.

The other point is that the type of

regimen is usually specified by the investigators, and we do have a Phase III randomized trial in Europe and their preference was melphalan and TBI. So it really is -- the trial here was not to look really at a specific regimen, but just at the unselected and selected infusion.

CHAIRPERSON VOSE: Dr. Berman?

DR. BERMAN: Did you break down your groups in terms of stage of disease at diagnosis? I am particularly questioning why four patients weren't able to be mobilized correctly? Were those people with advanced stage disease at the start?

DR. JACOBS: There was actually only one patient that -- there was only one patient that didn't mobilize adequately. The other three patients were randomized. One had progressive disease that was found during baseline assessment. One patient elected not to go to transplant on this study but had an allogeneic transplant. And one patient actually died of sepsis during the mobilization prior to being randomized.

DR. BERMAN: But just to get back to the question of staging of disease in the two arms, did you look at that? Were they comparable?

DR. JACOBS: Yes, they were comparable.

CHAIRPERSON VOSE: Dr. Hartigan?

DR. HARTIGAN: In your looking at the baseline factors that were responsible for the time to platelet engraftment, you found that CD4 counts and platelet count at baseline were significantly related. Did you look at the interaction between the two?

DR. WHITE: Which interaction are you

talking about? The interaction between platelet count at randomization and CD34 count infused?

DR. HARTIGAN: Yes.

DR. WHITE: Yes, we did. There was no interaction between them. We have also done some further investigation of what is happening with these two factors, and we have looked at patients who took a longer time to engraft and ranked their CD34 count as well as their platelet count. What happens is that they really are somewhat independent in their effect on time to engraftment.

CHAIRPERSON VOSE: Additional questions? I had one. Jim, you mentioned that the patients had Interferon maintenance post-transplant, is that correct?

DR. JACOBS: Not in the Phase III.

DR. BERENSON: That was true in Phase II.

CHAIRPERSON VOSE: In Phase II, but not

this study?

DR. JACOBS: No.

DR. BERENSON: Yes, Julie. In the Phase II, we did employ Interferon, which was also done in the French Intergroup. Most of us these days are a little disconcerted using it based on some recent randomized trials that you are aware of. So we did

not use any maintenance in this Phase III.

CHAIRPERSON VOSE: In the Phase II study, was there any difficulty as far as toxicity for the patients outside what would normally be expected?

DR. BERENSON: In the Phase II study?

CHAIRPERSON VOSE: Phase II.

DR. BERENSON: There was difficulty for them to tolerate the Interferon definitely, both count-wise as well as constitutional symptom-wise. But that has been the experience of others using unselected transplants as well. And again, Keith or Ken, if you have other comments.

DR. ANDERSON: I would just echo that there is no standard maintenance therapy now in myeloma. The large meta-analysis that has been done looking at Interferon really has not shown an overwhelming effect for its utility. In the randomized trial of conventional therapy versus

standard therapy that is just about finished accrual in the United States, we are randomizing responding patients who get either conventional or high-dose therapy to Interferon or not, hoping once and for all to answer this question. But since there wasn't a standard at the time the Phase III was designed, there was no maintenance built into this trial.

CHAIRPERSON VOSE: Okay. Thank you.

Additional questions or comments? Okay. Why don't we take a 10-minute break, and then we will resume with the FDA's perspective. Thank you.

(Whereupon, at 10:07 a.m. off the record until 10:23 a.m.)

CHAIRPERSON VOSE: If everyone can take their seats. We would like to go ahead with the FDA perspective, and Dr. Litwin will present that.

DR. LITWIN: Thank you for the music. I am Dr. Stephen Litwin, and I will open the CBER review. This is a supplement to an existing license device, the Ceprate SC stem cell selectional concentration system. The sponsor, as you very well know, is CellPro. The product essentially are a collection of CD34 selected cells selected from autologous peripheral blood which will be hematologically re-engrafted after myeloablative chemotherapy in patients

with multiple myeloma.

This was a highly interactive review, and I would just like to briefly mention the contributions of Wendy Shores, who looked at the transferability of the device for peripheral blood as opposed to bone marrow, for which it is licensed, and some other aspects of yields and such. And Dr. Edward Max, who

will very shortly present a critical review of the PCR technique. Also, Dr. Gupta, who did all the statistical analyses for us.

The current package insert indication for the device is for CD34 selection of autologous bone marrow. The clinical indication is a lowered incidence of DMSO infusion-associated complications, mostly cardiorespiratory. And there was a caveat in the current indication that the infusion of less than 1.2 million CD34 cells per kilogram is associated with delayed platelet engraftment.

The proposed indication is that the labeling be extended from autologous bone marrow to autologous bone marrow and peripheral stem cells. The clinical benefit would have as additionally a 100-fold reduction, that is a 2 log or greater reduction in the number of tumor cells present in the hematologic autograft. And to the caveat about a minimum number

of CD34 for bone marrow is added a further labeling that the infusion of less than 2 million CD34 positive cells per kilogram of peripheral blood cells or cells collected from peripheral blood is associated with delayed or slower platelet engraftment.

A few words about the CBER review. Rather than repeat a lot of the descriptive elements which

Dr. Jacobs has so carefully presented, the CBER review will focus after a very short descriptive two slides on three elements; the collection and the processing of the cells and the impact that that has had on various outcomes, the efficacy analysis, which Ed Max and myself will present, and finally the safety analysis, which will mainly focus on engraftment.

A few words about the experimental design.

131 multiple myeloma patients were enrolled. All of them had to have a diagnosis of intermediate or high cell mass. All of them had to lack progressive disease at any time during their course. And all of them had to have had no more than three months prior alkylator chemotherapy. After mobilization, they were randomized in a balanced fashion. The Ceprate selected arm patients numbering 67 underwent leukophoresis and followed on the same day by the processing through the Ceprate device. The control

arm of 64 subjects simply underwent leukophoresis, and the two underlined cell products here are the ones that were infused into the patients. After high-dose chemotherapy and the autologous transplant, safety was followed for the first 100 days, particularly post-transplant. The two periods of time of major interest to CBER were the collection and the processing of the

autologous cells during which time the efficacy was determined by examining a subset, that is the 28 patients out of the 67 or 66 in the Ceprate selected arm, for whom an immunoglobulin sequence could be developed. And the second safety period, that is the period 100 days post-transplant.

The efficacy endpoint was reduction by 2 logs or more of tumor cells in the infusate after the Ceprate selection. And the safety endpoint was comparable neutrophil and platelet engraftment.

The next six slides deal with the cell collection and processing. You should keep in mind, first of all, that the guidelines for the collection and the end of collection of cells for the two arms differed. And secondly, that the amount of CD34 progenitor cells mobilized from the blood in individual patients in the selected arm varied greatly, and both of these had an impact on the

outcomes.

There were two leukophoreses minimum requirement. The first criterion or guideline applied both to the selected and the unselected arms, and that was that there was a requirement that a minimum of 5 x 108 total nucleated cells per kilogram be in the leukophoresis referred to as the PBPC product. The

second criterion applied only to the selected arm. It required a minimum of 4 x 106 total nucleated cells per kilogram in the Ceprate selected, that is the enriched product. The assumption was that half of these cells would be CD34 positive, and therefore every patient in the selected arm would receive at least 2 million CD34 positive cells. This did not work out in every case.

Looking at the leukophoreses to begin with, there were more leukophoreses in the selected arm, 3.0 mean, with a standard error of the mean of 0.2, as opposed to the unselected arm, in which they were 2.3 with a standard error of the mean of 0.1. The median was the same and the range was slightly longer for the selected arm.

It would be anticipated that the total nucleated cells per kilogram would also be increased in the selected arm because there were an increased

number of leukophoreses. This is not as marked as one would have anticipated, but the trend is present.

The next slide looks -- oh, I am sorry. This is the slide you have seen already but in different colors. This is a distribution of the leukophoreses. You can see that 50 percent of the control patients required no more than 2

leukophoreses, whereas 36 percent of the selected arm patients were able to be satisfied with two leukophoresis. And this difference, that is, a requirement for more leukophoreses in the selected arm, is present at all points of the distribution.

This looks at the progenitor cells, the CD34 cells that were collected. If we look at the upper portion of this, you can see that three analyses can be done. If we just look at the comparison of the leukophoresis product, there were more CD34 positive cells collected in the selected arm, presumably because of the increased number of leukophoreses, 14.3 million, as opposed to the control or unselected arm, 11.7. If on the other hand we look at the two steps that each patient in the selected arm underwent, that is, the leukophoresis and then the Ceprate selection, there was a reduction of about 50 percent of the CD34 cells lost during the selection procedure. And the

number of infused enriched product, that is, Ceprate selected cells, was 7.1 million. And finally we can compare in the darkened boxes the number of actually infused CD34 positive cells. There were less in the selected arm, 7.1 million per kilogram of body weight, as opposed to the unselected arm, 11.7. It should be noted that these numbers, the numbers of infusate CD34

cells, are relatively high for autologous transplants.

The same analysis can be made using the data for CFU per kilogram. I won't go into this except to say that the trend is in the same direction, but the data are much less dramatic. The reasons for this may be that the CD34 measurements were done at a central site, whereas the CFU measurements were done at individual sites and the assay is known to be highly variable.

The third impact of the cell collection strategy was that there were 14 percent of the patients or 17 patients who received less than 2 million CD34 cells per kilogram. They are listed here. There were 10 in the selected arm and 7 in the unselected arm. The numbers of CD34 infused are shown.

If we look at the first column under selected for ANC engraftment, keeping in mind that the median day to ANC engraftment is 12 days, it is possible that there

is some prolongation. But this is -- it is not possible to statistically analyze this. If we look at platelet recovery, there are four patients who had delayed platelet recovery, that is, longer than 20 days, which is beyond the 95th percentile of the patients in the unselected group. Three of the four patients are in the selected arm and one in the

unselected arm. And the same three patients who had a delayed platelet recovery, when examined by platelet count -- and these are platelet counts at the 6-month visit after transplantation -- the same three patients continued to have platelet numbers that were substantially less than would be predicted for the remainder of the group. The last patient actually was the only patient in the study group who could be considered as an engraftment failure for platelets. This was an individual who continued to have platelets under 20,000 after transplantation, and on day 119 was given the depleted fraction, that is, the pass-through fraction, which was used as a back-up in the case of engraftment failure. And this patient at 180 days still continued to have low platelet counts. In the unselected arm, the single patient seems to have a normal platelet level.

In summary, then, the impact of the

collection processing strategy on the outcomes were three-fold. There were more leukophoreses and more total nucleated cells collected from selected arm patients. On the other hand, there were less CD34 cells infused into these selected arm patients, presumably due to loss during the selection process. And there were 17 patients who were infused with less

than 2 million CD34 positive cells, a limited number of whom, 4 exactly, had delayed platelet recovery.

I am going to introduce now Dr. Ed Max, who is going to give us a critical review of the assay.

DR. MAX: I'm using the slides rather than the video. I am just going to go through our analysis of the PCR assay, which forms an essential element to the efficacy determination in this trial. As Dr. Berenson mentioned, the PCR provides a potential for a unique assay for the patient's own myeloma because the multiple myeloma cell will have an immunoglobulin rearrangement involving a V, a D, and a J. So if a sense primer is positioned within the V region and an anti-sense primer positioned within the D/J region, then one can develop an assay that would be specific for the patient's immunoglobulin gene. The sponsor reports one multiple myeloma cell per 100,000

nucleated cell sensitivity of the assay, and the strategy for quantification was to make successive dilutions of a half log starting with 100,000 cells and use five duplicates at each dilution, and then to calculate the number of myeloma cells in the starting population by Poisson statistics based on the number of the five duplicate PCR amplifications that were

positive at each dilution.

I am going to very briefly go through the kind of data that was submitted to us so you can see the kind of nitty gritty of the assay. This just represents an individual patient's immunoglobulin gene. Here is the sense primer. Here is the anti-sense primer. For each patient, the germ line gene corresponding to the myeloma gene was looked at in comparison to the myeloma sequence and the design of the primers included an effort to maximize the somatic mutation in the primer so that the primers would have less likelihood of amplifying a product of the same immunoglobulin V region germ line.

The DNA samples for the various cell samples accumulated during the trial were encoded with letters here chosen by a random code. And we are going to look at the results for one patient, the first patient in the trial, where the leukophoresis

was coded with a V and the CD34 enriched sample was encoded with a P.

This just shows that for each patient there was an attempt to optimize the conditions of amplification and a particular amplification protocol was chosen. I am showing you now the protocol for an individual gel, where you can see the P sample was

dilution A, which is undiluted sample representing DNA from 100,000 cells. Five replicates here -- here is dilution B and here is dilution C. As mentioned, each sample had a beta-actin control, an internal control for the DNA integrity. We will come back to that because we had some concerns about that control. Here is the V sample and bone marrow samples were run as a positive control.

I am going to show you the actual appearance of the gel. Here is a sample of five replicates. Four for this P sample were detected at the starting dilution, and then here is the next dilution where it went down to 2 and then to zero.

These gel lanes were observed by three independent observers who identified the number of lanes in each dilution which were positive, and those observers generally agreed with each other and most of the time agreed with us. We looked at every lane of

every gel submitted for the CD34 enriched and the starting PBPC product and samples of the CD34 depleted product.

The number of positive wells at each dilution was then entered into a Poisson statistical analysis program called DIL-SOLVE. Here are the numbers. I am sure it is too small for the people at

the back to see. For this particular sample that we looked at before where the initial undiluted sample gave 4 wells out of 5 positive. The next one was 2. On the basis of this, the program calculated a number of target cells per well at the starting concentration representing 100,000 cells. And also calculated an expected number of lanes positive for each of the dilutions. And in general, these were quite remarkably close to what was observed. And in general, the progression of the numbers of positive wells at the successive dilutions were pretty much as one would expect for Poisson distribution, although there were several samples where the positive wells trailed down from 5 slowly over successive dilutions, indicating probably some problem with the assay. Although, of course, some statistical fluctuation would be expected.

