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WORKSHOP ON EVALUATION OF IN VIVO EFFICACY OF PLATELET TRANSFUSION PRODUCTS AND PLATELET SUBSTITUTES

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Monday, September 28, 1998

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The workshop convened in Wilson Hall Building 1, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland, at 8:00 a.m., Jaroslav Vostal, M.D., Ph.D., Chairperson, presiding.

PRESENT:

KATHERINE C. ZOON, Ph.D., Director

PRESENT (Continued):

JAROSLAV VOSTAL, M.D., Ph.D., Chairperson

THOMAS REID, M.D., Ph.D., Co-chairperson

TRACI HEATH MONDORO, Ph.D., Co-chairperson

SPEAKERS PRESENT:

MORRIS BLAJCHMAN, M.D.

ARTHUR BODE, Ph.D.

JEROME CONNOR, Ph.D.

KATHRYN DAVIS, Ph.D.

JOSEPH C. FRATANTONI, M.D.

JAMES GEORGE, M.D.

LAURENCE HARKER, M.D.

STEIN HOLME, M.D.

CHITRA KRISHNAMURTI, Ph.D.

JACK LEVIN, M.D.

CONAN LI, Ph.D.

SARAH MIDDLETON, M.D.

GARY RASKOB, M.Sc.

MARJORIE READ, Ph.D.

R.P. CHANNING RODGERS, M.D.

STEPHEN ROTHWELL, Ph.D.

CHARLES SCHIFFER, M.D.

SHERRILL SLICHTER, M.D.

EDWARD SNYDER, M.D.

C-O-N-T-E-N-T-S

C-O-N-T-E-N-T-S (Continued)

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

(8:08 a.m.)

CHAIRPERSON VOSTAL: Good morning. I wonder if we could get started today.

My name is Jaro Vostal, and I'd like to welcome you here for the workshop on platelet efficacy in vivo.

And the opening remarks will be given by Dr. Kathryn Zoon, the Director of the Center for Biologics.

DR. ZOON: Thank you, Dr. Vostal.

It's a pleasure to be here this morning. When the committee asked me to come and give opening remarks, I'm always very pleased to do so. This is one of those areas that we deal with in blood and have been dealing with for many years, and while we've made progress, I think we all recognize that there's a need to make even more progress in the area of platelet transfusion therapy.

And I think this workshop actually is very important in putting, again, additional focus on this very important subject.

Now, the historical aspects of platelet regulation have been ongoing for many years, and in fact, it's close to 20 years we've been having meetings regarding platelets. Many of these meetings have been, like today, co-sponsored by NHLBI and in this case the Army, but in other cases other branches of the military, recognizing the importance of platelets in their ability to save lives.

The challenges with platelets over the years, while much progress has been made, actually focus us today in really looking at

the evaluation of the in vivo efficacy of platelet transfusion products and platelet substitutes.

In that respect, we've come a long way from 1980, when we were first talking about the evaluation of platelets for transfusion and looking at in vitro testing of platelets and their correlation with clinical efficacy.

Subsequently, back in 1986, there was an NIH consensus conference on platelet transfusion therapy, and there again we discussed safety efficacies and clinical use of platelet concentrates.

Over the past eight years, there have been three workshops, all dealing with platelets, and these have all been jointly sponsored by the FDA, the NIH, and, in particular, NHLBI, and various parts of the military, and I believe that today we are really making the progress that we need to improve the delivery of safe and effective platelet products.

Today the goals of this workshop are the following:

To define the clinical efficacy of a platelet transfusion;

To review the current methodology for measuring platelet clinical efficacy;

Discussing similarities and differences between intact platelets and platelet substitutes;

Looking at animal models that are used for measuring platelet substitute efficacy;

And discussing the design of clinical trials to establish clinical efficacy for platelets and platelet substitutes.

The information that we garner from the presentations today will assist FDA in developing standards to evaluate novel platelet products to insure their safety and clinical effectiveness.

I want to just thank the organizers and all the sponsors for helping us put on this workshop, and I wish you the very best and much success in the days to follow.

Thank you.

(Applause.)

CHAIRPERSON VOSTAL: Thank you, Dr. Zoon.

Well, I think we can get started with the program. We have a pretty exciting day ahead of us.

What we would like to do is start off with the clinical section, and that will be chaired by myself. That will be followed by a section on animal models, and that'll be chaired by Dr. Tom Reid from the Army Institute of Research, and that will be followed by the manufacturers' perspective, a section chaired by Dr. Traci Mondoro from the Center for Biologics.

And then we would like to have a panel discussion, and I'll try to keep a lid on that.

First of all, I would also like to thank all of the speakers that took the time and effort to come over here and make this meeting possible.

To get started, we're going to start with Dr. Charles Schiffer from the Wayne State University School of Medicine. He's going to talk about the clinical use or clinical assessment of platelet transfusions.

DR. SCHIFFER: Thank you very much. Thank you.

If find this to be a rather difficult talk because when I chaired the FDA Advisory Committee on Oncology Drugs, I found that the FDA staff had this incredible ability to ask these very, very simple "why is the sky blue" type questions, which as a committee we always found extremely difficult to answer, and in essence, what I'm being asked to do is justify or explain why I've given 20 to 25,000 platelet transfusions over the last 20-some odd years, when I'm going to tell you, if I ever get my slides, that bleeding is a very, very uncommon occurrence.

I would thank the organizers for allowing me to come back to this part of the country. I'm now living in Detroit, working at the Karmanos Cancer Institute, but I had the opportunity to turn the tables and actually stay at my son's house in Baltimore last night instead of the opposite, the way it's been for so many years.

Since this is a federal meeting, I've got to promise to tell the whole truth and nothing but the truth.

(Laughter.)

DR. SCHIFFER: But as I indicated, this is, I think, a very difficult task, and I thought I'd try to pose some very simple questions.

Why do we give platelet transfusion? Well, obviously if someone is bleeding and thrombocytopenic or platelets that don't work, we're trying to stop bleeding. The fact of the matter is that that's a rather uncommon indication for platelet transfusion, and the overwhelming majority of platelets are given in our belief that we are

going to prevent bleeding which might have occurred if we didn't give the platelet transfusion.

So what kind of bleeding do we want to prevent? Do we want to prevent all bleeding, minor bleeding, major bleeding? And there are all sorts of categories that people define this.

Clearly, we want to prevent death from bleeding, and often I think what we're doing is what's shown on the bottom line.

Notice that what's not on this slide are count increments, corrected or otherwise, bleeding time, corrected or otherwise. What you want to do as a clinician is something along this slide, and then if you follow logic to its extreme, what you're trying to do is design clinical trials administering hemostatic products that would address these questions, and the question is: is that possible?

There are all sorts of different types of bleeding. Skin bleeding like this is ugly, but never killed anybody and goes away.

Retinal bleeding like this is really ugly and you'd rather that it didn't happen, but it's actually mostly a consequence of severe anemia, and we did a study many, many years ago in which we showed fundi like this that looked like pizza pies in almost all newly diagnosed patients with leukemia, none of whom suffered visual sequelae.

I don't have a slide of a central nervous system bleed, but when you get right down to it, it's the fear of that event which prompts most platelet transfusions, and, again, that's an extremely uncommon event.

The good news is that clinically severe bleeding is very uncommon, and even though we all write papers that it happens more often, and I think it does, with fever, sepsis, and coagulopathy, I think it really only begins to increase appreciably in patients with severe coagulopathy and anatomic defects from which they might bleed.

Death from hemorrhage is very, very rare, and I'll show you data about that, and it's difficult to quantity hemorrhage when it occurs. As a consequence, I think, and these are data from the TRAP study, and if you just look at this slide, in this study of more than 500, close to 600 patients, the rate of hemorrhagic death was one percent, and at that time the study included some patients with progranulogytic leukemia, and that's probably in whom these deaths occurred or in severely alloimmunized patients who were failing to enter remission.

So preventing death is almost impossible to demonstrate at least in a clinical trial aspect. We didn't, I don't recall, quantitate Grade 3 and 4 hemorrhage, but Grade 3 and 4 hemorrhage is pretty uncommon also with liberal use of platelet transfusion.

Now, this is the classic straight line from Drs.Harker and Slichter. Why don't people bleed? You'd think they'd bleed to death because below counts of 100,000, your bleeding time prolongs. Below 50,000, I've never been able to get it quite on the line like this, but we used to stop at 30 minutes because it got boring. They just kept bleeding.

The bleeding time for most of the men and maybe some of the women in the room is close to 20 minutes because we're taking

aspirin because we want to live forever, and yet we're not walking around concerned that we're going to have spontaneous central nervous system hemorrhage.

Dr. Levin is going to tell us what's wrong with this in terms of its clinical predictability, but I think the miracle actually is that people don't bleed, despite the fact that they have prolonged, sustained platelet counts with indefinite bleeding times rather than that they bleed all the time.

Now, in the TRAP study, we gave platelets prophylactically at counts of about 20,000, and if we had a hemorrhagic death rate of less than one percent, why not just do it?

Well, in addition to the expense, what you're attempting to do is reduce hazards of platelet transfusion, a partial list of which is shown here. I think the one that's at the top of the slide is probably the one that's the least appreciated by clinicians because it's nonfatal and it just represents a bother in the afternoon when you've got to go see someone who has this reaction.

This scares the hell out of patients. Once they have one, they're very frightened about their subsequent platelet transfusion. It often results in hospitalization. It's by far the most common, if you will, side effect of platelet transfusion. It's clearly related to the number you receive, and any reduction or elimination of transfusions would reduce that.

And the others I'm not going to go into. Bacteremia occurs, but it's rare. Circulatory congestion is an underestimated problem, but these patients are often receiving seven or eight liters

of fluid. Fewer platelet transfusions reduce problems associated with that.

What's not on this slide, which is clearly a side effect of platelet transfusion, is alloimmunization, but that is not dose related, and reducing the number of transfusions is not going to reduce the incidence of that problem.

But going back to the goals slide, can you design clinical trials to address these clinically relevant goals? I think prevention of all bleeding is impossible. I think prevention of minor cutaneous bleeding is impossible. Major bleeding and death from bleeding, this would be statistically impossible. If you combine them, it's probably still impossible, but I put "formidable" there, and obviously this can't be quantified.

And because of this, it's been recognized for the last 20 years that you don't do clinical trials in platelet products addressing the most important clinical endpoints. You can't.

Now, I think it's relatively easy if we accept history to have criteria for acceptance of platelet products that sort of look like this, in which all of the cells have a relatively normal morphology. These are fresh blood separated PRP in which you can sort of compare, as I'll show you in a few minutes, with what could be considered perhaps to be a standard platelet product.

I believe that the purpose of the conference today is these were frozen platelets from my youth, but some of these look good. Some of them look like total junk, and we're going to hear about people who are transfusing stuff that looks like total junk with

the hypothesis that somehow this contributes to hemostasis, and that's the real problem, that is, the traditional measurements of increments and things are not suitable when you're transfusing membranes or lyophilized or whatever, that will not produce a platelet increment. I think that's why we're all here today. I'm not sure what the answer is.

We knew some of these answers. I don't think we know exactly in given individuals and under particular clinical circumstances what platelet count is necessary to maintain hemostasis, and in particular, what level provides a buffer against important and sometimes fatal clinical events.

This, I think, is an important question for this conference. That is, do all of the platelets have to be viable and functional, platelet membranes lyophilized, et cetera?

And if you give this stuff, are the results possibly going to be different in severely thrombocytopenic patients, that is, patients who have essentially no endogenous circulating platelets, or patients who have circulating platelets albeit at a lower level when you have the potential interaction between something that can have some hemostatic effectiveness and some residual normal platelets.

Those may be different clinical circumstances and hence different, if you will, margins of safety should these products have some suggestion of efficacy.

Now, I was going to put up the one from the United States, but there was a more recent one from the U.K., and we all know that our British brethren are better at use of language, but a remarkable

thing about prophylactic platelet transfusion is how people still equivocate, and I put in italics the equivocation.

There was a general agreement that a platelet threshold of 10,000 is as safe as higher levels for treating most patients without additional risk factors. These risk factors including sepsis, concurrent use of antibiotics which everyone's on, and other abnormalities are indications for a, quote, higher threshold. Higher threshold numbers are also needed to cover basic procedures.

A lot of equivocation, a lot of gray, presumably appropriate. We've clearly pushed the number down from 20,000 to 10,000. It can be lower, and clearly in patients who have non-clinically active thrombocytopenia aplastics and myelodysplastics, it can be lower.

But the point is even in this recent consensus conference, which summarized really the results of three or four randomized trials of platelet transfusion, there's still a lot of gray in the recommendations that are being made.

Now, what's been used through the years are surrogates because of the difficulties in performing clinical trials using the relevant endpoints, and the surrogates have been count increments, which obviously represents platelet viability with perhaps the best in vitro correlate being platelet morphology; functional being bleeding time because of the difficulties in finding enough patients who have quantifiable bleeding, the one or two patients per year with gross hematuria whom you're going to see, and there are a variety of in

vitro things that one can correct, and Mo is going to tell us about rabbit ears and someone else about baboons.

But the bottom line is once you screen products in rabbits, you still have to do people ears, and this, I think, will serve as an interesting way perhaps of screening platelet products, but not proof of clinical efficacy in people.

Now, in terms of increments, Dr. Davis is going to tell us what's wrong with this, but I think the principle is that since you are going to be giving different size transfusions to people of different size, you need some ways of standardizing your results unless you plan to administer hundreds of platelets and assume that everything is going to even out, and Kathryn will explain this to you, but this is as good or bad as anything else, but you do need some comparative to standardize here the results.

And this is what we've used, in fact, for years for non-licensed products and non-licensed techniques, and that is post transfusion increments. We assume that people who were alloimmunized and received random donors and didn't get an increment, that these people benefitted from these transfusions.

I'm convinced that they did, but I'll be damned or pressed to prove it to you in a way that mouse scientists would believe.

Now, how do you evaluate platelet products? Do you compare your new product, and I'm talking about viable platelets; do you compare it with turkeys or do you compare it with perhaps the best product? And this isn't really a turkey.

But in whom? Do you do survival in normals, or do you do what I think you eventually have to do, is something in patients?

What kind of study design? And we'll be hearing something further about this, that is, paired observations in the same patient, which gives you some savings potentially in terms of patient number, or do you just give a bazillion transfusions and do means and standard deviations?

This is a study we did a long time ago that I can't even remember for whom, and I think it was the one that resulted in approval of seven-day platelet storage, but it represents one study design in which we took patients who were clinically stable, without fever, who were not alloimmunized, screened for that.

We knew they could respond to platelet transfusion, and we expected that they had severe, prolonged thrombocytopenia because of their therapy, and they received three different types of platelet transfusions: fresh platelets, which you could do at the time, and that was before viral testing, and these were literally hot out of the body; three-day stored, which was the standard at that time for the maximal storage; and seven-day storage, and measured corrected increments all in the same patient.

And I can't remember. We should have, but we probably didn't, randomize the order of these too, and there was no difference here. There was a significant difference compared to fresh platelets, and the same results obtained for 24-hour increments.

Note that there were 12 or 16 transfusions or sequences of transfusions, and I believe at the time it was this that merited

approval of seven-day platelets, which were then taken off the market because of concern about bacteremia.

Now, is this the right study design? I don't think 15 observations certainly is. I think that's many too few, but what should the comparison be? I'm not going to answer it. I'm going to pose the question.

Should it be between the longest possible duration of storage that exists now, or should it be between a fresher product? That's perhaps a question that we can debate, but this was the decision that was made by the FDA at that time.

Another thing to remember is that the results that happened immediately after transfusion may not entirely be all that occurs with a platelet transfusion. This was a study that we did God knows how many years ago using frozen platelets in which we did crude aggregometry with only one agonist at the time. The platelets didn't aggregate.

After transfusion, an hour after transfusion, we obtained platelets from the patient. The bleeding time, which was infinite here, was 18 minutes, and the platelets aggregated somewhat.

The next day the platelet count was lower, but so was the bleeding time and the in vitro function was better, suggesting that at least with some products, there could actually be improvement in function after some period of time circulating in the host.

What does that mean in terms of platelet transfusion products? Do you assess them only here? Do you assess them there, or do you assess them at both times?

Don't hold your breath because the answer is not on this slide, and I would talk if I remembered what was on this slide.

(Laughter.)

DR. SCHIFFER: Try again. No, you don't want to do that again, do you? Well, let's see the one afterwards.

I think this slide is not going down because it's the one I wasn't sure about, whether or not I wanted to put in.

(Laughter.)

DR. SCHIFFER: It described a surgical -- yeah, that's right. We shouldn't have found it.

There have been a lot of trials done in the surgical setting, that is, in the CABG setting, of a variety of hemostatic and anti-hemostatic things, antibodies, platelets and stuff, and I don't believe this because I don't think this is a good venue, but it at least brings up the question of the fact that if you prove something, in quotes, in thrombocytopenic patients using perhaps the model that I showed you before, does that mean that the same thing is true in surgical patients and vice versa? And I don't think we know the answer to that.

A substantial fraction of the patients -- I'm sorry -- of the platelets we use or misuse occur in this setting.

If I was in this industry, which I'm not any longer, and I had a product, I'm going to modify that product. I'm going to change my technique. I'm going to try to make it better over time, and one of the things I'm not going to want to have to do is fund expensive

clinical trials, whatever clinical trial we agree upon is appropriate after this meeting.

Actually these 30-patient clinical trials are a lot less expensive than the large trials that I'm accustomed to, but nonetheless, they cost, and they're difficult to do, and I'm going to do lots of manipulations over the years, and what I would love is some surrogate which is verified at least once or twice with a clinical endpoint, which I could plug in all of my modifications so that I can test things serially so that I would have an expeditious, but efficient way of deciding what product that I want to or modification that I want to test in clinical trials.

And we're going to hear again about the next -- I'm not that old, but this must be the fourth generation of in vitro bleeding time machines I've heard about, and I hope that this one might be the right one because I think that, as I say, we need a screen for continued modifications of platelet products and eventually hopefully even a credible endpoint for clinical results for transfusions because of all the difficulties that we've had in defining that.

Well, I don't know if this was an appropriate lead-off talk or not. I've tried to leave more questions than answers because I don't think there are very good answers to these questions, and at the end of the day we're obviously going to have to compromise because we're not going to be able to answer the question in the way we would do it if we were rat doctors, that is, with a clinical model of bleeding, and we're going to have to arrive at some sort of acceptable surrogate.

Thanks for your attention.

(Applause.)

CHAIRPERSON VOSTAL: Thank you, Dr. Schiffer.

In the interest of time, we're going to try to hold off questions till after the first three speakers.

So the next talk will be given by Dr. Sherrill Slichter, and it's entitled "The Research Methods for Assessment of In Vivo Platelet Efficacy.

DR. SLICHTER: Okay. Well, I'm going to try and maybe take off where Charlie left off. What I'm going to discuss is an algorithm, if you will, for evaluating platelets in vivo using research methods, and I'm going to start with what we consider to be easier kinds of model systems and then go into those which are, in fact, more difficult.

So to start off with, we usually use paired autologous radiolabeled platelet recovery and survival measurements in normal volunteers, and I'll show you why paired infusions are important in these studies. You can do either concurrent labeling with indium and chromium or do sequential transfusions with the same isotope.

Now, here's normal recovery, around 60 percent. So this looks at increment corrected for blood volume and the number of platelets transfused, and then we look at the survival of the cells in circulation being somewhere between eight and ten days, and one of the things that we've looked at is something we've called total platelet viability, which is just multiplying recovery times the survival and dividing by two.

So basically you get the area under the curve, and so this allows you to have a sense of the total product and make comparisons with other products.

Now, this is a slide in which we did two studies in patients. We were interested in comparing the results of the different aphoresis machines. So each normal individual donated on two occasions with a two-week interval between the transfusions, and I've ranked here the results of these studies, which were five-day stored platelets based on total platelet viability, and shown down here the results I just showed you from the previous slide with a 54 percent recovery and 8.1-day survival.

And what I want to point out to you here in these studies is, for example, here's TM and TM. So two different machines. This study was the only study in which there was a decrease in post storage pH of less than six, but even so, this individual's platelets really store poorly.

Here's JS in sequence. Here's GG, who had platelets that stored the best.

We don't have any idea why there is this really relatively large heterogeneity in how well individual donors' platelets respond to storage conditions, but I think it's important that we recognize this because what it means to me is that if you want to evaluate Product A compared to Product B, the best experimental design is to do paired infusion studies using the same normal volunteer to look at the two products under evaluation, and there are several ways you can do this.

For example, this is a platelet aphoresis storage study in which one bag was stored for three days and labeled with one isotope. One bag was stored for five days and labeled with an alternate isotope.

It's important that the isotopes be varied in terms of whether they're used for the three-day or five-day storage, and with this experimental design, we clearly showed a statistically significant decrease in recovery and survival of the five-day compared to the three-day storage.

Another experimental design is this study in which we were looking at five-day storage of either platelet concentrates or aphoresis platelets, and in this study we simply drew a unit of whole blood from the donor. We spun down to get platelet rich plasma, reinfused their red cells, and then put them on an aphoresis machine.

And so here in the same individual at the same time, we had a platelet concentrate and an aphoresis product, stored them both for five days, labeled one with one isotope, one with the other, and basically showed no difference in the two products given to the same normal individual.

So there's a lot of ways that you can vary this. In the first study that I showed you, we just did sequential studies on two different machines, but I think the concept that it's important to use each normal individual as his own control when making comparisons between different products.

Now, the next thing I'm going to discuss is transfusions into thrombocytopenic patients, and as Charlie pointed out, this is,

after all, the gold standard. This is what we're really interested in.

And what we noticed here is that here is normal data, recovery and survival, and here is thrombocytopenic patients with platelet counts of at least less than 70,000, and what you'll note here is that although the recoveries are basically the same in thrombocytopenic patients as they are in normal individuals, there's clearly a significant decrease in the survival of platelets in circulation.

And so we were very interested in why is the survival shorter when you become thrombocytopenic, and in studies with Steve Hanson what we determined was that platelets are lost from circulation by two mechanisms.

They're either a senescent removal in the RE system, and in addition there seems to be a fixed number of platelets which are lost randomly every day conceivably in a hemostatic function.

So when Charlie was discussing providing hemostasis to patients, what we're really interested in is fulfilling this requirement, apparently platelets have to support the endothelium and to prevent blood loss through an intact vascular system.

And so when we looked at a group of 16 normal individuals whose platelet counts were in the normal range, as shown here, and another group of patients who were thrombocytopenic to various levels using either autologous radiolabeled platelets or donor platelets if there was no evidence of alloimmunization, it was determined that the maximum platelet lifespan was about 10.3 days, and that there was a

random loss of platelets of about 7,000 platelets per microliter per day, and that at platelet counts of less than 100,000, there is a direct relationship between platelet count and platelet survival.

And the reason for that is shown on this slide: that as your platelet count decreases, this random loss of platelets represents an ever increasing percentage of your circulating platelet and directly reduces your platelet survival.

So it's important to remember that although the increment will be -- should be in the normal range in thrombocytopenic patients, the survival will depend on their circulating platelet count.

And evidence for that is really shown in this slide, which is a study looking at the results of different doses of platelets, and what you see here is that the recovery is basically the same regardless of the dose, exactly what we would have predicted. The increment obviously goes up as you give more platelets, and also the days to next transfusion goes up, not surprising from what I've said, that the survival of the cells is dependent on the circulating platelet count.

And so there's now been some interest in trying to give more platelets so that you reduce the transfusion frequency, and this clearly is one approach.

Another approach might well be that if you calculate that you use about 7,000 platelets per microliter per day and you calculate the average individual's blood volume, you can supply that number of platelets by giving the equivalent of about one platelet concentrate a day.

So in order to be on the safe side, maybe you should give like two platelet concentrates a day. You wouldn't have to do platelet counts. You wouldn't have to worry about anything unless they had bleeding.

So I think the whole issue of dose of platelets and what's the most cost effective way to provide platelets is still open to question.

Now, once we get through the normal volunteer studies, as I said, we go into patient studies. I'm not going to say more about this. You can, as in the normal individuals, do concurrent indium and chromium labeled platelet transfusions if you're looking at two different products.

You can use sequential transfusions of radiolabeled platelets with the same isotope, or you can use unlabeled platelets because in this situation, the patient has zero or very small numbers of circulating autologous platelets so that it's relatively easy then to evaluate just by doing counts.

And certainly by doing radiolabels, you can get oftentimes a more accurate measurement, continue to follow the survival of the platelets of interest even though the clinician may have given another platelet transfusion.

We don't have any evidence that the labeling procedure per se injures the platelets with the current way that we're storing them, but there's always that question, and clearly in patients we don't give radiolabeled products routinely.

So there's some pros and cons for using radiolabeled versus unlabeled platelets in thrombocytopenic patients to evaluate transfused products.

Now, the next issue is that we are not only interested in the number of circulating platelets, but also in their function because platelet hemostasis represents a combination of both number and function, and so function measurements in thrombocytopenic patients have generally been evaluated by doing bleeding time versus platelet count measurements performed pre and serially post transfusion.

And I concur with Charlie's statement that he has found some evidence with some platelet products that they don't function normally immediately post transfusion, but there is some in vivo repair. So that's why if we don't get the expected relationship between bleeding time and platelet count at one hour, we usually do a four-hour post infusion to see if there's been any improvement and then look at least at the next morning at platelet count/bleeding time relationship to determine the durability of that product in the patient's circulation and can follow a bleeding time platelet count determination actually daily until the next platelet transfusion if there's reason to be interested in making those measurements.

Now, we have used for a very long period of time a standardized IV bleeding time where you elevate the blood pressure cuff to 40 millimeters of mercury. You make a standard length and depth of incision using a Bard Parker blade attached to a part of a

surgical knife and then making a one millimeter deep, one centimeter long incision and start a stop watch.

And if you do this at platelet counts greater than 100,000, the bleeding time is four and a half, plus or minus a minute and a half, and as Charlie indicated, at platelet counts between 100,000 and 10,000, there's a direct inverse relationship between bleeding time and platelet count.

And in your handout, I gave the equation, but it's been pointed out to me this morning that the parentheses is in the wrong place. So it should be 30.5 minus the platelet count divided by a constant rather than the constant divided into 30.5 minus the platelet count. So I apologize for the error in the handout material.

At platelet counts less than about 10,000, the bleeding time becomes unmeasurable and is usually greater than 30 minutes and becomes an unreliable measurement.

And if you do platelet transfusion studies in thrombocytopenic patients, this is some very old data. These are fresh platelet transfusions. The open circles are measurements made within two and a half hours of infusion. The closed circles are measurements made beyond two and a half hours from infusion.

You can see that there's some improvement in platelet function over time in some of these measurements. In the lower part here are transfusions that are stored for either 24, the triangles, or 72 hours, the circles at room temperature, again, the open figures are bleeding times within two and a half hours of infusion. The closed circles are after that period of time.

You can see that there's some evidence of function in some of the transfusions even immediately post transfusion, but in the majority of them, they are improved over time, and that at the lower platelet counts, the bleeding time, in fact, becomes unmeasurable.

So I think you're going to hear from Dr. Levin that the bleeding time does not predict post surgical bleeding, but we have had consistent experience that it does, in fact, show a correlation between bleeding time and platelet count when you're talking about thrombocytopenic patients in transfusions of platelet products.

Now, the last way to assess platelet function that I'm going to spend a little bit of time on is 51 radiochromium labeled daily stool blood loss measurements, and what's done here is that a sample of blood is obtained from the patient. The red cells are isolated, radiochromium labeled, reinfused into the patient.

Daily blood samples are then taken from the patient to determine what the level of circulating radioactivity is, and then all of the patient's stools are collected on a daily basis, and knowing the amount in the blood and the amount of activity in the stools, one can actually make a quantitative measure of the amount of blood loss in the stool.

And we have used this as a way to ask and answer the question: how much spontaneous bleeding is there through an intact vascular system, and how does that vary depending on the product that's being transfused?

And so these are studies that were done in a group of thrombocytopenic patients who were not being transfused at varying

platelet counts, starting from the lowest to the highest. As you can see, in these patients who had very low platelet counts, the bleeding time was generally unmeasurable at greater than 30 minutes, so would not allow you to assess hemostatic efficacy at these very low counts, which is often exactly where we're interested in measuring hemostatic efficacy.

If you then look at the patients who have platelet counts of less than 5,000, the bleeding time is generally increased somewhere around 50 plus or minus 20 mls per day, and this is the observation period here.

Once you get between about ten and 20,000, the bleeding time is about nine minutes, plus or minus seven, and at platelet count -- I'm sorry -- between five and ten it's about nine minutes, plus or minus seven, and at platelet counts above ten, it's basically within the normal range, which is less than 5 mls per day.

So this, in fact, on the face of it looks like a way where one can make measurements at very low platelet counts and conceivably see differences between bleeding risk based on the patient's circulating platelet count and thereby some measure of the hemostatic efficacy of those platelets in circulation.

And this is just the data from the prior table plotted and clearly shows at levels above 10,000, no evidence of an effect on stool blood loss; some variability here; and then clearly a substantial increase in bleeding risk at platelet counts less than 5,000.

This then looks at some of these patients in different situations. So here is baseline measurement in a patient who had about 8,000 platelets, the same platelet count for all of these observations. The patient was put on prednisone because there was some evidence that you improve vascular integrity on prednisone.

In fact, the bleeding may have increased on prednisone, and then adding a semi-synthetic penicillin, even more increase, and of interest to us, that when platelet transfusions were given, but they were still on this same therapeutic regimen, platelet count increased to 66,000 with the platelet transfusions. The stool blood loss then falls into the expected range for this platelet count, so suggesting, in fact, that this stool blood loss measurement does reflect the changes in platelet count and, therefore, improvement in hemostasis in spite of continuing to receive the medication.

Here's a patient with close to 17,000 platelets, a large amount of stool blood loss on prednisone. Here's one that goes up on cloxacillin, and one goes up on prednisone to prednisone and ampicillin.

And so if you plot this data on the prior slide, this is then the prednisone data. This is the semi-synthetic penicillin data, again suggesting that this measurement is able to show adverse consequences of things that are done to the patient that affect their hemostatic situation.

Now, this is a study by Gaydos, published in 1962, and this is major hemorrhage. So GI, GU, the kinds of things that we are, in fact, worried about.

This is a study which probably was the reason why 20,000 was chosen as the prophylactic platelet transfusion trigger, but if you read the original article, the authors say that you can't define a trigger level between five and 100,000, and in fact, their data suggest that the risk is at less than 5,000 the same as would be predicted by the stool blood loss data.

Now, we are recently in the process of doing a platelet transfusion trigger trial using stool blood loss as a measure of efficacy. This is the eligibility criteria. Any patient, no matter what their disease process, who's expected to have a less than 5,000 count for at least five days so that they can be randomized between the three arms of the study, which was five, ten, and 20.

Excluded were patients who were on other or had either anticoagulant or antithrombotic treatment or evidence of DIC or some other plasma coagulation abnormality besides a low platelet count, and we also excluded APML patients because of their increased bleeding problems and also CML patients because they tend to have a big spleen.

And I'm just going to show you some preliminary data from this study. This is the trigger level, five, ten or 20. These patients were transfused. When they were transfused with six units of pooled random donor platelets that had been stored for four to five days, so that we were trying to give the potentially poorest quality product that we had available, we made clinical observations about whether other things were happening, but did not allow the clinician to transfuse at other than their trigger level for reasons such as fever, rapid drop in platelet count which other studies have used as

an indication to change the transfusion trigger, which I think a lot of those things are not based on data to suggest the trigger should be changed.

What you see here is that the red cell transfusions are probably not going to be different between these arms, and when you look at red cell transfusions per day, less than 20,000, these are not probably going to be statistically significant differences, although I would emphasize to you that we're not finished with the study, and so the statistics have not been done.

Stool blood loss averages about between nine and 12. Broad ranges in all groups so that there were some bleeders in the groups, but we interestingly tried to radiolabel their red cells as soon as we could once they agreed to participate in the study, and so most of these patients who had high stool blood loss had high stool blood loss even with counts substantially above their trigger, suggesting that they may have had a GI lesion.

This table shows those patients who received less than two transfusions above their trigger. There were three patients in this arm and two patients in this arm who received more than two transfusions above their trigger.

One of the problems with this study is that we couldn't blind it because all of the docs simply had to call the lab in the morning and look at the platelet count. So you'll notice that nobody dropped out of this arm as being transfused above their trigger. That's because our clinicians are comfortable transfusing at 20,000, and clearly this was a different ball of wax for them.

These are the reasons why the five patients were excluded. The first patient in the 5,000 arm had a pericardial window. I think transfusing at higher levels is important for that.

This one, Charlie talked about nervous physician. This is basically what we had here.

This patient had LPs and vaginal bleeding, probably a reason to transfuse at a higher level.

This one had massive hematuria.

This one had hematuria GI bleeding and central line, but some of the patients in the 20,000 arm had similar kinds of abnormalities, but were not transfused at higher levels.

This is now the same data, but now looking at platelet transfusions, days at platelet count less than 20,000 basically the same between the arms. Here's platelet transfusions per day, CCIs, and when you get down here to the people who were really transfused at their trigger level, they used about half as many platelet transfusions per thrombocytopenia day in the five and 10,000 arm; no difference in CCI.

And I plotted a couple of examples of patients with the same disease at the different trigger levels. The dashed line here is the trigger level for that particular arm, and the number of days with platelet count less than 20,000 and the number of transfusions was six.

Here basically the same number of days of thrombocytopenia, substantially fewer transfusions. Here the patient had only one platelet transfusion.

Here's patients with AML, ten days, five transfusions. This is plotted on a different time scale here, 23 days, about twice as many days, the same number of transfusions, and here's an AML patient who got two platelet transfusions.

And if you blow up that data, it's of interest because one of the things that we found out is that this drop in platelet count is clearly often very gradual, and I would submit, as probably most of the people in the room would concur, that to have 16 days of thrombocytopenic in an AML patient and get only two platelet transfusions is, in fact, an event worth describing.

And then this is just data from three recent prospective randomized studies looking at 10,000 trigger versus 20,000 trigger. Major bleeding was the same, and the studies reported hemorrhagic death of very low numbers, no difference, but clearly a difference in terms of not red cell transfusions required.

Can you -- could somebody focus this slide, please? Could you focus the slide? Thanks.

But there was a difference in terms of platelet transfusions and substantial cost savings at the lower transfusion trigger level.

So I think the stool blood loss measurements may, in fact, allow us to make hemostatic observations. In some of the preparations that we are talking about today, things in which we can't measure platelet counts, we may be able to measure bleeding times, but stool blood loss, in fact, may represent a way which will allow us to look at a variety of preparations which we are not able to make our usual

kinds of either in vitro or in vivo measurements at least in terms of looking at changes in platelet count, but may allow us a way to determine hemostatic efficacy.

Thank you.

(Applause.)

CHAIRPERSON VOSTAL: Thank you, Dr. Slichter.

The next speaker will be Dr. Rick Rodgers, and his title is "Analyzing the Clinical Performance of the Bleeding Time: A Technical Prelude."

DR. RODGERS: Good morning. This will take just a moment to set up the overhead projector. Can everyone hear me?

Good morning, Jack.

These are a few brief moments here to make what I call a technical prelude to the talk that follows. I'm going to be talking about the methodology that was used in a meta-analytical review that was published in seminars of thrombosis nemostasis about nine years ago now by Jack Levin and myself, and he'll cover the actual clinical findings.

Now, in looking at any sort of clinical test, there are at least three important questions you can ask of results, and I'm going to be talking about methodologies that are rather general in nature, but I'm going to make a few simplifying assumptions to simplify the presentation.

First of all, let's assume that we have some predefined population of subjects. Let's assume, for example, they could be uremic subjects, and we're going to further categorize or subdivide

this population into two subpopulations: bleeders and non-bleeders, and we're going to measure bleeding time.

Now, bleeding time, of course, is a test that occurs on a continuous scale. We're going to dichotomize the results by creating some sort of a threshold or cutoff point. Bleeding times above this cutoff are going to be declared, quote, abnormal, and bleeding times below that cutoff are going to be declared, quote, normal.

Now, one question that we can ask in a study population like this is: what are the means of these two subpopulations, bleeders and non-bleeders?

Let's assume, for example, we have one population here. This might be the non-bleeders, and it's a slightly skewed Guassian-like distribution, but, in fact, again, the methods I'm talking about make no assumptions about the underlying distributions.

Let's assume we have a second population of bleeders, and the bleeding time is increasing as we go right along the scale. So you can see the mean bleeding time is higher in the bleeders in this hypothetical case, and it's lower in the non-bleeders.

Now, one question we can ask is: what are the means of these populations and are they distinguishable?

And the point is that these means can be arbitrarily close, but we can sort of overcome their closeness by just increasing the sample size for these two subpopulations. So that if you want to establish a statistically significant difference between means, just increasing N allows you to do that for almost arbitrarily close values of those means.

Now, a mean, of course, is a statement about a population, a group of individuals, and this does not tell us anything about the ability of this test, the underlying test we're looking at, to classify individuals as a statement about a population.

Let's look then at a situation where we have, again, these same two distributions, and now we're going to ask a second type of question. Given that we know we've identified these populations, how does the test perform? So that we know about the underlying populations, and we want to study the test. We're studying the test now, not the subjects.

I put a decision limit at an arbitrarily high level here, and we're going to actually plot two values: sensitivity and specificity. These are test parameters that are of great importance. What are they?

If I just put my pen down here, and that's the cutoff here, this is the bleeding population. The sensitivity of the test is the fraction under that curve, the fraction of the known bleeders who haven't had normal bleeding time tests. Ideally we'd like that to be 100 percent, right? If we put the cutoff here, then, in fact, it would be 100 percent because all of the bleeders would be categorized as having this abnormal test. That's sensitivity. It's also called the true positive fraction.

There's another parameter, specificity, which refers to the non-bleeding population, and the question there is: what is the fraction of non-bleeders with a normal test result?

Now, again, if I put the cutoff here, the value is 100 percent because everyone here is categorized as having normal bleeding time. If, however, I put the cutoff here, you can see that maybe half; the value is going to be roughly half.

So if we take the other side of this curve, this part of the curve, this is a false positive fraction, and I'm going to talk about true positive fraction and the false positive fraction now.

False positive fraction is nothing more than one minus the specificity. Now, it turns out that we can make a plot, something called a receiver operating characteristics plot, and we create that by essentially sliding the decision limit from right to left over these two distributions and plotting the false positive versus the true positive rate.

And for the cutoff up here, we have 100 percent specificity, that is, zero false positives and zero true positives. So there's a plot on this, this heavy dot in the lower left-hand corner.

If, however, now we start sliding that decision limit to the left, we're starting to pick up some true positives now, and you see that we can plot another point on this curve for this cutoff. It's right up here, just going straight up the left axis because so far we still have zero false positives, and we're starting to pick up some true positives.

We can continue sliding that cutoff limit. Now we're in between these two cleanly separated distributions, and you can see we

have a 100 percent true positive rate and zero false positive rate, perfect separation of these two populations.

This obviously is an ideal point. This is where we would like all tests in the world to operate. Sadly it rarely happens, ever happens.

Now, if we continue sliding the decision limit further to the left, we start picking up false positives. So now the curve starts going off, veering off on the upper axis of the plot.

And finally I slide the decision limit all the way to the left, and we've got 100 percent true positives, but we've also got 100 percent false positives. We're classifying everyone as having abnormal tests.

Well, this ROC is a very concise and powerful way of characterizing the performance of a test, but again, it doesn't tell us anything about individuals.

Now, let's look at a slightly more realistic situation. Now the distributions overlap, and sadly, this is the way things happen in the real world. You just never find tests in clinical practice that are perfectly separated, where you can perfectly categorize populations, individuals within populations.

Let's create an ROC of this situation, and again, I'm going to just slide this decision limit from the right here to the left, and we're going to plot an ROC. Again, we start out here in the lower left-hand corner, and as we slide our decision limit to the left, we start climbing up the left axis again.

Now we're here in the middle where we're starting to -- we haven't picked up all of the true positives yet, and we're starting to pick up false positives. So you can see we deviate now from the axis.

We continue sliding it to the left. Again, the frequency of both true and false positives is increasing, and finally all the way to the right.

So you can see now this difference between the ideal test in the sense that in the ideal test we just shot straight up here, the ideal operating point, and then straight over here. Real tests follow these curvolinear relationships.

Now, let's look at a situation where both of the distributions are perfectly overlapping so that you really can't distinguish the two populations. The ROC, as you can see, again, if we're looking at false and true positive fractions, they're going to be equivalent at all times. So as we slide the decision limit from right to left, the ROC simply is a straight line going from the lower left to the upper right-hand corner of the plot.

So in summary, an ROC looks like this. Non-informative test, straight line; perfect test zooming up here to the optimal operating point where you have perfect separation, perfect classification of individuals; and most real tests follow some kind of intermediate curve.

The area under this curve is often used as an index of how the test performs, but again, now, everything I've said thus far is studying the test, not studying individuals.

So the third sort of question that we can ask about a test is can it be used to classify an individual now. Everything I've said prior to this moment we've known what the populations were. They were pre-defined populations. We knew what their condition was, and we're looking at the way the test behaved.

Now, let's assume we have an individual that comes in. We do the test, and we try to guess. Is this person from a bleeding population or a non-bleeding population?

So now we're studying the subject rather than the test. In that situation, we use different variables, different factors. We look at the true and false predictive values of the test.

Now, the positive predictive value of a test is the fraction of subjects with the disorder of interest that actually have an abnormal test. Oh, I'm sorry. The fraction of people who have normal tests who actually have the condition of interest.

So, for example, if the positive predictive value were 50 percent, half of the people with an abnormal bleeding time would bleed.

The negative predictive value is the fraction of individuals with normal tests who did not have the condition of interest.

Now, the sensitivity and specificity that we looked at on these curves enter into the computation of positive and negative predictive value, but not by themselves.

The other factor that enters into the computation is prevalence of the disorder, the a priori probability that a given

individual drawn at random from the study population is in the abnormal population.

So, for example, in uremic bleeders, if in a particular population of uremic bleeders that you're examining 30 percent of the people go on to bleed, the prevalence of bleeding in that group is 30 percent.

And it turns out that tests with even extraordinarily high sensitivity and specificity for low prevalence are not going to perform that well. Also, the predictive value goes down as the prevalence goes down.

Why is that? Let's go back and look at our populations, our overlapping populations, again. Well, here I've drawn the areas under these curves as if they're equal. So this represents a study population in which the prevalence of bleeding is 50 percent. Both the size of these curves, the area under these curves is equal.

If you take the fraction of the total area, this is a fraction of the total area. It's 50 percent.

Suppose now it were only ten percent, and we draw basically the same sort of curve. Lower. I think you can begin to appreciate now that the relative importance of false positives becomes much higher in this setting than in the setting of the high prevalence.

So as the prevalence drops, the positive predictive value drops as well.

Well, I had some additional remarks to make concerning the linearity of the relationship between bleeding time and platelet

count, but I think I'm going to defer those and pass the baton to Jack Levin, and we can bring those up later if he thinks that's appropriate.

Is it an appropriate moment to stop for questions here or do you want to just --

CHAIRPERSON VOSTAL: After Jack Levin.

DR. RODGERS: Okay, all right. So, we'll have questions after Jack Levin.

(Applause.)

CHAIRPERSON VOSTAL: Thank you, Dr. Rodgers.

Okay. The next speaker is going to be Dr. Jack Levin, and this title is "Skin Bleeding Time as a Measurement of Platelet Efficacy."

DR. LEVIN: I realize that my topic today is potentially controversial, but nevertheless, I hope I will convince you that the bleeding time is not useful for the prediction of bleeding.

I want to emphasize at the outset that my presentation, the work that Dr. Rodgers and I have done, does not deal with the use of the bleeding time as a tool to diagnose certain hemorrhagic disorders, that is, the use of a bleeding time as a diagnostic test, nor am I questioning the observations by many in this room that various preparations of platelets and platelet substitutes shorten a prolonged bleeding time.

However, data supporting the increasing application of the bleeding time to predict bleeding are lacking. A major basis for this belief that the bleeding time can be used successfully in this manner

results from the fact that for many clinical conditions, for example, uremia, the mean bleeding time found in the affected population is different from normals, as Dr. Rodgers has just explained.

However, differences in populations as demonstrated by epidemiologic studies cannot necessarily be translated into an ability to predict whether bleeding will occur in an individual, as you've just heard, who happens to have a long bleeding time because of the typical overlap between the normal and the population.

Now, a review of the literature by Dr. Rodgers and myself, based on 862 publications that discuss the bleeding time and included 664 papers that actually had bleeding time data, allowed us to apply receiver operating characteristic analysis and to indicate that several major assumptions concerning the bleeding time are not supported by currently available data.

And our review and analysis of data have led to the following conclusions, and we can leave the lights on for my slides.

Firstly -- you can laugh when I'm done, not when I start -- firstly, that bleeding from a cut in the skin does not necessarily reflect risk of bleeding elsewhere, which I will get to; that the level of risk associated with a given bleeding time is not independent of the cause of the abnormality. There's no evidence that the bleeding time is a predictor of risk of hemorrhage in individuals and no evidence that the bleeding time is a useful indicator of the efficacy of therapy.

Now, O'Loughlan performed skin and gastric bleeding times in two groups of subjects that either had been receiving aspirin or

one day, Group 2, or for multiple days, Group 3, and although aspirin produced a significant prolongation of the skin bleeding time, you'll see that the gastric bleeding time, which was defined as bleeding after a gastric biopsy, was not altered whatsoever.

Ewe compared arm and thigh bleeding times in uremics, and although all of the uremics, 16 patients, had the classical bleeding times of greater than 20 minutes, only five had bleeding times of greater than ten minutes when it was performed on the thigh, and only eight of the 16 had thigh bleeding times of greater than eight minutes, despite the shorter control level of the thigh, and I'll get back to this later.

Now, in addition, the bleeding time is not a specific indicator of platelet function because it is affected by local tissue factors and components of the blood coagulation mechanism.

For example, abnormal bleeding time have been described in a wide range of coagulation disorders not felt to be platelet related. For example, prolonged bleeding times have been reported in patients with deficiencies of Factor V, VII, VIII, IX, X, XI, and XII.

One also has to take into account the disorder in which the bleeding time is associated. For example, the clinical significance of a prolonged bleeding time in someone with a collagen disease is almost certainly less significant clinically than in a patient with uremia.

And the point I want to make is that there's no basis for assuming that the level of risk associated with a given bleeding time is independent of the cause of the abnormality of the test.

Now, a particularly excellent example of significant alteration of the bleeding time in the absence of the necessary effect on intrinsic platelet function is provided by the effect of red blood cell mass upon the bleeding time.

It has been clearly documented that correction of the hematocrit, whether by transfusion or administration of erythropoietin, remarks a marked shortening or even total correction of the bleeding time in patients with uremia. This study by Livio demonstrated that the marked increase in proportion of abnormal bleeding times in patients with uremia when their hematocrit was less than 30, in contrast to the pattern of abnormal bleeding times in uremics when their hematocrit was greater than 30.

This slide is from a paper by Hernandez which describes the inverse correlation between bleeding time and hematocrit in patients with uremia. Most impressively is the effect of transfusion on the bleeding time of uremics, and as you can see, when transfusion raised the hematocrit to 30 percent or greater, the bleeding time was markedly shortened or even normalized, whereas, in contrast, when the hematocrit was not elevated to 30 percent, there was no effect on the bleeding time.

Now, this strongly suggests that rheological factors well known to this audience play a major role in the prolonged bleeding time seen in uremics, and therefore, anemia, rather than an intrinsic abnormality of platelet function, may be primarily responsible for the prolonged bleeding time seen in patients with uremia, as well in other disorders.

And of course, as is often the case, Duke of the Duke bleeding time recognized and clearly described this phenomenon in 1910. However, as this editorial from Lancet indicates, although the effect of hematocrit on the bleeding time was described, it was and has generally been ignored.

Now, this inverse relationship between the hematocrit and the bleeding time is also present in non-uremics, and pertinently, Dr. Blajchman has reported an identical relationship in normal rabbits.

There is essentially no evidence that the bleeding time is a predictor of the risk of hemorrhage. As already stated, there are many mechanisms that can produce an increased bleeding time, and therefore, the test needs to be interpreted differently in each instance.

These are data from my laboratory which demonstrate a complete lack of correlation between bleeding time and chest tube output during the 24 hour period following coronary artery bypass surgery.

These findings have been confirmed by an excellent study by De Caterina in Blood, who studied 40 patients undergoing elective CABG surgery who did not have any history of bleeding and had not received any nonsteroidal anti-inflammatory drugs, and there's no statistically significant relationship between chest tube drainage and the bleeding time in these patients or, perhaps more importantly, between the bleeding time in transfusion, which of course is really the critical issue.

The authors concluded, "We do not recommend the use of the bleeding time test in this setting to predict perioperative or postoperative bleeding," and her conclusions have been strongly supported by a recent position paper published by the College of American Pathologists and the American Society of Clinical Pathologists.

Now, analysis of a study of 1,000 consecutive renal biopsies reported from the Mayo clinic by Diaz-Buxo in which two percent of the patients developed a perirenal hematoma, which was the only complication described, reveal that the positive predictive value of an abnormal bleeding time was, at most, four percent.

Therefore, the bleeding time did not provide clinically significant information about the risk of a hemorrhagic complication in this patient population, and others, of course, have also recognized the lack of a predictive value of the bleeding time, this from the surgical literature reflecting the surprise of the surgeon that despite thrombocytopenia and a prolonged bleeding time, surprisingly no patient had any bleeding tendency.

Now, importantly, application of receiver operating characteristic analysis to the data available in the English language failed to identify a single study in which the bleeding time was performed prior to the development of bleeding that demonstrated that the bleeding time was a useful predictor of bleeding.

Now, Dr. Rodgers has already thoroughly explained to you the characteristics of this analysis. However, I want to remind you that this ROC curve is a plot of true positives versus false positives

as the decision limit for a dichotomous test, such as the bleeding time, is altered.

Now, ROC analysis, as I will show you shortly, was applied to the following data which appeared in the papers of Simon here and Barber, and first I'll show you the raw data from these two papers.

Simon studied the effect of prophylactic administration of four units of platelets after completion of coronary pulmonary bypass in CABG surgery. The platelet transfusions generally, but not uniformly, shortened the bleeding time in those patients who received the platelets, and the mean bleeding time before and after is shown by these short Rs.

So here are the treated patients. Here are the untreated patients.

There was no correlation between the postop. platelet count and whether there was bleeding as defined classically by chest tube drainage or between the bleeding time and bleeding as monitored by the chest tube. The controls are shown by the closed circles.

Now, Barber evaluated the significance of a prolonged bleeding time measured pre-operatively in 1,941 surgical patients, 110 of whom had prolonged bleeding times. There were 39 evaluable patients. Twenty-one of the 27 with prolonged -- I'm sorry. Twenty-one of the 27 with prolonged bleedings times who underwent major surgery were felt to have no abnormal bleeding. Their blood loss was less than 500 mL.

Pertinently, among the six who lost more than 500 mL and who had long bleeding times were people who underwent hip replacement

and spinal fusion, procedures which are well known to be associated with considerable blood loss, and to a point, ROC analysis as described by Dr. Rodgers, shows this 45 degree angle curve, indicating that the bleeding time was not informative and of no value in predicting hemorrhage.

Now, ROC analysis of all available data relating the bleeding time and bleeding in uremics at the time of our study is shown on this slide. Putting aside usually not being able to determine whether bleeding was clinically significant or minor, a very troublesome point in much of this literature, one can see if one sets the level of bleeding time prolongation that produces a true positive rate of about 75 percent, that is, 25 percent of patients who were clinically defined as bleeders would be missed, you would also have to accept a false positive rate of approximately 25 percent. That is, 25 percent of non-bleeders would be misclassified as bleeders by the bleeding time.

And I don't think this is satisfactory, and of course, any attempt to increase the true positive rate to a higher level, of course, concomitantly is associated with a marked increase in the false positive rate.

Now, the extensive use of DDAVP in an attempt to reduce bleeding following coronary bypass has provided an excellent opportunity to support the following conclusion. That is, there's no evidence that the bleeding time is a useful indicator of the efficacy of therapy.

Multiple double blind prospective studies have evaluated the effect of DDAVP after termination of coronary pulmonary bypass, and I've been able to identify eight studies based on a total of 926 cases. Seven of these eight studies have failed to demonstrate that DDAVP reduced postoperative bleeding, but the point is shown by the particularly informative studies of Rocha and Salzman.

Now, Rocha studied 100 patients. Although DDAVP did not reduce significantly the transfusion requirement -- in fact, it was moderately higher -- you can see that it did produce a statistically different shortening of the bleeding time in these patients.

An important contrast is the study by Salzman in which he reported that the administration of DDAVP produced a marked decrease in blood loss, 1,300 mL versus 2,200 mL, but had no effect whatsoever on the bleeding time.

In summary, there were four papers which reported the bleeding time following the administration of DDAVP. Two failed to show reduction of bleeding or shortening of the bleeding time. One by Rocha failed to show lessening of the bleeding, but showed shortening of the bleeding time and one by Salzman showed lessening of bleeding, but no shortening of the bleeding time.

In summary, there's not a single paper to my knowledge that has shown both significant shortening of the bleeding time and clinical decrease in bleeding.

Now, two other clinical settings emphasize the effect of inappropriate utilization of the bleeding time for prediction of bleeding. Regional anesthesia is very commonly used in obstetrical

anesthesia, and although the risk of a spinal hematoma, a much feared complication, is very rare and, in fact, is absent often in series of 10,000 patients, it's nevertheless of appropriate concern to obstetrical anesthesiologists because the bleeding time can be prolonged and pre-eclampsia, and thrombocytopenia occurs in some of these patients.

It's been authoritatively recommended in the anesthesiology literature that the bleeding time be used to screen pre-eclamptic women and that epidural anesthesia not be given if the bleeding time is prolonged.

However, based on an estimate from Rolbin that his group had performed spinal anesthesia on over 5,000 thrombocytopenic patients, I calculated that he had performed epidural anesthesia without complications on approximately 350 women whose platelet counts were less than 100,000 and whose bleeding times would have been predicted to be prolonged.

And a recent study by O'Kelly indicated that if ten minutes were used for the cutoff of a normal bleeding time, the false positive rate of seven percent that they generated in normal persons would have resulted in 70 women out of every 1,000 who needed an epidural being denied this safe and effective form of obstetrical anesthesia.

And I think the implications of these data for the use of platelets or platelet substitutes is obvious.

There are other clinical data available that when synthesized strongly indicate that the bleeding time is not predictive

of bleeding, and the example of this is performance of liver biopsies. As you may know, the bleeding time is not routinely determined prior to performance of a liver biopsy. However, many patients with liver disease are thrombocytopenic, as shown here.

Here are 100 patients with liver disease. Platelet counts are shown here. The bleeding time is shown along here. You can see although it's longer in general, there's enormous scatter of the bleeding time in these patients.

Now, as a pertinent aspect of this field and also further evidence of lack of correlation of the skin bleeding time with bleeding elsewhere is this study by Ewe in which he studied the liver bleeding time, which I'll define in a minute, versus the thrombocyte count, and we can assume that patients here would have long skin bleeding times.

Now, the liver bleeding time was done by the performance of a liver biopsy under laproscopic observation, following which the surface of the liver was rinsed with saline and the bleeding time determined when the blood stopped oozing from the capsule, and you can see, despite a large group of patients who certainly would have had long skin bleeding times, many people had liver bleeding times, the organ of interest, which were perfectly all right.

Now, a very important technical issue in the use of the bleeding time to predict bleeding or to evaluate platelet preparations is its lack of reproducibility. Now, this is a study which shows the coefficient of variation in a very thorough study of DeCaterina.

This is inter-observer variability that is the CV that resulted from the two bleeding times done by two different operators, and you can see whether the bleeding time is expressed in the usual way or by some of the other techniques the authors used to try to get some clinically useful data. The CV is quite large.

And even more striking was the intra observer variability. That is, when the bleeding times were done by the same person, you'll see, if anything, the coefficient of variation in some of these instances even became worse, and I think it's important to stress that this was in a research study where all of the bleeding times were done by two people who were the authors of the paper. So they were skilled and had a vested interest in doing this as carefully as possible.

There also remains the issue of variability of results dependent upon the anatomical region used for performance of the bleeding time, and in this paper by Dr. Bode, in which freeze dried canine platelets were used, he comments that their use lowered the jugular bleeding time, but the ear bleeding times were less corrected. And since the data were not provided, I have concluded that it means that the ear bleeding time was not significantly shortened.

And this raises the important issue of exactly what anatomical area, if any, is appropriate for the performance of the bleeding time.

And then on my last slide is this statement from a very nice summary of Dr. Alving and her colleagues of a conference which was held in Washington about two years ago covering generally the same area. And in their summary of the conference, and you can read this

for yourselves, the authors concluded that at the low platelet levels of five to 20,000 that Dr. Slichter just described, the bleeding time is not a discriminating measurement.

In summary, the bleeding time debuted in an era when clinical tests were utilized without scrutinization of their predictive value, in my opinion, it provides an excellent but an unfortunate example of how a laboratory test unsupported by adequate evidence of clinical applicability can persist in the diagnostic armamentarium.

This test reflects complex and poorly delineated pathophysiologic measurements and, as many of you know, has important aspects of variability. The results of the analytical study of Dr. Rodgers and myself have been confirmed by subsequent publications and by Dr. Alving's summary of the literature that was published after our paper was published.

And, therefore, although the bleeding time has been commonly used to monitor the effects of various preparations of platelets and platelet substitutes, I do not think that such data can be assumed to demonstrate clinical efficacy as defined by the FDA. And it should not be overlooked, as has been alluded to by two of the previous speakers, that relatively few people with severe thrombocytopenia and markedly prolonged bleeding time have clinically significant bleeding.

Thank you.

(Applause.)

CHAIRPERSON VOSTAL: Thank you, Dr. Levin.

I wonder if we could have this morning's speakers come up to the podium here, and we'll have a question and answer session. We have about 20 minutes to ask anything that's on your mind.

If anyone from the audience is asking questions, we'd appreciate it if they would go to the microphones on both sides of the rooms because this session is being recorded.

DR. ALVING: I have a question. Alving. I actually have two questions.

One would be a general question. What is the minimally acceptable platelet substitute that you think could find its way into clinical use -- and I'll address it to Dr. Schiffer/Slichter -- given the fact that most platelets are transfused for prophylaxis?

Does such a product have to circulate in addition to function? What do you think would be something that would be usable at your institutions?

DR. SCHIFFER: I don't know. I think the problem is that many of them don't circulate, and then that you're looking for a hemostatic model, and as disgusting as that model is and as difficult as those patients are to find, Sherrill's stool model might be a good one.

The problem is it looked like when you're dealing with more active patients, the numbers didn't look as tight as they did in the original studies. Is that true?

DR. ALVING: But would you pass out from your blood bank, for example? The clinician wants something. Are you going to say, "Here's something. You know, it works for five hours, ten hours, but

it may be useful"? Is it something maybe we have to infuse continuously?

You know, you've thought about this, right?

DR. SCHIFFER: I think you don't know. That's, I guess, what this conference is about.

I mean, you'd want to do the things that I've said, that is, to prevent major, major bleeding, and I think you need some evidence as to, one, that it does that, and I believe we can ask Jack about what he thinks about the bleeding time in that regard.

And secondly, because the kinetics of any benefit are unlikely to be the same as with platelet transfusion, you're going to have to do repetitive studies to determine the interval at which this stuff has to be infused.

DR. SLICHTER: Well, I think you're going to have to -- you know, to give the product that you're discussing, Barbara, I think you're going to have to say because we already have things that are better than what you're talking about, okay?

DR. ALVING: I don't know what I'm talking about.

DR. SLICHTER: Well, you're talking about --

DR. ALVING: I want you to tell me.

DR. SLICHTER: -- some product which may not increase the platelet count so you can't determine efficacy by that measurement; may provide evidence of hemostasis by some test or mechanism, but doesn't last very long. And, you know, that's not as good as what we've got.

So the issue then becomes at least to my mind: is there some reason why you don't have available to you what we currently have? I mean are you out in the fields of, you know, some war situation or something where that product truly is not available to you, and so are you talking a short-term situation where this product is better than nothing, or does that produce provide some other benefit which the current products don't?

Maybe it doesn't transmit a new variant CJD which nobody can determine is transmitted by transfusion anyway, but you know. So I think you're talking about risk-benefit ratios.

In other words, have you got a special circumstance where this is as good as you're likely to get for your patient? And in that situation, that may be a beneficial product. But I think under ordinary circumstances, unless your product does something better than what I've currently got, I'm not sure why I should be using that product.

DR. ALVING: So you say we may be talking about niche products really.

DR. SLICHTER: Well, yes. I would guess so, yes.

DR. ALVING: And I'd like to ask just one question to Dr. Levin. I think you've done an immense service. You've saved millions of people bleeding times. Let me just put this in a positive way.

Is there any time when you would use a bleeding time apart from you've already made your disclaimer of looking at the possible congenital bleeding disorder, but let's say you've got a uremic in the

intensive care unit. The hematocrit is 30. This patient is in uremia, and they want to go off and do a tracheostomy.

Is there any usefulness for a bleeding time to assess should we give estrogen, DDAVP, fill in the blank, or would you say there is no clinical situation in critical care units or out in let's take the uremic population for use of a bleeding time?

DR. LEVIN: No.

DR. ALVING: Okay. Good luck. Me, too.

DR. LEVIN: Well, I think we have to come to grips. I mean, Dr. Schiffer pointed out very wisely the literature is full of hedges, and because everybody has always hedged, we've made very little progress in important aspects of transfusion medicine.

I think that not just our work, but others have shown the bleeding time will not predict bleeding in an individual, and therefore, we should not use it.

DR. ALVING: I think you're going to be cutting on this.

DR. LEVIN: If the test is no good, you shouldn't do it. And I think then I stick with the answer is no. And we have done this in the hospital in which I work. We essentially don't do bleeding times anymore.

DR. SCHIFFER: Jack, I wouldn't dispute any of that, but with regard to the purpose of this conference, we're dealing in general or talking about people who have much lower platelet counts, much longer bleeding times, and we're talking about giving them something that will change that.

And what I think the FDA is asking is whether that change, the information you gain from that change, even though it doesn't predict bleeding in that individual, will give you enough inference that that product has hemostatic benefit that you're willing to or Dr. Alving is willing to use it.

DR. LEVIN: Well, I --

DR. RODGERS: You're asking a different question. So I'll interject if I can.

The question you asked is a very good one, but you're asking a population question. You're verging on Question 1 of what I discussed. Is there a difference between a bleeding and a non-bleeding population in terms of whether or not they received a certain treatment?

We're addressing the issue of can you predict bleeding in individuals, and it's meaningful to do a bleeding time if you're looking at the behavior of the bleeding time in a defined population, and you're interested in a mean.

DR. SCHIFFER: I think that's what this conference is about. It's not whether you're going to predict bleeding in a uremic individual, and population statistics never tell you about an individual anyway.

I think the question to be asked about the bleeding time is whether a new product can be shown to have some efficacy, some hemostatic efficacy, and then we could all value that.

DR. RODGERS: Let me interject again.

But then again you have to ask the question: are you interested in measuring what you're really after, which is bleeding, or are you interested in studying a surrogate, a hypothetical surrogate for bleeding, i.e., the bleeding time?

If we're interested in bleeding, we should be studying bleeding. If you're interested in studying the bleeding time, then, yes, measure the bleeding time. But the point is that there's very little evidence, no evidence that the bleeding time correlates with things that are of importance in the real world, i.e., human bleeding, clinically important human bleeding.

DR. HARKER: Laurence Harker from Emory.

I think it's been a very interesting and important morning, and I think that Charlie has done us a real favor to point out the fact that spontaneous bleeding from thrombocytopenia is distinctly uncommon, and therefore, that it is impossible to use conventional controlled trials to get answers with such low frequency events.

It then is very clear that under those circumstances, that the need at the moment is for prophylaxis, prophylaxis in settings where thrombocytopenic bleeding would be a disaster. And it's worth providing a lot of presumably unneeded prophylaxis in order to try and prevent those few events.

So the setting where it's being used, it justifies the fact that there is prophylaxis made available, even though most of those patients would not need it unless they had a lot of the complications that these folks get. Not only do they have a low

bleeding time, but as Sherrill has pointed out, they could get along fine with 5,000 platelets, but if you now start to add the chemotherapy and the antibiotics and the disease state, you suddenly get a very complicated situation where the thrombocytopenic bleeding becomes of great concern and something you'd like to prevent.

And under those circumstances, you can't use the real controlled trial for real events, and if you still believe it's important to treat those people, a surrogate is going to have to be needed in some way, and what has been used has been the platelet count.

And the platelet count has probably resulted in huge dispension of platelets that probably have not been needed in the majority of those patients.

Now, to get back to the point about what kind of a surrogate might be useful to help us to go through this morass, the bleeding time was never designed to predict bleeding. It was designed to assist global platelet hemostatic function.

So that if you're going to use it, it has to be in a setting where the platelet count is the determinant or the platelet function is the determinant as to whether an individual might bleed or be at risk.

And in the majority of patients who go to surgery, the platelet count is the last thing on the list that they may need. And so that showing that, yes, there is variability in a bleeding time because it is subject to vasomotor effects, is subject to skin effects, is subject to variation in who does it, and all of those

things are true, that you can obfuscate ending up with nothing, and sure enough, you end up with nothing.

There is not a correlative indication as to why under those circumstances the bleeding time would be predictive. It wasn't designed to be predictive.

But if you take a situation where the platelet is the determinant and you can carry out the situation where the patient or the animal can serve as his own control and then you can show that there is an effect on the basis of modifying platelet count or function, that this is a very reasonable surrogate to try and make that measurement.

In fact, it is possible that since platelet hemostasis is complicated with adhesion and recruitment through a pathway that is thromboxin A2, a pathway that is ADP, a pathway that is thrombin, that it would be very useful to have some notion of having all of that rolled in together to give us some measure of how the platelet responds to a hemostatic challenge.

And, in fact, under those circumstances an in vitro test might be very nice or -- sorry -- ex vivo, where you would actually have a simulated bleeding time test, say, a collagen membrane or some kind of membrane, and then take blood from the patient and see how long it takes for it to clot.

Then you would not have all of the variables of the vasomotor circumstances that complicate the use of an already compromised test, the bleeding time. But in all fairness, I don't think it's quite appropriate to obfuscate with all of the data that

have come through demonstrating noise to say that there is no value in trying to identify an appropriate surrogate to assess overall platelet function under those circumstances.

So I think this is essentially what stool blood loss represents, is an alternative to this, here using the GI tract as the measure. The bleeding time has some advantages because you're challenging hemostasis with the venous pressure being elevated, and therefore, you can increase the sensitivity, but you also increase the noise.

And the reason you don't get an equivalent measure with a liver and a kidney test is that there is no increase in venous pressure, and so you haven't challenged it, and it isn't going to be sensitive in the same range that you might need it.

So with all of those caveats, it's very clear that we don't have the good test of how overall, global platelet function is going to work. It would be nice if there was an ex vivo test. I thought at one time we had one, but somehow it's disappeared into the sunset. And so we still don't have a way of trying to assess how well will platelets in blood perform a hemostatic function in terms of occluding a break. I wish we did.

But somehow we're going to have to come to terms with the fact that some surrogate will represent what represents a reasonable risk. Probably the one thing that everybody could agree upon would be that stool blood loss would certainly be a meaningful way.

That is not an easy system. It requires a quite remarkable cohort of patients. You can imagine what it would be to

try and have a stable of severely thrombocytopenic patients around that you could study various sample "x" in, not a simple approach.

Anyway, I wanted to make the point that I think that there is merit in that it's not helpful to obfuscate just in order to suppress the merit in order to make a point. The argument you make is that it doesn't predict bleeding. It's true. It doesn't. It wasn't designed to do that.

And I think that the application in that setting is something that is unfortunate and has led to some misunderstandings.

DR. FRATANTONI: Fratantoni, Rockville.

Understanding that there is a controversy between the applicability of the bleeding time, nonetheless, in the presentations there was a discussion of correlating bleeding time with bleeding patients.

Bleeding time tends to have been used over the years as a definition of a bleeding patient. If you're not going to use the bleeding time or even if you are, could we agree upon some sort of working definition of the bleeding patient for the purposes of this workshop?

DR. SCHIFFER: Well, there are lots of definitions. The WHO has one. Each of the cooperative groups have one for their leukemia and transplant trials, and then they go from zero to four or mild to severe to death.

They're moderately different in terms of what some groups consider to be serious, and you know, you could do a clinical trial, although it would be, as I suggested, incredibly statistically

laborious in terms of sample size if you pooled death plus severe plus moderately severe.

I think it would be beyond the ability of society to test these half dozen new platelet products though if you did it that way, but there are definitions.

DR. FRATANTONI: People are talking about the desirability of some sort of surrogate measure, be it ex vivo, in vitro. If you're going to validate any such measure, you have to validate it against some sort of clinical reality. And if you're going to do that, you have to define the clinical reality.

DR. SCHIFFER: Well, the clinical reality is that most significant bleeding occurs when something else is going on, whether that something is a coagulopothy or an anatomic site. The anatomic site can be the entire, and often is, the entire GI tract, but it's much too complicated is my sense.

DR. LEVIN: But doesn't Dr. Fratantoni's question emphasize what you pointed out, that, in fact, practically none of these patients are bleeding? Isn't that underlying the problem of defining --

DR. SCHIFFER: Sure.

DR. LEVIN: -- the patient and what the test should be, that they have never bled and that they don't bleed?

DR. SCHIFFER: Well, that's not true that they've never bled and they don't bleed. It's just that the frequency is such that it's difficult to do.

Other things have changed, too. With stem cell transplant where you have the most anatomic disruption, your period of thrombocytopenia is much shorter. It's ten days, two weeks max., and it might even become shorter in the future.

So, you know, there are some clinical realities that are favorably affecting this, as well.

DR. CORASH: Corash, San Francisco.

A couple of things. Fortunately hemorrhage is quite rare today because we do transfuse a lot of platelets. Some of us in this room probably remember when platelet transfusions were more difficult and less effective, and we had lots of patients that did have hemorrhagic disease.

Many other things have also changed, such as antibiotic regimens, preparative regimens for transplantation, and obviously chemotherapy regimens, and even the stage at which we now diagnose patients with malignant diseases and how fast we initiate therapies. So many, many things have changed.

Sherrill, going to your studies about stool blood loss, I think I heard you make the recommendation that the stool blood loss model would be a very effective means for evaluation of hemostatic efficacy, but obviously we have limited experience at this stage of the game because the studies are hard to do, and we don't have the benefit of 20 years of ROC analysis to look at this test today.

And looking at your data, it seemed to me, if I understood correctly, that the sensitivity of the stool blood loss only is adequate when we get down to very low platelet counts. And the

question is: given the fact that patients have many complicating factors and clinicians, rightly or wrongly, become as we say nervous and do platelet transfusions, is it possible to really have stable populations of patients with the complicated therapies that we give today where we can actually make these types of meaningful observations?