This slide illustrates the basis of the

calculation. I would just like to go through it in detail just to show you for this one patient. We are looking now at the three samples, V, P, and C representing the PBPC product, the starting product, the CD34 enriched product, and the CD34 depleted product. There were three sorts of numbers that entered into the final determination of the number of

myeloma cells in each sample. First, the total number of nucleated cells were counted on the basis of a hemocytometer. As you can see for this patient, there was a very substantial number of depletion in going from the starting nucleated cells in the PBPC product to the CD34 enriched sample. And this ranged from approximately 200 to 400 depletion. So what this means is that as was pointed out earlier, if the column did not selectively bind to the myeloma cells, one would expect approximately a 200-fold depletion of myeloma cells just on the basis of the fact that the total nucleated cells in the CD34 enriched sample were very extensively depleted. But then, of course, the PCR assay was done to detect whether there was any further depletion, and in fact there was for most of the samples, although not all of the samples. There were three or four samples where the ratio of myeloma cells per total nucleated cell was either about the

same in the CD34 enriched or actually slightly higher.

Two of these patients represented the individuals that did not achieve the 2 log depletion of myeloma cells in the CD34 enriched sample.

So then the DNA was prepared from each of these samples and was quantified. So the first measurement that influenced the final assay result was

the total nucleated cell count based on the hemocytometer. The second was the DNA assay. And that was performed using a fluorometric assay. Based on that DNA determination, an amount of DNA corresponding to 100,000 cells and successive dilutions of those cells were, as previously mentioned, alloquoted and dispensed in replicates into the various tubes. This shows the dilution and here are the number of positive wells out of the 5 -- 4 out of 5 and 2 out of 5 as shown earlier. Based on these numbers using the Poisson distribution analysis, a calculation was made of the percentage of myeloma cells in the total nucleated cell count. This number was then multiplied by the number that had been determined on the basis of the hemocytometer reading to give you a final estimate of the number of tumor cells in that sample per million cells.

So as you can see here, the percentage of

tumor cells was, in this case, substantially depleted from .02 percent to .0013 percent. And when these numbers were multiplied together, you get a very small number, which for this particular patient represented a 3.745 log depletion of tumor cells.

This is a summary of all the patients. You have seen these numbers before. 28 patients, a

subset of the total number of patients in the randomized to the CD34 selected arm, had a product which was capable of being amplified. 24 of these patients had a product that showed evidence of the multiple myeloma cell in the PBPC product. Of these, in 11 of the patients, the myeloma cells were undetectable after Ceprate selection, or 46 percent.

Now this represents an amended version of a figure that was submitted by CellPro showing for each patient the starting log number of tumor cells in the PBPC product, and this shows the CD34 enriched sample after processing through the Ceprate. The original figure actually showed those 11 patients as going to zero. We have replotted these using the calculation based on the detection sensitivity because the assay is, of course, limited by the detection sensitivity of the PCR assay.

This slide actually shows the final

results just plotted as a log tumor depletion. What I have plotted here in black represents the number of multiple myeloma cells in the CD34 enriched samples. And the open circles just represent those patients for whom no myeloma cell could be detected in the product. What I have used here as a calculation to estimate the log depletion is that number of cells which represents

the minimal detection level of the PCR assay. So, in fact, these are potential underestimates. You can see that the average log depletion is in the range that CellPro has stated. There are definitely two patients who did not meet the goal of 2 log removal of myeloma cells in the assay.

Now I would like to mention one concern that FDA had apart from the question of the assay accuracy and the log error, which CellPro has estimated as about a half a log. An additional issue we felt that raised some concern is that the control that CellPro used, the beta-actin control, as an internal control for the integrity or the amplifiability of each DNA sample was inadequate. And the reason for that is that amplifying the undiluted DNA at 60 PCR cycles for beta-actin, this is basically a non-quantitative assay. It is insensitive to potential template problems that might have decreased

the amplifiability of that sample, either due to poor DNA quality or the presence of PCR inhibitors. 60 cycles basically overwhelms the system.

Therefore, CellPro has not ruled out the worst case scenario, and that is that systematic template problems specific to the CD34 selected sample might have led to an undercount of multiple myeloma

cells in that sample, and such an undercount would simulate myeloma cell depletion by the Ceprate device.

We raised this issue to the sponsor, and we have had kind of a back and forth over the past couple of months about how worrisome this concern might be. Whether it is just a theoretical issue or whether it is ruled out by the data that they present. Their first response was that no multiple myeloma signal has been lost due to any hypothesized systematic undercount since the myeloma cells assay in the two fractions, that is the CD34 enriched plus the CD34 depleted sample, add up to the myeloma cells in the starting PBPC. We looked at this and actually it is difficult to make a case that that is correct when you look at individual patients. I will just show you this patient we have looked at before. Here the starting PBPC product showed 13 million myeloma cells. And in the CD34 depleted sample, there were 94 million

assayed. Obviously one can't sum these two fractions and get 94. Here in this patient, we have the opposite problem as was pointed out earlier. This represents, I guess, one of those two patients that kind of fell outside the cluster in the log plot shown earlier. Here there were 363 -- I am sorry, this represents one of those patients. Here is the

opposite problem. We had 363 million in the PBPC product, and the sum of the two fractionated samples is quite low. So clearly analysis of individual patients will not show the kind of conservation of myeloma cells that might have ruled out the possible systematic undercount of myeloma cells in the CD34 depleted sample.

Well, CellPro came back with another suggestion which was to average over all the patients. They looked at the ratio of PBPC product, the starting, plus this sum. Theoretically, this sum should equal the PBPC product, so this ratio would be 1. The log would therefore be zero. And so if this was examined over all patients, they found that this value was not significantly different from zero, that is, the 95 confidence limits included zero as expected if there was no myeloma signal loss. We felt that this kind of analysis really failed to test whether

the data are inconsistent with an undercount of myeloma cells. And, in fact, if you examine or if you assess that possibility and multiply the number of determined myeloma cells in the CD34 enriched fraction by 250 and add that to the myeloma cells in the CD34 depleted sample, one finds that this log is still not significantly different from zero. So the data do not

exclude a 250-fold undercount.

The reason, of course, that this addition is insensitive to multiplying one of these addends by 250 is the fact that the number of myeloma cells measured in the CD34 enriched sample was just very, very small compared to the error in these numbers, which CellPro has estimated at .5 log, but is perhaps larger than that.

So in view of our concern, CellPro submitted to us data on a single patient in which they tried to address the concern about possible systematic undercount of myeloma cells in this CD34 enriched sample. They did a quantitative PCR amplification of beta-actin using the same kind of strategy of dilution analysis and Poisson statistics, showing for that single patient, amplification efficiency was nearly equivalent for the starting PBPC and the CD34 enriched sample. However, in the data they submitted to us,

the efficiency was only about 5 percent of what was predicted, and perhaps even less than that. We had some question about their assumptions for these numbers. CellPro suggested that this low efficiency might have been due to a PCR protocol that was optimized for shorter amplification products, products in the range of 100 to 200 base pairs versus the

product that they amplified for beta-actin, which was 650 base pairs, and might have required longer PCR extension time.

So I would like to summarize our final assessment and hope that the Advisory Committee will consider our concerns. The PCR assay has demonstrated that the Ceprate selection leads to a substantial depletion of myeloma cells. Usually it is greater than 100-fold, that is, in 22 of the 24 evaluable patients based on the numbers they submitted. We still remain somewhat concern that the possibility of a systematic undercount of myeloma cells has not completely been ruled out by CellPro, and the reasons are the beta-actin control that they presented to us was only on one patient and it did give an unexpectedly low beta-actin amplification on that one patient. Finally, we have sort of a theoretical concern that even that beta-actin amplification

doesn't really test the integrity of the myeloma cell DNA because it is done on the bulk CD34 enriched sample, which is obviously made up mostly of non-myeloma cells. It is conceivable that DNA deriving from the myeloma cells might have some impairment in its amplifiability. But FDA feels that testing for this would not really be feasible.

So in conclusion, there is no reason to believe that such an undercount would have occurred. It is a theoretical possibility that CellPro failed to provide adequate controls against. It is only a theoretical concern and the data that they submitted certainly supports depletion for most patients that reaches the target of 100-fold depletion.

DR. LITWIN: I'm going to add some additional data on the efficacy analysis. I will try not to repeat what Dr. Max has gone into in great detail. These are simply the numbers of tumor cells. This is the number in the leukophoresis product on the 28 patients in whom a probe was available, and this is the number in the Ceprate selected enriched product. The distribution was asymmetric and the values are given as quartiles, 25th, median 50, and 75th. If we look at the numbers, you could see that the median patient would have between 2 million and 3 million

tumor cells in the first leukophoresis product. All of these data are done on the first leukophoresis product. The numbers for the post-device depletion product are much lower. This is equivalent to about 700 cells if accurate.

The determination for the depletion was done as a log geometrically. These are the figures

with the same distribution expressed for the leukophoresis product and the Ceprate selected product. The median depletion was 3.10 logs. The mean was 3.29. This met the prospectively discussed endpoint of greater than 2 logs depletion.

The next slide shows the distribution of the log depletion. This is very much similar to the vertical slide that Ed showed. Most patients had between -- the log depletion is shown under the X axis. Most patients had between 3 and 4 logs depletion or 2 and 3, but there were some patients who had less than that and there were a number of patients who had more. These are the 24 patients in whom the log depletion was measured.

Finally, I think most of this has been discussed. I will only emphasize the fact that 11 of the 24 patients could be purged to non-detectability, and 13 continued to have detectable tumor. The

average -- we also looked at the log depletion of total nucleated cells. The average depletion was about two-fold as opposed to the 3-plus log depletion we have just discussed for tumor cells.

I will turn now to the safety analysis starting with neutrophil engraftment. CBER chose to determine the engraftment parameters for neutrophils

and platelets using the mean and median days to duration of engraftment and the Kaplan Meier analysis rather than for the neutrophils the landmark analysis that Dr. Jacobs has presented. And I think Dr. Siegel has already commented on the previous communications between us and the sponsor and the reasons that we consider this more useful.

The selected arm had 7/10ths of a day longer mean duration to neutrophil engraftment, 12.4 days as opposed to 11.7 days. The median day of engraftment was 12 days for both arms at the point that 50 percent of the engraftment events had occurred. Both arms were 12 days. The Kaplan Meier analysis looks a little unusual because of the highly compacted period of time over which the engraftment occurred. Testing using non-parametric tests for the differences between the two arms shows a low P value, the null hypothesis being that there is no difference

to suggest that there is a possibility or a difference between the two arms favoring the unselected arm.

Turning to platelet safety endpoints, I think the data are more dramatic. The median day -- we use platelet recovery rather than platelet engraftment, which is, I think, some of the data that you saw shown by Dr. Jacobs. Because CBER considers

it a more suitable endpoint with respect to the fact that it is not as dependent on transfusions. The data is very similar between the two. The median day of platelet recovery differed by one day. It was one day longer in the selected arm. The mean day differed by several days. The P values once again are low. The null hypothesis is that there is no difference, and this strongly suggests that the difference we see, that is, the prolongation of engraftment of platelets by a few days in the selected arm is real. The next slide shows the Kaplan Meier analysis. You can see that when 50 percent of the events had occurred, the two arms are pretty much the same. If we follow it further out, it is obvious that -- the red, by the way, is the selected arm. I know it is hard to see.

It is obvious that a number of patients in the selected arm had probably several days prolonged engraftment. And at a little over 40 days, all of the

patients in the control arm had platelet recovery, whereas there are a number of patients who continued out. We have not -- we stopped this graph for the sake of description at 80 days, but there was a patient who I described already who at 119 days still lacked platelet recovery. And this was the single patient who was given the back-up or depleted product.

The analyses of this indicates that the P values are once again, strongly supporting a difference between the arms, that is, a prolonged engraftment for platelet recovery -- prolonged platelet recovery in the selected arm.

There were a number of platelet safety endpoints. The platelet transfusion events and days of platelet transfusion events are listed. The mean showed a difference. the selected arm was 4.4 days, the unselected arm 3.2. The median, which you have seen already, I believe, is 3 and 2 days. The ranges are shown below.

A number of other secondary endpoints that were related to engraftment were also done. They included incidents of infection day 0 to 100, incidence of severe infection, days of hospitalization, days of rehospitalization, number of days of growth factor given post-transplant. All of

these secondary endpoints, as summarized by Dr. Jacobs, were the same in both arms.

Late engraftment events were also looked at, and these are platelet late engraftment events.

CBER did an analysis by taking from the list files all patients who at 100 days after transplantation -- I am sorry, 6 months after transplantation had 100,000 or

less platelets. When there was more than one determination, as there often was, we took the first determination. These 17 patients that we had were further subset. There were 17 altogether, 13 in the selected arm. By having equal to or less than 75,000, et cetera, for 50 and 20. The preponderance of selected arm patients, about three-quarters, is noticed here.

The next slide continues this analysis. We were concerned with the possibility that the data that we were looking at in terms of late engraftment or late recovery or incomplete recovery -- it is a little hard to judge at six months -- was related to risk factors. And the two that were considered were infusion of less than 2 million CD34 cells per kilogram. This is simply the control down here. And having equal to or less than 75,000 platelets at the time of mobilization. The latter data are not shown.

They don't -- they are not very clearcut. But we did both analyses. If we just look at the upper section, you can see once again the preponderance of selected arm subjects. If we look at those patients who have less than or equal to 50,000 -- now this is 6 months post-transplant -- all four subjects were infused with less than 2 million cells, and in fact all four were

selected arm subjects. If we look at those who have less than 75,000, of which there were 8 subjects, 6 of the 8 were infused with less than 2 million cells. 2 of the 8 had over that. And of these 6, 5 out of the 6 were in the selected arm.

These data suggest that the infusion of less than 2 million per kilogram of CD34 positive cells could be a risk factor, particularly in conjunction with the Ceprate selection.

Continuing the safety analysis, there was a central review of progression of disease. Patients were evaluated at baseline at 100 days, at 6 months, and at 12 months. At baseline, almost all the patients were either in remission or were stable. There are no substantial differences in the numbers of patients here. At 12 months, there were 19 patients in each arm who showed disease progression, 29 percent and 30 percent respectively.

A similar analysis was done for deaths.

At 100 days, 6 months, and 12 months, once again there are no significant differences in the number of deaths between the groups, 12 percent for the selected arm and 9 percent for the unselected arm respectively.

During the course of the CBER analysis, a number of labeling issues arose, and three are listed

here. The one of major concern, which I suspect is already obvious, is the question of whether infusion of less than 2 million CD34 positive cells per kilogram is a risk factor. Four pieces of information bear on this. The 17 patients of the 131 who were studied who were infused with less than 2 million cells had a limited number, 4 specifically, of slow platelet recovery. I have just presented the late engraftment data, which is 6 months after transplantation. There is published literature that strongly supports the fact that patients infused with less than 2 to 5 million CD34 positive cells had a slower rate of engraftment than did those infused with above that. The sponsor has presented the data already on logistic regression analysis which shows that among those variables which they concluded were connected with engraftment events, the two that were most prominent were the number of CD34 positive cells

infused and the number of platelets at the time of mobilization.

There were two other issues. We asked the sponsor to provide a literature search on the expression of CD34 on malignant cells. They supplied that of 66 publications. The publications show essentially what I suspect most of the Committee and

the audience is already aware of, that is, that CD34 is seen for the most part on undifferentiated immature cells and cells of a leukocytic series. That aside from leukemic cells, it is rarely seen among cells of solid tumors. However, there are several reports, including one last year in 1997 in Blood, which does report very strong data on the appearance of CD34 on myeloma cells. So that it can happen and it does happen apparently.

Finally, the question of reduction of tumor burden in other than multiple myeloma cancers. The literature review of the 15 papers has already been alluded to by Dr. Jacobs. The sponsor also in response to our request has provided Phase I and II data on two studies. One was from myeloma and the other from breast cancer. They both are consistent with a lot of the data recorded. The myeloma study indicated that about half the patients could be purged

to non-detectability and that there was a reduction of tumor burden by 3 to 4 percent in that report. And the second paper on 15 patients out of a larger group of breast cancer patients reported a reduction of tumor burden by one and a half logs, but that may be very dependent on the methodology.

The last two slides I would like to

summarize my comments and reviewers comments. Primary endpoints were satisfied as prospectively designated using data from single protocol CP0060-02. There was a marginal delay in neutrophil engraftment and a definitive one to two day delay in platelet recovery.

The term marginal as used here is based on our data, which indicates that there is a 7/10th's of a day delay in mean and that there is a low P value for supporting differences in the Kaplan Meier analysis for neutrophil engraftment. Late engraftment suggested a limited number of selected subjects had poor or delayed platelet recovery. The infusion of 2 million CD34 positive cells per kilogram may be a risk factors. There were concerns, which Dr. Ed Max has discussed, about the quantitative aspects of the PCR-based assay of the tumor cells.

The disadvantages of the proposed use of Ceprate selection includes a small increase in the

number of leukophoresis, a decreased number of progenitor cells which could be infused, and possibly slower and incomplete engraftment, that is, referred to as late engraftment problems. The major advantage is reduction of tumor cells in the autografts. There were limited data provided on tumor purging in clinical settings other than multiple myeloma and

follow-up studies of disease progression and patient survival should continue after FDA action. Thank you.

CHAIRPERSON VOSE: Thank you, Dr. Litwin. Let's go ahead and open it up for any questions from the Committee. Dr. Auchincloss?

DR. AUCHINCLOSS: May I ask Dr. Max about his concerns, which statistically look perfectly reasonable. But is there any biologic example where such systematic undercounting of cells has occurred by PCR analysis as versus positive selection?

DR. MAX: I don't know of any example. And as I said, we have no particular reason. We would assume that the cells would behave more or less identically -- the cells that have stuck to the column and the cells that have passed through. FDA prefers not to base approvals on unproven assumptions, however.

DR. AUCHINCLOSS: But then you went on to

say that there was no way they could prove their assumption as I understood you. That the test of the multiple myeloma cell DNA was impossible to perform.

DR. MAX: I think that test is maybe not quite impossible, but unfeasible to ask to look at every patient. What I think is feasible and would rule out let's say PCR inhibitors, for instance, is to

look at the beta-actin in a quantitative fashion as they have done for one patient to assess effects that might be having an action on the myeloma cells as well as the bulk DNA. And we understand that they have looked at with a different pair of primers and two additional patients and have gotten more satisfactory results, which maybe CellPro would care to describe. But FDA has not seen those data.

DR. AUCHINCLOSS: Does the fact that they did in fact in those three patients end up with the same proportion of tumor cells as in the initial sample -- does that lead you to conclude that, in fact, they can measure tumor cells when they are there?

DR. MAX: Well, maybe I should clarify what they have done. It is not a question of assaying tumor cells in the original sample. It is a question of assaying the beta-actin, which is an internal

control for --

DR. AUCHINCLOSS: No, but I am talking now about multiple myeloma cell detection. And they were, in fact, detecting a similar proportion to their starting population in three patients. That was part of the complaint about how accurate the assay really was. But there they are apparently measuring what

would seem like a reasonable number of cells. So they must be able to detect multiple myeloma cells if they are there.

DR. MAX: I am not sure I follow.

DR. AUCHINCLOSS: No. That may be a lousy argument. Let me conclude by just asking you this. Is it fair to characterize your concern as raising a remote possibility, this degree of systematic undercounting?

DR. MAX: I would say since we don't know how remote it is, I would say it is a theoretical possibility.

DR. BROUDY: I have a question for Dr. Max as well. Part of the concern was that the number of tumor cells in the CD34 positive and then the CD34 negative fractions did not always add up to the number of tumor cells in the starting fraction, but you presented very clearly how they calculated that by

multiplying the number of cells times a percentage of tumor cells. Did the total numbers of cells always add up? Could part of the disparity in the numbers be due to systematic or random errors in counting? Perhaps they undercounted or overcounted and then multiplied and that is why things didn't add up?

DR. MAX: Yes. I think -- I can't give

you an absolute -- I can't tell you quantitatively, but there were some cases where there was a failure to add up just in terms of the total number of nucleated cells. And that is why I emphasized that there are really three independent determinations in two samples. So we are talking about six numbers that combine together for that final log removal. And that is the DNA assay, which has its own potential problems, the counting on a hemocytometer, and the PCR assay. I think it is probable that the -- based on my own experience that the error in the PCR assay is the one that is most worrisome. But really we have no way of knowing.

CHAIRPERSON VOSE: Dr. Anderson?

DR. ANDERSON: Yes. We have seen the data where it doesn't add up. Let me just ask, of all the various patients, how many times did it add up?

DR. MAX: Well, I would say that there are

only two or three patients where the numbers added up in a way that you would hope to see if the assay was quantitative. There were -- I don't have the numbers completely memorized, but there were about maybe half a dozen patients in which the counts in the depleted sample were, let's say, in the range of 7 to 10 times that in the starting sample. There was one patient

where the amount measured in the depleted sample was 200-fold and another close to 1000-fold of what had been present in the starting sample. And then there were similarly on the side of errors in the other direction where the myeloma cells were much lower in the depleted sample, and therefore the total of depleted and enriched was much lower. Those numbers were also -- I don't, again, have the numbers in front of me, but I would say there were three or four patients where the numbers looked the way you would be very happy to see if they were your own experimental results.

DR. ANDERSON: But what this sounds like is FDA doing what FDA ought to do, which is to really look at very strict criteria to determine if there is a systematic error that basically discounts the results. And I gather your conclusion is that having analyzed it very thoroughly, that the sponsors have

not proven their point, but the FDA has not disproven it either?

DR. MAX: Well, what I would like to emphasize is that what we feel is the error in the assay is larger than one would hope. On the other hand, the margin of error that would discount the sponsor's results is such that this assay may be

sufficient to prove their case. So that if instead of a half log error, let's say the error was more like a log, then there might be more than the two patients -- two patients fell below -- on their best estimate fell below the target of 2 logs removal. Now if you asked how many patients fell such that their 95 percent confidence interval was below their target, obviously that would be greater than 2. And depending on their log error -- if their log error was sufficiently high, then that number would rise significantly.

DR. ANDERSON: What would be the possibility that the same set of errors could have resulted randomly in two patients being under, but that in fact none of the patients were under?

DR. MAX: I am not sure I --

DR. ANDERSON: In other words, what I am saying is because of the randomness in the numbers, it is theoretically possible that 64 out of 64 all had a

depletion rather than 62 out of 64.

DR. MAX: Yes. I think -- yes, that is certainly true. I mean if one, instead of asking what is the 95 percent confidence limit that they were all above, you could say --

DR. ANDERSON: What are the chances that they are all below.

DR. MAX: Below. And that would be quite low.

DR. ANDERSON: Okay.

DR. MAX: But I don't think that their error is so great -- that we have evidence that their error is so great. The data that was shown by Dr. Berenson, I believe, of the log log plot would indicate that there is some correlation between the myeloma in the starting PBPC sample and the CD34 depleted sample, which implies that these are not random numbers.

DR. ANDERSON: I guess where I am coming from, and then I will stop here, is that based on that plot, what you just mentioned, where there are the two outliers to the upper left that you pointed out and there appeared to be one outlier on the right, the rest of them all seem to be reasonably close to the line, which implies that -- and there are enough

points on that line -- that it would imply that the assay is good enough.

DR. MAX: That is what I think is true. That is, of course, excluding this theoretical possibility of the undercount in the CD34 enriched cells. Because that undercount would not have affected that curve.

DR. ANDERSON: Right.

CHAIRPERSON VOSE: Additional questions? Mr. Katz?

MR. KATZ: Well, I guess if I were a patient that was imminently considering a transplant, I would be looking at what is the potential benefit of this to me and what is the potential risk of it to me. It seems from the discussion here that we will not agree conclusively on benefit. And there is an area of benefit that hasn't even been discussed that mentally appeals to me just listening to it in saying that we are measuring tumor cells, cells that have gone all the way down the chain of differentiation and whatever else happens to make them a myeloma cell. But if there is evidence to say that progenitor cells that aren't yet identifiable by this assay or however as myeloma cells might actually be selected out as CD34 negative, that would be appealing. But it seems

like no one is going to be able to prove that one way or the other. So I would be more concerned about the risk side of this. I think your recommendation in the document says it correctly in that there should be a commitment to study the outcomes because clearly no one has any data on the real outcomes in terms of survival because the patients really are looking at

that, not at specifically what is happening in engraftment. As long as they are not going to die during the transplant. But also that the risk factors are addressed. Because I am not hearing -- it sounds like there may be some need to very closely monitor these late engraftment events. I am not sure what is implied in an approval in terms of a commitment to monitor that very closely. I am not sure what constitutes a reportable event to the FDA if this gets approved. And also from listening to the discussion, it seems like maybe there are some strictures around if you look at the harvest and you conclude that you are not going to inject or infuse 2 million cells, that maybe we shouldn't be using strictly the enriched product if that is a risk. I don't know.

So I guess my concern to the group would be -- and I think we are focused on whether we can absolutely prove the benefit, and I don't think we

can. If we can control the risk, it seems like something that should be available to the patient community and something that should be available for study if we can manage the risk.

CHAIRPERSON VOSE: I think a lot of those issues relate to things such as the study was not designed to look at overall survival, so we cannot

really say that. A lot of the risks things and follow-up information that you mentioned are things that we can discuss when we discuss the labeling issues and also the post-marketing studies that we would suggest.

DR. FLOYD: I have a number of questions. First I would like to make a couple of comments about devices in general. We are talking here about a device that is affecting a biological specimen. The first question that popped to my mind when I read through this is that everything I learned as a young biologist being trained in growing up seems not to have been considered in the design of this experiment. Now I say that because one of the things we have done here is we have subjected half of a population -- essentially half of a population to a treatment, but we haven't subjected the other half. Basically, the unselected arm of this experiment simply had

leukophoresis period. Their cells were not subjected to essentially a dummy device, if you will. The only way this experiment could have made sense from my perspective on evaluating a device is if those cells had gone through the same Ceprate machine that had a capsule that did not have a CD34 binder on whatever the binding medium is in this case. That kind of

experiment would have gotten rid of many of the questions that keep coming up in this particular submission as well as the others that I have sat on for this particular group. I would like to see what happens with a dummy device, if you will, because otherwise we don't have any way to evaluate the effect here. It is a real issue.

The other issue that has not been discussed that I have seen, and frankly I haven't gone back and done a literature search on all literature about binding devices, and I know there is a fantastically large literature in that area, one of the other pieces of information that I have not seen here whatsoever is what the actual repeatability of devices provided by the company are with respect to capture. In other words, if I open 10 different packages of a capture cartridge, what is the range of variability in capture of each of those cartridges and

what is the range of release using whatever mechanism they are using. As I understood it here, we are using a stirring bar of some sort to dislodge the bound cells. What range of human variability do we have in real patient populations of release from that kind of mechanism? Those are the kinds of concerns I have. And at this point, I haven't heard any answers to any

of those things.

CHAIRPERSON VOSE: If someone from the company would like to comment on the second issue regarding variability between actual devices?

DR. TARNOWSKI: I am Joe Tarnowski from CellPro. I am Vice President and Chief Technical Officer. Regarding your question about reproducibility, there are two answers to your question. First you said a controlled study using a non-binder. We have done studies with mobilized normal donors where we passed material through our column without the antibody, and what we find is no difference in the distribution of the cell population as it passes through the column. You do have some hang-up in the tubing pathways, et cetera, where you have some loss of cells in general, and this is just a physical manipulation. So I think the answer to your control arm is that we have done those in

laboratory experiments and we wouldn't predict any difference there.

As far as reproducibility of the system, we find that capture is very high in terms of we capture about 75 percent to 80 percent of the cells using the monoclonal antibody. We see some cells that pass through in the unbound fraction, and these

probably are lower density cells that have lower CD34 antigen distribution on its surface. Those cells don't seem to be readily captured by our antibody because of affinity differences and they pass through and are in the depleted bag. The release mechanism is very efficient. We use a non-chemical, non-interactive release. We use a physical stirring mechanism. And the bond that breaks between the cell and the antibody is quite efficient. In the laboratory, we see a very tight range of about 50 to 60 percent using mobilized normal donors. In the patient population, of course, the distribution is a little bit broader because it is dependent on the incidence of CD34 positive cells in the starting material. We find that when patients have a mobilization of .5 percent or greater, our efficiency and recovery is much higher than the low mobilizers. So some of the data here in terms of the wide ranges

you might see is based on the incident at which you can mobilize. And I think that makes good sense for the label claim that we are saying 2 million per kilo is a good target that you want to choose. Any other questions that I might be able to answer?