DR. SLICHTER: Well, Larry, you know, we don't have a lot of data. I am suggesting because I think I get the sense that all of us are kind of grasping for how do we really evaluate the hemostatic efficacy of these products.

That, I think, is as good a way as I have seen and have worked with. You're correct, Larry. I mean, we were looking for a way to measure hemostatic efficacy at levels at which we couldn't use the bleeding time because the bleeding time was unmeasurable.

So the stool blood loss seems to give some sensitivity at very low platelet counts, and therefore, I think it may be a useful way to determine efficacy of products which, in fact, we can't measure an increase in the platelet count.

But I think all of us would agree that, you know, we transfuse a lot of platelets prophylactically now so that we don't see the kind of bleeding we used to see when we either didn't have platelets or didn't have platelets that were any good.

But I basically am convinced that most patients if their platelet count gets to be less than 5,000, they are, in fact, going to bleed and have a bleeding risk. So I think that these products which are coming down the pike, which we may not be able to use increases in

platelet counts because they don't increase the platelet count; they're membranes or they're things hooked to particles or whatever; that we're going to have to have some way to determine whether, in fact, these things do provide hemostasis.

And, you know, in our current study we are looking at, you know, was the patient febrile, what antibiotics they're on, and da-da-da-da, and we haven't analyzed all of that information. But I think if you accept the fact that if you have no platelets you're going to bleed. And if you infuse a product which, in fact, by stool blood loss or bleeding time or whatever measurement you want to use shows that you don't have bleeding, then I think you can make a suggestion at least that that product is efficacious.

And as I showed in one of the early slides from our very early study, if you gave a platelet transfusion to these people who are on prednisone, a semi-synthetic penicillin, you got their platelet count up, their stool blood loss, in fact, decreased.

So I personally do think, Larry, that it may be a way that will help us through this dilemma of how do we evaluate hemostasis if we can't increase count with some of these products, and I think it needs to be able to show hemostatic efficacy, Larry, with all the things that's happening to the patient because those are the patients that we deal with.

I mean one of the things that we did with the stool blood loss study that we're currently doing is we did exclude patients who had plasma coagulation abnormalities because I didn't want to add that on at this point, but we took everybody else, and you know, they are

AML patients. They are breast cancer patients. They are severely thrombocytopenic. They're having fevers and all the kinds of things that these very sick patients have, and hopefully this will be a way to allow us to make an assessment.

DR. CORASH: But I think that, you know, the N in your studies is very small, and part of that --

DR. SLICHTER: It's very small.

DR. CORASH: And part of that, if I remember the data, the sensitivity is only there -- we only see consistently increased stool blood loss when we get down to platelet counts below 10,000 per microliter.

DR. SLICHTER: Yes.

DR. CORASH: In the zone between ten and 20, stool blood loss is not informative.

DR. SLICHTER: That's right.

DR. CORASH: So the question is --

DR. SLICHTER: It's not informative. It did not show a difference, Larry, in the first population of patients who were stable, aplastics, not sick as sin, not on 8,000 medications. That original stool blood loss study was really patients who were on no medications, were not being transfused. It was at a time done many years ago when there were a population of patients that we just couldn't support with platelet transfusions, and so we did not.

DR. CORASH: Yes. I guess my question though is: how many clinicians will give us how many patients that we can study under these types of conditions? Because I don't think that we can find

enough patients with enough controllable variables so that we can analyze the data at the end of the study and get a large enough N in some meaningful lifespan of the investigators so that we could actually do the study.

That's what really concerns me, and many of the people in this room were here in 1981, and we're doing the same homework, and it's because the problem is tough.

DR. SLICHTER: Yeah.

DR. CORASH: Not because we're not --

DR. SLICHTER: Trying.

DR. CORASH: -- smart enough. We're all getting a little smarter, but that's really the crux.

I mean, I think this is attractive, but when I look at the complications of doing these types of clinical trials and what clinicians will allow us to do to their patients, I'm very concerned that we will never be able to do this type of clinical trial in anybody's meaningful lifespan.

DR. SLICHTER: Well, Larry, you know, I don't strongly disagree with what you've had to say, but I think we may need to do it one step at a time. In other words, our clinicians -- most of these, you know, take the patient out of the study, transfuse at a higher level, occurred when we first started the study.

Our clinicians are now saying, "We don't want to transfuse at 20,000. We're going to transfuse at ten." They may go down to five. So I think it's a matter of making the clinician comfortable

that they don't need very high platelet counts in order to prevent bleeding, and then we are going to have to work with them, Larry.

I mean, I think there's going to have to be some preclinical data that's been done in some of the animal model systems or with some of the in vitro measurements before the clinician will allow us to give these products.

But if we don't have some clinicians that are prepared to allow us to transfuse it to their patients, the FDA is never going to license them. So we might as well not even be working on the problem if we don't think that there is going to be some way that eventually we can show efficacy in patients.

DR. LEVIN: In terms of documenting effectiveness, and I think you have to be very careful about how you use the word "efficacy," but in terms of testing I'm surprised nobody has commented on the assay that originally established the effect of platelets, and that is the loss of red blood cell in the lymph of either dogs or rats.

Now, that's a solid in vivo assay in which you can document in a matter of minutes the transfusion of an effective hemostatic product, in that instance platelets. It immediately stops the appearance of red blood cells in the lymph.

Now, this is an in vivo study. It strikes me it's measuring hemostasis, and I'm surprised that it hasn't reappeared in the literature since it played a very important role in the initial establishment of platelet transfusions.

And one other. Just getting back to the efficacy issue, I think one of the general problems here is the difference between a test result that changes following your intervention, bleeding time or whatever, and the clinical efficacy.

Dr. Fratantoni and I have been to a lot of meetings based on hemoglobin based red blood cell substitutes, and I think I'm reflecting the FDA's position reasonably accurately when I say although these substitutes carry oxygen and will circulate, that is not accepted as demonstration of clinical efficacy, and I think there's a real parallel between the struggles in the HBOC and the problems that we're talking about today.

DR. RODGERS: I just want to make one really quick comment here. There was a very lengthy comment to an earlier question here dealing with this issue of how do we find a surrogate for the bleeding time.

It should be made very clear that the work that Dr. Levin and I did addressed the bleeding time as it's done today, and from the point of view of sort of a clinical information scientist, I must say that bleeding time is a pretty stupid test, and by that I mean to say that it's quite possible we could come up with a better version of it.

For example, it's very clear that there are gender based differences in the mean of the bleeding time. It tends to be longer in women than in men. It could be that by a combination of individualized reference ranges, different reference ranges for genders, controlling for diet, drug use, and doing something like a delta, a before and after test. You could come up with some kind of

provoked bleeding time that would have more clinical predictive value than the current bleeding time.

Just doing a simple provoked bleeding time on a person drawn at random from the population, it's pretty clear that is pretty useless.

CHAIRPERSON VOSTAL: If we could take just one more question, and then we'll have a recess.

DR. BLAJCHMAN: Mo Blackman, Hamilton.

As has been pointed out by several speakers this morning, the bleeding time test, and for that matter the stool blood loss, is a screening test of global hemostasis, and as, Jack, you pointed out, other factors, hematocrit, level of coagulation factors affect this test.

So it's not surprising that when you apply an ROC curve to a screening test, it's not going to be specific. And to make the point as strongly as I possibly can, I would point out to you that if one applied ROC statistics to the HIV I screening test, that no one in the world would consider stop doing, you would find exactly the same thing, that it's not a very effective test for finding people of HIV because of the false positives.

Another example. Each one of us when we came here when we got on the plane were screened for metal looking for a gun. If one applies ROC statistics to the quality of that test, one would find that it's a miserable test.

So I think we use the bleeding time test as a screening test that one uses or one can use to assess hemostasis. It doesn't

mean that it's necessarily going to be predictive of subsequent bleeding in a patient, but I think it's still useful in my view of assessing that global hemostasis, and an intervention that corrects that may still be useful clinically.

DR. RODGERS: I'm sorry, but I'd be happy to run numbers on any of those cases you mentioned, metal detection and the HIV detection, but without looking at the numbers, I'd have to say I disagree with your blanket statement that those would be proven worthless tests by this methodology.

I mean do you have something better to substitute for 50 years of experience in biostatistics? If you do, I'd be glad to use the methodology.

DR. BLAJCHMAN: I'm not criticizing the ROC test. I'm criticizing -- I'm simply pointing out that the results that you obtain are to some extent a self-fulfilling prophecy.

DR. RODGERS: The results we obtained were a simple statistical analysis of actual observed data in the literature, nothing more, nothing less.

In every case, yes, you could say in our study there were biases built in it, and in every case the biases built in make the test look more useful and make the results look more favorable toward the test than they would have against the test.

For example, I mean, the case you pointed out where bleeding in uremics didn't distinguish between trivial and clinically severe bleeding. That would have made the test look much more powerful, in fact, than it was.

CHAIRPERSON VOSTAL: Okay. I think this is a good time for a break.

Thank you very much to all of the speakers.

(Whereupon, the foregoing matter went off the record at 10:11 a.m. and went back on the record at 10:28 a.m.)

CHAIRPERSON VOSTAL: If we could get started for the second session.

We're going to get started on the second session, and we have more excitement coming our way, but first, Dr. Schiffer has asked for two minutes of our time for a couple of more thoughts before we get started with the rest of the session.

DR. SCHIFFER: If I may, something that occurred to me while the discussion was going -- the question and answer was going on, and let me pose it this way. I personally think that if you're dealing with intact, viable platelets, there's precedent for how to measure efficacy, and increments is a pretty good surrogate. You could argue about that, but the FDA has accepted that in the past.

The real issue is with these other products. Let me just pose this very practical question, and we can get back to it as time goes on.

I think we would all accept that the risk of bleeding increases at counts of 10,000 or less. We know that clinically, and it's much less, let's say, at a count of 30 to 40,000.

We also know that there's a difference in the bleeding time at those two levels, although we've heard that that may not be the best test.

If you take someone who has a count of 10,000 and has this bleeding time, this very, very long bleeding time, and you give them platelet dust, and their platelet count doesn't change, but their bleeding time becomes more similar to the person who has a platelet count of 30 or 40 or 50,000, would we regard that as something that has -- and you do this repetitively obviously -- would we regard this as something that's of benefit to our patients?

And let me just throw that out, and we can discuss it perhaps as the day goes along because I think what we want at the end of this is some practical models of how to approach these questions as well.

CHAIRPERSON VOSTAL: Okay. If we can continue now with Dr. Ed Snyder, and his talk is going to be "In Vivo Platelet Survival -- Labeling, Methods, and In Vitro Predictors."

DR. SNYDER: Thank you.

What I'm going to attempt to do today without trying to bring coals to Newcastle is to show how in vitro studies may or may not correlate with in vivo studies, what some of the in vivo radiolabeled survival data are. And then maybe just speculate a little bit on how some of this, if any of it, might apply to some of the newer platelet products that may be coming out.

This is a picture of what we're really trying to do. We're trying to get platelets that are in good shape, stored for long periods of time, and transfused and do a good job. And obviously these are platelets that have undergone a release reaction and would not function very well. If these products were infused, they would

primarily provide mostly the opportunity for febrile reactions and chills and so forth and very little efficacy.

Yet there are we now know glycoproteins and so forth on the surface that may be of some value, and some of the companies that are here today and certainly some of the interest among many of the people in the audience as to whether there is something retrievable on these platelet surfaces.

How do we know that there's a storage lesion in the first place? And I show this slide frequently by Hogge in Transfusion, 1986, which showed transfusion increments. This was a one-hour corrected time increment. These were fresh platelets compared with platelets that were stored in two different blood bags, but three-day platelets, and seven-day stored platelets, and there was a difference between fresh and both three and seven day, which was significant, but there was no difference between one hour corrected time increments for platelets that were stored three days versus seven days, and similar results were seen for 24 hour CCIs.

This implies that something happened to the platelets between fresh, which is something that we can only dream about these days, and day three or day seven, but once the platelets had been prepared, there was very little damage, further damage, as evidenced by the corrected time increment that occurred during storage.

And this has generally been attributed to the lesion of preparation which relates to the centrifugation. In the United States, platelets are given a soft spin and then a hard spin onto the plastic bag to make the platelet concentrate, as opposed to European

buffy coat technique which spins the platelets hard on a softer cushion of red cells.

But the fact is that there is something that is different about a fresher platelet versus the platelet that is stored, and the same would be true for five-day stored platelets, as well.

Well, how does one analyze platelets in general, regardless of how they're stored, whether they're pumped or irradiated or psoralenized or methylene blued or gamma irradiated. And the techniques that have been used primarily for the FDA and have involved in vitro analysis followed by radiolabeled autologous survival and recovery in vivo and then in vivo platelet transfusion studies in what we can euphemistically refer to as a standard sick person, which is someone with leukemia generally who is ill but is not actively septic, is not in extremis at the time. And there's often a window period when they can be transfused and should be transfused appropriately with two doses, the test as well as the controlled product, and CCI data, for what it's worth, collected.

And those are the three phases which are still in use today, and any different products would need to probably follow that type of analysis.

It breaks down when you get to things like infusible platelet membranes or freeze dried platelet debris, if you will, that doesn't give you an increment. I don't mean "debris" in a pejorative term. I apologize, but you can't look at a corrected count increment, and you can't look at a survival necessarily in the same way, and that's where we need to decide how we can approach this.

This is a paper that came out in Transfusion Medicine Reviews by Murphy and others. It reflects the Best committee's assessment of in vitro assays. Everybody is looking for the one wonderful assay. The winner and still champion is -- the Mark McGwire, if you will of assays -- is pH with 127 reports.

(Laughter.)

DR. SNYDER: And it is probably still the best, but unfortunately it's only good if you look at everything like at 6.3 pH and below versus 6.4 and above. It's not a linear relationship. It tends to be bimodal.

And there are the Whitman sampler of a zillion other assays which I don't think I need to go into. There is a second slide which continues it on so you can all see it, which looks at every aspect, everyone looking for, as has been reported, the Holy Grail.

There is none, and there probably never will be as far as a simple in vitro assay that can predict in vivo survival. There just is none. The best you can come up with probably is a pH somewhere below 6.3, giving you a bad recovery.

Well, a poor person's pH meter could be looking at swirl. This was published by Bertolini in Transfusion. Those to the left of the zero are degrees or swirl, the lack of swirl, negative swirl, if you will, and those to the right are positive swirl.

Swirling refers to the ability of a platelet in a discoid form to refract light, and platelets that have undergone the disk to sphere transformation are spheroid and do not refract light. So presumably these are in the disk form or are in the sphere form,

rather, and do not refract light, and these do, and looking at the pH to try to correlate, you can see that the pH clusters around six and a half to seven and a half, and these have swirl.

The problem in using swirl, which would be a method for just looking at the bag without having to actually enter the bag and lose the unit, isn't very helpful because if you threw out all of those units that didn't swirl, you'd be throwing out a lot that had appropriate pH and presumably were not infected.

It's just that it's an imperfect test. There have been -- the Bellhouse Corporation -- I don't know if it still exists -- had a machine which is supposed to codify this into red, yellow, and green lights that tell you whether the platelet had a lot of disks or whether it didn't or whether you shouldn't transfuse it.

It's an imperfect assay, but generally if you see swirl, it probably means your pH is okay. If you don't, you really can't tell very much about it. Again, it's not a good assay to predict what's going on in vitro, let alone what's going on in vivo.

A lot of studies were done. This is an old slide looking at beta thromboglobulin release, which I still fondly remember, and we looked at beta thromboglobulin for three days. These are individual units of platelets. And as you can see, the longer you store platelets, the more the beta thromboglobulin means value rises.

Of course, there's a tremendous variability which we now know is not only biologic variability, but variability in platelet production technology in preparative technique. Some people like to resuspend the little platelet clumps as if every little nubbin has to

be erased from the face of the other. Other people just let it gently resuspend on a rotator.

The people who are probably rubbing all of the aggregates away would probably cause more of the release reaction, and those that are doing it more gently probably less. And there is certainly a lot of biological variability which we now know among individuals.

Looking at radiolabeled -- looking at CD-62, and that became available. This is a paper that Rinder published in Transfusion. It's the same kinds of results showing percentages of cells, showing a positive CD-62, a P selectin or PADGEM or GMP 140, showing an increase in, again, variability among the different individual units.

Knowing that's the case as far as in vitro assays, there are a million of them. You can use whichever ones you prefer, but it doesn't replace in vivo studies.

This was a symposium that was put on in 1986, and Gary Moroff and Toby Simon got together with us, and we invited many of the people that are still in this room. And someone asked me once are we smarter than we were 14 years or 20 years ago, and the answer is, well, we're still here. So I guess we must have learned something.

(Laughter.)

DR. SNYDER: It was Woody Allen that said showing up is 80 percent of life or something like that.

(Laughter.)

DR. SNYDER: In any event, this symposium, which I refer to you, still has a lot of very valuable information, and I've called

some data from that to look at where we are with radiolabeled survivals.

This slide I realized I didn't have a picture of and Xeroxed yesterday afternoon at 4:30. So I apologize for its rather crude, Stonehenge type look, but it gets the point across.

(Laughter.)

DR. SNYDER: This is a paper by Steiner.

The key point is if we're going to look at radiolabeling as having any benefit to telling us about infusible platelet membrane, formalinized membrane or other types of platelets, we have to know where the isotope binds. And then we can help a priori determine if it will relate to the new products coming across.

This paper by Steiner, 1970, in Blood showed that chromium-51 bind primarily to the cytoplasm, little to the microsome, some in mitochondria, and about 25 percent or so to stromo, which is easily washed off. And we know that the cytoplasmic constituents are primarily nucleotides, ADP and ATP.

And chromium enters the cell as a hexovalent molecule, ion, and then gets reduced to a trivalent, where it stays in the cell and it associates primarily with nucleotides. And the mitochondria probably was related to some mitochondrial nucleotides.

So if chromium is going to be of any value, it needs presumably, from what my understanding of the literature is, nucleotides to be present.

What about indium? Indium-111 is the other one that Thacker and others, Joist, had pioneered.

This is a paper by Hudson and J. Lab. Clin. M, made in '81. They wanted to find out where indium bound, and indium bound primarily to the cytosol fract, and this is a sucrose density gradient also done on the Xerox machine at the last minute, for which I apologize, as was this.

The percent indium in subcellular fractions and Fraction A was the cytosol. And it's known that it binds to 46,000 Dalton material in the cytosol fraction. And indium oxine gets in because the oxine allows the indium to get through the membrane. Once it gets in, however, it dissociates. And the free indium pretty much binds to this 46 KD cytosol fraction, and the oxine is broken free.

It doesn't bind very much to alpha granules or dense granules as opposed to dogs, where it tends to a greater degree. So if the platelets undergo the release reaction, you don't have a lot of release of indium, but if you have platelet lysis or so forth, then you might.

So here's a cytosol fraction for indium, and that's where it appears to bind.

The other benefit of indium, by the way, that you can do actual imaging studies showing -- here, this is a study by Andy Heaton -- showing the distribution of indium labeled platelets after infusion in the spleen. You obviously have to use much higher doses for splenic imaging if you wanted to do that as well, two to 300 mcgs.

Normally you can get away with it used to be 30 to 50. Now I believe that the better centers are using 15 microcuries of

indium to inject because they're using a larger sodium iodide crystal. So you can get a better imaging with lower radiation amounts.

So in trying to look at correlations among various aspects, we'll look at some correlations knowing that there's indium and chromium, and those are pretty much the only two labels that platelets are used for routinely. I will get to biotin, lest you think I forgot that, in a few minutes.

Looking at correlations, are there any ways we can see trends that may be useful? Well, the first one, which is pH versus BTG release, is there any correlation? And here we see a lovely scattergram which has a line through it just to show you that you can generate a mathematical curve, but it doesn't really do much to let you know in any way what the relationship is.

Correlation coefficient is R, and that tells you the relationship obviously between two variables. The percent or the degree to which one variable is dependent on another, however, is R squared. So that's the key.

So you'll see a lot of studies where they show you very high Rs or Rs that are like .6 or something, but that's only really an R squared of .36, which means 36 percent of the variability in X is explained by Y, which is not very much. You can flip a coin ofttimes and get 50 percent. So that's something to consider.

There is no correlation between the release reaction and the degree, although if you look at this, at the pH 6 and below there are only high ones, 40, 50, 60, 70 to 90. Whereas if you get into the normal pH range, you see a lot more below, less than 40, let's say,

although you see some up there as well. So there is some benefit to be derived from this, but it's not a general correlation. If you know the pH, you know what the release is going to be or vice versa.

Other activation studies were done. We looked at percent LDH discharge, which was an evidence of lysis of the platelet membrane versus beta thromboglobulin release or CD-62, whatever. And again, the R squared is .36, which shows basically that you can't predict lysis on the basis of what may happen with the release reaction either.

And this, again, a paper by Harvey Rinder and his group looking at percent recovery of Indium-111 labeled platelets versus percent activation, and although there was a correlation here, there's pretty much two outliers.

If you take this point away and this point away, it becomes more of a scattergram. So generally we get the same kinds of results. There are trends, but nothing that you can say if we look at percent activation, we don't have to do in vivo recovery because we can predict. You really can't, certainly not enough that I think the FDA would want to use for licensure purposes. My own thoughts.

This is a slide which shows you the kinds of things you can get with radiolabeling, and they're fairly powerful. Here's percent recovery. This is time of storage, and platelets that are labeled with 30 -- I'm sorry -- irradiated with 30 Grays or not and stored for five days and then infused, and I think you get a pretty good sense of comfort that 30 Grays of radiation does not decrease survival compared to nonirradiated platelets.

This is the kind of power that you get from a radiolabeled survival study, which you can't get by looking at an in vitro assay and trying to extrapolate.

This is a paper that Scott Murphy and others published, which basically changed the way people do business and the way we store platelets. This looked at in vivo recovery, and there is no gold standard for platelets the way there is a crimson, if you will, for red cells. Seventy-five percent survival 24 hours after injection at the end of the storage period, that's what you need for red cell survival to get approval.

There is no such thing for platelets, and 40 percent has generally been chosen by the people who work in the field.

Looking at this, Murphy and his group looked at platelets that were stored on a variety of agitators. There was a large amount of interest many years ago about agitation, platelet agitation. There's still interest in agitation these days.

(Laughter.)

DR. SNYDER: That's a social commentary. I won't go further.

And what Scott found is that elliptical storage, a 6 rpm elliptical rotator gave you decreased in vivo recovery to the point that it was unacceptable, and in addition, the ferris wheel of three days didn't do very well, but the tumbler did very nicely at five days, and the flatbeds generally, the to and fro agitation did better when 50 mL for five days.

So what this basically did was it ended the use of elliptical rotators. People decided that the sheer stress presumably was the problem, but this, based on in vivo recovery data, allowed decisions to be made as to what was appropriate and what wasn't, although I don't think the FDA was involved in this. The field, I think, just took it on itself to say that was not an appropriate form of agitation for platelets.

Here is a -- can you focus that? Is there a human up there? Thank you -- beta thromboglobulin versus two hour recovery, still trying to find some raison d'etre for BTG, and I think I've got the lowest R squared that I've ever reported, .006.

(Laughter.)

DR. SNYDER: Which is remarkable.

Again, there's no point. I need not say more, and those of you who wondered what LDH was doing, it was a little better at .075.

You can't do in vitro studies and try to correlate it with in vivo data. It just does not work, even though diazo slides look lovely.

Gail Rock published in 1986 in that -- it was published as a supplement to Transfusion -- the radiolabeled study that I showed you the title page, "Correlation Coefficients Between In Vitro and In Vivo Assays in Platelets," and she looked at survival and recovery.

Everyone has their -- every of these assays has a devotee, the Kunicki assay, the Bolin assay, Holme and so forth. They all did studies looking at survival and recovery, and basically there are no -

- she concluded that there were no correlations that were strong enough to allow one to say that either pH or hypotonic shock or size dispersion were good.

There's a recent paper that came out in Transfusion by Holme and others looking at extent of shape change and hypotonic shock response. For extent of shape change, the -- I'm trying to get -- it doesn't really much matter -- the R was .7. So you had a 50 percent R squared, and for hypotonic shock, I believe it was point -- it came out to 32 percent, .6 or something like that.

So what you're left with is 50 percent and 32 percent of the variability in either extent of shape change or hypotonic shock or osmotic recovery will give you what's going to happen in in vivo. Again, it's not an appropriate assay to evaluate what you need to, showing again in 1998 what Rock and others had shown in 1986, showing that no matter how sophisticated your technique is, the assay just has inherent problems, and I think I've beaten this horse to death.

Now, Holme in the British Journal of Haematology, Stein Holme and others in 1993, looked at survival of indium versus chromium. Are they equivalent? What are the data that indium and chromium labeling are equivalent?

Indium has a much shorter half-life and has benefits in that regard.

Looking at zero days of storage -- this is the bottom line here -- the mean recovery of 65 percent for fresh platelets versus 63, indium and chromium, and 194 versus 184 hours.

After five days of storage, it goes down to 44 and 48. Now, that's with that 40 percent cutoff. You know, below that, you figure the platelet is not doing very well. Forty-five versus 48, no difference, and you had a slightly lower survival, 156 and 155 hours, again, indium versus chromium. Together, no difference, but lower than -- less than fresh.

And looking at ten-day platelets, we're lower yet still. So this kind of data is available. I've got a lot of other slides, as well.

This was another one that Holme and Heaton did in British Journal. This was a landmark paper because this showed that you could do double label studies. That was a key. You didn't need to look at indium and then two weeks later or three weeks later after you had degraded the baseline inject again because things happen to donors.

One donor we were doing a study on that was not a double label. I remember we had labeled him with the control and were ready to do the test, and he walked in to get injected that afternoon. He had a Bandaid on his finger, and I said, "Mario, what's the Bandaid?"

He said, "Oh, I dropped a bowling ball on my finger," and I took it off and his nail came off. He had a massive infection in his finger that he wasn't going to tell us about. Had we injected him, we might very well have had a lot of problems, including explaining sepsis and so forth.

These things could be eliminated if you could label one person at the same time for test and control, and that's what Heaton and Holme pioneered, and what they did here was they labeled two units

of identical platelet concentrates, PC-1 and 2. The PC-1 was chromium, which meant PC-2 was indium, and this is chromium. PC-2 was indium. This was indium. So that the PC-2 was chromium.

The bottom line is they got the same results for recovery and for survival, showing that you could inject the chromium and an indium labeled into the same person and by doing appropriate counting to sum peak and the appropriate peak for chromium, sum peak for indium, and the chromium peak and making corrections for overlap, you could follow two cohorts in one individual.

Again, the percent recovery, again, for chromium and indium when corrected for red cell and white cell shows you a survival, post infusion survival, again, that showed that you were getting equivalent results, and they were comparable.

This is, again, Gail Rock looking at the kinds of studies. It was mentioned earlier the need to do paired studies. I believe Sherrill commented on that. What Rock here showed is that if you take eight donors and you do unpaired studies, you'll be able to take, assuming a control of 9.6 as the normal platelet lifespan, that you can show a ten percent difference with eight unpaired, whereas you can find a five percent difference if you use paired studies. That's 9.1 versus 9.6.

Taking that as the normal control, for unpaired you'd need much more or twice as much of a divertance from the control in order to pick it up.

So if you want to get subtle differences or just any differences, paired studies are better. Contemporaneous studies, doing them double label is the best way to go.

Some people have questioned whether it really makes much difference if there's only that five percent. That's not what I'm here to discuss. I'm here to present the data, and it can be applied by the manufacturers and the investigators to their studies.

This is a slide that Andy Heaton just gave me moments ago. It's still warm, and what it shows basically here's the zero line, and this is the differences in survival and differences in recovery. And what this is showing is in pink are people who were labeled with indium and chromium at the same time, and in blue are people who are labeled with indium 21 days after they had been injected the first time.

And what you see is that there's a much tighter CV in those who are labeled concurrently, and there's much more scatter, a lot more scatter obviously in the blue, which were labeled 21 days later, more scatter than you would expect just from random variation.

The implication is that there's a biological difference in platelets 21 days after they're injected. Things change in the donor, so many things that you really may miss changes because of the scatter if you do studies that are 21 days apart as opposed to doing concurrent studies at the same time in the same individual.

So that I think we're getting to the point that that is an important thing to bear in mind, and it may be the only way to go, although I'm not prepared to say that.

Looking at how you evaluate these studies, percent survival, Bob Bolin, again, in that Transfusion article looked at percent recovery and mean survival for all of these models, and the gamma or multiple hit, recovery and survival, the percent recovery is the extrapolation of the multiple curve back to the Y intercept. Thirty-eight percent, somewhat higher than linear, lower than exponential. Survival, again, is somewhat lower than linear, but that seems to be -- Scott Murphy, I know, was an advocate of linear and T one-halves, but the gamma, I believe, is now pretty much accepted as the way to go.

If you look at the number of hits, goodness of fit, the gamma, again, had the most number of fits where the model satisfied the data, and I won't begin to try to explain that, the mathematics behind that.

Okay. Now you ask what about biotin. Everything is lovely with indium and chromium. What about biotin?

Well, this is a paper by Alberio in Platelets, which is an excellent paper I suggest you might want to get a hold of. This is the structure of biotin. It's 244 molecular weight vitamin. If you treat it with an N-hydroxy succinimide, you can derivatize it, and then it allows free amino groups, the epsilon amino group of lysine specifically -- not specifically -- but in large degree, and allows you to have this bind to proteins. Thus, you will bind surface glycoproteins and you will bind proteins, and that's how biotin works, and it is evaluated by its reacting with strepavidin in a very strong, strong bond.

This paper from Franco in Transfusion, 1994, shows that you can do double labeled studies with biotin as well. What they did was they biotinylated platelets -- this is log fluorescence intensity -- at two levels of derivatization. This was a lower level; this was a higher level. It was infused, and this is initial. This is four hours, day one, two, and three. And there's less biotinylated platelets detected over time, as you would expect. But you can still see the two peaks, this one being the higher biotinylated, I think about 5.8 percent, and this being the lower biotinylated level.

So by determining how heavily you biotinylate the platelet, you can do the double labeled studies using the same label but just with two different intensities rather than two different labels.

Franco's paper again looked at does the high and low biotinylization give you adverse results. Does it have a negative impact on recovery and survival?

Recovery was 69 percent. These are in rabbits, and 72 percent with the high biotin, and the survival in days was 2.68 days versus 2.54, showing that low and high biotinylization does not adversely affect at these levels, does not adversely affect platelet function and survival.

And this shows a percent of platelets remaining after injection, showing the survival of biotinylated platelets. This is a whole series of individual dogs, and this is now dogs. And this 168 hours' survival in dogs pretty much is what you see for chromium and

indium. So they're comparable, the results that are reported in the literature.

Heilmann, who actually did the initial work for doing in vivo biotinylation, actually would biotinylate whole blood and then reinfuse it, and you biotinylated the entire cohort of platelets, not just a small sample. And this just shows, again, survival in hours, but this is using the multiple hit model, the gamma function, which tends to give you a little lower value. But 145 hours is similar to what's been reported in the literature, I believe, for dogs using other isotopes, using isotopes.

How do you follow these? These are obviously flow diagrams. This is a 2F9 monoclonal canine anti-platelet antibody, and this is a FITC phycoerythrin conjugated strepavidin. So on this axis we're looking at platelets. On this axis we're looking at biotinylization.

And what you see here, this is without biotin, and this is with biotin. Without biotin, these are primarily red cells that are not biotinylated. These are primarily platelets, and this, again, is looking at the 2F9.

Looking at the ordinant here after biotinylation, the red cells have been biotinylated. Here are your platelets over here.

So if you now transfuse the platelets and see what you get in recovery, I think this, again, from Heilmann and BGH. This is, again, the platelets going out this way and biotinylization here. This shows red cells that are within the patient. This shows biotinylinated red cells which were post transfusion.

These are platelets that were in the patient that were not biotinylated, and these are the biotinylated platelets. So this allows you to look at cohorts of old platelets versus fresh. These were presumably native platelets that were not infused, and these were platelets that were infused. So you can follow actually transfused platelets and see how they do very nicely with flow, which is another very good benefit of biotin.

However, all good things must have their down size, and Dale, George Dale, published in J. Lab. Clin. Med. that there are people who have antibodies to biotin. He looked at 60 individuals because they had looked at biotin antibodies in people in their lab and found very high levels.

They arbitrarily named one of their studies L8 as being one and compared everything to the level of biotin in that one individual, L8 over here. And they found that there were six individuals, two, four, six individuals, who had levels of biotin antibody that were five times that of the control. And there were a whole slew of people that had antibodies that were not up to five times, but a lot of people had antibodies to biotin.

Presumably it came about from eating foods or from infection or some other way. These people, not all of them, but I think four of the six, worked with NHS and were biotinylating and apparently got much higher titres. The other people presumably got it from food exposure or others.

In addition, studies that were done by Hou. This was an abstract in Blood in 1997 in the non-presented abstract book. I only

mention that so you can find it, not that I have a comment about whether it should have been there. Biotin -- you have to be very politically correct these days. I'm trying very, very hard, those of you who know me.

(Laughter.)

DR. SNYDER: Biotin concentration, millimolars. The higher the concentration of biotin at this level, aggregation to ADP was obliterated. Aggregation to adrenalin was obliterated, and here's the mean fluorescent intensity showing that you can -- this explains how you can get, you know, various cohorts of biotinylation and be able to distinguish them in that paper that I showed you earlier.

But what this shows is that too high a concentration of biotin will cause damage in platelet function. This was similar to work that was published by Kattlove and Spaet.

Dr. Ted Spaet who trained me in hematology, may he rest in peace, studied this in chromatin and found that very high levels of chromium, much higher than you needed to radiolabel platelets also affected function by blocking the release reaction.

Similarly, you don't need to use these high levels of biotin, but if you do, realize that it may have some impact on your platelet function, and you need to be aware of that and just keep this in mind as you go ahead.

I think I've just got a couple of slides left.

What about the future? Well, we had all had very high hopes for mega karyocyte growth and development factor or

thrombopoietin. We now know that it was very antigenic and has been withdrawn from the market.

There were lots of possible uses for this material. How would you go about studying it? If we were going to, well, you would for that kind of materials, you'd use standard radiolabeled survival studies, which we were going to do, but the study was stopped, or you could use your various assays.

This was just a slide from a paper that's coming out showing that thrombopoietin or MGDF, rather, when incubated with platelets ahead of time, will potentiate the aggregation response. This is ADP aggregation in platelets that were pre-incubated with MGDF, and the red marker are platelets that were not pre-incubated, and then the black and the blue are lower levels of ADP. This is pre-incubated with MGDF and this not, showing that MGDF will potentiate reactivity to ADP. It doesn't stimulate the release reaction, but it can potentiate the reaction.

Still the basic kinds of things, but how does that relate to infusible membrane and so forth? Well, here's a picture of an infusible membrane from a paper by Chao, and what is it? It's outdated platelets, washed, frozen, thawed, heated, sonicated, lyophilized, and retains GMP1B.

Well, since it doesn't have nucleotides, presumably it wouldn't do very well with chromium, and I don't think indium would bind because those proteins are probably washed away, but it does have surface glycoprotein. So technically it could be biotinylated.

How you would study that in vitro and in vivo, especially looking at binding, I don't have an answer, and that's something that can be brought up in the discussion by people who are dealing with this, but from my read of this, biotinylation may be a possible method of evaluating this, again, concerned about antibody and toxicities and so forth.

So in sum, this is the last slide. Where are we left with? We're left with indium and chromium. I think we're much more sophisticated, thanks to the work of many of the people in this room; that indium and chromium are both acceptable. Controlled, paired, randomized studies should be done, at least ten samples collected, you know, at various time periods so for the multiple hit.