DR. FLOYD: Well, I am not surprised at all at your comments about the biological way the

device works. What I am suggesting here is that in this kind of study, there are always these unanswered questions about why you get delayed engraftment. Whether it is 7/10th's of a day, one day, 10 days, whatever. What else is happening to this sample? What else binds? All of us who have been involved in research and the research laboratory know that when you deal with cells and/or proteins and/or glycoproteins and all sorts of other compounds, anytime they are subjected to a place where they can stick by and clump or whatever, most likely they will. In this particular case, we have a group of patients, all of whom have obviously signed consent agreements to undergo a study. One group, however, has had the standard traditional clinical treatment, that is, leukophoresis and then re-engraftment, if you will. The other group has gone through a device. And what I am suggesting here is that the only way to tighten

up this data is to run both groups through a device, one of which is CD34 positive and the other one which is not. And I don't see that kind of study here, and it is the kind of study that I would really like to see happen.

DR. SIEGEL: But if we were to do that study, presumably in the ones that went through --

clearly in the ones that go through the CellPro, you would infuse those that adhere to the column and then eluted. If you were to do that in the control arm, there would be virtually no cells there and it would be reasonable to assume that those patients would fail to engraft. Would you suggest that there would be value in the dummy device in infusing the population that did not adhere and comparing that to the results of the population that did adhere in the CellPro device?

DR. FLOYD: Precisely.

CHAIRPERSON VOSE: But I think the idea is to compare it to the standard of care, which is what they have done. They have compared it to the standard of care that is currently used. I understand the rationale and scientific behind that, but you need to compare it to what is done in the patient population.

DR. BROUDY: And could I make just a brief

comment. That is that I have fewer concerns about the column perhaps than you do. I think it is very clearly shown in the literature that the speed of engraftment is related to the CD34 content infused in peripheral blood progenitor cell transplant. And I think the reason they engrafted perhaps slightly slower, particularly for platelets, is that fewer CD34

cells were infused, and that has been clearly shown even in populations of cells that have not gone through a column or some other device. So I am not concerned that the column is damaging the cells and I would be less concerned about the need for that type of control.

CHAIRPERSON VOSE: Dr. Auchincloss?

DR. AUCHINCLOSS: A question for Dr. Litwin. Again on the delayed platelet engraftment. It looked to me as if the people who got into trouble were the people who were already in trouble if you will. I mean before this study ever started, they were the ones who had low platelet counts to begin with . I guess my question for you is a) is that a reasonable interpretation of the data; and b) would you conclude from that that maybe more than 70,000 platelets or whatever the cut-off point was should be mentioned as a safety feature in taking part in this

kind of cell transplant?

DR. LITWIN: We looked at immediate platelet engraftment and we found limited numbers of patients with slow platelet recovery. The majority of the data that I think I showed you was this late event that is six months afterward. We looked at two risk factors. What is clear is a risk factor is infusion

of less than 2 million CD34. We also looked at having 75,000 platelets at time of mobilization, and the data were just too few to reach any conclusion whatsoever. I think I included that in the briefing, but I did not mention --

DR. AUCHINCLOSS: But my point is that the ones that were infused with less than 10 x 106 --

DR. LITWIN: 2 million per kilogram.

DR. AUCHINCLOSS: Looked like they were the ones who you could have predicted they were going to end up with a smaller number of cells because they already had evidence of diminished bone marrow reserve.

DR. LITWIN: That may very well be. We have no way of distinguishing that.

DR. AUCHINCLOSS: The question then is should you label this to protect yourself from going to those patients, i.e., have a platelet count of

100,000 instead of 70,000?

CHAIRPERSON VOSE: That is actually pretty well documented in the literature even for unselected transplant patients. That if they have a platelet count below 100,000 when you start mobilization.

DR. AUCHINCLOSS: I think this study went for 70,000.

CHAIRPERSON VOSE: Yes. 100,000 is kind of more standard.

DR. WEISS: And this is an issue we tried to address in one of the questions, but it is still confusing. And I was wondering if maybe the sponsor could address this. Because there are patients that entered the study, but they weren't randomized until after they were -- until just at the start of leukophoresis, and the minimum platelet count at the time that they collected platelets was supposed to be 30,000 platelets. That is the minimum number. And maybe Dr. Jacobs can address and try to clarify.

DR. JACOBS: There were two criteria for starting or initiating leukophoresis, and that was a white blood cell count greater than 1,000 and the platelet counts had to be greater than 30,000. There were only two patients that were below 30,000 that I gave exception to to go ahead and randomize because

their white blood cell counts were clearly climbing.

And there was one at I think 20,000 platelet counts and another one maybe at 26,000 or 27,000 platelet counts. And probably one of our clinicians could kind of address the standard as far as starting leukophoresis and taking patients to transplant with platelet counts.

DR. ANDERSON: Ken Anderson from Boston.

I would comment on that. I think the main finding of this study and supported broadly in the literature in a variety of cancers is that this threshold of 2 million CD34 positive cells per kilogram is necessary to get satisfactory engraftment long-term of all lineages. In terms of trying to identify factors beforehand that might predict for inability to collect those cells, I think the best evidence is that prior treatment is where it is at. And in particular, we have learned over the years that certain classes of drugs, alkylating agents and nitrosoureas in particular, which are known to be stem cell toxins, can clearly -- or other factors such as extensive radiation -- can abrogate our ability to collect sufficient numbers of cells later. So, in fact, when this trial was designed, that was considered and that is why there was a limitation on the amount of

alkylating agent exposure patients could have had prior to entering this trial, these three cycles.

DR. AUCHINCLOSS: So your recommendation to the FDA would be to include that in the labeling and the warning of the product?

DR. SIEGEL: There are just a couple of things I would like to comment on here. One is that

the platelet counts that you have been looking at in these analyses and that you referred to as baseline platelet counts are the platelet counts after mobilization with cyclophosphamide and G-CSF, and in general are lower than the platelet counts on study.

And it is those platelet counts after mobilization at the time of randomization and leukophoresis that seem to correlate with and be somewhat predictive of who is going to have delayed platelet engraftment. Just a point of information. They still could be used to determine whether or not to use this device.

DR. AUCHINCLOSS: That is all I am looking for. What is the predictor of who is not going to come up with 2 million cells.

DR. SIEGEL: Right. But it should be clear that that is what we are talking about, not the platelet count when you present for the conditioning regimen.

The labeling that we have for use in this device with bone marrow also indicates that when low members of CD34 cells are infused, there is a delay in platelet engraftment. And this study is consistent with that. And in that study also, there was more delay in platelet engraftment in the treated arm than in the non-treated arm, suggesting strongly that it is

related to use of the device. It is not listed as a contraindication. I think people need to way -- to look at the data and whether or not a delay in platelets is likely to be a critical issue in that indication.

Finally I would note, and I think the record is clear on this -- it was stated in the company's -- I think it probably has been clear since then -- something that seemed a little misleading although true in the company statement that there is no significant treatment arm effect on platelet engraftment after you adjust for CD34 cells received and for a baseline platelet. And while that may be true, it needs to be noted that the treatment arm is an important determinant here of the number of CD34 cells received. That in fact it halved the number. So that statement might imply that there aren't effects beyond the fact that the device reduces the

number of CD34 cells, but it doesn't suggest that the device, per se, is not responsible -- or the use of the device for those delays in platelets.

And that reminds me actually of another point regarding Dr. Floyd's and Dr. Broudy's comments, which is that there aren't hard data as to whether per CD34 cell the device does better or less well in

engraftment. There is a very soft suggestion I noticed in the data that was up that if you looked amongst those who had less than 2 million cells at a platelet cut point of 50,000 -- and as you saw, there are many cut points and there are a lot of multiplicity issues -- but there are about 5 of the 10 in the treatment arm that had delays or problems and 1 in 7 in the other arm, which I am sure is not significant, but I don't think we can say one way or the other for sure whether all the effects are attributable to reduction in CD34. Even if we could, it is important to note that we couldn't say that if you just did more leukophoreses and got more CD34 cells, the problem would go away. That is probably something one shouldn't presume. Reductions in CD34 cells may also correlate with issues regarding bone marrow reserve, prior chemotherapy, and the actual functional efficacy of the CD34 cells, and it exists

as a theoretical and not terribly unlikely possibility that patients who have fewer CD34 cells and don't engraft well that if you kept whipping them with some drug or more leukophoresis until you got more CD34 cells, they might still not engraft as well as those people who had a lot of cells to begin with.

DR. BROUDY: I'd just like to make a

couple of brief comments. About your concern about whether patients with extensive prior alkylator therapy, there should be a comment in here. I guess my feeling is no. And the reason is that it is the clinician's judgment and also because there are many predictors one could use, and one is the number of CD34 cells per ml less than 2,000 CD34 cells per ml prior to mobilization, for example. This predicts a group of patients who will mobilize poorly. So there are many, many predictors one could use, and I guess I would prefer not to see them all spelled out, but I think one should spell out the 2 x 106 CD34 cells per kilogram because I think it is very clear that patients infused with fewer than that do engraft more slowly.

I would like to make one other comment in response to what Jay has said, and that is that CD34, even though we are going to propose to use it I think

here as a committee, is an imperfect measure because it is a very small subset of the CD34 cells that actually engraft. What you are measuring with the CD34 cells is the progenitor cells mainly, whereas it has clearly been shown in the mouse model by Weissman and the Systemics folks and some others quite recently that it is actually the stem cells and not the

progenitor cells that are required for early engraftment in the first 14 days. And those cells we have no way to measure. And CD34 does not really measure those cells. So it is an imperfect measure anyway.

CHAIRPERSON VOSE: It is a surrogate. Unfortunately we don't have a better one to use at the present time. I agree that it is going to be impossible to list all of the factors as far as putting what we should and shouldn't do for selecting patients, and we have to use our best surrogate, which right now is the CD34.

DR. AUCHINCLOSS: But if I can just ask you a little bit further on that. I believe that 2 x 106 is the right number, but you only know that after you put them through the column whether you have it. So to tell people, hey, if you didn't get 2 million, you are in trouble and they have already used the

device --

CHAIRPERSON VOSE: You need to continue to do additional leukophoresis.

DR. AUCHINCLOSS: Well, that is the question that Jay just brought up. I am not so sure that just continuing to do leukophoresis is really going to provide you with what you want. So don't you

think it is wise to tell people this device may get you into trouble if you set out with a set of predictors that tell you you are not going to get very many CD34 positive cells out of it.

DR. SIEGEL: Well, you can continue to leukophorese and you also have the option as to whether in those future leukophoreses, you separate them or not.

DR. AUCHINCLOSS: You wouldn't separate them perhaps. And I guess there are ways around it.

DR. SIEGEL: But you also have the back-up there.

CHAIRPERSON VOSE: You have the back-up.

DR. AUCHINCLOSS: But I agree with you fundamentally that it comes down to clinical judgment.

CHAIRPERSON VOSE: Each clinical situation is so different that it is hard to generalize I think. Dr. Silver?

DR. SILVER: I have a question about the back-up cells. Is there any advantage to being infused with the back-up cells? This is really a clinical question. If they have been depleted of CD34, are they useless or what function do they -- and was there any data in this study that in those few patients that did get back-up cells, was there any

immunologic benefit?

DR. STEWART: Keith Stewart from Toronto. Only one patient was infused with the back-up, which was in Toronto, and it was ineffective.

DR. BROUDY: I guess I would say if they recover 60 percent of the CD34 cells, some percent of the CD34 cells will still be left in the flow-through.

CHAIRPERSON VOSE: Additional questions?

DR. AUCHINCLOSS: Yes, one big question for Jay really and the FDA. I think it is a rerun of the conversation we had in this Committee a hear and a half ago, the last time we looked at one of these issues. As highlighted when Dr. Litwin started his presentation, he said this device is already approved. And my question for you basically is as a device, I believe this device does what the company says it does. It removes tumor cells and gives CD34 cells that are safe. You are really asking them to give us

efficacy or give us a sense of efficacy in clinical treatment. Do you really think it is wise to judge this on a clinical treatment efficacy as opposed to it is a device that does what the device says it is doing?

DR. SIEGEL: Well, that is a tough question to answer. We haven't, obviously, asked nor

have we seen evidence of efficacy in clinical treatment in the sense that that would be -- would constitute survival and outcome data I would think. In fact, the data you are looking at is our data as to whether it does a specific function, remove CD34 cells. This device and most devices, although obviously it is variable from device to device, but most devices carry with them a certain -- well, I don't know if it is most devices, but certainly some devices carry with them a certain downside to their use. And I think we have seen that aside from the issues of platelet engraftment impairment and a very minor but statistically significant neutrophil engraftment impairment, there is the issue of more leukophoreses. It just took more to meet the standards that were imposed before. So I think the correct question in the context of this device is is the overall picture appropriate? Can what we presume

about the function of removing CD34 cells is that adequate against what we know about the impact on engraftment and number of leukophoreses to consider this device safe and effective for use? Devices do have an effectiveness standard. It isn't always applied in quite the same manner as drugs because the indications often are not the same as for drugs. And

it is hard to general about what we should be asking of a device except to say that I think it should be in context of a risk benefit analysis as it is for all our products.

DR. AUCHINCLOSS: Jay, you need to be clear that there has been efficacy asked of the company in the sense that this product was originally approved based on diminished first dose infusement effects. Correct? And basically they are coming back to you now and saying in 1994, your committee said that a 2 log or maybe more than that reduction in tumor cells could also be used as a surrogate marker for efficacy. Now be careful about what the committee really said in 1994. We can read back to that at some point if we need to. My point is I think that that marker for efficacy is probably flawed. I don't think this device is actually providing benefit to patients, at least as far as I can tell so far. And I think you

get yourself into trouble by asking them to show efficacy in a clinical sense, whereas what you really can do is say, all right, make a device that does what your device says it does and let clinicians decide whether it is worth using it.