The gamma model should be used, and post labeled biotin should be reported, and this is basically what was published in 1986 by Mark and Simon and many of the people in this room have contributed to that.

As far as where we go with membranes and other types of portions of platelets and so forth, we will just have to wait and see. There are not a lot of things that you can do, and if you take away the bleeding time, one wonders how you are going to assess many of these projects at all.

And I'll leave that for the discussion and for other speakers.

Thank you very much.

(Applause.)

CHAIRPERSON VOSTAL: Thank you, Dr. Snyder.

If you're interested in obtaining a copy of an abstract of Dr. Snyder's talk, he has left copies at the front desk.

An urgent message has just come to Dr. Len Friedman, and I have a message if you'd like to have it.

Okay. The next talk is a two-part deal, and the first part will be presented by Dr. Jim George, and the title is "Design of Clinical Trials to Evaluate Clinical Outcomes of Platelet Products."

DR. GEORGE: Thank you, Dr. Vostal.

I must begin with an explanation of why the title of my talk is called Part A. When Dr. Vostal called me some weeks ago to ask if I could present here a discussion about clinical aspects of evaluation of these products, I recognized that that was a difficult, formidable task.

Also though I could recognize, knowing the participants, what some of the other comments were going to be. I could recognize that the surrogate markers were going to run into heavy traffic. They were going to take heavy hits. I knew the bleeding time was going to be bludgeoned. I knew that --

(Laughter.)

DR. GEORGE: -- other studies of platelet survival were going to be difficult.

And so what I suggested to Dr. Vostal was what he needed at this discussion was somebody who wasn't in this Claude Reines round up the usual suspects -- like I put myself in that category -- but somebody who was trained in clinical trials, and I recommended my colleague at Oklahoma, Gary Raskob.

And so he allowed us to share this, and I think that what I will try to do is to present my perspective on the clinical issues related to benefits and potential risks of platelet products, and then Gary will describe in more detail, expand on my conclusion that in spite of some of the comments we've heard, also which I could anticipate -- Dr. Harker's categorical denial that randomized clinical trials are possible, Dr. Corash's comment that not in our lifetime -- but I will conclude that I feel that the only way these products are going to be evaluated and known for their efficacy and risks is through innovative uses of clinical trials with clinical endpoints of bleeding and thrombosis. So that's the beginning and the end of my talk, and now the middle.

(Laughter.)

DR. GEORGE: Oops, laser pointer.

A lot of what I say has already been anticipated and discussed by the previous speakers and so I can run through some of these observations very quickly.

I will focus on what I feel are the potential benefits and the potential, though very theoretical, risks of platelet products.

I think the benefits are obvious from the fact that prevention of bleeding requires very few platelets. We've heard that repeatedly and requires minimal platelet function, and we can learn this from clinical examples of patients with severe thrombocytopenia or patients with severely impaired platelet function.

In term of the potential risks, infectious risks of platelet products or platelet transfusions are obvious, and I won't

dwell on that. There is a theoretical risk from one in vitro study that some of these products could potentially even exacerbate bleeding, and there are some data that suggest a potential risk for thrombotic risks.

To start with potential toxicities and a potential increased risk for bleeding, there could be a hypothesis that transfusing less effective or potentially inert platelet material could interfere with the assembly of a platelet aggregate, and the data for that are only in vitro and only from this one study that I could report results from Lisa Jennings.

Her in vitro experiment is very analogous to the familiar mixing study that we use in coagulation where you're looking for a coagulation inhibitor. In her study, the data came from a clinical observation on platelet transfusion in a patient with Glanzmann thrombasthenia a patient with an inherited functional disorder, and the observation that even when the platelet count was increased substantially, the bleeding time was not corrected.

And she followed this with this in vitro study looking at percent aggregation with a very high dose of ADP and showing that with normal platelets even down to 50,000 platelets per microliter, there was substantial platelet aggregation.

But when you mixed normal platelets with either EGTA platelets which were nonfunctional or Glanzmann thrombasthenic platelets which are nonfunctional in ADP aggregation and you held the abnormal platelets constant at 250,000 and looked at gradients of normal platelets, if you had a four or five-to-one mixture of abnormal

versus normal, it significantly inhibited or impaired the assembly of a normal aggregate.

There are no clinical data that any products exacerbate bleeding, but I think there is the theoretical consideration that this may be possible.

More likely is a potential risk for thrombosis with products because clearly the platelet products which are being developed are related to lipid vesicles, platelet microparticles, products related to platelets which retain some hemostatic and potential coagulant activity.

And these may have exposed procoagulant surface, and as we all know from the traditional hemostasis diagram of coagulation, distinct from the typical platelet functions of adhesion and aggregation, platelets or the phospholipids on platelets are key for the assembly of prothrombinase enzymes to stimulate fibrin formation.

Now, again, the clinical data on this is not strong, but theoretical is the issue that the platelet microparticles developed when platelets are activated or stimulated carry the bulk of the platelet coagulant activity.

In this study of some years ago by Peter Sims and Therese Wiedmer, they looked at the flow cytometry demonstration of platelet coagulant activity defined by the binding of activated Factor V as a quantitative assay for the role of platelets in coagulation.

In control inactivated platelets where platelet size is shown by the forward scatter, and these are platelets, and the binding

of an antibody to Factor Va is shown on the ordinant. Here is Va binding, and these are platelets that are intact.

Once these platelets were activated in this in vitro experiment by a complement complex, then microparticles were formed, and the distinction in size between intact platelets and microparticles is seen.

The binding to activated platelets of Factor V is increased, but on this log scale, approximately 90 percent of the binding was to the microparticles, suggesting that in platelet coagulant reactions, the microparticles are more effective in intact platelets and raise the question that developed microparticles as surrogate products or artificial products for platelets may be powerful in terms of promoting coagulant reactions.

This could have adverse and thrombotic effects. The data here are not from any of the platelet products that we currently envision, but again, older studies on the prothrombin complex concentrates that are used to treat patients with Factor IX deficiency hemophilia b and other coagulation deficiencies.

It's been known since the advent of these products that there are some patients with risks for thrombosis, and in an in vitro and rabbit study by Alan Giles, the lipid phosphotidyl choline, phosphotidyl serine equivalent of these products in a coagulation thrombingeneration assay was compared to thrombogenicity in a rabbit jugular vein assay, and it appeared with a reasonable Ed Snyder R value that this is a correlation between what is the active thrombotic

potential of these products as a potential risk for clinical thrombosis.

This is not an old issue. The issue last week of Thrombosis and Hemostasis reported a cluster of five thrombotic deaths from a prothrombin complex concentrate.

Turning from the issue of potential risks, which are theoretical, to the potential benefits, which I think are real, we can focus on the fact that very, very few platelets and very limited platelet function is required to mediate successful and safe hemostasis.

Data from years ago from Michigan on patients with ITP suggests what we've heard in several talks earlier this morning, that even if your platelet count is less than 10,000, bleeding by this score, going from zero to minor purpura to major life threatening bleeding is not inevitable, and above a platelet count of 10,000 is distinctly rare.

And we know this from many clinical observations, and I'll give you just two anecdotes from patients we follow with ITP.

This would be a characteristic patient, a woman now 26 diagnosed 14 years ago, has never had major bleeding. The only bleeding she's had is menorrhagia. She had the usual splenectomy. She's required IV IG twice. Her platelet count varies from unmeasurable, as recently, up to a high of 33,000 over a period of 14 years. She's a healthy, active social worker in an Oklahoma hospital, and she testifies to the fact that minimal platelet material is required.

Another patient whom we follow, now 35 years old, diagnosed 15 years ago, treated with all the usual stuff here, again is a healthy, active mother of a junior high school daughter with no bleeding other than purpura, except for three devastating occasions in '94, '97, and '98, when she had intracerebral hemorrhage without thankfully any sequelae and continues to be healthy and active.

The problem then in demonstrating efficacy of platelet products is this. If she had been treated with something to improve hemostasis, it would have been deemed a success every day of the past 14 years or 15 years, except for three days. On three days it would have failed.

These are rare events, but they occur, and were all here because we believe platelets are important.

We've already seen reference in Dr. Slichter's talk to this study of how many platelets are enough in terms of these transfusion trigger studies. I put the data here for the 10,000 versus 20,000 German study, that the 10,000 trigger had 20 bleeding complications, the 20,000 no different.

And, again, you've already heard that when major bleeding episodes occur, they're very rare. They happened to all occur in the 20,000 trigger group, and they seem more related to other issues than the thrombocytopenia itself, as three of the major bleeding episodes occurred in patients in whom we would consider having a safe platelet level.

What about platelet function? I put up here just three selected anecdotes from a review ten years ago on patients with

Glanzmann thrombasthenia, and these are patients specifically selected because they had profoundly abnormal platelet function, absent GP IIb-IIIa, infinite bleeding times, and this first patient here is actually famous for having an isoantibody against GP IIb-IIIa for greater than 15 years, and they have almost never bled.

Now, patients with Glanzmann thrombasthenia can bleed, but these patients are not an exception. Never has had epistaxis or gingival bleeding, transfused twice for a duodenal ulcer, these two brothers with severe thrombocytopenia transfused as a child, transfused once for a hand hematoma, and these are not people with transient antibiotic or aspirin induced platelet dysfunction. They were born this way, and they have lived a lifetime this way, the message being that minimal platelet function is required and redundant systems of platelet hemostatic activity are operative.

So the conclusions then of this part are that the number must be profoundly decreased before bleeding occurs. Function must be profoundly abnormal and even then may occur without spontaneous bleeding, and the good news then for the products is that minimal replacement therapy may be sufficient.

But I think that clinical trials will be required because major bleeding is rare. Therefore, efficacy will be difficult to evaluate, and most patients will not have major bleeding even with placebo.

Thrombotic complications are dangerous potentially, and most patients may not be at risk, but there may be subsets of patients who are more profoundly at risk.

And, finally, I think the benefits from these products can be substantial. The risks are hypothetical, and I think the only way we can gain confidence is by innovative approaches to clinical design of trials which will demonstrate that the benefits are at least as good as platelet transfusions, and the associated benefits of different products may provide substantial advantage.

Thank you.

(Applause.)

CHAIRPERSON VOSTAL: Thank you, Dr. George.

We're going to continue with Dr. Gary Raskob in the design of clinical trials.

MR. RASKOB: Thank you very much, Dr. Vostal, for the opportunity to participate, and I also want to thank my colleague, Jim George. I think I want to thank you, given the controversy I've heard, for getting me involved in this area, which is a bit different to the area I usually work in.

I'm going to start off my presentation by giving the punch line first, and then I'd like to make two prefacing comments, one for the representatives of industry who are here and working at the hard area of developing these products, and the other for FDA.

The punch line, I think, is, to build on what Jim said, in that I think for definitive Phase III pivotal studies, we're going to have to use true clinical endpoints, and I'm going to build a case as to why that is.

Now, for the people in industry, before you switch off or leave the room or get anxious about how much I'm going to cost you

over the next years, I would like to say to bear with me through this because there's some good news for you at the end.

To the FDA, I'd like to say that I've been impressed this morning by how infrequently I've heard the word "safety," and I think one of the key issues is safety because we have to remember the clinical context in which we're dealing with here, and that is the administration of an intervention where the success is judged by preventing a rare occurrence.

When we deal with that setting, the requirements for safety that are posed are much more stringent than many other clinical settings where we have high risks of bad events if we don't give interventions.

So I think that has to also be kept in mind here, and I think I will build a bit on that as I go through.

Now, we've heard the word "surrogate endpoints" many times, and I just thought that as a basis for further discussion I would just like to give a definition of what I think is a surrogate endpoint, and I've chosen the definition that I think is about the best I've found in the literature by the FDA's own Dr. Temple, that we're talking about a laboratory measurement or physical sign used as a substitute for a clinically meaningful endpoint that measures then directly -- the clinical meaningful endpoint measures directly -- how a patient feels, functions, or survives.

There are now well established criteria in the literature and many writings about what is required to establish the validity of a surrogate endpoint, and there are two key criteria. The first is

that the surrogate in its use, the effect of the intervention on the surrogate must predict the effect of the intervention on clinical outcome. It's not enough merely to show a correlation between a surrogate endpoint and clinical outcome.

Importantly though, with a high degree of concordance, there has to be this relationship, that the surrogate, the effect of the intervention on the surrogate will protect the effect on the outcome, and that's very difficult to demonstrate.

Even one of the well thought and well established surrogate endpoints, the open artery in thrombolysis did not do so in recent studies.

Now, even if we can accomplish this, a second requirement is also required, and that is we must capture the net treatment effect, and even if that is possible for effectiveness, I will pose that it will be very difficult for us to capture the harms, potential harms of treatments through many of these surrogate measures.

Now, in the handout material that I've given you, I've listed this reference which I think is an excellent paper by Drs. Fleming and David DeMets, talking about the whole issue of surrogate endpoints in clinical medicine and in clinical trials, and I've taken this diagram from their article, and I'd just like us to consider just briefly reasons why surrogate endpoints may fail.

If we look at Panel B, this is a case of where an intervention may affect the surrogate endpoint in a pathway where the surrogate endpoint is very well linked to the true clinical outcome, but there are many other causal pathways of the disease process

leading to clinical outcomes that are not affected by the intervention, and so overall, the surrogate endpoint, while it may appear that an intervention is very effective, may not predict the ultimate effectiveness of an intervention on clinical outcome.

Conversely, if we look at Panel C, an intervention may not have an effect on a surrogate endpoint, but may impact the clinical outcome through other pathways that are important through which the surrogate does not operate, and so for people in industry who may be making decisions about pursuing or discarding programs and interventions, you run the risk of using a surrogate endpoint and discarding an intervention which may be potentially effective in this scenario.

Finally, an intervention may have impacts on clinical outcome which operate entirely independently of these other pathways, and so at least, for example, some of the potential harms of either drugs or biologic products as we're talking about today, the unanticipated effects may work through these pathways, which aren't known based on our knowledge of how the surrogate relates to clinical outcome.

Now, I'd like to just give a couple examples -- there's many more -- of cases that we now know where it was well thought, based on the biologic knowledge and pathophysiologic knowledge at the time, that surrogate endpoints would be good predictors of clinical outcome.

We're aware of the case of suppression of ventricular arrhythmias as a surrogate for preventing sudden cardiac death and the

cardiac arrhythmia suppression trials clearly show that treatment with the specific anti-arrhythmic drugs, encainide, flecainide, moricizine, resulted in increased mortality, despite the fact that they clearly suppressed arrhythmias.

Secondly, as another example, exercise tolerance was thought to be a very good surrogate in the setting of chronic congestive heart failure, but two interventions which had significant impact on exercise tolerance were shown in these studies here, the study of milrinone in heart failure, here floziquinan in heart failure. Both of these studies showed increased mortality in patients treated with the new intervention.

And of course, tumor size may not reflect the ultimate benefit to a patient in terms of either survival, and certainly may not predict the potential harmful effects of potentially very toxic medications, and we can go on and on and go through many, many other examples which are well outlined in the article by Fleming and DeMets of why surrogate endpoints have not worked in most instances that they were used in clinical trials.

So my conclusion would be that of Drs. Fleming and DeMets, and that is that for definitive Phase III trials, except for rare circumstances which I don't believe are met in the case of platelet substitutes, the primary endpoint should be the true clinical outcome.

Now, Jim's gone through what he thinks and believes are some of the key outcomes, and I agree with that, and I'd like to just again not for the purpose of duplication, but just to highlight a few

methodologic issues that we should look at in terms of clinical trials related to measuring outcomes.

Major bleeding has been used as an outcome in many studies, and I think here there's really not a major problem in my view in coming up with good, standard definitions of major bleeding, and indeed, there have been studies, for example, in the anticoagulant literature which have assessed the agreement between observers and the reproducibility of specific definitions of major bleeding, and those when put to independent blind adjudication panels and those agreements are very high.

Minor bleeding, all I want to say about that is I don't think we should discard that as necessarily unimportant, and it may depend on the degree, and I think here with minor bleeding we have to distinguish clinically overt bleeding, such as epistaxis or mucous membrane bleeding, from perhaps petechiae in purpura, but this may be important to patients, and I don't think we should merely discard that. It's clearly less important than major bleeding.

Thromboembolism will be the least challenging, I think, in terms of coming up with definitions. Those clinical manifestations are often very clear, and we have objective tests that can establish the diagnosis. So I think that that is not going to be an issue.

Infection, I think, similarly will not pose major challenges. Death and cause specific death, on the other hand, may pose some challenges, and so, for example, we saw data earlier reporting the rate of hemorrhagic death at one percent or less, but the overall mortality was significantly higher, and so, for example,

how do we attribute the cause of death accurately and without bias? And this is a very important element that if we're going to use cause specific mortality, it has to be considered in the design of these studies.

I would even ask you to consider the case of a patient who has a very low platelet count, who has underlying coronary disease, who has some bleeding which may not even meet necessarily criteria of major bleeding, and then shortly thereafter dies from myocardial infarction. Did bleeding contribute to that patient's death?

These are questions that require methodologic approaches to control for and to handle rigorously in clinical trials. There are ways to do that, and I think I would caution you about interpreting some of the data in the literature if those approaches have not been applied to those outcomes.

Quality of life, I think, may or may not be important in certain clinical settings. The issues there are that we are getting increasingly getting better at developing techniques to measure that. Will general tools that are available to measure quality of life be applicable to the case of patients who require platelet products, platelet substitutes?

And finally, but certainly not least in this current health care climate, is cost effectiveness, and so we need data from rigorously done experiments in order -- measuring the appropriate outcomes to make valid assessments of cost effectiveness, and is what we're doing worth it relative to the application of those health resources in other areas?

Now, another reason that I wanted to list all of these outcomes was to make the final point on these two slides.

Yes, the primary focus is major bleeding and the prevention of major bleeding, but in many instances that we're thinking about in terms of developing platelet substitutes or derivative products, our goal may be, in fact, to be as effective, but there will be practical advantages, ease of use, and other features.

And so these other outcomes are also important because they will tip the risk-benefit assessment in terms of the relative advantage and disadvantage of certain products versus platelets, for example, and so I think that has to be kept in mind.

Even if we could develop an excellent surrogate according to the criteria I mentioned that reflect major bleeding, these other outcomes may still be important in the decision making.

Now, my last two slides. One is to summarize what I think are the design challenges, and then the final slide would be to at least, I think, make some recommendations that might help us towards thinking about ways to get at this problem.

Measurement of minor bleeding may or may not be important, depending on the clinical context, but certainly the bias that's inherent in the potential to measure minor bleeding. Nevertheless, I think we can develop methodologic approaches to deal with this. It will require some innovative approaches, particularly for studies which are not blinded, if it's impossible to blind studies.

Quality of life really means can existing tools which are general measures be applied to this particular setting.

Number three is really the big issue, I think, and it's the one that's been talked about a lot this morning, and people have made comments about the feasibility and impossibility of studies.

I think we're dealing with in many settings here equivalence trials for effectiveness, perhaps equivalence for safety or advantages for safety, but other practical advantages in terms of ease of use.

We're dealing with rare events. So these are challenging in terms of statistical concepts and design of studies, but it can be done, and it has been done.

Interventional cardiology has been doing this with designing equivalence trials with devices within ranges, type ranges of confidence intervals for outcome events, like the ranges we may be meeting, and so I think we need to step back a bit, and before we make a conclusion that certain trials are impossible or not feasible, we really need to sit down, to look at the assumptions we're making for these outcomes, to try and determine what we might know about what these rates of events are, and we might not know if we really look hard at the literature. We may not have good estimates of what they are.

But I think before we conclude that certain studies are not feasible, we really have to run the calculations looking at innovative approaches to developing the sample sizes, and much work has been done in the past 15 or 20 years in the statistical area of equivalence testing.

I would also say that if we consider all of those outcomes that I listed, that given the benefits we may have on one or more of the outcomes other than major bleeding, this may give us more variance in the range we would accept in our definition of what is equivalence on major bleeding.

So it's a balancing of advantages and disadvantages, and I just don't think that simple surrogate endpoints will give us the information we need to make those decisions.

So my recommendations would be that we have to use true clinical outcomes for the pivotal studies. We should develop standard bleeding definitions so that we've learned a tremendous amount in clinical trials of anticoagulant therapy of how we lost tremendous information early on from non-standard definitions of bleeding, and if we can have standardized definitions of bleeding that can be used consistently in different trials, this then will make us much more able to use tools later on, such as meta analysis, for example, which may be needed at some point.

Sorry. Okay. It didn't like what I was saying, I guess.

The final point is this. I think we also have to be innovative in how we approach this from a drug regulatory point of view or a biologic regulatory point of view, and I think that there is not much to be gained at least at the present time with our present state of knowledge from small Phase II studies with surrogate endpoints.

And so I think why should we make the sponsors in industry go through the constant expense of that type of study for lack of

information that we're going to get, and I would propose that we go to, for lack of a better term, combined Phase II/III trials where the outcomes are provided right in the very early stage, and where innovative approaches are used in special monitoring of sequential testing that may allow us to discontinue one arm or the other at an appropriate time during the study if certain conditions have been met.

A lot of work has been done in that area as well, and so I think with those comments in mind, I would like to end off by saying that we could benefit a lot from stepping back from the problem and perhaps putting at least as much energy and focus into developing innovative trials in developing these designs as we are into the concept of surrogate endpoints.

After all, we had this when we were meeting like this in '81. So it's at least worth some intense efforts at trying to solve the problem rather than saying the studies are impossible and we can't do it.

Thank you.

(Applause.)

CHAIRPERSON VOSTAL: Thank you, Dr. Raskob.

So if we could have the three speakers from this session come up to the podium for the question session.

If we could keep the questions and comments kind of short, we're running a little bit over.

DR. SLICHTER: Slichter, Seattle.

A couple of questions for two different speakers. Ed, the first one is directed to you. You mentioned the labeling with biotin,

that a lot of people, in fact, may have antibodies to biotin for a variety of reasons.

Was there any evidence to show that having an antibody to biotin, in fact, affected the survival of biotin labeled platelets?

DR. SNYDER: No, and that point was made. I'm sorry if I didn't emphasize that. The conclusion of the studies was that it would not affect the labeling in any way, but may have some effect more on assays that involve biotin which would be unrelated to what you're referring to.

So it appeared to be true, true, and unrelated.

DR. SLICHTER: Okay. Thanks.

DR. GEORGE: But if I could add something, since those studies were done by George Dale at Oklahoma. I think even though the data are not published, he's not done platelet studies in humans, but he's done red cell studies in humans, and we got very extreme differences in terms of red cell survivals, and that's what triggered the search for anti-biotin antibodies in the first place.

DR. SLICHTER: Okay.

DR. GEORGE: Was to provide an explanation for why the red cell survival seemed quite different in me, for example, versus other laboratory workers.

So I think it's still open that the antibodies could impact survival.

DR. SNYDER: Was there a correlation with decreased survival?

DR. GEORGE: Not clear.

DR. SLICHTER: Okay. The next question relates to the clinical design of the studies that you were discussing, that you thought instead of using surrogate markers we should go to Phase II/III combined clinical trials and use a, quote, innovative approach.

Do you want to help us with what you think that -- I mean, that's -- do you want to help us with what that innovative approach might be?

MR. RASKOB: I think the innovative approach is to do that because it has -- historically the way we've done development traditionally has been Phase I, II, III, IV, and all I was trying to say is I don't think we should lock into that.

I think in this context there's very little to be gained by small Phase II studies. In fact, there's tremendous risk for making wrong decisions about pursuing interventions further due to lack of effect on a surrogate.

So I think we need to bring clinical outcomes once we get out of Phase I into the studies very early on, and it really then becomes an issue of being able to sequentially monitor those with known techniques to allow a certain intervention or regimen to be discontinued or once we have evidence which may be clear, for example, in safety endpoints.

DR. GEORGE: And if I could piggyback again on Gary for that, maybe help interpret what I understand Sherrill's question.

What I've taken from what Gary has taught me is that the key issue here is that you don't have to prove that anything is better than a platelet transfusion. You have to demonstrate the equivalence,

that it's as good, and with rare events, you may just have zero or one or two events in each arm, but if you can demonstrate this concept of equivalence, there may be in the substitute products so many more advantages that that will make it a preferable option or an improvable option.

DR. SLICHTER: I'm not a biostatistician, but having participated in the CMV study where we were trying to show equivalence between CMV seronegative and leukoreduced white cells in transmission of CMV by transfusion, I mean, the number of patients that we had to get in the sample size was enormous. So --

MR. RASKOB: What was it?

DR. SLICHTER: -- I must not be -- what?

MR. RASKOB: What was the sample size?

DR. SLICHTER: Like 250 patients in each arm because there was a low event, you know, of transfusion in CMV seronegative products. So if you're trying to show the same benefit by a leukoreduced product as a CMV seronegative product, when that is a very low event, the sample size was very big.

So I guess I don't track how we're being helped by showing an equivalent study in a low event situation.

MR. RASKOB: Well, I'm enheartened because I thought you were going to say 40,000 patients.

DR. SLICHTER: Well, but if you talked to --

MR. RASKOB: But 250 patients per arm is not a big study.

DR. SLICHTER: Well, but if you think you're helping the manufacturers in this audience by telling them that they need a sample size of 500, I think most of them have already passed out.

MR. RASKOB: But that is what's required to get the scientific answer, and the issue is these are not rare disease areas where these products are being used. So as I understand it and in some of the contexts I've seen earlier, it's certainly not -- you know, if we were talking about ITP, a randomized study of 500 patients is a challenge. We're challenged to do one of 100 patients.

DR. SLICHTER: Well --

MR. RASKOB: But if we're talking certain other settings, these are not infrequent patients.

DR. SLICHTER: I mean, we have people who develop very low platelet counts in a variety of clinical settings. So we've got lots of patients, but still the costs of doing a study and the time required to do that for a sample size of 500 is -- I mean, the TRAP trial was about 500 patients. It took us three or four years to accrue and complete that study.

So, again, I think, you know, if the manufacturers haven't passed out, they're not going to make it back after lunch.

MR. RASKOB: It's going to require networks and multi-center studies, but studies in the range of 500 to 1,000 patients in this situation should not be unfeasible.

DR. SCHIFFER: Let's get back to the real world. I mean that was very, very eloquent and perhaps correct in a theoretical sense, but I think that the reason that this is a problem is not

because the participants recognize that a Phase III clinical trial is not possibly the gold standard, but also the recognition that it's extremely difficult to do for any variety of reasons some of which I'll mention.

You didn't pay us the courtesy, for example, of even doing a sample calculation on what it takes to eliminate a one percent death rate, controlling for the many clinical variables that are associated with it.

One reason for doing Phase II trials and one reason you're going to have to do Phase II trials is you're comparing potentially the removal of an effective therapy, and there's no way on earth that you're going to convince physicians to remove an effective therapy without some evidence that's not pre-clinical, but is clinical, of a hint of efficacy.

Phase II trials and Phase I trials are terribly imperfect in that regard, but we rely on them for some suggestion of efficacy before we're willing to remove effective therapy from people, and here you're talking about not Phase II trials in cancer where there is no effective therapy, but where you have a very good therapy, that is, therapy that produces a less than one percent mortality rate.

The other reality is that this is an uncommon disorder. We have six or seven potential platelet products out there. That doesn't even include the ones that are real platelets. The general population are patients and adults with acute myeloid leukemia.

I chaired a cooperative group that did trials in this area. It takes us four to five years or three to four, depending on

the question, to accumulate three or 400 patients for questions that we consider to be really relevant to advancing the therapy of the disease, not equivalence questions.

We rarely would countenance doing studies that addressed equivalence questions because the resources and the expenses that are associated with it were so great, and therefore, we attempted to put our energy towards questions that we thought would advance treatment rather than questions that would make things approximately the same with one less case of hepatitis at the other end.

I think that's why there is this interest in surrogates, because of the frustrations and the expenses associated with organizing large trials. It's not out of ignorance, but it's perhaps out of necessity.

Now, it may very well be that we'll hear at the end of the day that the surrogates all stink and we'll be back to square one, but there has been a considerable amount of thought, I think, on participants in this field about the ideal way to do such studies.

MR. RASKOB: Yeah, to address those three points, first of all, I didn't want to in any way give the impression that I thought there was not an adequate amount of thought by the participants. Clearly they've been working in a very difficult area, but if we take the three points you made, I didn't present a sample size calculation because that requires, I think, more details about assumptions of what event rates are important to exclude or not, depending on the different clinical contexts, and you put exclusion of a one percent

mortality, given the potential other benefits that may or may not be the appropriate boundaries.

And so those assumptions can profoundly influence the sample size. I just simply wanted to make the point that I think that we should at least run some of those calculations for different settings and look at whether it may be feasible.

The second point about removing ineffective therapy can be done as efficiently or better by a Phase II/III hybrid study as it can by a Phase II alone. So we lose nothing there by going to that type of design.

And the third point is that even if what you propose is correct and we can develop surrogates, remember if we're talking about rare events, we'll need sufficient patients to evaluate significant safety issues, such as thromboembolism.

So, for example, if we save one major bleed per 100 or five per 1,000 but we cause a stroke, are we benefitting people by substituting new interventions?

So those type of things need to be, I believe, at least attempted to be studied. We're not going to answer all questions with one study, but we want to try and study those using experimental methodologies that measure those outcomes.

DR. SCHIFFER: One last comment about the surrogate. In fact, if we take that logic to its extreme, we have no proof that platelet transfusion works, and if we want to put that on the table, I doubt that most clinicians would be willing to. I certainly wouldn't.

But, you know, it's taking things back in time. If you want to make the assumption that platelet transfusions have some effectiveness, then you do perhaps have some sort of target to go at because I think taking what you're saying all the way to its logical extreme really means that because we have never actually ever studied platelet transfusion, period, with the rigor with which you're demanding.

MR. RASKOB: Well, and if the event rates are as rare as you propose or others propose, then maybe that is what needs to be done. So I don't think that is an extreme, and I think the reason a number of the people are developing these products is for the tremendous practical advantages, and maybe what is needed to be done is whether there is a real benefit of platelet transfusion in a definitive trial.

CHAIRPERSON VOSTAL: We're running about 20 minutes over. I'm wondering if we could take just one more question, and I'll take Dr. Murphy over here because we haven't heard from him yet.

DR. MURPHY: Well, it's not just one question unfortunately. I think that what I want to say is coming to the defense of surrogate endpoints, as well.

This field has not done badly because of the correlation that's been observed between results in thrombocytopenic patients with radioisotopic studies, and the radioisotopic study is clearly a surrogate endpoint for a clinical event.

I have to come to the defense of in vitro tests relative to in vivo tests and disagree strongly with my friend Ed Snyder with

the way he interpreted the paper in Transfusion that I was a co-author on with Stein Holme and Gary Moroff.

It is true that there's a low R value between hypotonic shock response and extent of shape change and radioisotopic studies, but that derives predominantly from the fact that if you label fresh platelets from ten or 20 normal individuals, there's a tremendous variation in the in vivo recovery related, I think, to miscalculations of the blood volume and differences among normal people in the size of the splenic pool.

If you do the kind of paired studies that you suggest with in vitro studies, like hypotonic shock, you'll find that you can discriminate small differences just as you can with paired studies with isotopic techniques.

Also, in defense of surrogate testing, I think that the risk of thrombocytopenic bleeding in patients who are severely thrombocytopenic for a significant period of time, I mean zero and one, which would be characteristic in marrow transplant if we didn't transfuse them, is really pretty high. I think there's a lot of feeling here that this is not a risky situation.

And when I hear about ideas about not giving platelet transfusions to see whether they're really needed in situations like that, it makes me very frightened, indeed.

Finally, just going back to the in vitro tests, I think one thing which I think the conference will not deal with is I think we do an extraordinarily poor job in quality control in our platelet products in the real world. All we do is measure pH and platelet

count in one out of 100 products, and I think we're not doing anything to really assess the quality of what goes out of our blood centers.

And I think things like swirling, imperfect as they are, or some other in vitro studies, I think, would be a step forward.

So my question, Ed: you don't really mean that about our --

DR. SNYDER: Oh, no, I didn't really mean that.

I guess the point is if you realize that there are deficiencies with some of the assays, and there are things you can do to minimize that and maximize the information you get, such as the modifications you mentioned, that's appropriate.

What I was doing was evaluating the data that had been published, and I still think that it's sort of like bricks in a wall; that if you put together all of the in vitro assays, and they're all basically showing a positive result, that gives you a pretty good confidence that you can move on to the radiolabeling level, that you could have a product that actually works.

So I think there is a lot to be said for in vitro assays. Looking at them as an individual, you're on weaker ground, but I agree with your comments, and I didn't mean to over-interpret the nature of that.

So thank you for that.

CHAIRPERSON VOSTAL: Okay. I'd like to thank the speakers for their discussion, and I'll hand the chair over to Dr. Tom Reid.

DR. REID: Let me make a suggestion that we break for lunch now and come back in an hour and a half.

All those opposed, raise your hand.

(Laughter.)

DR. REID: Okay. It's now noon. At 1:30, that was the original schedule. One hour? Okay. One hour.

(Whereupon, at 11:59 a.m., the meeting was recessed for lunch, to reconvene at 1:00 p.m., the same day.)

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

(1:01 p.m.)

DR. REID: If we could all start getting in our seats so that we can resume. All right. It's all in order. Okay. If you could ask the people out in the hallway to come join us.

Okay. My name's Tom Reid. I'm at the Walter Reed Army Institute of Research, and I'm happy to chair this next session on animal models, looking at platelet function and survival.

Before we get started, though, I want to address an ignored population from this morning's discussion, and that's the trauma patient, and clearly that's what DOD is mostly interested in, is trauma.

The question you may ask: are platelets important in trauma? And I think in the cosmos as defined by Penrose and Hawkings, I think just about everybody would say yes. Maybe a handful that would disagree, but I think all of the surgeons you talk to and just about anybody else would agree that platelets are important in stopping the bleeding associated with trauma.

What's the DOD interest? Well, pretty much fourfold. One, logistical issues. We can't get platelets to where we need them. When we do need platelets, we take whole blood, but we call the walking blood bank, and that has its own problems.

The thrombocytopenia that we see in trauma patients is clearly less severe than you've all heard about this morning, but there are problems with the thrombocytopenia in the surgical patients. This can be dilution, dilutional thrombocytopenia, or consumption.

Dilutional thrombocytopenia really is not a big problem. It's really the consumptive thrombocytopenia. This can occur because of the extent and the location in the body of the trauma, the extent and location of surgery and debridement in the patient.

These patients have a the shortened lifespan in the circulating platelets.

There's something called diffuse microvascular bleeding that occurs postoperatively in these patients. The patient may have been heroically salvaged just to find out a couple of hours later just from about every orifice they are bleeding. This is associated with the consumptive process.

There are clinical guidelines for target platelet counts in trauma, and depending on what kind of trauma you have, there are different targets. This initially came out of our experience in Southeast Asia, but has been extended to the recent guidelines by the anesthesiologists' societies.

This morning we heard about the prevention of spontaneous bleeding, but what I'd like to have you consider in addition to the prevention of spontaneous bleeding from thrombocytopenia is the treatment of bleeding associated with trauma and in surgical patients.

So a couple of questions I'd like to throw out. What are the aspects of platelet function that are important to stopping bleeding? Dr. George had addressed this in part this morning. I guess one corollary question would be: to prevent spontaneous bleeding in our cancer patients, would a platelet substitute just

having a membrane that could serve as a surface for clotting proteins, would that be sufficient?

Another corollary question: is the product required to prevent or stop mucocutaneous bleeding in severely thrombocytopenic patients the same as that required to treat the bleeding complications of our surgical patient?

So with those couple of questions and that background, I want to introduce and invite Dr. Morris Blajchman to come up and talk about his rabbit models.

DR. BLAJCHMAN: Thanks, Tom.

I'm pleased to be here and tell you about some of our studies. Some of the data I'll present I've presented elsewhere, and in fact, we've recently published a review of some of the whole area of platelet substitutes and novel platelet products. So I'll try and highlight the work that we've done in this area.

Now, apropos of some of the discussions that took place this morning, I feel that the ultimate reason to transfuse platelets or platelet substitutes to a bleeding thrombocytopenic patient is to improve their hemostatic function. I think patients with thrombocytopenia are at increased risk for bleeding, and it's important to correct that, and this is a self-evident truth, and I think the fact that some patients with low platelet counts don't have severe bleeding episodes doesn't mitigate against this reason.

Now, what I want to do over the next half hour or so is to describe some of the work that we've done using a thrombocytopenic rabbit model. This rabbit model has been used in my lab at McMaster

University for about 15 years or more to evaluate hemostatic efficacy of various substances, hemostatic substances like corticosteroids.

We've also looked at platelets and more recently at platelet substitutes. So I'm going to review this work with you not because you're not aware of it, but to point out that -- and this is relevant to the discussion that took place about the clinical use -- the need for clinical trials.

I feel very strongly like Gary Raskob that there is a need for clinical trials. Whether one can do those clinical trials effectively remains to be seen because of the sorts of patients that thrombocytopenic patients usually are.

There are ethical issues of using new products when you have a starting product that's efficacious, but at least at the preclinical level, I think that the sort of model that I'm about to describe may be useful at least as a screening test to determine which products should go on to clinical trial, and I won't get too involved in the issue of how to do that clinical trial.

Now, basically we're dealing with the treatment of the management of the fractory thrombocytopenic patient. There are a whole host of treatments that are available to these patients because if they're not refractory or alloimmunized, then you can use standard platelets.

But if you have a refractory thrombocytopenic patient, then you have to use a variety of interventions that may or may not be useful. HLA or cross-matched compatible platelets have been shown by some to be useful. ABO compatible platelets have been shown to be

useful. There are techniques to remove antigens from platelets that can cause them to be nonrefractory.

In addition to that, there are a whole host of pharmacological agents that can be used to treat bleeding episodes in thrombocytopenic patients or even prevent bleeding: EACA, DDAVP, aprotenin.

Corticosteroids was mentioned by Sherrill Slichter this morning as enhancing at least the GI blood loss. In our hands corticosteroids correct the bleeding time in thrombocytopenic rabbits, and similar clinical data are available in the literature.

Estrogens have been used over the years.

Now, where these various agents should be used in the context of the management of such patients is unclear, and whether, in fact, in some instances, whether these patients -- these interventions work at all is clearly not established yet.

Now, the other things that we can do is in the refractory thrombocytopenic patient who has impaired hemostasis, we can raise the hematocrit in the anemic patient. I'm going to show you some data on this.

Then there are a whole host of novel products that are platelet derived. These include platelets that have been cold stored so as to prevent some of the problems; frozen platelets. Lyophilized platelets have been used. There are some very interesting developments and close to clinical use at least over the next couple of years of psoralen UV treated platelets.

So those are platelet related products, and then there are other products that are either platelet derived, like infusible platelet membranes, and an interesting product manufactured by two companies now, one in the United States and one in Britain, and you'll hear from -- I'll talk about the American preparation, and you'll hear later this afternoon about the British preparation of fibrinogen coated albumin microspheres, and then there are some liposome based agents that I really won't spend any time talking about because there's very little written about them other than the ones that have not been efficacious.

So what I'm going to do is show you some of the data that we've accumulated over the last five years or more showing you some of these effects in our rabbit thrombocytopenic model.

Now, what we do in this bleeding time model is we initially make the rabbit thrombocytopenic, and this has been done by gamma irradiation of the rabbits. The rabbits subsequent to the gamma irradiation are injected with a heterologous anti-platelet serum made in sheep, and this combination of intervention produces a profound thrombocytopenia where better than 95 percent of the rabbits that we have after they've been irradiated and injected with the heterologous platelet antiserum have a platelet count of less than ten.

We then put the ear into a saline bath and Kepta 37 (phonetic), then make an incision to avoid macroscopically visible vessels.

We then reimmerse the ear into the saline bath, and then we watch for visible bleeding, and then we do two determinations to determine -- to make a determination of the bleeding time.

These is a cartoon showing this. The 930 rads or centigray followed by sheep anti-rabbit platelet antiserum.

This is a plot of the platelet count in a group of rabbits where we've done this. So irradiation takes place on day zero. The platelet count starts dropping at around day four, and at about this point in time -- it varies a little bit from experiment to experiment. It's not crucial when you inject the platelet antiserum, but the platelet count may be between 50 and 100,000. When the inject the platelet antiserum, the platelet count drops to less than 10,000.

And then around day 11 -- it varies a little bit from rabbit to rabbit -- the bone marrow starts functioning again. The dose of irradiation that we use is not a lethal dose of irradiation. It's a sublethal dose.

Now, this is a picture of a rabbit. The rabbit is anesthetized, and you can see a platform with a hole in it, and the rabbit ear is in the saline water bath. This is a band to keep the temperature of this saline bath at 37 degrees. There's a magnetic stirrer on the bottom. You can't see it in this picture. That stirs the fluid.

The next thing is a close-up, and you can get a sense of the flow of blood away from this incision, and actually using this approach, you can readily see the endpoint of the bleeding time.

Several in the room here have been to my lab and actually have seen some of these things and could have seen it in operation.

Now, one of the things that we evaluate -- in fact, this evaluation was done quite a number of years ago, and Jim George was involved in these determinations, we looked at the hemostatic function of young and old platelets, and the main reason that I'm showing you this data is that we can differentiate using this bleeding time technique the hemostatic function differences between young and old platelets.

What we did, and this shows you rabbit platelets have a smaller medium size than human platelets, approximately half, and you can see following irradiation, the size drops, and when recovery takes place, they're quite large.

This are old platelets because the narrow shut down. These are young platelets because the marrow is just regenerating. So taking platelets from the rabbit or doing bleeding times during this point and this point will give you the -- show you the difference between the hemostatic function of young and old platelets.

And this essentially is shown here. The yellow dots are the bleeding times as a function of platelet count for old rabbit platelets shown in yellow and young rabbit platelets are shown in blue.

And clearly you can show that young rabbit platelets have better hemostatic function. So in a platelet count of, say, somewhere around 50, young platelets will have a bleeding time of somewhere around, between two and 300. The corresponding -- sorry. It should

be going this way -- the corresponding hemostatic function or bleeding time for old rabbit platelets will be approximately 600 to 700 seconds.

Now, I want to show you the data that was mentioned this morning by Jack Levin that shows the hematocrit and bleeding. This was published in the British Journal of Haematology three or four years ago, and as you lower the hematocrit of bunnies -- this is done in normal, non-thrombocytopenic bunnies -- you can see that the bleeding time gets prolonged.

So a hematocrit of .2, the bleeding time might be about 250, 300 seconds. At a normal hematocrit of around .4 or 40 percent, the bleeding time is around 100 seconds.

So hematocrit makes a difference to the bleeding time.

When we were doing this study, we wanted to check out to see whether the hematocrit makes a difference in thrombocytopenic animals, and in this study we made some bunnies moderately thrombocytopenic. In fact, we didn't inject any platelet antiserum. We simply irradiate them, as I remember, and what you can see, a difference between the bleeding time in bunnies who are anemic, and the only impact here is the platelet count. You can see a marked elevation in the bleeding time, almost a doubling of the bleeding time when the platelet count is around 90,000.

When you raise these animals were then transfused up, and if you transfuse up the animals, you can see you can significantly at the same platelet count, significantly shorten the bleeding time.

So hematocrit plays a role particularly in the thrombocytopenic. I think clinically this type of evidence is not used very often. We tend to let thrombocytopenic patients remain anemic, and sometimes it's useful, and this has been shown clinically. You can raise the hematocrit and improve the hemostatic function.

Now, I'm going to turn from that into a variety of alternatives to conventional platelet concentrates that we've looked at, and these include frozen platelets, cold stored platelets, lyophilized platelets, IPMs, and some non-platelet derived substitutes.

But, first of all, I have to tell you about what happens to human platelets when they're infused into rabbits. Well, what happens is shown here, and if you take normal rabbits, infuse platelets, the platelets essentially are gone within ten minutes.

So what we did then is use a maneuver which blocks the RE system using ethyl palmitate at a dose of one gram per kilogram. This doesn't completely block the artery system. You can use higher doses, and splenectomy doesn't make a difference.

And if you do that, you can prolong the survival of human platelets in the rabbit to about six to eight hours, and during this period of time, if you take a thrombocytopenic bunny and inject the bunny with human platelets, you have the majority of platelets that are in the circulation are human platelets, and you can measure the hemostatic function of those platelets.

So when we do assess the hemostatic function of human platelets, we use the following protocol. On day minus ten we

irradiate. On day minus one in the morning, we inject the platelet antiserum. The same day minus one in the afternoon we inject the ethyl palmitate, and then the next day infuse human platelets.

We vary this a little bit for convenience purposes or for other purposes. So these can be done at day eight and so forth.

We always take a blood sample for platelet count at the time that the ear bleeding time is performed, but we don't know what the platelet count is until after the bleeding time is done. So essentially the platelet count -- the bleeding time is done by an operator who doesn't know the platelet count of the rabbit at the time that the bleeding time is being done.

This is what fresh human platelets looked in this microvascular, and I used the young and old, old and young platelet curves to show that. So each of these dots represents the infusion of liquid stored or sorry. Actually these are fresh. So within 24 hours human platelets that were infused into the body, and they fit very well where you would expect them to. At least I do.

We've also stored human platelets, and you can see stored them for five days, and the various symbols represent storage and essentially, again, the human platelets have hemostatic function at the sort of place where you'd expect them.

We've also done cold stored platelets, and in this model we're able to show that one day old, four degree stored platelets in the liquid state function normally. However, platelets stored beyond 24 hours do not function at all.

And incidentally, we abort all of the tests after 15 minutes. So 900 seconds, it could be -- in fact, we've done quite a number of rabbits, probably about 20 rabbits now, where we've done bleeding time in thrombocytopenic rabbits and didn't stop the bleeding time at 15 minutes, and they will go on to bleed for about an hour.

We've looked at frozen human platelets, and this is some human platelets that have been stored in six percent DMSO. We've used other doses of DMSO that equally show similar sorts of effects.

We've done experiments, but I'm not at liberty to tell you because of confidentiality agreements. We've looked at frozen platelets that have been produced in other ways, and these and some of the other methods that have been used do not show functionality, hemostatic function in this model.

I won't show you any data. We've looked at cold stored platelets, and every type of preparation of cold stored platelets that we've looked at have not shown hemostatic function.

One of the interesting things we've done is worked with various companies or institutions that have tried photodynamic methods for the inactivation of viruses, particularly psoralen UVA radiation. There are companies that we've worked with, UVB, this agent, methylene blue, and phthalocyanines. I'll only show some data with one psoralen and UVA irradiation, and that's shown in this slide, which shows the hemostatic function of AMT-UVA treatment in human platelets that have been stored for five days.

This is the experiment, the platelet an N of ten, that no treatment was administered and the mean bleeding time for a platelet

count of 159 was just under 200 seconds. Just giving AMT or just giving the UVA gives similar bleeding times, and the combination of AMT and UVA does not appear to affect platelet function as assessed in this animal model.

When we're doing these experiments, we did one experiment in which this data -- you've raised the data you've just seen -- but we did one experiment in which we tripled the dose of UVA in this one experiment in 15 animals, and you can see when we did that, we achieved a reasonably good platelet count, but the hemostatic function was about threefold, two and a half-fold higher, at least the bleeding times, compared to the untreated or the conventionally treated UVA-AMT treated platelets.

We've looked at other preparations of UVA and psoralen, and they work equally well.

Now, we've also done some work with lyophilized human platelets in collaboration with Art Bode, and lyophilized platelets basically are fixed to paraformaldehyde. They're frozen and then lyophilized. These platelets when reconstituted are morphologically intact.

You will hear some more presentations about this this afternoon. They shorten bleeding time in thrombocytopenic rabbits, but not in VW diseased dogs.

Now, we've given various doses of these lyophilized platelets to the rabbits, zero, one, 2.5, five, and ten times ten to the ten per rabbit. As a control for this experiment, we gave 2.5 times ten to the ten platelets in the form of platelet rich plasma or

platelet concentrate actually, and you can see that the bleeding time -- there's a dose response curve, but the comparable dose of lyophilized platelets to the liquid stored human platelets, it's much greater.

This gave us a bleeding time of somewhere around 250 seconds. The same dose of lyophilized platelets gave us a bleeding time over 500 seconds. So while you see a correction, the correction is not equivalent to that seen with fresh or liquid stored platelets.

We've also done some work with infusible platelet membranes. We haven't done a lot of work with this product, but we have done some recently, and somebody went through this in their presentation this morning. It's basically prepared by freeze drying of outdated platelet, human platelets.

There have been Phase I and Phase II clinical studies that indicate some hemostatic activity in some, but not all refractory thrombocytopenic patients. It's interesting. This is a virally inactivated product that has a shelf life of three years.

What you can see here is this is the mean platelet count in 12 rabbits that were given either two milligrams per kilogram of IPM or four milligram per kilogram of IPM, and you can see a shortening of the bleeding time compared to greater than 900 seconds, and at a dose of four milligrams per kilogram, we got a platelet count of 456.

This is approximately equivalent to a platelet count for liquid stored human platelets of about 50,000. These two numbers are not statistically significantly different.

So we're getting function four hours after the infusion of these IPMs. That gives you in a rabbit that has a platelet count of, say, around 10,000 the equivalent hemostatic function to liquid stored human platelets that in rabbits that would have a platelet count of somewhere around 50,000.

In the last part of my talk, I'm going to describe some of our experiments with fibrinogen coated albumin, microspheres. These Thrombospheres, which is the company trade name for this material, is an albumin microsphere about one micron in diameter, which have covalently linked on the surface fibrinogen.

In the experiments that we've done, we've used controlled spheres. These are spheres that were also albumin microspheres, but do not have the fibrinogen at their surface.

So we've done experiments with these Thrombospheres using either controlled spheres or saline.

This is what these spheres look like under the electromicroscope. The mean size is about one micron, but you can see some smaller spheres and occasionally some rather large spheres.

When you do platelet aggregation in a mixture containing Thrombospheres and platelets, you see that the platelets co-aggregate with the spheres. The Thrombospheres by themselves do not aggregate these plates but need -- co-aggregate in the presence only of a platelet agonist.

This is a dose response curve of increasing doses of Thrombospheres in thrombocytopenic rabbits. In these experiments we did not use ethyl palmitate. There was no need, we felt, to RE block

these animals. So these are thrombocytopenic animals prepared by the combination of irradiation and heterologous platelet antiserum, and you can see a nice dose response curve.

For comparison, this is the bleeding time produced by an equivalent dose of one day old human platelets.

Interestingly, we have shown after a single dose of Thrombospheres we have an effect that we see at one hour, 24 hours, 48 hours, and even at 72 hours. The controlled sphere, which are in the yellow triangles, does not produce an effect, nor does the saline.

And you can see the N for these experiments is quite significant.

Now, one of the most interesting aspects of these studies is what is happening to try and figure out what's going on. We learned very early on that the Thrombospheres do not stay in the circulation for very long.

Now, this is to say we took some of these Thrombospheres and labeled them with I-125, and this is the recovery in the circulation of the labeled Thrombospheres.

First of all, only less than ten percent of the infused Thrombospheres stay in the circulation, and as you can see, they bounce around a little bit. If I put some more points, you'd see some bouncing, and then they're around for between one and about 16 hours, and by 24, 30 hours, they're gone from the circulation.

So there's no Thrombospheres in the circulation after about 30 hours. We can't protect them.

They're not in the blood. We start seeing radioactivity in the urine and feces, but most of the Thrombospheres stay in a noncirculating compartment.

I'm going back to the previous slide because essentially what I'm saying to you is that there are Thrombospheres around the circulation when we do bleeding times here. There are some, but very few present here, but essentially there's no Thrombospheres in the circulation at 48 hours or at 72 hours. Yet we're getting this hemostatic effect.

What's going on? Well, I don't know is the answer, but I've got some clues. This just summarizes what we know about these fibrinogen coated albumin microspheres. They shorten the microvascular bleeding time in thrombocytopenic animals. We've done some experiments where we've done some standard -- created some standard wounds in the years, and we've shown that they decrease blood loss from a standardized wound.

The hemostatic effect is clearly dose dependent and appears to persist for up to 72 hours. These FAMs are fibrinogen coated albumin microspheres do not aggregate resting platelets, and in work that we've done with John Vickers in our institution, we have shown that these Thrombospheres enhance ADP stimulated aggregation in vitro.

We, however, do not detect these microspheres in the circulation at 30 hours, but we've been able to show that 48 hours after their infusion into normal animals, there was decreased platelet

PIP2, which is a phosphyl inosatol phosphotitle serine, which is detected on ADP stimulation of isolated platelets.

To the experts that know about these things, this effect suggests that there's an enhanced platelet response to agonists which persists even though the FAMs are no longer in the circulation. This in some ways would be analogous to what Ed Snyder described that occurs with MGDF administration or thrombopoietin, and this may be the same effect that we may be see, a similar effect that we may be seeing here.

Just to try to deal with this, we've done what I consider an interesting experiment. We have treated animals with either controlled spheres or one preparation of Thrombosphere. Just pay attention to this.

And so these are normal animals that have been treated either with controlled spheres or two doses of Thrombosphere. We then follow this with an in vivo infusion of ADP.

This infusion of ADP, if you take normal animals and infuse ADP, you get a drop in platelet count, and the drop in platelet count is in response -- basically what you're getting is in vivo aggregation.

When you infuse controlled spheres or saline -- we've also done many experiments with saline -- you get an infusion following the ADP infusion of about 30 to 35 percent, the drop in platelet count.

When one looked at the fibrinogen coated spheres, the first preparation at 6 mLs per kilogram, we got a 63 percent drop in

platelet count, suggesting that those platelets are more reactive to ADP.

A lower dose of the same preparation produced the 48 percent.

We then took a different preparation of these Thrombospheres, a preparation that was not very effective in vivo in correcting the bleeding time, and that preparation when we did the same experiment produced the 40 percent drop in platelet count in response to in vivo ADP, and the lower dose produced virtually no drop in platelet count in response to ADP.

Now, I think what we need to do is we need to do some more -- we have a lot of work to do in this area, and one of the things that we need to do, we need to determine how the hemostatic function of novel platelet products and substitutes need to be evaluated, how they work, and they may work by ways -- even though they're designed to work in one way, they may work in a way that isn't expected, and this is true, I think, for the Thrombospheres.

We need to ascertain how to provide -- some criteria need to be established to provide safe and hemostatically effective platelet products and substitutes that don't have some potential side effects like thromboembolic phenomena, produce thrombocytopenia, and so forth.

We need to obviously study the putative mechanisms of these platelet products and the substitutes, and we need to also establish the effectiveness of some of the other approaches to the treatment of thrombocytopenic patients and the role that they play in

the treatment of patients like correcting the hematocrit, like the use of estrogens, DDAVP, et cetera.

I think I'll stop there, and I think that was my last slide.

Thank you very much for your attention.

(Applause.)

DR. REID: I'd like to invite Dr. Harker to talk about his baboon model.

DR. HARKER: This is an interesting cohort of people. It's a grey cohort by and large, which tells us that it's been the same cohort that's been going to these meetings for this question for some time.

This slide shows data that are by and large 15-plus years old, and as my companion at dinner said, "Oh, I remember your work from when I was a student." And he's not a young man.

(Laughter.)

DR. HARKER: So I think this is a perplexing problem that we clearly have not come to terms with, and the issues as I see them is that -- different slides -- the difficult is if we're going to try and deal with spontaneous events, then we're going to be looking at the ceiling function of platelets, and if we're looking at that, then it's going to require very low platelet counts or equivalent.

And the discussion that Sherrill had this morning about a selected number of patients where you might actually obtain data and get objective evidence for efficacy and then decide what kind of trials might be required if such be the case.

The second thing is if we're going to have a provocation model, it certainly has to be a platelet dependent model, and that having just any kind of bleeding episode is not going to do the trick, and perhaps that is well illustrated by the dilemma that one has looking at bleeding times in rodents by cutting off the tail.

That's not a very platelet dependent process. It's a coagulation dependent process. So it's important to be able to focus upon an issue if you're looking for efficacy on a process that is truly platelet dependent that you can test for.

As the day went on, I kept taking more and more slides out, and it looks like I just about got rid of them all.

The ceiling function of platelets I just wanted to illustrate for you as shown here with this Baumgartner photo micrograph in which a small blood vessel with endothelial lining is shown with the red cells within the lumen and at a site of widened intercellular junction that the platelet has neatly sat down to provide that ceiling function, and it doesn't take very many platelets to do that, and this is the source of that consumptive component involved in platelet survival time.

Well, if you're going to look at non-human primates, the advantage is that you can use the same probes as you do in humans. You're going to do flow cytometry, for example. You have all of the same kinds of configurations, and if you're going to use human platelets, at least you're closer in terms of being able to try and make the parallel.

The other positive feature is if you're doing primates and using autologous cells, you've got enough platelets so that you can do something with. Conversely, if you're going to use transfused platelets, then it requires a substantial number to be able to deal with.

So that in looking at viability, which is the first issue that has been posed, the choices are three, and actually for most purposes they are one, and that is the process of labeling with indium.

If looking at counts is truly an important issue so that you can look at the function also, the techniques that are available are myelosuppressive approaches, irradiation or a new one that has just become evidence in the course of the last brief period where you administer human thrombopoietin repeatedly, and the animals develop antibodies to these antigens, and they cross-react with endogenous thrombopoietin, and the platelet counts fall to some ten, 20,000.

This happens in about 80 percent of rhesus monkeys so that there's a model for getting a sustained thrombocytopenia that might be really quite useful under some circumstances where you could actually then use counts as well as function for thrombocytopenic animal.

I don't think there are very many data to go further other than that the observation is quite consistent, that administering human thrombopoietin will produce a predictable thrombocytopenia that is lasting and steady state in approximately three quarters of the animals that have been studied and reported.

That contrasts with the model that's shown here where a myelosuppressive agent is administered, and of course, you can get a lot of thrombocytopenia, but it's transient and it's changing and it's accompanied with alterations in other hematopoietic cells in the circulation.

So that it is a pancytopenia which obviously becomes more complicated.

Platelet survival times have been discussed, and certainly they are very useful in being able to show the effects of the influences regarding storage or particular administration. Demonstrating viability under these circumstances can be very important. In this illustration the intent was just to show that there's no effect by having an MPL ligand stimulus in those particular platelets either in vivo or ex vivo.

When platelet viability is being assessed, there are some important issues that need to be settled. One has for the last several years had the challenge that there are ways of modifying platelets during storage so that they will retain viability when readministered and allow you to store them at four degrees and thereby obviate all of the infectious potential complications.

And Tom Stossel, for example, has been one of the proponents of this plan and has developed his own particular storage solution, and the solution preserves very nicely the morphology that one can see, but it certainly does not do anything for the viability, and it reproduces the data that Sherrill produced many years ago when looking at humans, and it's exactly the same story in that the use of

this particular solution is not helpful in modifying that process. That 22 degrees stored platelets are viable and functional and four degrees stored platelets are not and the solution does not improve that.

So we're still on the hunt for that particular solution to the platelet transfusion dilemma.

When looking at assessments of platelet function in non-human primates, the list is pretty long and generally not all that helpful. The surgical blood loss imposes a defined surgical procedure, and all of the variables that that imposes, and one must be very selective before using that as a general approach.

GI blood loss might be usable if you had a sustained thrombocytopenic animal, which it's now feasible to do in primates, and the issue about template bleeding time has been discussed more than any of us wanted to have it discussed.

The aggregatory responses and flow cytometric expression are a specialized testing that may add to, but certainly do not substitute for any of the real measures which would be to assess some real function of staunching of bleeding.

There are some models that have been developed that are platelet dependent that one should think about in trying to put together a test system to see whether hemostatic benefit is derived, and one of them is hypothermia. The second one is extra corporeal circulation, and then the notion of having some platelet inhibitory effects that one can look at, and I'm prompted to suggest to you to consider the issue of cardiopulmonary bypass as a potential model.

This is clearly a platelet dependent responsive system as shown by the fact that that system readily picked up the effect of aspirin having been given to these patients preoperatively, and that by having aspirin/no aspirin, you can show a significant difference in the amount of chest tube drainage.

So that this is a platelet dependent process, and presumably that same model system might be usable to test a hemostatic agent under circumstances that's short term, very doable, and could be readily applicable in a relatively small number of patients.

This slide illustrates for you in non-human primates the effects of cardiopulmonary bypass shown on the left, compared to the effect of hypothermia, and what one does see, however, is that the bleeding time and the platelet specific secreted materials are present, and it assumes that these surrogates are reflecting the dysfunction that is transient.

This also is a potential model that one should think about at least in some of the preclinical development because it is a very platelet specific kind of process, and being able to correct that would give you a lot of confidence that you could develop a patient model that may then really be useful.

The final issue is in conjunction with some of those processes, besides the bleeding time, you can certainly look at alternative ways of measuring platelet function. One of the classic ways is platelet aggregation.

I remind you of the agonists that are here, that there is a dose response effect to be seen with respect to ADP, and that has

been very helpful in trying to look at differences and changes in function as illustrated by the studies that were reported in relationship to the administration of MPL ligands, which have the capacity to increase sensitivity, and that this can be quite convincingly shown if you now do a dose response for each of the samples that you have obtained from the subject.

In this way you can then plot that concentration which induces half maximal aggregation to give you maximal sensitivity, and that this then can be expressed as a single concentration that represents a substantial amount of data that give you assurance and reproducibility that otherwise is not available in aggregation studies, and you can show that there are significant shifts that occur.

And here's an illustration of how the amount of ADP that's required to induce half maximal aggregation is increasing, which is shown here, and that this is inhibitable by using a soluble MPL ligand to demonstrate the specificity of the actual process.

And, again, the same process can be seen if you look ex vivo, and here are data showing the expression of these numbers in terms of the concentration that will induce half maximal aggregation, and the ADP illustrates that there is a significant decrease just at the time when the platelets are emerging from the bone marrow at their maximal rate.

So there are a lot of new platelets. They are now being stimulated in vivo with the MPL ligand, and that this is inducing an enhanced responsiveness to the ADP, and likewise you can show converse

changes like this in patients and in animals that are undergoing hypothermia or cardiopulmonary bypass.

So I think there are a number of useful measures that one can think about. The one thing I would have you consider is taking those situations that are, in clinical medicine at the present time, that are platelet dependent and have been shown to have demonstrable differences that can be demonstrated using platelet dependent intervention, and that here the chance of being able to show something, for example, with cardiopulmonary bypass in terms of the chest tube drainage, I think, is a very real one because of the sensitivity and reproducibility and the clinical relevance of that challenge.

Thank you.

(Applause.)

DR. REID: Thank you, Dr. Harker.

Our next speaker is Dr. Rothwell, who will talk about human platelet survival in animal model.

DR. ROTHWELL: Thank you.

Before I get started, I'd just like to make a short note, and that's that the studies that I'll be talking about today are actually just one part of a collaboration that I've been conducting along with Dr. Chitra Krishnamurti, and so she'll be talking about her part of the collaboration or of our collaboration in the next talk, and so we really need to consider what I'm going to be saying as just sort of Part 1, and what she'll be talking about is actually Part 2 of the entire project.

I'd also like to take a minute just to acknowledge the expert technical assistance in dealing with the rabbits that I received from Peter Maglasang, who's worked in our laboratory, and I'd also like to acknowledge the support that we've received from Dr. Thomas Reid, who's the Program Director for the platelet project at Walter Reed Army Institute of Research.

Okay. So the title of the talk, then, is "Survival of Human Platelets in Rabbits," and when we started getting into the problem of trying to devise enhanced platelet products, one of the realizations that we had early on was that we would probably need some sort of a platelet, some sort of an animal model in order to validate and enhance the biochemical in vitro assays that were available for platelet function.

And so one of the things that we decided was that (a) we wanted to be able to look at the efficacy of platelets in vivo, but even before we started that, we wanted to be able to just track the survival and circulation of platelets in the animal model.

And the animal that we settled on was the New Zealand rabbit, and so the idea then was to have some sort of an experimental design in which you're able to track human platelets in the circulation of the animal.

Now, as we've heard previously, there have been a number of different ways in which one can actually detect and monitor platelets in either humans or animals. We have heard about biotinylization, loading of platelets with radioactive or fluorescent dyes.

These are all very good approaches to this problem, but we were sort of pulling back from these approaches because of several problems. One is all of these require at least some sort of modification of the platelet, and we were worried that since we were already going to be looking at platelet products that would be deviating from the normal fresh human platelet, we were somewhat wary about trying to add additional modifications on top of what we might be already doing.

The second problem was that if we were looking ahead and being hopeful that a product that we might be testing or developing might be getting to the point where it could actually be used in some sort of human trials, we wanted to be testing the same product that would actually be ultimately used.

And so we wanted to try to avoid any sort of additional modification that we'd be doing to the platelets.

Now, fortunately Mother Nature was on our side in this respect because we're using a two species model here with human platelets going into a rabbit, and so we reasoned, therefore, that if we used an antibody approach that would recognize one of the proteins that is found on the surface of the human platelet, that this antibody probably would not cross-react with a similar protein found on the rabbit platelets.

And the protein that we decided to look at was antibodies against glycoprotein IX, which is also known as CD42A, and there's Bectan Dickinson antibody available for this protein that has been very well characterized and used in many studies.

And so what we decided was that we would use this antibody to try to detect human platelets in rabbit blood after the infusion of the human platelets.

So the methodology then that we're going to follow is rabbits were first treated with ethyl palmitate, and we already heard Dr. Blajchman describe the use of ethyl palmitate in his rabbit model. The ethyl palmitate is an organic molecule which is avidly phagocytosed by the macrophages.

And then according to studies that were conducted by Smith and Stewart back in the '70s, apparently after the phagolysosome forms, the compound causes the lysis of this organelle, and the compound is then released into the cytoplasm of the cell along with the hydrolytic enzymes that were contained in the phagolysosome, and the cell basically self-destructs itself.

So this has proven to be a very efficient way of eliminating the macrophage population, as we've already heard.

Twenty-four hours after the administration of the ethyl palmitate then, we now infuse either fresh or eight day old human platelets into the rabbits.

Now, our definition of "fresh" in this study and the following talk by Dr. Krishnamurti is actually a one day old platelet because this represents the platelets as we get them after the serology has been conducted by the hospital blood bank.

We decided on using an eight day old platelet as the second preparation to look at because we decided that this would represent expired human platelets. It would be at least three days

past the normal five-day shelf life of platelets that are used in hospitals today.

But we decided that this would be even still a fairly minor modification. We haven't done anything like cooled or frozen or chemically modified the platelets.

So we were interested in seeing if our model would be able to detect what we thought might be relatively minor differences between platelet preparations.

Then finally, following the infusion, blood would be drawn at various time intervals, and the presence of human platelets in rabbit blood would be monitored by flow cytometry using a Bectan Dickinson CAT scan flow cytometer, or by fluorescence microscopy.

And as we can see in this slide, if we just take a preparation of pure human platelets and label them with the antibody, again CD42, you can see that the platelets are quite brightly labeled with the fluorescent antibodies, and then if you compare the platelets that we see in the fluorescent image to the plates that are seen in just the light image, we can see that all of the platelets are labeled.

And one of the advantages of the CD42 ligand is that all platelets are labeled regardless of whether they're in a resting state or an activated state. So this is sort of a pan-platelet label.

In contrast, if we take rabbit platelets and incubate them with the same antibodies, we can see that none of the rabbit platelets are fluorescent. So none of the rabbit platelets are labeled by an

antibody, and so our hypothesis about the utility of the antibody was borne out.

If we take a mix of rabbit and human platelets and label them with the antibodies, what we find is that only the human platelets are labeled, and the rabbit platelets remain unlabeled.

So this gives us the ability to separate out the rabbit and the human platelet by fluorescent microscopy.

If we look at the same sort of data in the flow cytometer, in the Panel A we can see that human platelets labeled with the CD42 antibodies give a nice, strong fluorescent peak. The dashed line here represents the isotype control antibody. You can see this is down in the unlabeled side of the scale.

In contrast, if we take rabbit platelets and incubate them with CD42, we find that again, as expected, they do not take up the or they are not labeled by the antibody, and they remain displaying a lower fluorescent or no fluorescent peak in the flow cytometer.

If we take a mix of rabbit and human platelets and incubate them with the fluorescent CD42 antibody, again, we find that the human platelets show a nice fluorescent peak compared to the rabbit platelets which remain unlabeled.

So following this kind of a treatment then, we're able to detect and actually calculate the amount of human platelets that are present in the various samples that we've removed from the rabbit following infusion with the human platelet.

Now, if we look at just the platelet counts without reference to any sort of immuno-fluorescent labeling, we can find that

our platelet count in the ethyl palmitate treated rabbits increase following infusion of the rabbits with the human platelets.

And I'd like to note at this time that the load of human platelets that we're infusing into the rabbits for each of these experiments is between two to three times ten to the tenth platelets per animal, and this represents about 25 to 30 percent of the normal rabbit platelet load.

In addition, these animals, they've been treated with ethyl palmitate, but they are not thrombocytopenic at this point. So they have their own normal level of human platelets, and you can see that that's reflected in the zero hour point of platelet count before infusion.

And you can see that under this model that out to at least 24 hours and in some cases as far as 48 hours, we're still able to see an elevation in platelet numbers.

When this is compared to animals that were not treated with ethyl palmitate, you can see that the CD42 labeling process was basically unable to detect human platelets in these animals even as early as ten minutes into the infusion period, and this is the same sort of data that we've seen in the previous talk by Dr. Blajchman.

And so consistent with this, we did not see an increased platelet count at any time during or after the infusion. So basically if you don't treat the rabbits with ethyl palmitate, the macrophage system of the rabbits is quite efficient and able to remove the platelets right away.

Now, using this system then, we wanted to see if we could detect a platelet and actually track the survival of human platelets in rabbits, and in this slide, we're comparing the survival of the two different kinds of human platelets that we were using. We have the fresh human platelets and the eight day old platelets.

And using as a normalized 100 percent value the number of platelets detected at 30 minutes following infusion, we're able to plot a survival curve for the human platelets in the rabbits, and what we find is that fresh platelets were detectable out at least to 24 hours and gave us a half-life of just about 8.6 hours.

In a similar study using the eight day old platelets, we found that the platelets failed to circulate must past about eight or nine hours, and that the half-life of these platelets was only about 2.9 hours.

And so what this graph shows us is that the system is, indeed, sensitive enough to be able to detect differences in survivability and to detect circulating platelets in two fairly similar human platelet preparations.

So in summary then, what we've done is we have examined the circulation of human platelets in a rabbit model, and we have confirmed that if the rabbits are untreated, that the human platelets are rapidly removed.

On the other hand, if we treat the rabbits with ethyl palmitate, then fresh human platelets will circulate for about 24 hours with an average half-life of 8.6 hours, and in comparison, eight day old platelets infused into ethyl palmitate treated animals

survived in the rabbits, but for nowhere near as long, and they had an average half-life of only about 2.9 hours.

So in conclusion then, we've described a rapid and an efficient method of monitoring the survival of fresh platelets or preserved platelets in a rabbit model using flow cytometry, and in addition to being rapid and efficient, this is an extremely convenient model, and it's one that does not require prior modification of the platelets before they're actually infused into the rabbit.