DR. SIEGEL: You are suggesting that we did ask them to show efficacy?

DR. AUCHINCLOSS: Yes. I think you have asked them to show efficacy for something that I am not sure is --

DR. SIEGEL: What sort of efficacy did we ask them show? Efficacy in --

DR. AUCHINCLOSS: I thought the entire response that the company was coming up to was we will show you 2 log tumor reduction and that will be taken as a surrogate marker for efficacy. I mean, isn't that --

DR. SIEGEL: Right. But in fact if you are saying we should ask them to show that the device does what it says it does, then if the device says that it reduces tumors --

DR. AUCHINCLOSS: I am not going to ask them to show me anything except that they reduce tumor number.

DR. SIEGEL: Well, that is all we asked.

DR. AUCHINCLOSS: And I don't want to use the device because I don't think that does anybody any good.

CHAIRPERSON VOSE: I think Abbey Meyers would like to ask a question on the phone here if we can hear her.

MS. DAPOLITO: Abbey, can you hear us?

MS. MEYERS (telephonically): Yes, I can hear you. Can you hear me?

MS. DAPOLITO: Go ahead and we will see what we can do.

MS. MEYERS (telephonically): Okay. It is along the lines of what Jay was just saying.

CHAIRPERSON VOSE: She was asking about there are many different types of malignancies, and is the device going to need to go through an approval process for each type of malignancy?

DR. SIEGEL: I think that is part of one of our questions to the committee. I don't think we have a determination on that.

CHAIRPERSON VOSE: It is a question for later in the discussion, yes.

DR. SIEGEL: I would like to interject here that Dr. Max has a flight to catch shortly on Government business. It was a very difficult

scheduling problem and we are certainly most appreciative of his efforts in that regard. But if there are further questions specifically of Dr. Max, he won't be available later in the day.

CHAIRPERSON VOSE: Maybe we could just -- I was going to say we could go through many of the questions. I think they address a lot of the things

that we are talking about.

DR. SIEGEL: I think Bob Vescio should address that with some additional data regarding the beta-actin just to reassure you.

DR. VESCIO: Yes. First of all, I want to apologize for not having the data sooner. There were a lot of time constraints and unfortunately my clinical responsibilities were such that I couldn't get this data in time. But I was hopeful that maybe if you have -- I can show you actually the beta-actin gels that were run in a quantitative fashion to again address whether there was any quantitative degradation in the DNA from one patient -- from the 34 enriched product versus the leukophoresis product.

Basically what was done is we took 3 patients -- it is carousel 2, slide 51 -- 3 patients took their leukophoresis DNA and their CD34 enriched DNA and basically quantified the amount of beta-actin

that was amplifiable using this Poisson PCR. This is not the right slide. Carousel 2, slide 51.

Basically what we found -- this is an example of one of the patients. Because the assay was set up and the PCR conditions were set up to amplify a small PCR product, when we took the initial beta-actin primers that were used as a positive control and

run with all the assays, those PCR primers were not really optimized to detect just one copy of the gene per cell and that was why the percent contamination rate was quite low. I redesigned primers that were more comparable to this PCR product size looking for tumor burden, and in fact kind of fortuitously these particular primers actually amplified two germ line genes within the patient, one of about 225 base pairs in size and one of about 112 base pairs in size. I don't have a pointer, but you can see there on the slide. And basically in this particular example -- this is an H dilution, so this represents DNA from 30 cells, DNA on an I dilution and there are five replicates, DNA from 10 cells, 3 cells, 1 cell, .3, and .1. And as you can see here, there is -- as one might expect when one starts getting to a statistical chance of having one cell within the PCR tube, occasionally one will find an amplifiable product and

occasionally one will find no beta-actin gene present.

Again, one can score the positive reactions from this upper band which represents one germ line gene, and also at the same time as a comparison control the number of replicates positive bands looking at this lower gene product. In this particular case, 124 percent of the cells had an amplifiable beta-actin

gene looking at the upper product and 88 percent of the cells had an amplifiable beta-actin gene with 100 percent obviously being what one would expect.

This is the analysis for the same patient on the 34 enriched product, and again you can see very similar findings. And as one might expect as one starts getting to 1 cell of DNA within the PCR tube, one starts to have positive and negative results, and that again falls off. And again in this particular example, the calculated contamination rate was 78 percent for the upper band and 217 percent for the lower band.

I have two more slides that we can just whip through just again for comparison. Again, here is the results for the leukophoresis product. Here is 158 percent. Here was 145 percent. And for the 34 enriched product, here was somewhat lower 32 percent. Here the bands are a little bit weak, but it was 127

percent. And the third patient was also quite comparable and I have the gels if the panel would like to look at those.

So I think if one looked at all -- basically in this particular case, there are 3 patients. If you looked at the 34 enriched fraction, looked at the leukophoresis fraction, the fact that

there is actually two genes that are amplified and assessable in each of these time points. Again, all the findings were within the half log error rate of the assay. Again, I hope that this kind of allays some of the fears that the panel may have that the DNA was qualitatively or systematically degradaded in the 34 enriched product versus the leukophoresis product.

CHAIRPERSON VOSE: Why don't we go ahead and go through the questions. And then if it is not answered at the end of the questions, we can have that additionally, Abbey. Because I think many of the questions address what we are talking about here.

Let's turn to the questions, then. The first one really has to do with what we have been talking about as far as the validation and performance of the assay system to detect the tumor in the phoresis product. Do we have any additional concerns or questions regarding the discrepancies that we have noted or the

lack of internal control? Dr. Silver, are you satisfied now with the information that has been presented? Dr. Auchincloss?

DR. AUCHINCLOSS: I didn't hear exactly how you phrased the question.

CHAIRPERSON VOSE: Just are you satisfied with the information as far as the -- do you still

have concerns regarding the assay system, the discrepancies we have discussed or lack of internal control?

DR. AUCHINCLOSS: Well, as I think has been brought out here already, there are two different concerns. One is is the assay really accurate to within half a log. And I suspect the answer to that is no. But that doesn't terribly bother me because I do not believe that there is much evidence -- there is any evidence, I guess, that there is a systematic error that would lead to the incorrect conclusion that they are depleting tumor cells by at least 2 logs. So I think that part is probably true. I am not sure if your assay isn't as sensitive as you think it is that you necessarily are infusing as many products that are tumor free as you think you are, but that is neither here nor there from the point of view of what the company says it is trying to do. So I believe that

with the data I have seen that their product does what they say it does to in general remove at least 2 logs of tumor and probably more.

CHAIRPERSON VOSE: Is everyone satisfied that the data suggests that there is at least 2 log removal?

DR. BROUDY: Yes, I certainly am. I would

have to say that I think Dr. Max's very careful analysis of all the data, how it was generated and looking at all the gels served to actually convince me that the company has done a very good job in studying as large a number of patients as was possible that they could amplify the product from that it does remove at least 2 logs of tumor cells. And I think they are to be commended for having done this very carefully and having analyzed the run-through fraction as well.

CHAIRPERSON VOSE: It is actually a very carefully performed study. It is unfortunate that there couldn't have been a higher number of patients that could be amplified to add to that number. But that is unfortunately a difficulty of the analysis.

Any additional comments? Okay. Let's move to the next question. There typically are not reports of CD34 antigen expressed from myeloma cells, but there

recently has been at least one report of that. To what extent does this cause any safety or efficacy concerns of this selection device in patients with multiple myeloma? Anyone want to comment on that?

DR. BROUDY: Well, I think it also depends on the cell surface density. What you can detect by flow cytometry is probably about 1,000 molecules per

cell. I am not sure how many molecules need to be detected for a cell to be retained by this particular device. But reassuring is the fact that in no patients in whom we saw the data was there any evidence that the myeloma cells were preferentially selected, at least in this group of 28 patients. So we couldn't exclude that possibility, but it would be a lesser concern of mine.

CHAIRPERSON VOSE: I think certainly the number of patients that would have this problem would be very small and probably a minority of cells as you discussed. So in this particular patient population, I wouldn't say it is a huge problem, although certainly it is a concern for a generalizability to other malignancies.

DR. SIEGEL: If they were preferentially selected -- if they were CD34 positive and preferentially selected, it would still be almost

inconceivable -- I better put that almost -- that you would actually wind up infusing more tumor cells than you would infuse if you didn't separate them. It is unlikely that you are going to -- certainly highly unlikely that you are going to infuse more than you took out in leukophoresis. It is not impossible. So I guess one issue that we talk around and that is

somewhat implicit in this question is what if you did somebody who was CD34 positive and you purified those tumor cells. So basically what you would be infusing would be a product that was enriched that had half of the original number of CD34 cells and half or maybe more of the original number of tumor cells, but then didn't have a lot of other things like T cells, for example. Is there any reason that if that were done -- if you did, in a sense, enrich for tumor cells, that that might be or would be a safety concern?

CHAIRPERSON VOSE: Of course, if we don't know if taking them out makes any difference, how do we know if addition them makes any difference? But I think there is a theoretical concern about this is going to be somewhat of a depleted product with immune effector cells, and if you are going to put in a product that is very enriched with tumor cells without immune effector cells, that could theoretically be a

problem. There is no way to know that. We don't have any data.

DR. ANDERSON: Yes, I agree. Basically you could imagine all kinds of theoretical possibilities that you would select out on your column a specific CD34 population that is more malignant than the standard and therefore make it worse. But all

these things come down to basically getting data and the point of the FDA is to look at the risk and to look at the benefit and to put appropriate labeling so that physicians are aware of the risk, and then one looks to see what happens in patients and a continued analysis. And in the use of the product, these theoretical possibilities will be worked out. We can't answer everything.

CHAIRPERSON VOSE: Dr. Silver?

DR. SILVER: It seems to me it would be appropriate -- possibly appropriate in the literature to say -- to note that some tumors or some myelomas have been reported or one at least has been reported to be CD34 positive. And in that case, the claim of 100-fold reduction would probably not apply.

CHAIRPERSON VOSE: To put that specifically in the labeling?

DR. SILVER: Yes.

CHAIRPERSON VOSE: We will leave that up to our FDA colleagues. Any additional comments on that? Okay. The next question relates to safety outcomes for engraftment. Discuss the effects of the Ceprate device on neutrophil and platelet engraftment. Does anyone have comments on that as far as clinical relevance to what we have seen today? Well, from my

standpoint, I would say that neutrophil engraftment was certainly adequate in both arms and platelet engraftment, although it appeared to be slightly slower in the Ceprate arm was really not clinically significantly different and did not represent a problem as far as patients are concerned. There were, however, several outliers, and that needs to be looked at with respect to the number of cells infused and things like that that we have already discussed. Additional comments? Okay. Question number 4 -- this was a question that our FDA colleagues wanted us actually to vote on. Are the findings of additional leukophoresis procedures, platelet transfusions, and in certain patients an impaired platelet engraftment acceptable given the potential benefits of a 2 to 3 log tumor depletion? Can we have some discussion on that first? This is kind of the $64,000.00 question.

MR. KATZ: I think that what we are

19 talking about in terms of additional leukophoresis

20 once you've got the set-up to do that -- you've got

21 the catheter and all the other good stuff -- and

22 transfusions is kind of kidstuff compared to going

23 through a transplant. So I think that if there was

24 even a prayer that it was going to improve the outcome

25 and survival, I don't think patients would look at

that very unfavorably.

CHAIRPERSON VOSE: And typically I think it was only one extra leukophoresis for most patients.

MR. KATZ: Yes. Once you've got the plumbing installed, it is pretty easy.

CHAIRPERSON VOSE: Dr. Broudy?

DR. BROUDY: I guess I would just like to point out that at least some of the extra leukophoreses were dictated even pre-column just to achieve the target minimal number of mononuclear cells. So I believe that probably some fraction of a leukophoresis extra was required to generate the number of cells required to go through the column. But most of it was just that that patient population happened to have perhaps slightly poorer bone marrow reserve for some reason that we don't know at the present time. I guess I would vote yes on this. I am quite convinced that they deplete at least 2 to 3 logs

of tumor, and while the major part of the clinical problem is residual tumor in the patient, at least this potentially offers a step forward. So I would vote yes on this.

CHAIRPERSON VOSE: Additional discussion?

DR. SIEGEL: I would interject that perhaps we could rephrase your comment to say that

this is not the $64,000.00 question, but the log 4.8 dollar question.

CHAIRPERSON VOSE: Thank you. Okay. So let's take this --

DR. AUCHINCLOSS: Let me -- I guess I need education. It sounds good, a 2 log reduction in tumor. It feels good. Is there any evidence anywhere, even a suggestion, that it is good?

DR. BROUDY: Well, I think this is by implication from the gene marking studies done by Dr. Brenner, a former member of our committee, in which he clearly showed that infused gene marked peripheral blood tumor cells can contribute to relapse. So I think though much of the problem in myeloma and perhaps some of the clinicians here who deal more with myeloma than I do since I do mostly lymphomas -- you know, a major part of the problem is relapse in the patient. But at least this has been demonstrated in

Childhood ALL that infused tumor cells can contribute to relapse. So I think it is desirable that we infuse fewer. Does anyone want to comment?

DR. AUCHINCLOSS: Do you think you need to go to zero or do you think you need to be less?

DR. BERENSON: Let me comment on that based on the Phase II. Obviously that trial was not

designed with all the caveats of the Phase III. But several of the patients with extremely high tumor burden, for example the one that had 200 million tumor cells before the column, she is now in remission four years out from that transplant. And I certainly believe the 3 logs we removed may have contributed to that. I can't prove that to you. And we have two other patients who had high tumor burden as well that are out now at this point. One of them nearly five years out. I can't prove that to you, but those are just anecdotal cases. But you wanted at least an inkling that there may be some help here.