And we envision this as being a very useful technique both for monitoring survival and for looking at future platelet preparations in the surgical model that Dr. Krishnamurti will describe.

Thank you.

(Applause.)

DR. REID: And Dr. Krishnamurti will now talk about the animal model she's developed with Steve.

DR. KRISHNAMURTI: Thank you, Steve, and thank you Tom.

As we've been hearing all day, one of the common goals of the pharmaceutical industry, as well as the clinical researchers has been the development of blood products that could replace fresh platelets, but regardless of the specific product formulation, the ability to document the function of the platelet is, indeed, critical.

To this end, we have developed an organ injury model in the rabbit to determine platelet function. My colleague, Dr. Rothwell, has just described a rapid and convenient method for

assessing the survival of human platelets in a rabbit model using flow cytometry.

In the present study that I will now describe, we have developed a rabbit kidney injury model to assess the bleeding loss in both normal and thrombocytopenic rabbits. That is the title of my talk.

Thus, the purpose of the study was to develop an organ injury model in the rabbit to test human platelets in vivo and to examine the efficacy of human platelets in reducing blood loss from the wound.

Platelets were obtained from the Walter Reed Hospital by doing aphoresis on the patients, on the human volunteers, not on patients, on normal donors, and this was done under a human use approved protocol.

The platelets were used one day after collection following screening for infectious agents, as Steve has already told you, and we have termed that as being fresh platelets.

These platelets were purified using Cell Sep in the presence of prostacyclin to inhibit activation of the platelets.

Typical platelet counts were six times ten to the tenth from an aphoresis unit of about 100 mL.

Some of these platelets were activated in vitro using thrombin because we wanted to later on determine if there was different efficaciousness between activated platelets as compared to fresh platelets.

The platelets that were activated were processed without the addition of prostacyclin. These platelets were activated with four and five units per mL of human thrombin for ten minutes at 37 degrees Centigrade.

The cells were always diluted to 20 mLs with normal saline before infusion into the rabbits.

Male New Zealand white rabbits were made thrombocytopenic by two consecutive injections of busulfam which was given on day zero and day three after the animals were anesthetized.

We found that the platelet counts dropped to below 40,000 per microliter by between 13 to 15 days. Therefore, two weeks later, human platelets were infused into animals whose reticuloendothelial system was suppressed by the administration of ethyl palmitate or EP.

One day after the animal had received EP, it was anesthetized using katemine rompum mixture, and through the airway we had an infusion of sodium pentobarbital that was given every half hour to manage the anesthesia until the end of the experiment.

A midline cut was made along the linear alba (phonetic), and the kidney was exposed. It was denuded of fat and placed on a preweighed parafilm boat, very sophisticated.

The left kidney was exposed and a slice excised from the superior pole after the infusion of platelets that was given over 20 minutes. We always made sure that the slice excised was between .4 to .5 grams, and this was always kept consistent.

The blood was contained in a parafilm boat and absorbed by preweighed gauze to assess blood loss. The blood was expressed as grams, taking the density of blood to be 1.053 grams per mL.

We found that the only way we could get measurable bleeding was if the cut was made through the medulla of the kidney. If a slice had been made along the cortical portion, there was inadequate bleeding.

The subsequent slides are the results we obtained. This slide shows the blood loss from kidney injury in normal rabbits. Normal rabbits were made into three groups. The first group received saline. The second group received ethyl palmitate followed by saline the next day. The third group received ethyl palmitate followed by fresh platelets on the next day.

The blood loss in the saline group was about 37 grams, and in the animals that received saline or fresh platelets, there was no difference, no significant difference between those groups and the ones that received saline.

This slide shows the blood loss from the injury in EP treated rabbits that were made thrombocytopenic. The first group of these rabbits received saline, and the blood loss was 79 grams.

In contrast, the animals that received fresh platelets, there was a significant decrease in the blood loss. Thus, in these thrombocytopenic rabbits, infusion of fresh platelets decreased blood loss significantly.

To determine if platelets that were activated by thrombin in vitro and then infused into thrombocytopenic rabbits that received

EP, we determined the blood loss. These are the two that were represented in the previous slide.

We found, again, to reiterate, that the fresh platelets significantly decreased blood loss. When animals received activated platelets, there was a significant decrease between those and the ones that received saline. There was no difference in blood loss between animals that received fresh platelets or activated platelets.

Furthermore, platelet counts in animals that received fresh or activated platelets but elevated in these thrombocytopenic rabbits, there was no change in platelet counts in those animals that received saline alone.

The percentage of human platelets infused into the rabbit was determined by flow cytometry. Blood was collected from the cut site and labeled with fluorescein labeled anti-CD42A, the marker for human platelets that Dr. Rothwell had already referred to.

The degree of activation of human platelets was determined using phycoerythrin or PE tag anti-CD62A which is a marker for human P selectin.

The percentage of activated platelets, that's the thrombin activated platelets that were infused into thrombocytopenic rabbits, as you can see here the percentage of anti-CD62A label was not increased in the rabbits after infusion.

When normal resting or fresh platelets were infused, again, there was no difference before and after infusion.

Moreover, when these platelets were activated in vitro using thrombin, they could be activated, showing that after

circulation of the platelets within the rabbit, they were not inhibited from activation in vivo.

This figure shows a histogram of the thrombin activated human platelets that were infused into rabbits. The left side shows the FITC with anti-CD42A.

This slides shows the human platelets before infusion, and this is the human platelets after infusion. The section on the right are the PE labeled anti-CD62A platelets. Again, these are the human platelets before infusion and these are the human platelets after infusion.

It should be pointed out that the number of events that were counted by the flow cytometer in the samples that were removed from the rabbit, that is, after infusion, included both the human platelets as well as the rabbit platelets. That's why there's a decrease in the actual amount, number of cells counted.

To summarize our results, there was no significant difference in blood loss between EP treated animals infused with saline or fresh platelets. However, infusion of fresh human platelets into thrombocytopenic rabbits resulted in marked reduction in blood loss.

The blood loss after infusion of activated platelets was similar to the blood loss following infusion of fresh platelets, although there was a significant decrease as compared to the saline controls.

Normal untreated platelets were not further activated after circulating in the rabbit.

Platelet counts of thrombocytopenic rabbits were increased after infusion of fresh and activated human platelets. However, there was no change in platelet counts after infusion of saline into thrombocytopenic rabbits.

In conclusion, this is the first report describing an organ injury model to investigate the efficacy of platelets in vivo. This rabbit kidney injury model was developed to assess blood loss in normal and thrombocytopenic animals infused with human platelets.

Blood loss was strikingly reduced in thrombocytopenic rabbits infused with fresh or activated human platelets.

This model can be used to assess the efficacy of any of the human platelets that are being made in the pharmaceutical industry.

Currently we are using this model to study different platelet preparations made in our lab, frozen platelets, cold platelets, et cetera, and we are trying to correlate the bleeding loss obtained in this in vivo model with in vitro functional tests for the platelets, for example, the forced development clot fraction and aggregation.

Thank you.

(Applause.)

DR. REID: If I could have the four previous speakers please come up to the podium to answer any questions.

It was interesting that Dr. Levin had mentioned the thoracic duct model in the dog. Dr. Sandler and I had talked about

this in the past, and that's also one of the models that we're in the process of developing.

While he's getting that in, Chitra, could you describe how you actually made the slice in the kidney? You mentioned you made a slice, but how did you make sure that the cut was the same?

DR. KRISHNAMURTI: Very sophisticated.

DR. REID: But it works.

DR. KRISHNAMURTI: We have a parafilm boat into which we put the kidney, and we have a plastic spoon with a hole in it, and we put it across the kidney so that the superior pole is exposed, and so every time we get the same cut. We slice out from there, the edge. There's a hole made.

DR. REID: Dr. Harker, a question for you in terms of the recombinant thrombopoietin and decreasing the platelet count in baboons.

Have you tried other animals and seen the same thing?

DR. HARKER: Other primates, yes.

DR. REID: Other primates.

DR. HARKER: Primates.

DR. REID: Do you think it would work in swine?

DR. HARKER: Well, a similar thing has been reported in dogs.

DR. REID: So using the human thrombopoietin in dogs?

DR. HARKER: Yes.

DR. REID: Okay.

DR. HARKER: Morris, tell me what is on these fibrinogen coated materials.

DR. BLAJCHMAN: Sorry?

DR. HARKER: What kind of proteins have been absorbed from the circulation? Have you got von Willebrand factor? Have you got fiber -- what's on there after it's circulated?

DR. BLAJCHMAN: Well, as you heard, they don't circulate for very long, and to be very honest with you, we haven't looked.

DR. HARKER: They should flow very well.

DR. BLAJCHMAN: They should.

DR. HARKER: Flow cytometry you could.

DR. BLAJCHMAN: But the problem is reagents to the rabbit proteins.

DR. LEVIN: This slide is taken from a paper by Woods in 1953, and this is the demonstration of enthrombocytopenic dogs, and this is a similar demonstration that's been shown in rats, that red cells appear in the thoracic lymph, which is shown on the right-hand side when the dogs are made thrombocytopenic.

And where you see the red cells fall is where the animals have been infused with platelets shown on the left, and within a matter of hours, the red cells disappear essentially completely from the thoracic lymph, and then you can track their reappearance as a platelet count, again fall, shown on the left.

And this same model has been shown in rats, and I think this should get some serious consideration because it strikes me it's physiologic and in vivo, and as I said quickly this morning, this was

the model that demonstrated for the first time that platelet transfusions were physiologically effective.

Thank you.

DR. REID: Just one additional aspect to that model. When Dr. Jackson and Dr. Harrington tested that in dogs, I think, in 1959 in the JCI article, they showed that fresh platelets decreased the red cells to normal levels in the thoracic duct lymph, but when the dogs were given a platelet preparation that the platelets had been processed in some way, the red cells did not go away after repeated infusions.

DR. SLICHTER: I think one of the problems with the thoracic duct model, my understanding is that it's not a trivial issue to, in fact, cannulate the thoracic duct. So I think that may be one of the reasons that it has not been used more extensively, because it clearly did show the results that Dr. Levin mentioned.

But I think it, you know, requires a good surgeon and maybe better surgical techniques than at least some of us internists have available to us.

DR. BLAJCHMAN: That's right. We've tried this same thing to try to duplicate those results and to try to do surgery, you need a fairly large animal to do it with. Rabbit is probably too small, but also if you're dealing with a thrombocytopenic animal with a platelet count less than 10,000, the surgery is not trivial, and the amount of bleeding you get during the surgery is not trivial.

I think it's very nice to show one experiment in one animal, but I suspect to get that one result, they probably did 20 animals.

DR. LEVIN: I don't want to defend their work because it's not mine, but I just don't want you to discard it too quickly because they did do the same work in rats, and this is one of at least three papers I know from three different groups that did this work.

DR. BLAJCHMAN: I'm not discounting the work. I'm just pointing out that the technical skills that are required to do that are not trivial.

DR. REID: I think as Dr. Sandler has pointed out, this study was done by people who really loved being in the lab, and so you had to be willing to spend a lot of time, but you can get around those issues about the thrombocytopenia by actually cannulating the thoracic duct ahead of time, before you give the busulfam, before you irradiate them, and we're also going to take the spleen out as well as one possible problem.

DR. BLAJCHMAN: Good luck.

DR. REID: Thanks.

PARTICIPANT: I just want the panel to respond to a question that I have here. Most of the studies you use EP to immunosuppress the animal. Do you think that will have an effect on the in vivo survival or the efficacy of any of these blot substitutes when you actually do the test in noncompromised animal model?

DR. BLAJCHMAN: You're asking me that question?

PARTICIPANT: Just if you know.

DR. BLAJCHMAN: The only time we use the ethyl palmitate is when the human platelets are used. We have not used ethyl palmitate for the IPM studies, nor the Thrombosphere studies. We have used them when we did the studies with the lyophilized human platelets and the various frozen human platelets.

PARTICIPANT: Yeah, the comment that I have is if you are using that to look at the efficacy of some of these platelet substitutes, in a situation where you don't have them, you know, compromised, if they have a shortened lifespan, would that still affect the efficacy?

DR. BLAJCHMAN: I think that's a possibility, but again, for the IPMs, for the Thrombospheres, we did not use RE blockade.

If you're asking if we use them would it prolong their in vivo survival, it might do, but we've never done those experiments.

DR. SLICHTER: I'd like to ask a question about the kidney model that you've developed. I assume that the reason why you've developed the kidney model is you're trying to, in a sense, reduplicate conceivably a trauma or a major rather than the microvascular bleeding that you see associated with the bleeding time.

My question relates to do you intend or have you done any studies to compare whether you see differences in response to various products whether you use the bleeding time model in the rabbit versus the kidney slice model which you've been discussing.

I think it would be of some interest to see whether what you need to control microvascular bleeding is the same and/or

different than what you might need to control the bleeding from the kidney, and have you done those experiments?

DR. KRISHNAMURTI: We haven't done them.

DR. SLICHTER: Or do you intend to?

DR. KRISHNAMURTI: We haven't. We haven't done any comparison. That's between Mo's model and --

DR. SLICHTER: Yeah, and yours.

DR. KRISHNAMURTI: No, we have not.

DR. SLICHTER: Do you intend to conceivably do them?

DR. KRISHNAMURTI: We have thought about it, but we haven't gotten down to doing it yet.

DR. HEATON: Yes, Andrew Heaton.

Dr. Krishnamurti, I was curious to know why did you use activated platelets. I noticed you used thrombin activated platelets, and my experience is they would have a very short circulating lifespan, and I was just looking at your flow cytometric plot. There was a very big fall-off in the number of platelets pre-infusion and immediately post infusion.

Were you hoping to get some accelerated effect through the use of thrombin activated platelets? What was your goal?

DR. KRISHNAMURTI: No. The reason why you see that fall is because we were counting rabbit platelets also in the number of events.

You're talking about the histogram, right?

DR. HEATON: Un-huh.

DR. KRISHNAMURTI: Yeah. The number of events have remained the same. So if you counted 5,000 events, that included the rabbit platelets as well as the human platelets. So that's why we get that extra little bump, the first hump that you see.

DR. HEATON: Yeah.

DR. KRISHNAMURTI: So it's because of that that you get a decrease in the height of the curve, but in terms of fluorescence it's the same.

DR. HEATON: Presumably you get a very short lifespan, and that would be cleared very quickly, yes.

DR. KRISHNAMURTI: The CD42A?

DR. HEATON: Yes, the P selectin activated ligand.

DR. KRISHNAMURTI: Do you want to answer that?

DR. ROTHWELL: Are you asking if there's a short lifespan of the activated --

DR. HEATON: Yes, and why you would use that. Were you hoping to get accelerated hemostatic effect?

DR. ROTHWELL: Well, one of the reasons why we decided to use that was because when we first started just looking at the fresh platelets, we sort of had the assumption that as the platelets were exiting from the wound, that they would become activated and we would see an increase in the level of CD62 on those platelets.

But when we actually did the experiments and ran them through the flow cytometer, what we found was that the level of activation of those platelets was pretty much the same as whatever it was when we put them into the animal.

So if they were 20 percent activated platelets, they were about 20 percent as we collected them from the wound. So there wasn't a big increase.

So then we just sort of made the next step, and we said, well, if we don't see them being activated as they come out of the wound, can we increase or affect the blood loss by preactivating them before we put the platelets in, and so basically that was the rationale behind that experiment.

DR. HEATON: Well, you have to be very careful to do a control because the rate of clearance is extremely fast with thrombin activated platelets.

DR. ROTHWELL: Well, but in the acute injury model that we're looking at now, we really only need them to circulate for 20 minutes, and then we go ahead and do -- or maybe 40 minutes.

DR. KRISHNAMURTI: Forty minutes total.

DR. ROTHWELL: So that they circulate during the time of the actual infusion so that we get to the end of the infusion and still have circulating platelets, and then the typical bleeding time ranges from about 15 to 30 minutes, and as long as they're still there during that time.

DR. BLAJCHMAN: Andy, if I could also respond to your question, I think the evidence that activated platelets are removed very quickly from the circulation, I think, or the issue is not sorted out.

We have also done similar experiments to these with human platelets that we put into the RE blocked animals and looked at --

what's his name? Michaelson, Mosenson? Michaelson -- looked at activated platelets and P selectin and showed in a primate, if I remember correctly, and showed that the survival of activated platelets were not shorter and, in fact, that P selectin comes off during the circulation.

We have asked that question using human platelets in the rabbit model and have found identical results, that the human platelets in the rabbit stay around as long as human platelets nonactivated stay around, but that they, over the period of time of about 12 hours which we looked at, they lose P selectin from the surface.

DR. ROTHWELL: Well, I don't think the circulation is exactly the same because our eight day old platelets for the most part were quite high in terms of the expression of CD62. So if we use that as a level of activation, then they were probably pretty activated.

And there is a difference between the circulation times, but they did circulate for quite some time, meaning hours as opposed to minutes certainly.

DR. BLAJCHMAN: The circulation time of old platelets is not necessarily reduced because of the activation.

DR. ROTHWELL: That's true.

PARTICIPANT: I think these xenologous models are interesting, but I'm perplexed by one thing maybe the three of you who have done these rabbit experiments could answer, and that is that I think that these human platelets are being washed before you transfuse

them. I suspect that that's why you're using the Cell Sep methodology, to get rid of the human plasma proteins.

Since we've all been taught that von Willebrand factor is important for adhesion, are we now learning that it doesn't matter what the source of the von Willebrand protein is and that, therefore, that's not important?

I'm very perplexed how that -- what role that plays.

DR. ROTHWELL: Well, I mean, we are washing the platelets. So --

DR. KRISHNAMURTI: We wash them, yes.

DR. HARKER: Yeah, but what would be taken off? Do you know? Do you have some idea how thorough the washing is? It's hard to get Factor V off. It's hard to get --

DR. ROTHWELL: No. We don't really have a quantitative number for that. In fact, we don't have any number for it.

DR. BLAJCHMAN: And the rabbits have endogenous von Willebrand factor. So it's not as if there's no von Willebrand factor.

PARTICIPANT: Well, that's true, but then we're saying rabbit von Willebrand protein interacts with human platelets and works effectively. It's a pretty big leap.

I'm reminded of the old experiments by Howard and Firkin where basically if you take risticetin, which is a very potent von Willebrand factor, stimulating agent, if you will, and you put that into rabbits, they drop dead because of the tremendous aggregates they get.

I'm just wondering about the complexity of this system going from washed human platelets depending upon rabbit von Willebrand factor to give some type of effective hemostatic measure.

DR. SLICHTER: One other comments about your surgical rabbit model. Your rabbit model, as I gathered from your slides, you still have a residual platelet count in the rabbit of somewhere around 40,000.

DR. KRISHNAMURTI: Yeah.

DR. SLICHTER: So one of the issues that I think you need to be careful of is how much contribution the infused material requires the presence of some residual platelets in order to show an effect.

In other words, you know, when we're usually transfusing at least prophylactically patients, we're transfusing people who really don't have any autologous circulating platelets. So all I'm saying is that at some point you may want to do the experiments to make a truly thrombocytopenic rabbit model and then try your infusions to make sure that you're not getting some contribution of their endogenous platelets as a measured effect from what you're transfusing.

So I just think you need to be careful about whether you're really measuring what you think you're measuring since they're not severely thrombocytopenic.

DR. KRISHNAMURTI: Wouldn't saline controls be part of our controls as compared to the ones that we've infused with the

platelets? They would have the same low platelet counts as our treated ones.

DR. SLICHTER: I know you see a difference, but --

DR. KRISHNAMURTI: And the other thing is it's a very fine line between what we call severely thrombocytopenic. These animals have a tough time afterwards dealing with EP. Just before we treat them with the platelets we find we get -- they die. We get a high mortality rate if we take it down very low.

DR. BLAJCHMAN: No, I think the problem with cytoxan is that they're also having it leukopenic, and I suspect the deaths are related to the animals being sick from infections.

In our model, if you remember the kinetics for the platelet count, the drop -- what's the word I'm looking for? The bottom.

DR. KRISHNAMURTI: Nadir.

DR. BLAJCHMAN: The trough and nadir of the platelet count is about seven days after irradiation. The nadir for the white count after irradiation is about three days.

So by seven days, in fact, the white count is back to normal.

DR. REID: Okay. Last question.

DR. RINDER: Thanks. Harvey Rinder.

Have you had a chance to examine the contribution of the surface area of the fibrinogen coated microspheres? For example, maybe comparing them directly to infusion of cryoprecipitate.

DR. BLAJCHMAN: We haven't really considered that. Richard Yen, who's the inventor of these Thrombospheres, Richard, have you looked at that?

DR. RINDER: I'm just wondering the relative contributions of a higher fibrinogen in the local environment versus maybe the real contribution of the higher surface area.

DR. BLAJCHMAN: We've done some experiments where we've infused the equivalent amount of fibrinogen, and that has no effect. We tired one experiment, and I shouldn't be laughing, but we tried one experiment where we used fibrinogen coated polystyrene beads. That experiment was a disaster in that the animals died within minutes of getting this infusion.

So the question being asked is: does fibrinogen on another surface produce the same effect? And of course, we don't know the answer to that because of the way the experiment turned out.

DR. YEN: Richard Yen, Hemosphere.

In terms of whether there's a local effect versus the global effect of fibrinogen, I think, Mo, you may want to discuss a little bit, share a little bit about the results that you obtained from looking at the clots to see whether there are any localized spheres there.

DR. BLAJCHMAN: Well, one of the things that has perplexed us about the whole Thrombosphere story is the assumption, our assumption and the manufacturer's, is the assumption that the Thrombospheres act by enhancing hemostatic plug by participating in the hemostatic plug, and this is clearly seen in vitro.

We have looked at many, many, many now hemostatic plugs in animals given the Thrombospheres and have never seen a Thrombosphere in a hemostatic plug. We've looked at one hour, 24 hours, 48 hours, what have you.

I'd be interested to know whether the Andaris Group have done that and what they have found, but I'm convinced now that the Thrombosphere does not work in the way that we anticipated that it worked, namely by participating in the hemostatic plug.

DR. REID: Let me ask the last question. Dr. Blajchman has identified a disconnect between the functional ability of platelet substitutes versus their survival. You've seen platelet substitutes with short survival, but shortened bleeding time for some period out.

Has Dr. Harker and Dr. Krishnamurti or Dr. Rothwell, any of their models seen a similar finding at all?

DR. HARKER: No.

DR. BLAJCHMAN: No.

DR. REID: Okay. Thank you.

DR. BLAJCHMAN: I don't think people have looked very carefully at that.

DR. REID: Okay, and Dr. Mondoro will now take the last session.

DR. MONDORO: Good afternoon. My name is Traci Heath Mondoro, and I work at the FDA with Dr. Vostal.

Our last session is called the "Manufacturer's Perspective," and as the title of this workshop indicates, we are talking about platelet substitute. That's pretty obvious, but before

we get to our final discussion of the FDA posed questions about platelet substitutes, we're going to hear straight from the horse's mouth.

These people are the investigators and the developers of these platelet substitutes and have worked with them extensively, and unfortunately our time is short. So we will not have time for a question and answer period after the presentations. So if you have questions for these presenters, you'll have to find them afterwards and talk to them.

And also our schedule is out of order already. We're going to have Dr. Katherine Davis, who was the last listed speaker, will be speaking first, and then everyone will be bumped down a slot.

And due to the short time of these presentations, if I could ask Drs. Fratantoni, Holme, Middleton, and Connor to come and sit down front so you can immediately come to the microphone while Dr. Davis is coming up.

DR. DAVIS: Maybe while she does that, I will give you informed consent first. I'm not a manufacturer, and I'm not going to talk about substitute platelets.

I'm a professor of biostatistics from the University of Washington, and I was director of the data center for the TRAP trial, and I'm going to talk about something to do with CCI.

I was a late entry to this procedure. So I'm a little out of order in more ways than one.

Well, yes, that's it.

Okay. I'm going to talk about the use of the CCI and the PPR as measures of efficacy, and I'm going to use TRAP as an illustration to show you some of the things that we learned about using these measures from the TRAP database.

To remind you, the TRAP was a trial sponsored by the NHLBI. It was in AML patients who were receiving initial induction chemotherapy. It was a randomized trial. The patients were randomly assigned to unmodified pool random donor platelets, UVB irradiated pool random donor platelets, filtered pool random donor platelets, or filtered aphoresis random donor platelets.

TRAP was based on the first eight weeks following induction chemotherapy or during induction chemotherapy and included all transfusions. In this trial I'm not intending to present TRAP results. I'm using TRAP only as an illustration, and so I'm using only the first transfusion and only 585 patients who had the first transfusion and had the increment measured.

The CCI and the PPR, percent platelet recovery, are both ratio measures. They're really quite similar measures. Both of them have the increment of post minus the pre-count divided by the dose, and then CCI is multiplied by the body surface area, whereas percent platelet recovery is multiplied by the blood volume.

Statisticians don't like these kinds of measures in general, and I want to show you some problems that are associated with using these measures and suggest maybe a better way to do things.

In this slide, which are the results of just the first transfusion for pool random donor platelets comparing the filtered

products to the UVB products, you see some of the problems are interesting effects of CCI and PPR.

If we look at just the platelet count increment, we would say that the UVB looks a little bit better than the filtered product. I mean, they're very close. I'm not doing P values. I'm not doing comparisons. This is strictly an illustration.

But UVB has a better increment than filtered, and also if we use the increment to measure clinical refractoriness we might say that UVB was a little better in terms of refractoriness than filtered, using just the increment.

But if we used the CCI and the PPR, these ratio measures, it's reversed. Using the CCI and PPR, we would say that the filtered platelets were doing a little bit better than the UVB platelets.

And if we use these measures to determine clinical refractoriness, we might say that the filtered were a little better than the UVB.

So clearly, if you're a UVB manufacturer out there, you want to use this measurement, and if you're a filter manufacturer out there, you want to use this measurement.

This is not a good situation, and so let me show you something that really is a better way to do the same thing. In TRAP, being a randomized study, this is clearly the best measurement. Since it's a large study, the people are from random assignment of similar size. The doses would be similar as far as the dose that's obtained from the donor goes, and so the platelet count increment would be the best way to look at it in a randomized study such as TRAP.

But I appreciate the interest in reducing the variability, making a more accurate measurement, which the CCI and PPR are trying to do by putting in dose and patient size as an adjustment.

So I'm going to do a comparison of using a ratio measure to using a regression measure and use for this example these other two groups from TRAP. One is 138 pooled random donor platelets and the other is 152 patients who had filtered pooled random donor platelets. They're just the same except one's filtered and one's not.

If we use a ratio -- and here I'm just using the ratio of the increment to the dose, leaving out body surface area or blood volume. So it's just the ratio measurement. PPI -- CCI and PPR are the same in this ratio.

If I use just this ratio and compare unfiltered and filtered, for unfiltered we get 8,210; for filtered, 7,780. They're very similar, and by a T test not significantly different, P of 0.45.

Now I do a simple regression analysis and come up with this. In the regression analysis I've put in filtration, dose, and an interaction between the two, and here instead of .45, I get a really quite high level of significance for filtration. I get .01 instead of .45, really quite different by this analysis.

Also, dose is significant, but the interaction is significant. The interaction being significant means that the effective dose is different for filtered and unfiltered platelets, and that's the source of the apparent disparity between this analysis, the .01, and the .45 significance in just using CCI.

There are two different equations, and let me show you the equations that are generated by this analysis. For the unfiltered platelets, the increment would be estimated to be about 23,000 plus about 3,000 times the dose.

For the filtered platelets, the basic increment is minus 910 plus now about 8,000 times the dose.

So both of them are going up with dose, but this one, the filtered, are going up almost three times as much for the dose. It's sort of saying that for filtered platelets on a per platelet basis, you get maybe more bang for the buck per platelet, but you lose platelets in the filtration. So your starting mark for the unfiltered is bigger.

Thinking back to that other slide where I showed just the comparison of the increment plus the C -- to the CCI, the same thing was happening there. For the CCI that we used just for the dose, filtration looks better because you're getting better per platelet transfused.

But on the average, you have more platelets in the UVB product. So overall you get a better total increment for the UVB.

You see, there are two parts to it, and CCI obscures all of that.

Here's a graphic illustration of the situation. We have a dose here going from two to eight, which is the range it was in TRAP. The platelet count increment.

For the unfiltered platelets, you get an increase in increment with dose, but you get a more rapid increase with dose for

the filtered platelets. If you use CCI, you don't see this. You see a simple comparison that says these two are not different.

Well, they are different, but it depends on the dose.

I included this slide to show you that for this apparently fancier analysis, you really don't pay a price in sample size. If you used a very simple analysis and just looked at the platelet count with no adjustment at all, say you required 100 patients for that kind of analysis. If you used the count increment instead, you are accounting for what the patient started out with. So you'd only need 72 patients. You've increased your precision somewhat.

If you use CCI and PPR, you're also accounting for dose and body size. So you increased your precision even more, and you'd need only 60 patients.

But with the regression model -- and here I've included a measure of size in it -- you need 59, slightly more or the same. So at any rate, you're certainly not paying a price by using regression instead of using CCI or PPR.

So in summary, the regression analysis is much more informative, and you can in the regression analysis separate the effects of dose and then properties that are associated perhaps with the product, platelet viability, leukocytes, whatever it is that at least in this analysis seems to be making a platelet after filtration be better than a general unfiltered platelet, but you can separate these two effects, and you don't have to increase your sample size to do so.

And now since after I presented this part -- and also I didn't say that this is work that's been submitted to Transfusion. Sherrill Slichter and Larry Corash are my co-authors on this

I have one more slide not on this topic, but for TRAP which I think is also useful.

This is a plot now of the first 25 TRAP transfusions. This is the mean platelet count increment, mean over patients of the first 25 TRAP transfusions.

I really don't think this is well appreciated, that the count increment decreased markedly from the first transfusion down to about the 15th transfusion, and here is there is more variability and also drop in sample size.

This decrease you see here is not an effect of people becoming alloimmunized because if you leave out the people who become alloimmunized, you see this same decrement.

And I show this because I want to make the point that one transfusion is not like another. In these studies, you need to account for the sequence of transfusion and also make some sort of connection between transfusions within the same patient. There's a new technique called longitudinal regression analysis that if you talk to your friendly neighborhood biostatistician, I'm sure they can help you out with that one, but that adjustment needs to be made.

And also, in these studies, in the clinical studies in patients, I don't believe that the paired analysis is the best way to go. In particular, if you do do a paired analysis, you very definitely need to take account of this order effect because the first

transfusion a priori is liable to have a higher increment than the second transfusion.

And in TRAP, because there's so much variability since these patients are being treated, the correlation between transfusions within the same patient is not so strong that you actually would improve your sample size by doing a paired study in this type of patients. You actually use a lower sample size, using independent patients instead of pairs in this situation.

I realize this morning most people were talking about more controlled trials and trials in normals where you were talking about using pairs, but in this situation pairs potentially introduce a bias and probably, at least from TRAP, not reduce your sample size.

And that's all.

(Applause.)

DR. MONDORO: Thank you.

Next we have Dr. Joseph Fratantoni, and the title of his talk is "Regulatory Approach to Platelet Substitutes: Lessons Learned from Red Blood Cell Substitutes."

DR. FRATANTONI: Thank you.

I also am not a manufacturer. In fact, this will be some regulatory history and some regulatory philosophy.

I'd like to just touch on three topics from my people of the regulation of platelet products at CBER since about the early 1980s. Many people in the audience are familiar with it, but those of you who aren't a quick run over.

I'd like to talk about the lessons learned from the experience with red cell substitutes, and then talk about possible applications to the new platelet related products that we've been hearing about.

In 1981, there was a workshop that people have talked about and actually proceedings which were published in Vox and in which people with an assortment of interest and expertise in platelet storage and platelet physiology talked about applications of various in vitro and in vivo measurements to platelet products, and at that time we were talking about platelet stored for transfusion.

In '81, there was issued written guidance which described a three stage level of testing: laboratory evaluation of platelets essentially to demonstrate that the platelets were alive using a barrage of tests; autologous in vivo survival, recoveries to demonstrate the circulation of the preparation; and then the clinical validation, which was not really a strict clinical trial, but clinical demonstration that the platelets would give hemostatic support to patients who needed therapy.

The use of these guidelines over the past 17 years now resulted or at least permitted the approval of a number of new containers, the second generation platelet containers, and the cell separators that had been in use.

These were intended for the application of platelets stored for transfusion where there were minimal changes from the normal state, and they really are not suitable for the products that have been markedly altered, as we've heard several times today.

Red cell substitutes. There's certainly been scientific interest in developing a red cell substitute for a number of years. The field received a stimulus by the military about 1980 when there was a decision to develop what the military referred to as a resuscitation fluid for use in treating combat casualties and chose the hemoglobin material.

And there was another burst of stimulus after the AIDS era had come, largely pushed by public fear regarding blood.

There were attempts to use both the fluoro- carbons and hemoglobin based products, and certainly while these were being referred to at times as blood substitutes, at times red blood substitutes, they clearly were markedly different from the red cells.

Again, because of the nature of the stimulus, the direction that most of development took was as a red cell substitute, and as was mentioned earlier today, the trials that were required were those that would show a global effect similar to what a red cell would show.

And designing these trials, designing trials to demonstrate these products substitutes that these products function as red cells has been extremely difficult, and I can't help wonder whether more limited goals, perhaps use in limited organ perfusion or use to show that an ischemic organ could be supported in certain specific pathologic states, may have permitted more rapid approval of some of these products, although the indications clearly had been much more circumscribed.

So you wonder if you could do it over again what you would do, and of course, you can't do it over again because, as the philosopher said, life only makes sense when it's examined in retrospect, but unfortunately we have to live it in the forward direction.

But what could we do about platelets, about the platelet products of the type we've talked about today?

The platelet is certainly more complex than the red cell, multiple functions. Jim George referred to it as this multiple redundancy in the platelet which tells you it must be important because it's got so many fail safe mechanisms built in.

But it would seem now that a platelet substitute is even less likely to be obtainable than a true red cell substitute, and again, I wonder whether limited product goals and regulatory requirements might be the way to go here, and that a few products we'll refer to as adjuncts to hemostasis or some other euphemism rather than platelet substitute; whether the testing and perhaps even the approval would be less complex.

If this is going to happen and people who are developing products, people who are financing the products would have to consider the long-term biological, medical and financial aspects of perhaps developing these for very discrete clinical situations, very discrete clinical models where the efficacy could be shown more easily.

Do I have any suggestions for what those might be? I've talked about some. I don't have any that I could talk about right now, but certainly to give a glib answer to a question as complex as

that, again, I give you my closing quotation that for every complex problem there's a simple and obvious solution which has invariably found to be wrong.

Thank you.

(Applause.)

DR. MONDORO: Thank you, Dr. Fratantoni.

Our next speaker is Dr. Stein Holme, and the title of his talk is "The Residual Lifespan Parameter for Quantitation of Platelet In Vivo Viability by Radiolabeling and Infusion into Normal Volunteers."

DR. HOLME: The purpose of my presentation is to show that the residual lifespan parameter is a more useful and more informative parameter for measurement of platelet in vivo survival of a product than the currently used survival parameter.

The survival parameter that is used today for quantitational of in vivo viability of a platelet product is the so-called numerical expected lifespan, and this parameter was originally developed to measure platelet survival in patients, and by definition it is the birth cohort lifespan of platelets that are newly released from the bone marrow.

And as I mentioned, it's used to determine platelet survivals in thrombocytopenic patients in order to determine the platelet turnover rates and also events in the circulation system, such as senescence and random distractions.

It's not really meaningful to use this survival parameter to measure the viability of a platelet product. Here we are more

interested in looking at storage condition or processing conditions in vitro which will have an effect on platelet in vivo behavior.

A more appropriate and more informative survival parameter in this respect is the residual lifespan. By definition, this is the mean residual or mean remaining lifespan in the circulation of the labeled and infused platelet population. This is often referred to the cross-sectional sample or sample population, which is similar to the product population.

And as I will try to demonstrate in this presentation, by using this concept we can obtain a more appropriate and more informative information about the viability of a platelet product.

This is a hypothetical population of so-called numerical population. The horizontal lines on this slide here represent the lifespans of 11 platelet subpopulations, and the length of the lifespan is the expected lifespan.

The average numerical expected lifespan is taken by adding all of these subpopulations up and then divided by the number of subpopulations.

This is the so-called cross-sectional population which is the population or sample population taken at a certain time, and this population is different than the numerical population because, as we can see this vertical red line here, the platelet subpopulation that has the lowest lifespan is not included in the cross-sectional populations, and this is typical.

When you take a sample population or cross-sectional population, it will contain platelets with relatively longer lifespan than you will find in the numerical population.

The residual lifespan or the cross-sectional population is shown to the right of this vertical line, and here on this slide I have arranged the cross-sectional subpopulations according to their lifespan, and this is the typical platelet survival curve which is obtained by radiolabeling and infusion.

And the mean residual lifespan, the average lifespan of the total population is then obtained by taking each of these subpopulations and divided by the number of subpopulation, in this case ten.

And this is similar to taking the area below the survival curve and divided by the percent recovery. That gives you the total viability of the platelet product and thus is a much better measurement of platelet viability than using the numerical expected lifespan.

This slide shows a typical survival curve for fresh platelets, and the mean residual lifespan is then the area below the survival curve divided by percent recovery, and in this case it's about 5.5 days.

So this means that after infusion, the infused platelet lives on the average of 5.5 days.

The cross-sectional -- the total lifespan of the cross-sectional subpopulation is twice this. So it would be 11 days, and

this is substantially longer than what we see in numerical expected lifespan.

The numerical expected lifespan is determined by taking the initial tangent to the survival curve, and where this tangent intercepts with the X axis, it's the numerical expected lifespan, which is normally for fresh platelets around 8.5 days, which is then, of course, substantially less than the cross-sectional lifespan.

I will in the remaining part of this presentation give you some examples where by using the residual lifespan concept we can get more informative information about the viability of stored or a platelet product.

This slide shows the survival curve of fresh and five-day stored platelets, and the decrease in the recovery by the stored platelets is about 21 percent. However, in terms of total loss of viability, which is the loss of mean residual lifespan, there is about 36 percent loss.

That means that with five days stored platelet, stored under currently optimal conditions, there is about 36 percent loss of total viability.

By combining this concept of residual lifespan with mathematical function that incorporates the concept of senescence and random destruction, we can get additional information about the viability of our stored product.

The survival curve of the five-day stored product can be obtained in this case by taking the residual lifespans of the fresh platelets and subtract from each platelet a lifespan of two days.

So what this suggests is that with platelet stored at optimal conditions at 22 degrees there is about a two days' loss of in vivo residual lifespan, which is basically in vitro aging.

This slide shows the survival curve of cold exposed platelets as compared, again, with fresh platelets, and what is typical for cold exposed platelet is a proportional reduction of the platelet residual lifespan.

So the survival curve for the cold stored platelets is the same as if you took the survival curve of the fresh platelets and then had a 50 percent reduction in the residual lifespan for each platelet. So this means when platelets are exposed to cold, that each of the platelets -- their lifespan is proportionally decreased with a certain percentage.

And finally, this is survival curve of fresh and cryopreserved platelets. In this case, we have about 44 percent nonviable platelets. However, the residual lifespans of the cryopreserved platelets that survive in circulations are unchanged.

So in summary, the residual lifespan parameter, we will get more accurate and more informative information about the viability of our product since we can measure the percent of nonrecovered, nonviable platelets in the product. We can look at the reduction in the residual lifespan of the viable circulating platelet in the product, and also we can determine the reduction in the residual lifespan of the total platelet population in the product to get an estimation of the total loss of viability.

And further, by combination of the residual lifespan parameter with mathematical formulas, we can determine the percentage of nonviable platelets in the product that is caused by random destructions versus in vitro aging, and also we can determine the reduction in the average lifespan of the circulating platelets by the platelets in the product that is caused by in vitro aging versus random proportional reduction in lifespan.

Thank you.

(Applause.)

DR. MONDORO: Thank you, Dr. Holme.

Our next talk is by Dr. Sarah Middleton, and the title of her talk is "Evaluation of In Vivo Efficacy of a Platelet Substitute, Synthocytes -- the Challenge."

DR. MIDDLETON: Thank you very much.

I'd just quite briefly like to tell you about the work we're doing to develop a platelet substitute which has already been referred to, which is fibrinogen immobilized human serum albumin microcapsules, and we are trying to develop this product at Andaris potentially for the prevention and/or the treatment of bleeding thrombocytopenia, which is in itself a fairly difficult concept I'm beginning to understand.

One thing I have to tell you is this product is currently in exploratory development, Andaris, and what I mean by that is that senior management aren't going to be at all impressed until I've actually proved that this product will work in human subjects, and

until that point I have obviously a finite amount of resources with which to work, and that's important.

This product is made by -- essentially we make albumin microcapsules to define the size with a very tight size specification by spray drying human albumin.

We then immobilize the human fibrinogen onto those capsules. It's not covalently linked. It's performed with no chemistry involved, and essentially is quite a simple process.

Just to point out, a previous questioner asked about the amount of fibrinogen on these capsules. Now, in our case, the amount of fibrinogen is very tiny. We estimate that in the maximum dose that might be infused into a patient, they would receive a total of nine milligrams of immobilized fibrinogen as compared to the ten grams already approximately circulating. So we don't really think that the fibrinogen itself contributes very much to the overall pool in terms of pre-fibrinogen.

I have to say we characterize these capsules by measuring the amount of fibrinogen on the capsules using an ELISA technique.

Our only in vitro measure of efficacy is by adding thrombin to the capsule and measuring the time for aggregation, and that's all we've been able to develop up till now to look particularly for activity in vitro.

We, like everybody else, have gone to thrombocytopenic methods, and one of the questions that I actually would like to ask is we've decided to use rabbits mainly because rabbits seem to be the accepted model. One of my concerns has, particularly for a product

like this, is do rabbits -- is the relationship between rabbit platelets and human platelets sufficiently close to mean that this is actually a satisfactory model for this sort of preparation?

We have looked at three lots of rabbit models, two using antibody to delete rabbit platelets, and this one which I'm just showing the results of uses the cytotoxic busulfam, which you've already heard described as well.

What we show with this product is that we get this very significant reduction in bleeding time, in template bleeding time, in thrombocytopenic rabbits which have been treated with busulfam 12 days prior to the study and nine days prior to the study, two doses of busulfam.

What we see is in a platelet count that's less than 20 times ten to the ninth per liter, we see a significant reduction in ear bleeding time at one mL per kilogram and .5 mL per kilogram of the Synthocyte preparation.

In contrast, the control bleeding -- the controls, which are saline controls and control microcapsules which have no fibrinogen absorbed, are still prolonged. This effect is still sustained at 180 minutes, although perhaps to a slightly lesser extent.

And we've also looked at the surgical incision in these animals whereby we did an abdominal incision, a standard incision in the abdomen wall, and looked for blood loss over 15 minutes by absorbing, pressing gauze onto the wound and measuring the amount of blood that was lost.

As you can see, we actually similarly see a significant amount in blood loss from the surgical incision with the Synthocyte product, but not with the controls, and this, again, was seen up to 180 minutes.

Again, to try to answer another question, we have actually looked at biopsies of these wounds now to look to see whether we can see Synthocytes in the wound. I'm afraid I'm going to disappoint you just to say that those results are still in the post, as it were, and we have done the work, but I haven't seen the data yet. So watch this phase.

My concern about using rabbit platelets, I guess, sent us off to look for another model where we could actually look at the effect in human blood. We have tried to look at the effect of Synthocytes on platelets, human platelets by aggrogometry, and the results are very much pretty messy to say the least.

We have a lot of donor variability, and it's very difficult to get a qualitative -- a quantitative estimate of efficacy, never mind making it qualitative.

So we're now using a perfusion chamber like a Baumgartner chamber, to examine these, and this technique turns out to be one of the best techniques that we've got at our disposal, and we're currently looking at trying to make this at least quantitative.

Essentially it allows us the freedom to look at the effect of Synthocytes on a number of different surfaces where we can mimic blood vessels under a number of different conditions in terms of coagulation and other things that may be important in the activity.

This is just to show you, in essence, that in the presence of HSA capsules, passing them over extracellular matrix we see where mixed with platelets in human blood, we see just the platelets laid down on the surface and no capsules present.

In the presence of Synthocytes in the same system, we see that the Synthocytes seem to be interacting with human platelet and essentially augmenting the platelet plug, which is what we're -- the thrombus plug which is what our aim is with this product.

I can also tell you that the result tends to suggest now that in the absence of thrombin there seems to be -- the interaction of Synthocytes with platelets seems to be very limited.

We are now moving on into -- we completed a preclinical program to show a far as possible the safety of the agent prior to moving into our first clinical studies to prove this concept. We have opted to work straight in thrombocytopenic patients for reasons we think of safety because we do not wish to be infusing these into people with normal platelet levels.

We have tried to find in the first instance for a safety study to look at, quote, stable thrombocytopenic patients who are not bleeding, and we've selected a patient population with aplastic anemia, MDS -- sorry -- myelodysplastic syndrome, and ITP.

We are having to use the multi-center study to get enough patients to complete this. So we have at least five clinics in the U.K. who are going to help us with this study.

To try and get some feeling for efficacy, we are falling back on the bleeding time in this instance, and we will only be

monitoring the bleeding time in the high dose group because clearly it's not necessarily ethical to start looking at bleeding times where you would not necessarily expect efficacy to occur, and we are using the rabbits as a guidance for this bleeding time measurement.

In terms of a second study, we're actually going to try to look at the product to see whether it will actually stop patient -- bleeding in patients who are bleeding. We are scouting around for suitable models. We're talking to clinicians to try and find out what would be an acceptable model and one where we can actually see the bleeding is occurring so that when we infuse the product we can actually see something happening.

At the moment, we are led to believe that potentially bleeding from a Hickman line following insertion of that line can occur. A way around this is to actually -- we're actually conducting a survey now to find out exactly when that bleeding occurs. Are platelets given? What are the effects, and these sorts of things?

On the final overhead, it really is used -- just shows the issues that we have found on developing this program. I haven't time to say anything really very much on this, but I'd just like to draw your attention to the last point, the identification of positive control.

We're a new company in this area. So we tend to challenge preconceived ideas, I think, but I have already heard it said that do we actually know that platelet works.

Platelets do actually work, and something that's happened recently in the U.K. vis-a-vis human serum albumin, which has been

used for 40 years and everybody said it worked, somebody has now done a meta analysis and published in the British Journal of Medicine that, in fact, albumin doesn't even work, but in fact, it's positively dangerous.

So I just hope we're not going to get into this situation.

Thank you very much.

(Applause.)

DR. MONDORO: Thank you, Dr. Middleton.

We'll just have one more talk before our break, and that is from Dr. Jerome Connor, and the title of his talk is "Analysis of the In Vitro and In Vivo Functional Activity of Human Plates Cryopreserved with ThromboSol: Development of a Platelet Storage System."

DR. CONNOR: Well, good afternoon.

I'm going to be somewhat different in that I'm not going to be talking specifically about a platelet substitute, but about a new method for preserving standard platelets and normal, functional platelets.

We have a platelet preservation system. It's based on the biochemical stabilization of platelets to allow them to be stored without the standard storage lesion events that we see.

We have programs looking at cryopreservation of platelets, refrigerated storage of platelets, and ultimately looking at some freeze dried formulation of platelets.

What I'd like to focus on today is the cryopreservation system. It's our most forward reaching project, and I'd like to show you some of the in vitro and in vivo data that we've generated here.

We are in a unique situation as compared to the platelet substitutes in that we are looking to preserve a fully functional, normal platelet. It does give us some advantages in terms of how we go about our testing, what we use as our controls in that we don't have to look at the surrogates. We can look at comparison to a standard platelet.

So what we are looking for is the ability to get a fully functional platelet following cryopreservation. What we are striving for is to have a formulation that would be directly transfusable. Thus, you wouldn't have to have wash steps and other manipulation of the platelets that are damaging, and thirdly, we want something that's logistically simple, that can integrate very well into standard blood banking, and that can work simply in the clinical situation.

What I want to do is sort of take you through the development of our program, a brief description of the stabilization formulation, and then the standard protocol that we have followed to do in vitro analysis.

The advantage of that is that it allows us to do screening and development of the formulation that we are trying to establish.

We went on and did some preliminary hemostasis, which is obviously a bit more stringent testing than a single functional test, and then finally a pilot clinical study to look at circulatory in vivo parameters of these platelets.

Well, ThromboSol was based on the approach that the endogenous platelets in circulation are maintained under a constant inhibition by endogenous molecules in relation to the blood stream. When you harvest these platelets during aphoresis or random donor unit preparations, put them in storage bags, you're obviously removing them from this endogenous storage system.

What we attempted to do was to mimic this endogenous inhibition event by stimulating specific second message vector systems in the platelets to help protect them against the storage lesion events that occur during storage.

Our formulation consists of three components: amiloride that inhibits the sodium proton pump and blocks the release of calcium going into the stores; adenosine and sodium nitroprusside, which are cyclic nucleotide stimulators which stimulate cyclic ANP and cyclic GNP which are endogenous inhibitor systems to prevent activation.

One of the advantages of having this biochemical stabilization is we've had the ability to reduce the requirement for cryoprotectant for DMSO down to two percent.

Another advantage of this system is that it is very well integrated into the blood bank. It's logistically simple to do. The formulation is dissolved in the DMSOs with the single direct addition. This can be done from a satellite bag, by sterile docking. It can be done by an injection through a sterile port.

You directly insert the unit into a minus 80 degree freezer in an aluminum cassette. There is no controlled rate freezing. It's a very straightforward freezing step, and then thaw.

You just stick it into a 37 degree water bath, and your platelet unit is ready to go.

Obviously our goal is to have no wash step following thaw. We feel that solution can be directly transfused, which would eliminate the normal thaw-wash steps that are required with the six percent DMSO.

The question that's been raised, and it's an important point, is what do you compare this to. What do you use that should control samples?

Obviously the gold standard is a fresh platelet. A fresh platelet does not exist. I mean, it's been mentioned before. All platelets that we get are usually about 18 to 24 hours old by the time you get them from the blood bank and do all of your biohazard testing.

So while fresh platelets is your gold standard, a fresh platelet really is a 24 hour old platelet that's been through normal blood banking systems.

The second control sample that we compare things to are five-day stored platelet. Obviously these are the optimal transfusion platelet, but in the clinical setting we quite often do see three, four, and five day old units transfused. It is the five-day limit set by the FDA. So we use this as our second criteria of comparison.

And finally, the last criterion of comparison is a standard six percent DMSO cryopreserved platelet, which while it has been used is not used very much in the field, and we know that there are problems in terms of recovery of cell number, in vitro functional activity, and in vivo functional activity.

In terms of in vitro analysis, I think that it's been mentioned that obviously there are many different types of tests that can be done. They all tend to test a single functional parameter. There's no one that is indicative of how well it's going to do in vivo.

I think if you can get an overall characteristic of your platelet, you can get a good feel as to whether or not it will do well in vivo. Certainly there is some correlation to cutoff values. I think that's been seen in the literature from Dr. Holme's work that certain values, like the extent of shape change and hypotonic shock, that if they're above a cutoff threshold that they're certainly going to be effective even if there are not R correlation values that are perfect.

Obviously it's important to get good recovery of cell number, and I think percent discoid is a good indication of the -- morphology is a good indication of how good your platelet is.

Vax analysis of surface markers, obviously it's important. You don't want to lose 1b in many of the surface markers.

P selectin, it does indicate that you have activation, but we've heard today that may not be indicative of how well it does in circulation.

What I want to do here since I really am on short time is to look at how our platelets compare to the three controls, the fresh platelet, the most stringent; the five-day stored, liquid stored platelet; and a six percent DMSO cryopreserved. These are our

criteria, and most of the actual number of data have been published in this Transfusion paper.

If you look down the list, you'll see that six percent DMSO do very poorly compared to ours. We see loss of cell number. We see loss of discoid. Shape change and shock response is very well reduced in these, though you do see good aggregation. You see no loss of 1b, and you see a lot more expression of P selectin.

Five-day liquid stores, you see some loss of cell number of percent discoid during the five-day storage that's not seen in these cryopreserved platelets, but in terms of the other functional activation criteria we see no loss here.

Obviously the five-day stores lose aggregation. That's not surprising. It doesn't indicate any. We know following transfusion they tend to reverse this and get their aggregation back.

We do see significantly more expression of P selectin in these five-day stored platelets.

Obviously compared to fresh platelets we don't expect our platelets to hold up to that criteria, but I think two categories that are important is we do not see any loss of cell number. We see greater than 95 percent recovery of cell number in all cases following thaw, and we do see some drop of percent discoid. The fresh platelets are somewhat better, but statistically in this paper, you'll see that the percent discoid is not statistically less than the fresh platelet control, though obviously in terms of functional activity we tend to see about a 50 percent dropoff. These are, as I said, 50 percent

better, and we do obviously see significant increase in our P selectin.

So we felt that using these in vitro screenings, we could conclude that we're obviously significantly superior to six percent DMSO systems, equivalent to a five-day stored platelet, and obviously we are not up to the gold standard of fresh platelets.

We moved on and looked at some hemostatic models because we felt that this was a more stringent test that required multiple platelet functions of adhesion, aggregation, coagulation effect.

We looked in three systems. We've done some work with the PFA 100 model, perfusion model, for the ability to -- perfusion model that adheres to a collagen matrix; a Baumgartner perfusion model; and then we've done some preliminary work, though limited, with the thrombocytopenic rabbit model which was with Dr. Mo Blajchman.

And, again I would just like to sort of touch base on these. The PFA 100 model, we looked at the PFA index, which is a measurement of both the amount of blood flow and the time it takes to close the wound, and we get slightly better than five-day stores and 50 percent less in fresh.

In the Baumgartner model and thrombocytopenic model, we see similar things. We see much better than six percent, equivalent to five, and 40 percent, and I want to push forward because I want to get to this.

We went on to the in vivo model. We did the model as explained by Dr. Snyder, developed by Dr. Holme, and we used small

volumes of radiolabeled samples. These were paired, and we did percent recovery and survival time.

This is the standard model that was done at M.D. Anderson Cancer Center under a physician sponsored IND. We took healthy volunteers. We cryopreserved half of them with ThromboSol, half with six percent DMSO. We radiolabeled one with chromium-indium. This was randomly done.

They were mixed, infused, and we do blood counts at two hours, 24, and continuous times for ten days. Percent recovery, and survival times.

This was analyzed using the multiple hit gamma function analysis. That's considered the best method, and as you can see, the six percent DMSOs we see a very great loss of percent recovery, down to 28, and the ThromboSol cryopreserved we see 40 percent recovery at -- this is extrapolated obviously to time zero, which is significant -- 2.005.

The survival time, the six percent is 152 hours to 166. That's not truly significant, but it is slightly higher. That's not surprising, as Dr. Holme showed us. Once a platelet is in circulation, a cryopreserved platelet, we don't expect to see it fall off.

So in conclusion we say we've shown significant and statistical improvement versus the six percent DMSO system. We appear to be equivalent to a five-day stored platelet, and if you look at some of the in vivo recovery times in the literature of five-day

stored platelets, they do fall in that low 40 range. So the in vivo data does appear to correlate.

We definitely are not up to fresh platelets. We get a 40 to 50 percent recovery versus that, but we do integrate well into the standard blood banking, and I think based on the formulation, we have the potential to have direct transfusion. Direct transfusion would increase our recovery rate since obviously the in vivo data required specific wash steps.

And just to touch base on, you know, our further work, we're going to do a clinical study where we look at a patient population that's having chemotherapy where they'll have multiple rounds of chemotherapy. This is osteosarcoma.

The advantage of this is that we can compare during the different rounds of chemotherapy a standard blood bank platelet, a standard STU that's 24 to 48 hours, to an autologous STU that was donated prior to chemotherapy that's been cryopreserved with ThromboSol, though based on the last talk, we may want to revise how we do our CCIs.

The indication is that we can, through different rounds of chemotherapy, compare their autologous cryopreserved to a standard blood banking platelets, and we'll randomize in what order they get that, and it should allow us to do some very good comparison to the standard treatment.

Thank you very much.

(Applause.)

DR. MONDORO: Okay. I think we'll take a short break and be back at ten till four, and we'll finished up the manufacturers and move on to the panel discussion.

(Whereupon, the foregoing matter went off the record at 3:41 p.m. and went back on the record at 3:52 p.m.)

DR. MONDORO: If everybody could please take their seats, we're going to continue the manufacturer's perspective. If Drs. Li, Read and Bode could come down front, please.

Our next speaker is Dr. Conan Li, and the title of his talk is "A Dynamic Flow System for Assessing Function of a Platelet Substitute."

DR. LI: Thank you, Dr. Mondoro.

Good afternoon, ladies and gentlemen. I'd like to discuss today an in vitro commercial device that measures hemostasis and how it applies to evaluating functionality of a platelet substitute, namely Cyplex or referred earlier in this conference to as IPM.

We've heard a lot today about the need for an evaluation method, a device for pre-evaluating platelet substitutes before we get into expensive human clinical trials, and I'd like to just summarize for you what I feel are some of the attributes that an effective in vitro or ex vivo system for evaluating platelets should have.

Firstly, it should assess global hemostasis because when you transfuse platelets, you also want to know the effect on other hemostatic events.

Secondly, it really should use native whole blood. We want to avoid the complications of anticoagulant as part of the transfusant.

Next, it should simulate bleeding, physiological bleeding, in that it should perfuse the sample in non-recirculating flow over an exposed subendothelial tissue surface.

And finally, we should be able to observe physiological flow rates, pressure, and temperature in such a system.

I'd like to introduce at this point the Xylum clot signature analyzer which has the following properties. It does assess global hemostasis. It measures platelet adhesion, platelet aggregation, and coagulation on one sample in one run.

Also, it tests native non-anticoagulated whole blood in flow under physiological conditions.

Now, I'm going to spend some time describing the CSA system, which consists of an instrument and a cassette. The instrument is the portion to the right of the black bar.

What happens in this system is you have an oil reservoir that is pressurized and causes the oil to flow through a tube at a fixed rate. The oil is injected into the disposable and goes up into a syringe that's preloaded with your blood sample. The oil being lighter than blood rises to the top and displaces the blood into a perfusion tubing that's in the cassette, and the blood actually flows into this pressure chamber, which is downstream and preloaded with oil.

So basically what you have is a column of native whole blood sample jacketed between two segments of oil, and the movement of that blood is measured by this pressure transducer which monitors the pressure in this pressure chamber, and that is a direct function of the flow of blood.

At the end of the test, the entire cassette, including all of the waste materials, blood, and oil is disposed of.

Here's a picture of the cassette, showing a scale, and it has two syringes for the punch and collagen channels, which I'll get into in a minute.

Here's a picture of the instrument system. It has two handlers for capability of running two cassettes simultaneously. So you actually can run four channels, two punch and two collagen channels.

It's a benchtop automated system, and we have five parameters that we're already able to explore on this system, of which two of them, the PHT and CITF are the ones that I'm going to focus on primarily today due to time, and these are your major platelet indicating functions.

Now, firstly, I'm going to get into a little bit about the punch channel. This cartoon shows the flow of blood in a half millimeter lumen which is maintained at physiological temperature, and this is the perfusing blood that I showed you in the cassette at the beginning of the test.

Now, at the moment of punch, the tubing is pierced by a fine needle producing 200 micron wide punch channels or holes, and the

blood beings to flow out of these holes, and this is indicating a high sheer area in which the platelets will be activated.

Now, let me just show you this drawing, which is a calculated line drawing showing the mechanics of flow, and these are the two punch channels, and you can see that there are areas of very high sheer rate, in fact, in excess of 10,000 per second, as the blood exits through these two punch channels from the holes formed in the lumen.

In terms of the instrument output, we have a pressure tracing with time, and initially the pressure stabilizes at a stabilization value. At the moment of punch, because you're losing blood from the lumen, the pressure drops precipitously, and then you see a spike in response as the pressure recovers back up to its initial stabilization value.

This is caused by platelets adhering and aggregating in the punch channels and causing occlusion of those channels so that you restore lumenal flow, and that's indicated by this time called PHT, or platelet hemostasis time, at which point the pressure has recovered to its initial stabilization value.

As you get lumenal flow, you also eventually in about 22 minutes or so have occlusion of the entire lumen, and this causes a drop in pressure altogether, but this is the platelet related function right here that we're going to be looking at more closely.

This is a cross-sectional micrograph of the lumen showing the two punch channels, and as you can see, well, these are a lot of red cells and white cells, but these areas here in the channel are

predominantly platelet thrombi, and this is a closeup of one of the channels showing that this platelet thrombus -- this is the direction of flow -- predominantly is what's causing the occlusion of that punch channel. This was taken after a run, this slide.

We did a lot of experiments on PHT on blood samples in the presence of various inhibitors, and as you can see, significant inhibition or prolongation of PHT above control occurs with platelet inhibitors, that is, heparin, which is a thrombin inhibitor, but also ATA, which is an inhibitor of high sheer induced platelet activation, ReoPro, antibody to von Willebrand factor, and GP1B.

All of these very potent platelet inhibitors primarily are responsible for prolonging the PHT, suggesting that the PHT is, in fact, a measure of sheer induced platelet activation.

Now I'm going to spend some time talking about the other channel, the collagen channel. This is a cartoon again that shows the construction of the collagen channel. It's comprised of a collagen fiber that is concentric with the lumen of flow, and with time platelets will adhere to the collagen fiber primarily through the 1A2A receptor, and build up a platelet thrombus, eventually occluding the flow in this lumen.

And, again, as indicated by the pressure output in the collagen channel, you have initial stabilization of pressure and gradually the pressure will decrease because of the platelet thrombus formation in the collagen channel on the collagen fiber until the flow is completely occluded, shown by the drop of pressure down the baseline.

And what the instrument reports and calculates is this CITF parameter, which is the time from the start of the run until the pressure has dropped 50 percent.

This is a micrograph -- I'm sorry -- a light micrograph of the collagen fiber after a run, and as you can see, there's a platelet thrombus that has adhered to the collagen fiber, suggesting that the platelets actually are playing a role in forming a thrombus on the collagen surface.

And, again, experiments that we've done with various inhibitors indicate that this is, in fact, a platelet mediated response. The ATA, ReoPro, the potent platelet inhibitors do, in fact, cause a significant prolongation of the collagen induced thrombus formation, CITF, which is at time to 50 percent occlusion.

Now I'd like to spend some time on the experiments with Cyplex. We took about 15 rabbits that were induced into thrombocytopenia with the chemical busulfam, and what we did was we measured the ear bleeding time, the PHT, and the CITF, which are the CSA parameters, as a function of platelet count, and these correlations are significant using a non-parametric Spearman rank order.

The P value is less than .05, confirming what we already know. That is, these three parameters are dependent upon platelet function.

In the next series of experiments we took 20 thrombocytopenic rabbits with platelet counts from 6,000 to 40,000 per microliter, and we injected Cyplex or IPM, and two hours later we

measured the ear bleeding time, the PHT, and CITF, and we can see from this chart that the bleeding time reduced significantly after Cyplex, and so did the collagen induced thrombus formation, but the PHT did not change significantly.

And this suggests that the means of operation of Cyplex, if you will, the way it works is through a mechanism that involves enhancing the platelet's ability to adhere to a subendothelial surface, namely, collagen or tissue, but it does not work, as this data suggests, through an enhancement of the high sheer induced platelet mechanisms.

And this is consistent with the fact that was presented earlier today that the Cyplex molecule or I should say the Cyplex particle does not contain GP2B3A, which is an innate mechanism in the high sheer activation and aggregation of platelets.

So, in conclusion, our investigation shows that the ear bleeding time, PHT, and CITF increase at lower platelet counts, showing that they reflect platelet activity.

Furthermore Cyplex lowers bleeding time in CITF in thrombocytopenic rabbits, showing that it enhances a platelet adhesion mechanism that operates at lower sheer.

However, Cyplex did not cause a significant change in the PHT in thrombocytopenic rabbits, consistent with the fact that Cyplex does not have the GP2B3A complex and also suggesting that the Cyplex does not work in the high sheer activation realm of platelet function.

Thank you very much.

(Applause.)

DR. MONDORO: Thank you, Dr. Li.

Our next talk with be by Dr. Marjorie Read, and her title is "Hemostatic Potential of Rehydrated Platelets."

DR. READ: My company is University of North Carolina, and I'm here briefly to give you a very quick overview of the work that Dr. Bode and I have been doing with rehydrated lyophylized platelets and using cross-linking agents.

Now, why am I not coming on? There we go.

And using cross-linking agents to prepare a freeze dried product, platelet product, that retains hemostatic properties as measured in these different assays relative to adhesion aggregation platelet plug and presuming a surface on which prothrombin is converted to thrombin.

In the Baumgartner, this is a shot of a Baumgartner adhesion study in which the -- I'm sorry -- in which the fixed platelets -- this is what I'm looking for -- in which the fixed platelets and the fresh platelets are pumped under an everted vessel segment, and as you can see, equivalent numbers of the rehydrated platelets adhere just as we see in fresh platelets, which you'll notice that we see less spread and fewer pseudopodia that are formed with these rehydrated platelets than you see with the fresh platelets.

However, we have noticed on additional studies on foreign surfaces and other blood cells that these platelets are perfectly capable of spreading. They do undergo spread. They do put out spiny protrusions and pseudopods, but at a much slower rate and with a less degree than do fresh platelets.

In studies relative to agglutination times, and just ignore this for the time being, in assays with erystocetin and botrocetin, we see identical agglutination times. The important thing that you'll see here is that these platelets do not aggregate with ADP.

However, equally important, in mixtures of fresh platelets and rehydrated platelets, they neither inhibit, nor do they retard fresh platelet aggregation. In fact, in mixtures of fresh platelet and rehydrated platelets, we find that these labeled, fluorescent labels of the CAJ (phonetic) 95 dye, these platelets are recruited and incorporated into aggregates that are formed with the fresh platelets in the presence of an ADP calcium. There are no aggregates; they still don't aggregate in the presence even with fresh platelets if there's no ADP added.

And we have found this to be concentration dependent. As we dilute the numbers of fresh platelets and increase the numbers of rehydrated platelets, we get smaller aggregates, but this is about ten percent fresh platelets or less, and they still continue to recruit the fixed platelet or the rehydrated platelet to the aggregate.

When we looked at mechanistic properties and wanted to look at preservation of platelets by flow cytometry looking at the percent of cells that stained positively for IIb/IIIa or Ib or IbIX with various antibodies, we find that nearly 100 percent of the platelets stained positive for these glycoprotein receptors.

And in specific assays which are specific in saturable assays using radiolabeled antibody and radiolabeled purified protein,

we find that the density, that the GP Ib density on the cells in rehydrated platelets is generally somewhat greater than fresh, about 139. The amount of vWF that is found is approximately 100 percent. The GP IIb/IIIa density, however, is reduced to about 42 percent, and in subsequent studies this runs between 35 and 45 percent, and the amount of fibrinogen that is found has been about 31 to 42 percent.

Now going to the procoagulant part, we find that in platelet rich plasma that's recalcified with one-sixth part of calcium giving a final of .027 molar we get a identical clotting times, that when we mix these platelets and in clots that are formed with them, again, we see that these platelets are caught up in the fibrinogen strands, and there seems to be some morphologic evidence that the fibrinogen strands are attached to the surface of these platelets.

We went to in vivo studies, some of which we reported in '95, in both rats and dogs looking at the bleeding time wounds and wound sites, occlusive thrombus participation in adhesion to injured vessel walls.

We were in the rat model in which we induced thrombocytopenia with an anti-rat thrombocyte antibody. We were able to knock the platelets out, extend the bleeding time to greater than 15 minutes, and when we replaced the platelet population with the rehydrated platelets, we were able to restore the bleeding time almost the same as it was in normal.

Since then we've done more rat. We have had approximately the same type of results, but we have also found that there is some

individual animal response. Some of them we couldn't correct at all, but most of them we got similar time.

This a fluorescent micrograph of the bleeding time wound in one of our occlusive thrombosis models in which we have a pinch injury. Actually we apply Goldblatt clamps so that we have a stenosis, and then we have a controlled injury.

We also looked at the -- and then we do ear cuts and look at that particular -- at the ear, actually the cut wound -- the biopsy is the word I'm looking for -- of the bleeding time wound, and we actually see that these labeled platelets then adhere right along the surface of the cut wound.

In the injured artery vessel, where in the injured artery we see -- this is a carotid artery -- that along where the wall of the vessel is torn and there is hemorrhage into the vessel wall, we find these platelets or in areas where they hemorrhage. They are in the small thrombi that tend to form in the lumen of the vessel, and in other slides that I didn't bring due to limited time, when there is no injury or in the control actual coronary artery that does not undergo injury, you don't see platelets of any kind, of course, because there's not a wound there.

Our conclusions then based on these studies are that we do see in the morphologic integrity with these platelets, which we've shown a number of times, they do have some pro coagulant activity, and things that I did not show, slides that I did not show relative to the pro coagulant activity is in the modeling which we activate these platelets with thrombin and put them in -- add prothrombin, and then

using a specific thrombin S22 chromogen, S2238, we find that we do have good conversion of prothrombin to thrombin.

If we do not activate the platelet even with thrombin or with a thrombin-collagen mixture, we get no conversion of prothrombin to thrombin.

We do find that the platelets are capable of spread. They're capable of inefficient ADP thrombin response. They have inefficient stimulation, as we call it, because they do not alone aggregate with the ADP. They have to have some fresh platelets present to do so.

They aggregate beautifully with botrocetin and erystrocetin, as one would expect since we have all of the Ib that we need. Functional recep., shortened bleeding time and participate in thrombus formation and adhere to injured wound sites.

These are some studies that we did or have been doing, and we have some ongoing studies that we are currently looking at relative to blocking fibrinogen binding and release of fibrinogen from these platelets that would show stimulation or the platelets being capable of stimulation.

We also are collaborating now with Dr. Tom Fisher who does a lot of cell signaling and calcium channel work, and he is currently looking at assays where we stimulate these platelets with thrombin and look at the amount of radiolabled tyrosine events that are present, and currently he is having positive results with that.

So our evidence to date looks like that these platelets are not dead, as one would think, since they are mixed with cross-linking agents. They are somewhat slow, but they are not dead.

Dr. Bode, I think, is going to give you the other half of this talk which is on some of his more current work with thrombosis.

(Applause.)

DR. MONDORO: Thank you, Dr. Read.

Our last talk before the panel discussion is from Dr. Art Bode, and it's "Rehydrated Platelets, Continued."

DR. BODE: I'd like to thank the organizers for letting me bring up the rear. I'll try to rise to the occasion.

But this is just a continued discussion of with Dr. Read has already introduced to you about cross-linked, freeze dried human platelets, and I wish I had brought a question mark to put up here. Preclinical testing of efficacy, question, because what we're talking about are still very much investigations rather than proof of efficacy even in a preclinical mode at this point.

So when I talk to you about data generated in a clot signature analyzer that Dr. Li just introduced to you, I'll talk again about the data that Mo Blajchman referred to in his review of everything that he's tested in thrombocytopenic rabbits, and then move on to an area where we're trying to develop a model that is looking at, well, really correction of bleeding time and recovery of platelets infused in dogs on cardiopulmonary bypass, and I'll be interested to hear Dr. Harker's remarks on this.