MR. KATZ: Well, I think this debate about whether removing the tumor cells at this stage is a bit of a red herring because the whole issue of whether you should transplant rather than pop melphalan prednisone pills, you can't prove that either. We wouldn't have trials ongoing for

transplant versus standard and early versus late transplants. So I think we are basically saying should we allow investigation of the logical extension of the transplant philosophy, which is knock out as many of the cells in the body as you can. Am I thinking incorrectly about that?

DR. AUCHINCLOSS: Yes, in my view in the

sense that there is nothing that I am wondering about -- and all I am doing is wondering out loud -- that prevents further investigation. Believe me, I am very much in favor of further investigation. The question is does the FDA want to put its stamp of -- they can label that this reduces tumor burden in the infused cells, which I think carries with it the implication, at least the way the FDA is handling this review, that that is good for you. I am not sure that I believe that it is good for you. I believe that they accomplish it. I just don't know that I believe that the FDA wants to say --

CHAIRPERSON VOSE: We have no clinical evidence that it is good for you, and the labeling has to say that it can do X, Y, Z that it says it can do. Unfortunately, we are not going to be able to police every physician who is going to use it. And I agree with you that that is a little bit of a concern. But

we have to specifically, I think, say that we don't have any evidence that it is good for you.

DR. ANDERSON: Let me take a brief crack, Hugh, at trying to answer you. This is basically looking at the philosophy behind doing the initial gene marking trials. The basic principle -- see if you would agree with this -- is that one does not have

to get the tumor burden to zero. One has to get it below the level that the body's immune system can counteract it. The difficulty is that we don't know what that level is. Now the justification for going forward with the marker trials initially was to get the sensitivity of the assay down into a level where one can start getting an answer or hopefully getting an answer as to what level of tumor burden below which the body can handle itself. So if you buy that argument -- you are nodding yes, so I gather you buy it up to this point -- then the logic is that the closer you get to that level, the better off you are, although if you are above it at all, then the possibility is that you are going to have relapses, just not quite as rapidly. So the issue is if you can't get below the level, then is simply putting off a relapse by a week or two weeks or three weeks, does that help, and the answer is no. But at what point do

you drop below the level, and the only way to find out is to keep dropping the tumor burden down until you get statistically relevant data. Do you agree with all that?

DR. AUCHINCLOSS: I do. But I am not sure it speaks to the question of whether or not at this point the FDA would want to imply that it knows this

level of reduction is important.

DR. ANDERSON: No, it can't. And that is -- I think our journal is quite correct. All the labeling can do is say what happens and not imply. Now the informed consent ought to make clear that a reduction in added tumor cells does not imply an improvement in either the survivability, time to relapse, et cetera.

DR. AUCHINCLOSS: You are talking about the physician's informed consent just in the ordinary course of doing the procedure, not something related to study. Because at this point, you are talking this out of study.

DR. ANDERSON: Well, that is true. A valid point. Which means it has to be in the labeling. And which is then a valid point whether the labeling should err on the side of being conservative and specifically state that there is no evidence that

this will reduce.

DR. AUCHINCLOSS: Jay, what do you think? What is your reaction to this?

DR. SIEGEL: Well, there is some sort of implication that if you indicate something as a marker of efficacy. Clearly, I think the labeling we would write would make it very clear, as we would expect

physicians to make clear to patients that there are no data indicating an impact in outcome. This is the dilemma that we brought to his committee, and we got very cogent comments and advice, much of which has been discussed, some hasn't. There was discussion, of course, of the difference between a tumor cell that is in the circulation and that is leukophoresed versus a tumor cell that might be, if you will, engraftable and give rise to tumor, the latter being something that can't be measured. There was a lot of discussion of the issue of the extent to which we know that tumor in the product contributes to disease. One view expressed was that to the extent there is also disease coming back from the patient, there was kind of a dual arm approach to get better therapies for what is in the patient and better therapies for what is in the marrow, and that you shouldn't hold the latter hostage to the former. If you don't allow marrow quality to

improve until you've got treatment quality to improve to the extent to where marrow quality matters, you won't get marrow quality to improve.

None of these I am forwarding as consensus statements or even as FDA opinions. These were all things that were said. I think what we took home from that, with all of that said, and what we talked about

with this company as this trial was designed was that reductions of tumor, particularly in hematologic malignancies, but reductions of tumor in general of specifically substantial amounts, and I don't know that there was consensus, but clearly we weren't talking about getting rid of half of the tumor cells as likely to matter, but substantial reductions of tumor were something that was to be considered a desirable outcome. Not something that was to be considered necessarily a proven measure of patient benefit, but a desirable outcome. And we specifically asked the committee how much problem with engraftment are you -- should we be willing to accept in a product that gives that desirable outcome. Because nobody had any problem with something that would do that and have no downside. The answer was, well it depends. It depends on the type of tumor, the likelihood that the marrow contributes, the log reduction, the sensitivity

of the assay, the proportion of patients who were reduced to below that sensitivity, the amount of delay, and the clinical significance of both the duration and the number of patients who did have delay of engraftment and the quality of the data and whether or not there were any data suggesting any impact on survival. So that is what leads us to where we are

and to this question. Now we have it depends and here we are and we need to make some decisions. I don't know if that answers your question, but that is all I've got to go on.

DR. AUCHINCLOSS: One thing to say is that you remember that meeting pretty well because I have spent the morning looking through those minutes and you got it absolutely perfect. That was not a committee meeting that I was part of. That was before my time. But I guess in a general sense the way I look at this is that the downside for this particular device and this particular disease looks like it is really on the verge of trivial and I think they are reducing tumor burden by at least 2 logs. I guess that means to go for it.

CHAIRPERSON VOSE: Okay. Why don't we vote on this question. Are the findings of additional leukophoresis products, platelet transfusions, and

impaired platelet engraftment acceptable given the potential benefits of a 2 to 3 log tumor reduction? Everyone that thinks this is acceptable, please raise your hand. It looks unanimous to me. And Dr. Floyd doesn't vote. Okay. Any other comments? Okay, good.

The next question, if approved, how should labeling address the risk factors associated with

engraftment delays? Should labeling advise that the number of CD34 cells post-selection be determined and recommend infusion of selected product only for patients who have at least 2 x 106 CD34 cells per kilo? And should there be any further studies done or other analysis of risk factors? From my standpoint, I would say that the labeling should say that, at least 2 x 106 per kilo just as we did for the bone marrow say 1.2.

DR. HONG: What would be the option?

CHAIRPERSON VOSE: The option would be --

DR. HONG: Just to pool everything?

CHAIRPERSON VOSE: Pool everything together. But specifically, I don't think you want to say -- you don't want to use the number that we use for bone marrow because in this circumstance, it would be inadequate. So you need to specify whether you are using bone marrow or stem cells for your product.

DR. SIEGEL: We are also asking in this question about the platelet. We have already heard comments in response to this question. We are not looking for a vote. But if there are any other comments on either of those that we might take in terms of thinking through as we work on this, I would appreciate them.

DR. WEISS: Or maybe a clarification. I am not so sure that we presented -- we actually did not present any analysis of platelet count. I think somebody asked earlier of Dr. White whether or not there is a correlation between platelet count at the start of the mobilization or at the start of the phoresis and the CD34 that you end up afterwards, and Dr. White said, no, there was none. But Dr. Auchincloss, I think, asked a very appropriate question. Are there ways before you actually start this whole procedure to try to predict and whether or not there are ways to look at it and other types of analyses. Are there other types of things that can be done to try to get a handle on that particular question?

CHAIRPERSON VOSE: Eugenie?

DR. KLEINERMAN: I assumed that was a question, and I just would like to reiterate what Dr.

Broudy said. I would like to see that put in the label, but I wouldn't use it as a cut-off. I think you need to give the clinician some latitude. There are all sorts of parameters that one takes into account and limiting it to patients who have 75,000 platelets I think may be a mistake.

MR. KATZ: I guess a question -- you

raised the issue before -- I think it was down there -- about whether additional leukophoresis would do any good if you were getting that kind of a yield that was below the 2 million. I guess the question that comes to mind is would you get a warning -- is there a predictable pattern of how you accumulate the cells and would you know that earlier in the collection and would there be any merit to sort of taking someone off the column early in the collection if that happened?

CHAIRPERSON VOSE: The problem from my standpoint is that patient variability is so high that it is really difficult to tell that except on a person-by-person basis. I am not sure that we can dictate something like that in a label. I don't know what anybody else thinks.

DR. BROUDY: Maybe they should say something like a sufficient number of peripheral blood mononuclear cells should be collected such that more

than 2 x 106 will be infused after the Ceprate column, and then making some assumptions that there are going to be 50 percent recovery, for example, from the Ceprate column. Because clearly you need to collect more than the 2 x 106 CD34 cells, at least twice as many as that, before the column, and then not making any comments about the platelet count or the number of

CD34's at the start or the extent of alkylator pretreatment and leaving all these things up to the clinician. But I do think that number of 2 x 106 CD34's per kilo post-column should be in there, and maybe the company has some thought about how that could be phrased to make sure that safety issue is met.

DR. KRIEGER: We already have that in the labeling that --

DR. BROUDY: Could you read that to us?

DR. KRIEGER: The labeling that we have proposed and submitted to the FDA already has in it the caveat that they should collect at least 2 x 106 CD34 positive cells per kilogram. That was the first slide. So this is the labeling that we have proposed and it was also the labeling that Dr. Litwin showed. The next slide actually shows the number. So we have shown here -- you see we have recommended that a

sufficient amount of peripheral blood be harvested to yield 2 x 106 CD34 cells per kilogram.

DR. BROUDY: But that doesn't give any guidance to the clinician about how many should be collected prior to the column.

DR. KRIEGER: We can put something in it similar to what we did in the clinical trial where we

say they collect 4 x 108 total nucleated cells.

DR. JACOBS: And we could do that either in the labeling or a cautionary warning as well in the labeling and it may be more appropriate for a cautionary warning for patients that may not have 5 x 108 total nucleated cells per kilogram at the time of processing.

CHAIRPERSON VOSE: I guess I am a little bit concerned about using the total nucleated cells because that doesn't always necessarily correlate. So I have a little concern about using that.

DR. SIEGEL: Indeed the design of the trial was such that they targeted 4 x 108 per kilogram and 10 of the patients didn't have 2 million.

CHAIRPERSON VOSE: Yes, Dr. Silver?

DR. SILVER: I wasn't sure what the role of the FDA is in this. Is it a foregone conclusion that there would be a sentence following the first

paragraph saying some sort of attempt to inform the clinician that there is no evidence at the moment whether reduction of tumor cells has a clinical benefit?

DR. SIEGEL: Is your question what the role of the FDA is in terms of what goes into the label? It is proposed by the sponsor, but we have to

approve it. We play a significant role in determining what is said where. What in particular were you asking about? Was there to be a warning to the clinician about the --

DR. SILVER: It seems to me appropriate that there be a note that says it is not clear whether a 2 log reduction in tumor in the transplant has a beneficial clinical effect. In the absence of such a statement, the claim proposed by the sponsor could be misleading, it seems to me. It sort of implies that it is obvious that there is such a benefit, and it is not really so obvious. So it seems to me that there should be some disclaimer to that effect.

DR. SIEGEL: You are advising that there be a prominent statement about the lack of information about that?

DR. SILVER: Yes.

CHAIRPERSON VOSE: Typically, if the Phase

III trial is described and it is described as not being adequately sized to show benefit or lack of benefit thereof is important information to have in there. Let's move on to the next question, which has to do with the generalizability of this information. Should this study just simply be -- should the label be restricted just to patients with multiple myeloma

or is it generalizable to other tumors, or should there be post-marketing studies of other tumor types? Some discussion on that? Abbey, would you like to comment on that since that was your question to begin with?

MS. DAPOLITO: You are breaking up, Ms. Meyers. We can't hear you.

MS. MEYERS (telephonically): Can you hear me now?

MS. DAPOLITO: Try again.

MS. MEYERS (telephonically): Hello?

MS. DAPOLITO: Yes, can you hear me?

MS. MEYERS (telephonically): Yes.

MS. DAPOLITO: Try again.

CHAIRPERSON VOSE: I think the gist of what Abbey said was that she didn't think that they should have to do studies in all different types of malignancies. The labeling should just say that it

separates the cells and not otherwise be specific.

Virginia?

DR. BROUDY: I guess I would differ from Ms. Meyers opinion on that. I would recommend personally that the label say depleted myeloma cells, because that is what has been I think elegantly and convincingly shown in this carefully done study by the

company. A potpourri of other trials were shown at the end, and they were characterized by much smaller patient numbers in the studies of, for example, lymphoma peripheral blood depletion studies. And I would like to see those studies done, particularly with BCL2 gene. That could be analyzed or looking for breast cancer cells. I would like to have it convincingly shown that this device also depletes breast cancer cells and lymphoma cells before generalizing this to other tumors.

CHAIRPERSON VOSE: Dr. Silver?

DR. SILVER: I think I disagree on two grounds. First of all, I don't think it was very convincingly shown in this study because of all the statistical problems and some patients in which the data wasn't consistent. But it is overwhelmingly likely from a biological point of view that a tumor that doesn't have CD34 on it won't be selected in this

column and the column allows you to reduce the number of cells you infuse by 100-fold. So almost certainly the number of tumor cells that are going to go back for a CD34 negative tumor is going to be down by about a factor of 100. And given that, I don't think it is appropriate to force the company to do additional studies. I think a statement saying something like

for tumors which are CD34 negative, this result might be generalizable to tumors that are CD34 negative. Because it is biologically very likely and a lot of work and a lot of money would have to be spent to prove it in each individual case.

CHAIRPERSON VOSE: Dr. Anderson?

DR. ANDERSON: Since it is difficult to hear Abbey -- Abbey, I am going to ask the next question that you would ask. So I am now the voice of Abbey Meyers. Will the label be such that third party payors will pick up the cost if this device is used in diseases other than myeloma? That was Abbey's next question.

CHAIRPERSON VOSE: Yes, I am sure it would have been knowing her so well. I guess that question has to go to Jay.

DR. SIEGEL: Well, obviously we are here seeking guidance from the committee. This is a

complex area. I think it is fair to say, among other things, that although one can arguably say and I think quite correctly say that more could be done in a variety of other tumors and perhaps more should be done in a variety of other tumors, that even at best it would be very hard to do in most tumors anything close to what was done here. PCR has a level of

sensitivity that is not apt to be -- for tumor detection that is not apt to be found with monoclonal antibodies, histochemical staining and other approaches that might be used. Certainly numbers could be done better and I think even relatively small numbers provide some assurances regarding the possibilities that Dr. Silver mentioned. The concerns -- if you look at a column that depletes cells by 2.2 logs -- that depletes a total number of cells by 2.2 logs, and then if you then assume you are CD34 negative and note that the number of CD34 negative cells goes down from almost 100 percent to about 30 percent, that is another half a log. You are starting with about a 2.7 log. And there is a reasonable a priori assumption if the tumor is CD34 negative that it will be reduced by about that much. However, we all know that there are cells that have non-specific sticking to all sorts of devices and columns and I am

not sure I am so comfortable buying that. So I guess what we are looking for is solid data or reasonably solid data in one tumor and is there enough elsewhere to make us reasonably comfortable with the type of assumption Dr. Silver mentioned. Certainly we can write a label in such a way -- we always -- you know, related issues come up at every meeting and my answer

always is that we try to be informative about the data rather than definitive in labeling. Certainly one of our options is to write an option that would both -- that would not restrict use to myeloma but would indicate the amount of data and the relative paucity of data in other tumor types. Another option would be one that would limit the indication. And I am not really here to tell you but to get input from you as to what might be more appropriate.

DR. ANDERSON: All right. I am going to follow on this because basically if Abbey were here, she would do this. What is the experience with what third party payors will do based on the labeling? If the labeling specifically says myeloma and then is sort of wishy washy about other things, will third party payors pay it for breast cancer, or does the labeling have to be specific that it is payable for other indications.

CHAIRPERSON VOSE: They won't even hardly pay for breast cancer transplants anyway.

DR. ANDERSON: I am sorry?

CHAIRPERSON VOSE: They won't hardly even pay for breast cancer transplants anyway, so that is probably a moot issue. Personally, I think that there is enough concern about CD34 positivity in cells. In

breast cancer it has been reported and also in some very early populational lymphoma cells. I think there is some concern there that we have to be careful about not just having a totally generalizable statement. We have to have some concern in the labeling in some way to reflect that.

DR. KLEINERMAN: Julie, in terms of paying, are we not looking at approving this for stem cell transplant, not just -- I mean, as a device to use for stem cell transplant and not just as a device to use to remove 2 logs of tumor? So, theoretically if it is approved as a device to select CD34 cells for stem cell transplant, you could use it with breast cancer and the log tumor reduction should be non-considered when it comes to third party payors. I mean, that is the way I would read it. That you are approving it as a selection device for stem cells, as we have done with another device. And in addition,

you may get this benefit.

DR. SIEGEL: Well, it would depend on how we wrote the labeling, I guess. That would be certainly a way that one could view it.

DR. KLEINERMAN: Then you could leave it up to the clinician as to whether they want to consider that.

CHAIRPERSON VOSE: Ms. Knowles?

MS. KNOWLES: Yes. I am aware of some examples in the Seattle area actually where there have been women who have sued their third party carriers to get this kind of treatment. Actually to have it done for them. They have been successful, but it has not been without a lot of effort on their part.

CHAIRPERSON VOSE: Abbey, did you have another comment?

DR. ANDERSON: Tell Abbey she has to shout.

CHAIRPERSON VOSE: Shout louder. Jay, did you hear Abbey's question?

DR. SIEGEL: If we approve it, will it -- I didn't catch the end.

CHAIRPERSON VOSE: The patent problem. If they approve it, will it be able to get on the market?

DR. ANDERSON: Is that an issue we can

deal with?

CHAIRPERSON VOSE: I think that is something we cannot address.

DR. SIEGEL: I think the device is currently on the market. Is it not on the market? I think if you want any more in-depth answer to that, you should ask the company to respond.

CHAIRPERSON VOSE: I think that is not appropriate for our meeting today.

DR. AUCHINCLOSS: Can I suggest a change in the wording for the proposed labeling in that portion that talks about tumors? I would say selection of PBPC can result in greater than 100-fold reduction in number of tumor cells present in the autograft if the tumor is CD34 negative.

DR. BROUDY: But how about saying that it has been demonstrated to result in a 2 log depletion of myeloma cells? That is what has been demonstrated. And I guess my concern is that breast carcinoma cells and epithelial cells are sticky cells, and I would just like to ask that the company do a study that quantitates the fold log reduction in breast cancer cells and lymphoma cells given the immense applicability of this potential device. I don't think that is asking too much. They have convinced me

already that it is safe. That CD34 selected peripheral blood -- mobilized peripheral blood progenitor cells selected by this device are safe. That they result in rapid neutrophil engraftment and only slightly delayed platelet engraftment. So I wouldn't necessarily require that they replicate all of those data, but I would like to see a carefully

done analysis of depletion of tumor cells for breast cancer cells and lymphoma cells. Because those other studies were just not as carefully done from the brief overview we had and from my own reading as this particular study.

CHAIRPERSON VOSE: Dr. Berman?

DR. BERMAN: I would disagree. I think that what the company has shown is that it is an extractable material, that is, the CD34 cells are safe going in. I think it is up to the clinical trials, all of which will be done in breast and lymphoma, to prove whether it is effective or not. But it is not up to the company. It is up to the remainder of the investigative community. And it will be used or not depending on those studies. But I think to require it for the company is wrong. I think it is up to us to prove it or disprove it. All we know is that the CD34 population is safe, and by the way, it can also

effectively reduce the log contamination.

DR. BROUDY: Oh, I wouldn't necessarily require it of the company. Perhaps I misspoke there. But I am concerned that the labeling not say that it reduces tumor cells in general. Because what we have seen in my view convincingly is that it depletes myeloma cells by 2 logs.

DR. BERMAN: But that will go into the labeling. The data are in myeloma cells. I am assuming that that will go in.

DR. AUCHINCLOSS: That will be in later. But for this particular up-front portion, there will be this sentence that makes the general statement. How about a compromise. Has been demonstrated to accomplish a 2 log reduction in tumor for a CD34 negative tumor and then require of the company a Phase IV post-marketing trial for breast cancer or your other tumor of choice.

CHAIRPERSON VOSE: I think I have to agree with Dr. Broudy. I mean the information we have, the good data that we have, such as it is, is in myeloma, and I don't think it is generalizable to the other tumors. There is too many differences in their physical properties. There are too many issues regarding CD34 positive stem cells in lymphoma, for

example. I think that is a concern.

DR. AUCHINCLOSS: Well, there is no question it is not absolutely generalizable, right? I mean, you wouldn't do this for a CD34 positive tumor, and probably there are other tumors. What we are trying to get away from is the Abbey concern. We really would like not to make this disease specific.

So if you made a general statement here, but not one that implies absolute generalizability, and then ask the company to come back and do additional studies, isn't that the best solution all around?

CHAIRPERSON VOSE: Other comments?

DR. KLEINERMAN: Yes, I would agree with that. I think we need to try to keep it as generalizable. Because I think we need to keep in mind that we want all these studies done, but part of the ability to do these studies is for patients to be able to pay for them. And I think that is a real concern. We can design studies, but if we can't get patients to enter them, we will never know the answer. So I think it is important that the label be designed in some kind of specific yet general enough way, either just by using a peripheral stem cell transplant that it is safe and effective in selecting CD34 cells. So that it can be picked up by third party payors, so

these other studies can be done.

CHAIRPERSON VOSE: But yet we do have to have some sort of caution, I think, somewhere regarding that the information we have is in myeloma.

MR. KATZ: I would think that the third party payors would seize on the disclaimer that we were talking about before about no proof of clinical

effect on the overall outcome, which I think is an important element of it. I think they would go straight to that. They wouldn't worry about the earlier statement. Wouldn't they?

CHAIRPERSON VOSE: You never know with those guys. Okay, any other discussion on that? Why don't we move to the last question then. Data collection for overall and disease-free survival in the study will continue if approved in addition to follow-up from the ongoing trial. Should post-marketing studies for evaluation of the effect of the Ceprate device on measures of time to relapse or reduction in recurrence rates on patients with myeloma be sought or other malignancies? Would the committee recommend any additional post-marketing studies? Dr. Auchincloss, you must have something.

DR. AUCHINCLOSS: Well, I just mentioned one. This is where I put in -- I mean, for sure we

want to know what the survival and recurrence of disease is. That is assumed. But another tumor, I think, is critical. And breast cancer is the obvious one, right?

CHAIRPERSON VOSE: Well, if you are going to do a post-marketing study, I would say that we at least need to do one that is large enough to be able

to see a difference in disease-free survival or survival or something.

DR. AUCHINCLOSS: Now you are talking something big from the company's point of view. If you start requiring a post-marketing study of the company that is powered to be able to show progression-free benefit, wouldn't that calculate to be something like 800 patients?

CHAIRPERSON VOSE: At least. I didn't --

DR. AUCHINCLOSS: I don't know that I think that is fair to ask of the company in return for the amount of labeling we have given them so far.

DR. SIEGEL: But think of the labeling they could get if they showed something.

CHAIRPERSON VOSE: But then why bother doing a post-marketing study at all? What are we asking them for?

DR. AUCHINCLOSS: To prove what they say

in their label is true. That is what we are asking them to do. In this case it would be to generalize it to more than one cancer. But I am not sure it is up to the company at this point to prove that 2 log or more tumor reduction is a good thing.

DR. BERMAN: That is the crux of the issue. I think it is to the rest of us as the

clinical investigators to prove it is a good thing or a bad thing. So I would not require it of the company. I think it is up to us. All the company has shown is that it is a safe product that can engraft promptly, and there is a log reduction in myeloma. Whether that is of any efficaceousness is up to us, and that will be answered 5 years from now when the randomized large studies have been done. But it is not up to the company to show that.

DR. SIEGEL: Do you anticipate the randomized large studies will be done?

DR. BERMAN: Yes, I do.

CHAIRPERSON VOSE: This could be a big undertaking. I don't know how such a large study could be done unless it was supported by the companies.

DR. BERMAN: Well, support is another question. Requiring them to perform it is something

else.

CHAIRPERSON VOSE: Additional discussion on post-marketing studies?

DR. JACOBS: Could I just add one thing?

We did have an additional 59 patients randomized onto the study. So we have close to 200, but still not enough. We also have a Phase III study that is

randomized in multiple myeloma looking at mephalan and TBI in Europe. So we possibly could look at 300 multiple myeloma patients. That is probably the best that a small company can afford to do. It may or may not show what is needed. But there will be follow-up on 300 patients.

CHAIRPERSON VOSE: I think one thing also to consider in the follow-up besides the disease-free survival is the long-term immunologic reconstitution of these patients as well. I think that is an important issue that we need to look at. Because there have been concerns about that as well. But I don't think that should really hold up anything that we are doing today.

DR. SIEGEL: You are talking about in the patients in the study?

CHAIRPERSON VOSE: Right. Any additional questions or comments? Okay. I think we are done.

We are going to take a 45-minute lunch break and then we are going to resume. Dr. Noguchi is going to start off this afternoon. Let's resume about 1:30.

(Whereupon, at 12:46 p.m., the meeting was adjourned for lunch to reconvene at 1:41 p.m.)

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

(1:41 p.m.)

CHAIR VOSE: Dr. Siegel, thank you.

We are still in the open session right now. Yes, we are going to proceed with Dr. Phil Noguchi, who is going to do the overview of the Laboratory or Cellular Immunology and Laboratory of Developmental Biology.

DR. NOGUCHI: I'll just go ahead and get started here, and I want to personally thank all the members for allowing us the opportunity to tell you a little bit about our research.

The division that I represent is called the Division of Cell and Gene Therapies, and just as an example of some of the product classes we have, basically, this is unlike taking just a single product, such as we've seen this morning with the CellPro device, where you are actually taking the

cells and you may expand them, take them from the body, expand them with interleukin 2, people are looking at other types of mesenchymal stem cells, some are even using sertoli cells for immunosuppression.

We also cover all the gene therapies using a variety of vectors to genetically alter cells, and we are getting into xenotransplantation using both

fetal tissues and trasnsgenic animals as well.

Now, in order to also just give you the other breadth of it, we cover a number of different diseases, among them genetic diseases, some of the more neurological, debilitating ones.

Cardiovascular disease, it's interesting, we think that gene therapies for that are going to be a very big area over the next year or so, and, of course, cancer and AIDS.

Now, the numbers of INDs is actually quite interesting. Over the last three years, we received in the area of cell and gene therapy about 100 to 120 INDs, which translates to about one out of every four INDs that comes to the center is a cell or a gene therapy IND, so we are seeing a lot of investigational activity and we hope that soon we'll be able to bring to this committee some actual products.

Now, the divisional structure, just

briefly going over that, Dr. Gerry Marti is in the Office of the Director, there will be three individuals who will be reviewed from the Laboratory of Cellular Immunology. This group has recently been reorganized to focus on issues of xenotransplantation, including both immunology and viral aspects.

We've reorganized Molecular and

Developmental Biology into one group, and Dr. Judy Kassis will also be reviewed today, and she'll be talking more about her specific interest in gene therapies.

And then the last laboratory, which is not being reviewed but has been reviewed previously, is the Laboratory of Molecular Tumor Biology.

Now, we always appreciate the opportunity for you to tell us how we are doing in our research, and given that we'd like to just focus on both what we are currently covering and some of the areas that we think will be coming down the pike. There will be other kinds of gene therapy vectors that we'll be looking at. We know lentiviral vectors are coming. Dr. Anderson, is he still here, is already talking about in utero types of protocols, where we'll do gene therapy in utero for some genetic diseases. There will be cell tissue and organ regeneration. We

already have some animal cloning, and we've already stated that human cloning, should it ever happen, would be an FDA regulated product.

So, basically, for this division what we can say is, what can be imagined will be done, and Dr. Eda Bloom will present her program now.

DR. BLOOM: Thank you.

It is my great pleasure to follow Phil and give you a more descriptive introduction, particularly, to the Laboratory of Cellular Immunology, and to give you an overview of both our regulatory and our research work.

To reiterate, the members of the laboratory that have been site visited include myself, Dr. Carolyn Wilson and Dr. Parris Burd, and our little Laboratory of Cellular Immunology is named as one that has been standing for a while, it is less descriptive of what the laboratory does now than what it once was. And, in fact, as you will see, the cohesive element that holds the three of us very tightly together in a collaborative network is that of xenotransplantation and the porcine endogenous -- and, clearly, the porcine endogenous retrovirus, but the idea of xenotransplantation and whatever endogenous retrovirus we need to look at.

The regulatory work in the laboratory is representative of that which is done at CBER. It includes review work, policy formation. We also, however, do research related and have done research related to adverse events and safety, as well as doing research applying our expertise, both in the anticipation of new products, and also in the

exploration of products that are currently under development and under IND. And, of course, as any group in an academic or similar atmosphere, we provide other services to CBER through various committee services.

The regulatory review activities in a little more depth include, of course, the product review of INDs, and I'm going to use the word "we" a lot, and when I use the word "we" I mean to say that one or more of us within the laboratory, that is, one or more of Dr. Burd, Dr. Wilson or myself.

We also mentor and advise IND reviewers. We have chaired and been members on various license application committees, as well as reviewing numerous post-market license supplements, which, as you may know, also have their own set of clocks and their own set of criteria that need to be met.

In addition, we have been inspectors of

manufacturing facilities to assure that such facilities conduct their manufacturing under good manufacturing procedures.

And, as you can see today, and have seen in the past, we make various scientific regulatory presentations to the FDA Advisory Committee panels.

This is a little bit of a rehash of a

slide that Phil just showed. The only difference is that, and this is totally cribbed from him, is that it also shows the growth of the cell and gene therapy INDs relative to the rest of the center.

The next slide focuses a little bit more on our laboratory per se, and as you can see, the reviewers in our laboratory provide a substantial share of the IND review within the division, especially, relative to the number of reviewers that we do provide.

As far as our policy activities are concerned, we have been drafting points to consider, now currently called guidance documents, for industry to be helpful in product manufacture. We have been involved in the drafting of the Public Health Service Guidance Document in xenotransplantation. We have also participated in both CBER and department-wide committees on xenotransplantation, prevented various

invited talks on the spectrum of regulatory subjects that the whole division deals with.

We have organized the FDA/NIH gene therapy conferences, which some of you I'm sure are familiar with, that have been held very successfully the past two years, '97 and '96. We have organized a Cystic Fibrosis Foundation conference, organized and led

CBER-wide viral vector working groups, which has served as a template for other working groups dealing with the types of products that our division deals with, and we have been panel members on public fora, such as the Commissioners Roundtable for Autologous Cells Manipulated Ex Vivo.

The laboratory responses to regulatory issues that our laboratory has been involved in include addressing the unexpected toxicities that arose in clinical trials of interleukin 12. We have designed paradigms and performed experiments for testing the presence of replication competent retroviruses, and particular emphasis on gene therapy products, and this slide says "developing," but, in fact, standards have been developed, a standard viral stock has been developed for use in safety testing, and we have developed technologies, isolated and characterized the endogenous infectious agent or

agents, probably more precisely agents, that are present in pig cells.

Now, I'm going to spend just a couple more minutes and go through each investigator's program.

Dr. Carolyn Wilson, program is Viral and Cellular Factors Influencing Retroviral Infection. She has two major studies within her program. In the

first study, she uses murine leukemia virus in order to study the viral receptor interactions, and one of the important emphases of her studies is, in fact, on how the retrovirus can enter the cells, an important first step in viral infection. In addition, she has been studying virus variation selection, using the now famous or infamous porcine endogenous retrovirus.

This next slide summarizes the relevance of her particular project to the regulatory mission of CBER. Notably, her xenotransplantation studies, using the porcine endogenous virus, has enabled a certain amount of risk assessment and, thereby, permitting FDA to make recommendations to sponsors of ongoing protocols using pig tissue for transplantation into humans.

Her studies in retroviral in the murine system have enabled studies expected to help develop new generations of retroviral vectors that, perhaps,

may provide a safer or more efficacious way of delivering genes in gene therapy.

And finally, her development of detection assays for recombinant complications -- complication, we don't mean that -- for replication competent retrovirus have enabled the means by which we are able to detect replication competent virus in retroviral

vectors and other products which may include such contaminants, such as monoclonal antibodies that are produced in mice, as well as in xenografts.

Just to briefly summarize progress by Dr. Wilson since the site visit, she has had an additional manuscript accepted for publication, she is currently expressing gp70, the envelope glycoprotein on amphitrobic murine leukemia virus in a vaccinia system and will be purifying this for use in binding studies, and in her virus variation selection program she has also had a very important paper accepted for publication, detailing the production of porcine endogenous virus by primary pig cells. She has isolated in a sequencing that envelopes CDNA for this virus.

Dr. Parris Burd's program has focused on the molecular biology of immunologically active cells. He has worked on cytokine networks in health and

disease, and in this study he has emphasized interleukin 12, interleukin 13 and chemokines. He has also developed PCT methods for analysis of archived histopathologic materials, which has clear and obvious relevance to studying potential reasons for adverse events using cytokines.

In the porcine endogenous retrovirus

studies, Dr. Burd is our resident molecular biologist, where Dr. Wilson is the resident retrovirologist, and his expertise has been necessary in the studies across species viral infectivity and activation.

As far as the relevance of Dr. Burd's research to the CBER mission, cytokine networks, as you on this committee certainly are aware, are part of the causal chain in disease processes, and provide key sites for the intervention and therapeutic approaches.

Also, we have, certainly, our share of adverse events that are referable to cytokine treatments.

In the porcine endogenous retrovirus study, it is clearly a prominent safety feature at the moment for use of porcine endogenous -- excuse me, for porcine xenografts, and Dr. Burd's establishment of a PCR method to assess the virus transfer to humans is something that will have a lot of relevance to our

ability to regulate these products.

Since the site visit, Dr. Burd has had two manuscripts accepted for publication, and he has submitted three additional manuscripts.

On the regulatory front, he has spearheaded an initiative to foster development of gene therapies for rare genetic disorders, and, in

particular, this program will effect outreach and education to the public and education of new investigators in the handling of therapies that may involve very few patients.

He has also been the co-organizer of an international conference on vectors for gene therapy that was held -- where is this, I think it was this month in Brussels.

My own program is centered around the cellular and molecular regulation of cytolytic lymphocytes, and I have those years up there because that's the time that elapsed since I was site visited at CBER, not the time period during which these projects particularly took place.

I have had three subtopics in my program. One is the activation and regulation of human natural killer cells, which is actually an extension of the project that I embarked upon in the early 1970s.

More recently, we've been studying the regulation of natural killer cells by oxidation reduction, and not just the regulation of their functional activity, but the regulation of their elimination through apoptosis as well.

In order to do this, we've been looking for effects on signaling pathways and have found

effects on signaling pathways as well as cell cycle progression as being key regulatory points by oxidation reduction.

A project that's winding down in my laboratory is the alteration of cytolytic T lymphocytes and the effect of age on CTL activity and generation. I list it here because it provides an important predecessor to the study that we are currently emphasizing, which would be the cellular immunity and safety issue in xenotransplantation, and where Dr. Wilson is the retrovirologist and Dr. Burd provides the molecular biology, I provide the cellular immunology and cell biology to that project.

The relevance of my particular program to the regulatory issues at CBER has been substantial, I like to think. Immune cells, as you are, again, I'm sure aware, comprise a large proportion of the somatic cellular therapies that are reviewed by CBER, in

particular, lymphokine-activated killer cells provide the prototype, and these are cells that you probably know are derived from natural killer cells. Cytolytic T cells are another subclass of somatic cell therapies, and gene therapy is also used frequently, lymphocytes as delivery systems.

My research program has led, or is leading

to, appropriate tests for lot release for certain of our products and has been important in the development of policy and particular guidelines that we have published for development of cellular therapies, as well as currently in xenotherapies.

Finally, within our program we have provided scientific data that have aided in the understanding of the adverse reactions to IL-12.

Finally, the progress in my own projects since the site visit is that I have had three manuscripts accepted for publication and an additional one is being submitted. We have tantalizing results that are, unfortunately, too preliminary to talk about, but, nevertheless, I have to mention them, regarding the effect of cytokines and various immune responses on the expression of porcine endogenous virus by lymphocytes.

The regulatory work since November has

blessed me with two license supplements for review, and I have acquired 16 new INDs to review.

And, with that, I'd like to introduce Dr. Judy Kassis, who will talk about her program and progress since her site visit.

DR. KASSIS: Okay. I'm going to keep this really short. My program is designed to study the

control of gene expression in transgenic animals, and we are the Division of Gene Therapy, and I think it's important for us to understand how transgenes are regulated in an organism. And, in order to have safe and effective gene therapies, like I just said, it is important to be able to predict how the therapeutic transgene will be expressed.

I study transgenic drosophila because it's a very fast system, it's a very easy system to study, and the system is, basically, one of an integrated transgene. You can think of this as a retrovirus, it's transposon with two repeated sequences, and you put your gene of interest in between them, and this transposon can get incorporated anywhere in the genome. And, what I'm interested in knowing is, how the genomic location influences the expression of this transgene.

So, there are two factors which can

contribute to the control of the integrated transgene.

One is the regulatory DNA in the transgene, and one is regulatory DNA flanking the transgene. For instance, this transgene carries information to be expressed in the lung, but since it's inserted near enhancers which cause it to express in the heart, it will be expressed in all three tissues, and we'd like to understand how

to better control transgene expression.

Also, during development and differentiation, genes are packaged into active and inactive chromatin, and, basically, we want to prevent a situation where a transgene, which we want to be active, is packaged into inactive chromatin, so we need to understand how this inactivation occurs.

So, in drosophila there's a group of genes called the polycomb group, which are involved in keeping genes off. In this schematic, early in development this gene is turned on by segmentation genes in this region of the embryo. Then, the polycomb group genes somehow recognize that this gene is off in these two regions of the embryos, and they stay with this gene packaging it into inactive chromatin so that this gene is repressed throughout development, so that in the in tact fly this gene is only expressed in this part of the fly.

If the polycomb group genes are mutated, this gene is expressed in other parts of the fly, and then you end up with flies with legs in place of antennae, and legs in place of wings.

So, I study a piece of regulatory DNA from a gene called engrailed, which has two unusual activities. First, when you make a transgenic

drosophila we use a marker, we use the mini-white gene. We inject white eyed flies, and when you inject this gene and you get a transgenic fly, the flies then have colored eyes.

The name of the gene was named for the mutation, when this gene is mutant the flies eyes are white, but the gene product makes the flies have colored eyes.

This vector inserts in the genome randomly and homozygous flies have two copies of this transgene. Therefore, they have darker eye color than heterozygous flies.

When you include this piece of engrailed DNA in this construct, the transgene now inserts in the genome in a more selective manner, and this piece of engrailed DNA causes the white transgene to be turned off.

So, the way this works is, for a normal

transformant, without the engrailed piece, you recover a transformant that has yellow eyes. You make it homozygote, it has red eyes. For the engrailed piece, if you recover an engrailed transformant with yellow eyes, if you make it homozygote, now it's two copies near each other in the genome and it has -- the transgene become repressed and you get white eyes.

If you have two copies far away from each other, you get, again, red eyes, but these two copies can either be very close to each other in cis or in trans, to get the transgene repression.

I'll just show a couple of slides. This is a normal transformant, this is a homozygote. This has two copies, it's got a darker eye color. For the transformant with the engrailed fragment, this has the heterozygote, this is a homozygote, the eye color is completely repressed, and now this I'd like to show because it gives a very dramatic example of the effect of the position of insertion in the genome on transgene expression.

In this case, the heterozygote has a represssion in part of the eye, just based on where it is inserted in the genome, and when you make the fly homozygous you get a patterned eye expression, only part of the eye color is repressed.

We'd like to understand how this type of thing happens.

And, the model for this is that the model for selective insertion is the same as the model for pairing sensitive silencing. They are DNA binding proteins which interact with the fragment, the engrailed DNA, proteins bind to that, they recognize

other proteins found in the genome, causing the transposon to be pulled to a particular region of the genome, and then you get selective insertion. For the silencing, this complex forms and silences the transgene expression.

Now, we've been dissecting this pairing sensitive silencer, and at the site visit I told about the isolation of a protein which binds to this conserved site, this sequence one, and the progress in my laboratory now is that we have candidates for DNA binding proteins which bind to this conserved site two. Both sites one and two are necessary for pairing sensitive silencing.

So, in summary, what I've found is a drosophila homolog of the mammalian transcription factor YY1 is necessary for pairing sensitive silencing. YY1 is encoded by pleiohomeotic, which is a member of the polycomb group of genes.

Pleiohomeotic is the first polycomb group protein shown to find a DNA, but we think that four additional proteins may also be involved in pairing sensitive silencing.

It's important to realize that this silencing fragment, we don't know what all the proteins are that cause this silencing, and we'd like

to be able to predict this to keep such a fragment out of transgenes.

I just want to make the point that all cloned polycomb group genes have human homologs, and I want to show one more slide which shows a model of silencing. Here I show these two chromosomes coming together, which turns off the transgene. It turns out that in particular locations in the transgene, in the genome, you can get interactions between these elements on different -- on widely separated insertion sites in the same chromosome, and it turns out that it's recently been shown by Jim Burchler's group that if you put six copies of a transgene in, that doesn't even have an obvious one of these silencing fragments, the transgenes will then come together and silence even the endogenous gene in a mechanism called co-repression, and this is mediated by the polycomb group genes.

So, I think the polycomb group, the action of these silencer proteins is very important for us to understand, to be able to predict how transgenes will be regulated in the organisms.

That's all.

CHAIR VOSE: Thank you.

Gail?

EXECUTIVE SECRETARY DAPOLITO: We just need a minute to clear the room. I think if the can vouch for everybody in the audience, and FDA, yes, and Dr. Siegel, you can vouch for everybody else as FDA on this side.

(Whereupon, the open session was concluded.)