Our clot signature analyzer does not input directly into a Power Point slide generator. So I'm going to show you the basic 25 cent printout that we have.

But in orienting you to what's going on here, this is the pressure monitoring of the punch channel. Here's the pressure monitoring tracing of the collagen channel, and this cassette was nothing more than non-anticoagulated whole blood quickly put into the syringes, put on the CSA, and you see the wave form that Dr. Li has already described for you.

If you add prostaglandin E1 to inhibit many of the platelet dependent processes in coagulation or hemostasis with whole blood, you do see, indeed, a remarkable shift in both the PHT, as the lead parameter for evaluation for us, or in the CITF or really the collagen related clotting time in that channel.

In trying to organize this to test surrogate platelets, substitute platelets, things that you put into a whole blood recombined environment, we had to look at what we call a blank.

We prepare fresh washed red blood cells and combine that with fresh autologous citrated platelet poor plasma, and if we're in the platelet modality testing, we can put platelets into that, recalcify it, and then put it into these two channels and look for the response.

If we do this without added platelets, you see again what we'll call an abnormal bleeding time generated in the punch channel, and a fairly abnormal collagen related clotting time in that channel.

And when we add that preparations of our lyophilized platelets, we do see a correction of the in vitro bleeding time in the punch channel and a shortening of the collagen into this clotting time as well.

The question I have is if I show another slide with a different kind of lyophilized platelet preparation and it's better in that parameter or in this parameter, does that mean we're actually approaching better clinical efficacy? As yet I think that's undecided, and hopefully that's something this group can evaluate.

Moving on to the data with Mo, these are the raw data that he showed you in his summarized bar plots where it shows you the platelet count that was achieved with infusion of either fresh human platelets or the human lyophilized platelets in the thrombocytopenic rabbits plotted versus the bleeding time after one hour, and in this particular plot, you see that we have achieved a circulating platelet count which is distributed like that of the fresh human platelet infusions, and we end up with bleeding times that actually by means are very similar.

The main difference though is if you use this plot as percent recovery rather than platelet count achieved, you see that we do have a lesser recovery of the lyophilized platelets in the circulation of the thrombocytopenic rabbits, therefore leading to what was said by Dr. Blajchman, that you need more of the paraformaldehyde process lyophilized platelets than you do the fresh, unfixed human platelets to achieve that bleeding time.

Now, this is what I'd like to spend more of my time if I can. Right down the hallway from our lab is the training room for the cardiac surgery residents, and they quite often put dogs on bypass as part of their training.

In doing that, the dog is under full anesthesia, and the chest is opened, they're cannulated and put on extra corporeal circulatory support for at least two hours.

We asked them to go in and ligate the spleen or take it out, and then also allow us the opportunity to monitor the dog during this pump time, and as far as not interfering with their surgical procedures, that's fine.

But all along the way we've been looking at the jugular vein vessel bleeding time as our main monitor of global hemostasis in these animals, and it really does look like a gusher when you strike it right, and then look at the effect of platelet infusions on the vessel bleeding time at various parts of the dog's procedure.

And these platelets are dog, canine lyophilized and fixed as we fix the human platelets, but with the lower concentration of paraformaldehyde.

I don't want to spend too much time on this other than to tell you that during the whole process of the dog on the pump, we wait for two hours until the bleeding times are getting remarkably prolonged and then do the infusions while the dog is still on that assisted circulation so that we do not run into problems of trying to get distribution of platelets in an animal that has a very low mean arterial pressure.

So once the infusion is accomplished, then the dog is taken off the pump, and we follow in a postoperative period bleeding times about every 30 minutes.

With that sense of data, let me just divide what we've seen into three study groups. The first study group is routine in that nothing extraordinary was added to support hemostasis other than our choice of lyophilized platelets, plasma, or nothing at all.

So after two hours of cardiopulmonary bypass for these dogs, the vessel bleeding time is remarkably prolonged over baseline, and in fact, in one particular dog, the sites that we had poked in the jugular vein began rebleeding spontaneously. We really had a disaster on our hands.

But if we infuse lyophilized canine platelets at least in these two animals, there was correction of that vessel bleeding time which was sustained over the postoperative period. If we did not infuse the lyophilized platelets, we did not see a return to normal baseline in the vessel bleeding time just because the dog came off the pump.

We also looked at situations where we infused the lyophilized platelets after the dog had finished the cardiopulmonary bypass, but as I'll show you those are less spectacular corrections.

In the second group, just to make things more complicated, every animal received one gram of Amicar, an anti-fibrolytic which is typically used in open heart surgery cases, and in this selection of four animals, you see the vessel bleeding time is markedly prolonged after bypass for two hours.

Three receive lyophilized canine platelets while still on the pump, and we saw restoration of a fairly normal bleeding time, and that's the average bleeding time over the full three-hour postoperative period.

And then again with lyophilized platelets infused when the animal was taken off the pump, maybe a less satisfactory result.

Here are the controls showing no normalization of the vessel bleeding time, and in fact, we lost both those dogs after they came off bypass.

The surgeons say make it more complicated. So we did. One gram of amicar, plus every animal received 300 milliliters of autologous canine plasma collected right before the procedure started, and in these particular animals, the vessel bleeding time was not quite as prolonged as a group after two hours of bypass.

These two animals received canine lyophilized platelets. There was a shortening of the vessel bleeding time, but I don't think it's quite as prominent, pronounced as we saw in the other studies, and in fact, one of the controls seemed to do quite well just with the regimen without the added platelets.

This shows that we've taken this model to the point where in trying to be more physiologic, we've reduced the platelet specific reactivities that we're trying to monitor.

But overall for the studies that we've done without going into the too complicated mode, we have seen as a group an effect of the infusion of the lyophilized platelets versus the controls that received only what the other parts of the study group had.

So I don't think I need to summarize this too much other than add to what Dr. Read has already said, and something we haven't really talked about at this conference is the safety issue, as well as efficacy.

So let me just interject that we know that the paraformaldehyde treatment which stabilizes the platelets also has very significant microbicidal potential, and maybe we can get that into the discussion during the panel talk.

I'd also like to thank the Navy for giving us the support and the relevance for these studies.

Thank you.

(Applause.)

DR. MONDORO: Thank you, Dr. Bode.

This is the manufacturer's perspective session, and I'd like to turn the next section over to Dr. Vostal for the panel discussion, and if all of the invited speakers would come and take a seat at the table.

CHAIRPERSON VOSTAL: Just the speakers from the first two sessions this morning because we wouldn't have enough room at the podium.

And we're hoping we're going to be able to continue the discussion, the lively discussion we had this morning, but in case there needs to be some stimulus, we prepared some questions that we could throw out.

Maybe I can start it here. One of the things we've been wondering about is whether there should be a minimum requirement for

in vivo recovery and survival of a transfused platelet product. Anybody have any thoughts on that?

PARTICIPANT: What was the question?

CHAIRPERSON VOSTAL: The question was should there be a minimum requirement for in vivo recovery and survival of a transfused platelet product. Sort of analogous to the red blood cell transfusion cutoff.

DR. BODE: Again, one of the contrasts that I don't think we've seen drawn very clearly at this meeting yet is the patient population in which these substitutes or platelet derived materials will be evaluated.

If it's meant to go into, as Tom Reid said, the surgical and trauma population, then maybe persistence of effect is more important than persisting platelet count, although I'd certainly grant you that in a prophylactic treatment regimen it would be quite the other way around.

So maybe we can have that.

DR. SCHIFFER: How was the 75 percent chosen?

DR. SLICHTER: Out of the ear.

CHAIRPERSON VOSTAL: Arbitrary.

DR. SCHIFFER: What's that?

CHAIRPERSON VOSTAL: I think it was an arbitrary decision.

DR. SCHIFFER: I imagine it might have been.

DR. GEORGE: Actually, the actual derivation was from post World War II in the Journal of Clinical Investigation. There was an issue devoted to the Army's evaluation of blood products, and in

looking at survivals with -- I believe it was with CPD or ACD, they were able to get -- there was a clear cutoff at 70 percent survival with ACD, and everything else was much worse.

So they listed it at 70 percent. It was in the 1947 issue of JCI. Actually there's one graph. I forget exactly. I think it may have been Boitler that did the original work.

DR. SCHIFFER: I guess that's really another way of phrasing what your -- if we're talking about viable platelets, I assume -- of what your comparison group should be. That is, should it be the best, as close to fresh as possible, or something that has more storage defect associated with it?

Because your decision in part is going to be based on how your new stuff compares to whatever you choose your standard to be.

CHAIRPERSON VOSTAL: Well, I guess we heard 40 percent as a cutoff. Forty percent recovery today was one of the numbers that was brought up. Would that be difficult to reach?

DR. SCHIFFER: I think it's the issue of what you want your standard to be. I think Dr. Murphy made a point that he has more concern about or equal concern about the stuff that's just being given out as we speak in terms of its quality.

DR. MURPHY: I think the point I was trying to make is that normal people with freshly labeled platelets, if they happen to be a low recovery type person, may only have a 40 to 50 percent recovery, whereas someone who happens to be a high recovery type person may have a much -- even better recovery after five days of storage.

I do think though that if we're trying to study real platelets as opposed to platelet substitutes, I think it's reasonable to have some sort of standard, but I think it has to be some sort of percentage of five-day stored platelets or 24 hour old platelets, something like that, a comparison of the test versus some control, and not some arbitrary figure.

I mean, the platelet world is quite different. I think with any significant step forward we're going to expect to see studies in patients which we never require in studies with red cells. So I think it's a quite different playing field.

DR. SLICHTER: You know, part of the issue is that over the period of time that some of us who have particularly grey hair sitting up here have been working in this field, you know, we've seen a lot of different products come down the pike. I think even the product that we currently are giving is not very good.

I mean the NHLBI has just now funded a study to try and see if we can't improve the efficacy of the current products or get more products or additional products.

I mean the reason why everybody is in this room is because I think we all recognize that what we've got is not where we would like to be, even though we've been working on it for a long period of time.

So one of the problems that I struggle with when you say what should we use as a standard, this is good, this is bad, that that implies that, you know, the red cell people have said, you know, if

there's not 75 percent left at 24 hours, we're not interested in what you have to give us.

I think one of the problems with that, and Jack and I were just sitting up here discussing, is that, you know, some of these products may give us something else. I mean they may give us no bacteria and no viruses.

Well, if you say to me or any other rational person, "Look. This thing does not give as good an increment. It doesn't last as long, but you're not going to get -- there's no infectious risk," and then you say to us, "What are you prepared to give up?" I may be prepared to give up a lot, or if I'm out in a battlefield casualty and I've got something that's not as good as what I've got on the shelf.

So, you know, I don't know that we are really able to put in a fixed requirement, but I think that if you say to me, "I've got a product which is not as good as what I've got now, and it doesn't have any additional advantages," then I'm not sure I'm prepared to provide that product to my patient unless it's substantially cheaper.

But I don't -- you know, so the cost effectiveness, I think, in this climate has to be brought into the equation, you know, and that's one of the issues, for example, that's surrounding the question about dose or trigger levels. I mean how low can we get and still provide hemostasis, or you know, do we get more by just giving a little every day or giving a lot and not transfusing?

I mean, so I think all of those questions have to be brought in. So I don't know that if you ask me directly and put my

feet to the fire. I'm not sure I would be prepared to give you a rigid answer about what I'm prepared to accept and not accept as a clinician, depending on what other advantages that product might supply to me in any given situation.

So when Barbara Alving -- I'm not sure Barbara is still here -- but she was talking about maybe there's a place for niche products, and I think that's clearly true. There may be situations wherein a particular alloimmunized thrombocytopenic patient that I'm not able to support with my currently available products.

Maybe something which has got the HLA antigens removed, but has a poor increment and a poor survival may for that patient make the difference between whether they bleed to death or they don't bleed to death, and so I would certainly hate to have you deny me the availability of that product for that patient, even though it may not meet the usual standards that we would like to see for the results of a platelet transfusion.

DR. SNYDER: Can I ask you if you were to receive an application for a pathogen inactivated red cell product that had less than 75 percent, would you -- what would the FDA's response today be to that?

CHAIRPERSON VOSTAL: We'd have to think about it.

(Laughter.)

DR. SLICHTER: Why is that answer not surprising?

CHAIRPERSON VOSTAL: But actually since that was brought up, let's say if there was a way to decontaminate platelets across the board, but that process reduced the performance of the platelets, I

mean, survival and recovery, say, 50 percent. I mean, would that be a valid tradeoff? Do you consider that tradeoff a valid tradeoff for a clinician?

I mean, that means everybody gets less, not looking at specific cases.

DR. BLAJCHMAN: I'd have to consider those platelets. I think you might have to consider doubling the dose to a particular patient to get a particular hemostatic effect, but I think just to say that that concentrate is not of value, I think, would be inappropriate.

CHAIRPERSON VOSTAL: Any questions from the audience?

I guess we'll just press on here. Here's a question that comes up every once in a while. I think it's been touched on today already.

Differing clinical situations may require different levels of hemostasis. I guess we're talking about surgical bleeding versus mucosal tissue bleeding versus prophylaxis. Would clinical efficacy of a processed platelet product or platelet substitutes have to be demonstrated in each situation?

DR. SCHIFFER: I think that there's likely to be a difference in the benefit from these platelet particles, whatever they are, lyophilized or whatever, depending on how many residual platelets there are around as compared to no endogenous platelets.

Now, I'm saying that. I don't know that, but it makes some sense, and there are certainly situations in which you do have platelets around. Even in the surgical situation where you have

severe thrombocytopenia, you have continued endogenous production which you don't or you have a minimum of in a chemotherapy situation.

So I'd suggest that for some products the answer may very well be yes, but that also may be the value of those products.

DR. SLICHTER: Well, you know, I think there's a difference between large vessel bleeding and small vessel bleeding, and I think the number of platelets that you need to just prevent the junctions between the endothelial cells from leaking red cells is, I think, clearly different than if you have a cut vessel, and then you have to make a platelet plug in order to be the initial defense, you know, and then fibrin has to come in and stabilize the plug.

So I think the number or the function or the efficacy of the platelets may need to be different depending on what the clinical situation is that the patient is faced with.

So some of these products, which are just particles and membranes and those kinds of things, may well, in fact, provide enough hemostasis in a non-ruptured vessel, but I think if you have a cut vessel, I think then what you may require may be different.

So if that's, in essence, an answer to your question, I think what may be needed in one situation may to my mind not be the same as what's needed in another situation, and so I would suggest that for the majority of these products, the less severe test is, in fact, the microvascular bleeding situation, and I think if they demonstrate efficacy there, then they have a role to play.

Whether they can then show efficacy in the other situation I think may well be no, but maybe yes, depending on if the patient has some of their own platelets.

So I think that the suggestion that these things may be better looked at, as Joe Fratantoni suggested, as not platelet substitute, but -- I forget what he said -- platelet additives or --

CHAIRPERSON VOSTAL: Hemostatic enhancing or --

DR. SLICHTER: Yeah, hemostatic enhancing or something.

So that they may have a role if you have some residual platelets of your own that these things may interact with and synergize with or facilitate, but may not be effective as a single agent without something of your own.

And that was partly the reason I asked the question about the kidney bleeding model that we heard discussed, because I think that they did have some residual platelets, and that may, in fact, falsely suggest that something may have an effect that they, I think, need to be careful that they're not showing an effect because they still have some residual cells.

DR. BLAJCHMAN: I'd like to say something, and this sort of in some way begs the question. We have in our armamentarium for the treatment of thrombocytopenic patients a whole host of agents, and I tried to address some of them in our talk, in addition to platelets, and these are licensed products.

Clinicians, and I'm guilty of this as much as any, use these products in some instances, but we really don't know whether

they work or not because they've never been tested in adequate clinical trials.

So to make a decision on how to use the new products, we haven't dealt adequately with the existing products. So I think we need to come to some sort of consensus at least how we learn to evaluate some of the existing products.

And Gary Raskob challenged us to this. What criteria are we going to use for evaluating these products? Never mind the new products. Let's may be start with some of the existing products.

DR. LEVIN: I think that point becomes particularly important since the transfusion trigger is rapidly decreasing, and I think it's reasonable to assume that it may be 5,000 in the next couple of years, but certainly ten, and therefore, you can't have it both ways.

If the trigger is now 10,000, then you have to admit that giving platelets prophylactically between ten and 20 isn't justified, and therefore, you can't use that as a comparison anymore.

DR. BODE: I was just going to say I was hoping that Mo would go back into the thrombocytopenic rabbit model and just say a little bit more about the comparison in microvascular ear bleeding time in something like a large vessel puncture bleeding time in case there really is different data or different interpretations to be concluded from it.

DR. BLAJCHMAN: We don't have a lot of experience certainly recently with microvascular bleeding in the large vessel.

Before we developed the microvascular bleeding time, we used to do punctures into the jugular vein. That's 20 years ago.

By and large, we have found that the jugular vein punctures and the microvascular bleeding time gave parallel results, but we really haven't looked very recently at that.

DR. SLICHTER: But have you hit an artery, Mo?

DR. BLAJCHMAN: We've never looked at an artery, but people don't bleed from arteries.

DR. SLICHTER: They don't bleed from arteries?

DR. BLAJCHMAN: No.

DR. SLICHTER: Our surgeons have them bleeding from arteries.

(Laughter.)

DR. SLICHTER: I'm sure they do.

DR. BLAJCHMAN: I'm talking about spontaneous bleeding.

DR. SCHIFFER: They do in Detroit.

DR. SLICHTER: Well, but they do --

(Laughter.)

DR. BLAJCHMAN: Guns are banned in Canada.

DR. SNYDER: Wouldn't practically -- let's say you submitted an application, and microvascular bleeding was what you studied, that it would be labeled as such, and the rest would just be off label use.

I think we won't even be old and grey. We will be moldy by the time we get studies done. I'm sure that CPD adenine hasn't been studied in neonates or in preemies. That wasn't what the study

groups were. So from a practical level, that's probably what would wind up happening. There would be a lot of off label use of a product that was approved for a certain indication.

That doesn't make it right, but at least it moves the country along a little bit, I think.

DR. SLICHTER: But I think in fairness, Ed, that's one of the problems that the FDA has, because they know that if they send something out into the hinterlands, and even though they label it as "don't use this unless you've got a hole in your tire," you know, they're going to put it in for gasoline.

And that doesn't mean that that may not be helpful, and off label uses often become on label uses, but I think that they feel some discomfort in allowing things to proceed.

DR. SNYDER: Yeah, but from a practical perspective, the people in this room probably constitute 95 percent of reasonable bibliographies on anything to do with platelets, and no one can come up with a percentage figure for what's an acceptable gold standard for a liquid stored platelet, and we'll just go around and around like gerbils in a cage.

Maybe I'm being from Mars. I feel the need to solve the problem as opposed to just appreciate the problem, but I feel -- maybe that's wrong. Maybe I should just say, yes, we all should get metaphysical about it, but there should be some way we can come up with a number or some appropriate indication just to get a start and then move forward from my perspective.

DR. MONDORO: Well, speaking from the venous FDA perspective, Dr. Slichter, you've done a good job of putting yourself in our shoes. We're going to be reviewing these products hopefully, and we don't have standards to go by.

And one of the questions we'd like to ask you because you're the people who could be using these products: how would you feel about a different set of standards for each product?

I mean if we were to use our common sense and think about these products and say, "Well, this is good for one thing, but not another," how would you feel about that? I mean is that reasonable?

I mean then the standards are sort of moving all the time, but in platelets that's not an uncommon thing.

DR. SCHIFFER: When we did anti-cancer drug evaluations, what we did was look at the evidence for what was presented and yea or nay, and a package insert was created that summarized precisely actually what the study was, and then the marketplace and science prevailed. That is, people used these things.

And they used it in lots of other things which they shouldn't have, as you know, and articles were written that say, "Hey, this stuff is actually good in bone cancer, too," or, "it stinks in colon cancer, but it's only good in lung cancer."

That actually feeds a creative process, and I'm not sure it actually has been the FDA's view that they have to protect against off label use and hence deny the availability of a product that they've decided is of value to a certain subset of patients.

DR. RASKOB: I would like to add to that. I understand the desire of the FDA to try and have some sort of uniform standards if possible. On the other hand, I think it makes more sense to have standards that are relevant to the particular clinical situation that you're evaluating because there may be different baseline risks and benefits and the burden of proof required in certain situations with the nature of unmet medical need and burden of illness and all of those other things makes sense that there may be different standards for certain different clinical situations.

DR. SLICHTER: And I think for certain products, depending on what, as I've said, what the tradeoff is, and so I mean, I'm sure the FDA would like some gold standard, but I'm not sure you're going to get it from this group because I think --

DR. SCHIFFER: I think you picked the wrong group.

DR. SLICHTER: Yeah, right.

(Laughter.)

DR. SLICHTER: Because you know, I went to trial with the HIV business, and at least in the State of Washington it's not a medical standard. It's what the guy out on the street thinks, and he thought we were just way out of line that we allowed a monogamous gay male to donate blood, and the early things were, you know, the FDA said multiple partners. So we allowed one.

Well, that one multiple partner -- in fact, the partner was not monogamous, but the guy on the street said, "Well, you're too stupid to be running your blood bank," and in fact, they found us

negligent even though we had a much more rigid standard doing what the FDA did.

So I presume that the FDA has advisory groups, and I think there are those of us in the room who would be prepared to sit down with the FDA and say as a clinician and as a scientist, I am, in fact, prepared to accept this product in this clinical situation, and it may have to vary with what the situation is.

Because platelets, in fact, are not as good a product as, I think, red cells are, and so we can't get to the same place that red cells are, and so we may have to accept some compromises, which it may depend on what it is.

DR. LEVIN: But you're going to have to be careful because the clinical situations are not going to be as clear cut often as we may make a table of them. A bad sentence. So you'd better not break it into too many parts because clinically people will not be able to really define whether this is Situation A, B, C, or D.

Dr. Slichter made an excellent point about microvascular bleeding versus large vessel bleeding, but even in that circumstance, clinically it may be very hard to judge what you're looking at.

DR. MONDORO: Let me play the devil's advocate for a new moments. If you were sitting in the shoes of the industry people here, how would you feel if you came to the FDA and we said, "Well, we don't have any standards. Well, go out and do whatever test you want, and then when you come back, we'll tell you if we like it or not, and we're not sure if we like it," if this sort of ambiguous response was

what you received after you had developed your product and put a lot of hard work in it?

DR. SCHIFFER: Well, you do have standards. You've approved -- let's just talk platelet products because the other stuff I think you should deal with separately.

You've approved phoresis machines. You have approved storage bags. You've approved lots of things. So you already have a playing field, and in general your playing field should remain level for the next product that comes along unless there's compelling reasons to think that you did it incorrectly or that there's new information.

So I think you already have some guidelines, and Joe summarized some of the history there.

And if you look at products, what happens, after they get approved -- I mean, again, I have experience on the cancer side of things -- they come in for very narrow indications, breast cancer refractory to three prior regimens or something like that, and of course, they get used widely.

And what happens is if you look at the pattern of use after something is approved, it's very, very high, and if you look at it over time, it comes down to approximately where it belongs, give or take, sometimes for different diseases and sometimes for different indications, but I think medically we learn a lot from that process.

And you have to decide as an agency whether you're willing to -- if you believe something works, whether you're willing to

deprive a population for whom it works of that product, and I personally don't think you should.

CHAIRPERSON VOSTAL: Joe, do you have a comment?

DR. FRATANTONI: I want to reassure Sherrill that the FDA is not naive and is not going to get blind-sided by off label use, and very often at the time I was with the agency, conversations were very directly addressing the possibilities of off label use when talking about the approval of a product, and it's part of the process.

And certainly looking at it from the industry's side, it might be the most economic way to get a product approved, is to get it approved for a fairly narrow indication, expecting that off label use will bolster your market.

The concern then is about safety, and so what the agency certainly has to be concerned about with off label use is that the population of patients that will use it will not be some unusual population for whom safety has not been considered.

That can partly be taken care of pre-approval, and more and more people are talking about post approval or Phase IV study, post marketing study, to try to track that.

The other point I just wanted to make just to reassure Ed for the logic of the 75 percent red cell recovery, what actually happened there, Ed, is in December of '92 there was a workshop on red cell evaluation, laboratory methods of red cell evaluation, hoping to find some method other than the 24 hour recovery to use as the gold standard.

A blue ribbon panel like this of red cell people came in, presented there data, and there wasn't anything. So we're stuck with the red cell recovery.

That same day of the workshop at the lunch break, the panel was up -- this is December of 1992 -- the panel was updated about what was happening with AIDS and at that time people still weren't sure that was being transmitted by transfusion, but it was getting worrisome.

A day or two after the workshop, Dennis Donahue came back and said, "We should be making a better product. Let's make it 75 percent." That's where it came from.

PARTICIPANT: You don't think that was '82?

DR. FRATANTONI: December of '82.

DR. SLICHTER: You said '92, Joe.

DR. FRATANTONI: Go back on the record. '82.

DR. BLAJCHMAN: I think it's appropriate at some point to solve Ed's problem, where he comes from --

(Laughter.)

DR. BLAJCHMAN: -- to start making some criteria based on in vivo recovery for the liquid stored platelet, aphoresis platelets, the standard random donor platelets, and perhaps add onto that some functional assay like the sort of assay that we've done as a hemostatic function.

Using that as a background, one could then start to set some criteria for some of the other products like lyophilized platelets, for example. It may be that they don't have and will not

have in all likelihood the sorts of recoveries of liquid stored platelets, but they may have efficacy in terms of a given dose in terms of having some hemostatic function.

So I think it's worthwhile thinking along those lines and what sort of surrogate test, if you like, might lead one to have a product when it's delivered to the patient to have certain criteria.

CHAIRPERSON VOSTAL: Let me just ask you this. That hemostatic function you're talking about, I mean, would it be enough to have an animal model or an in vitro test, or would that have to be an actual in vivo demonstration of hemostasis?

DR. BLAJCHMAN: I think the demonstration of the in vivo hemostasis in man is a difficult one because of all the reasons that we talked about.

Gary and I were talking about -- and he can probably talk better than I about this -- but the animal model, the in vivo bleeding time model, in rabbits has been used to validate various functions for low molecular weight heparin preparations in terms of its bleeding function, in terms of also for thrombus.

This led to the clinical studies, but before that the basis was animal type studies. So I think one leads to the other.

Ideally one would like a clinical study, but I think realistically at this moment in time, that's not likely to be feasible.

CHAIRPERSON VOSTAL: Dr. Raskob.

MR. RASKOB: Just adding to what Mo mentioned, I think it's a good example of where the animal hemorrhagic model predicted

the enhanced safety of low molecular weight heparin at high doses, whereas the 10A levels in plasma as a surrogate endpoint have not yet been predictive of either thrombosis or bleeding.

So I think that's an important point to keep in mind as an example.

DR. BLAJCHMAN: -- that in an animal model.

MR. RASKOB: Yeah. So getting to the point about taking it from the manufacturer's point of view, I think one way that the FDA can help is to be very explicit with them at pre-Phase III meetings and giving clear, explicit guidelines as to if you do this, then assuming everything is done well and within all appropriate guidelines, then that will likely meet approval.

And I think those type of advice would be, I think, appreciated given when someone asked you the question, you said, "We'd see." There was a chuckle, and everyone sort of understood what that meant.

So I think being very explicit at the pre-Phase III stage with some commitments, I think, would help them.

DR. MURPHY: Could I just clarify? Mo, you think that an animal model study would be enough to validate a product that doesn't circulate?

DR. BLAJCHMAN: No.

DR. MURPHY: Or can't be shown to circulate?

DR. BLAJCHMAN: My own personal bias, every time I've written an article using the animal model, I put the caveat out, as

most of you know, that this does not mean that we shouldn't be doing clinical studies.

The reality of that is that the animal model has been fairly robust in terms of predicting what happens. So in the absence of anything else and as a place to start, I think that may be someplace that -- in fact, this happened because, thanks to Joe Fratantoni, because of the availability of our animal model, many of the companies that have produced platelet substitutes in their assessment of hemostatic function have come to us.

Now, what has surprised me over the years is nobody else or very few other people have decided to set it up. Why that's happened I don't know, until recently. Now, there have been two or three labs around the world that have set up, but --

DR. SCHIFFER: Did they pay in Canadian dollars or --

DR. BLAJCHMAN: No, U.S. dollars.

(Laughter.)

DR. SLICHTER: But has anything been licensed, Mo, on the basis of the rat bleeding time model? I mean has the FDA said, "Oh, Mo says it's great. Stamp your forehead and proceed"?

DR. BLAJCHMAN: Well, all I can say, and Joe can correct me, when the new platelet containers came out, the data from the hemostatic function in the animal model played a role in that. The other part that played a role was the in vivo survival studies.

DR. FRATANTONI: Yeah, I think that's key. The animal studies would be part of the package, not the pivotal study, but part

of the package, and it's certainly what this group should be thinking about.

If you can demonstrate hemostatic efficacy in an animal model and demonstrate that you can give it to patients with impunity and perhaps with some indication of efficacy and with clinical safety, you wrap it all together, and you might have a package that would be approvable.

DR. SCHIFFER: Maybe you could poll the panel. Let's say it cures Mo's rabbits. It doesn't have acute side effects. What else did you say?

DR. FRATANTONI: Demonstrate that you can do the clinical --

DR. SCHIFFER: I'm not sure what that means. You can give it to people, but you haven't --

DR. SLICHTER: Well, we just got back to patients.

DR. SCHIFFER: But you haven't shown efficacy. Would the panel agree that that's a product that should be approved? That is, it didn't improve bleeding or something in patients.

I would cast the first vote and say no. I think it's a good screening test. You might want to know what the others think.

DR. FRATANTONI: For what it's worth, the FDA did say they would use this approach with fibrin sealants before across the board liberalizing the approach to fibrin sealants. When it was becoming demonstrated several years ago that it would look like it was going to be impossible to do a decent clinical study with fibrin sealants, the offer was made at a meeting in '94 that if people could come together

with a package, for example, showing hemostasis in an animal model, showing safety in a human, that this would be considered.

So there is some precedent there for that sort of approach.

DR. MURPHY: Could I just comment about something? There's a danger of boxing yourself into a corner if you insist that a closed platelet substitute do everything that platelets do. An example would be, I think, pretty well shown efficacy that Amicar is effective in controlling dental bleeding in hemophiliacs, but nobody claims that it's a Factor VIII substitute.

But if it does that and does it well, it should be approved for that purpose. So I don't think you should be looking for these to do everything that we think a platelet should do.

But then you get into the question I haven't heard the panel really describe how many platelet functions there are, which ones it's good for and which ones it isn't.

DR. SLICHTER: Well, but as we've already heard today, some of the -- you know, some of the platelets work in some assays and don't work in others, and as you say, Scott, do they have to work in every assay in order to do something?

And you and I know full well that some of the in vitro assay abnormalities, once you transfuse those platelets, they, in fact, are able to recover even that particular function once you take them out of the patient and retest them in the in vitro assay where they were abnormal before you transfused them.

DR. SCHIFFER: Let's do a very practical question, the one I tried to get at early this morning. We have all of these platelet products and these guys, and they sounded pretty interesting, I thought, this afternoon. Some of them sounded really cool, and so these people would like to know what they have to do.

I think that they have to show some hemostatic efficacy because they can't show increments because of the nature of the product. So how can they do that in a very practical sense?

I said something earlier this morning just as a toss-out, as a way to start it going. That is, and Dr. Levin's to my right, but I'm going to say it anyway, that if you make the bleeding time of someone who has a platelet count of 10,000 and it stays at 10,000 more like one of a patient with 40,000, you know, with appropriate pre and post and all of that kind of stuff, does that tell you that you have a product that's doing something that you might consider in certain circumstances?

And of course, you look for bleeding patients, and you see if you can make them stop bleeding and stuff, but are there discrete models that we can suggest to these people out there? Because they've got some very interesting things that could potentially be of benefit to patients.

CHAIRPERSON VOSTAL: Well, I think those are exactly the questions we are trying to get at.

DR. SCHIFFER: Let me ask that question. Is that a model, the one I suggested, that would make some sense? Jack.

DR. MURPHY: I think that's a model that makes some sense, even though Jack Levin probably doesn't agree.

DR. LEVIN: No, no.

(Laughter.)

DR. LEVIN: No, I don't think it's unreasonable to use the bleeding time or some time or some other -- I don't want to use the word "surrogate" anymore -- test as an initial look.

What concerns me is that the bleeding time in many people's minds has become equivalent to bleeding, and if the bleeding time is long, the assumption is that you have a bleeder. Well, that isn't true. So it's not --

DR. MURPHY: I think you've won the war more than you think you have.

DR. LEVIN: Thank you.

So I don't think it's inappropriate, not that it's up to me to decide, not to use the bleeding time as an initial screen, but I think there's a big disconnect between that test and the effect of an agent on that test and whether a patient is bleeding or not.

But I just would like to give you a striking clinical example that people don't really seem to factor into their thinking. Dr. Harker referred to coronary artery bypass patients before. By the time somebody is finished with bypass, the patient has multiple deficiencies of blood coagulation factors, is thrombocytopenic, and has a well defined qualitative platelet defect, and furthermore is fully anticoagulated, and yet very few of those patients bleed abnormally.

And there is some big surgical series where not a single patient that gets platelet transfusions. Now that shows you how misleading it can be to look at laboratory data.

There's not a surgeon in the world who would operate on a patient with the laboratory abnormalities that they produce every day in coronary artery bypass patients, never, and yet they basically do not bleed at least abnormally by the criteria we currently use, and that's an example to me of how misleading laboratory data can be.

It gets back to the need for who and what is a bleeding patient and how we can study them.

DR. BODE: Just to continue the discussion about open heart surgery patients, whether you define it as normal or abnormal bleeding, I guess, depends upon the size of the insult, and I think before the inauguration of drugs like aprotinin, open heart surgery patients postoperatively could be as much as two liters.

And if you showed even in today's modern pharmaceutical environment the introduction of another hemostatic agent which further reduced the total amount of blood loss in a patient whose life does not necessarily depend upon that as an intervention, but it is a test field, would that show you efficacy without it having to be a necessity and go on to other --

DR. LEVIN: But you're talking about a clinical observation now. That's different from a change in a laboratory test. That's the only point I'm making.

MR. RASKOB: If I was a company looking at this area, the general principles that I would use is I would first try to target

clinical niches rather than look at sort of very broad areas for approval, as was suggested.

The second thing I would do is first try to find an area where I'm going to superiority rather than equivalence, if that's possible. It may not be possible in this setting.

If we're going for equivalence on effectiveness, then I would next want to try and have a clinical setting where I can make some hypothesis of improvement in safety somewhere and be able to potentially measure that and show that.

Because if all we're left with is equivalence on effectiveness and safety and our major advantage is one of convenience and ease of use and other things, then it may be actually hard to get patients to consent to participate in such a study because of potential lack of benefit to the individual patient.

So I would try and go through that sort of series, find a clinical niche that may meet some of those criteria and work in that area.

DR. SCHIFFER: And it's not just patient consent. It's doctor consent. There are just so many studies you can do, and you want to be motivated, as I said earlier, to do a study, and you generally are motivated by studies that produce improvement.

Now, improvement can be in safety certainly, but improvement.

CHAIRPERSON VOSTAL: I'd just like a question to Dr. George. In terms of toxicity, do you think that the procoagulant

quality of some of these substitutes -- would they be required to be tested in a DIC model, animal model?

DR. GEORGE: Dr. Vostal, I thought I was going to get away without saying anything on this panel.

The question he's raising is that if there is any thrombogenic or thrombotic risk, do you have to select certain categories of patients to make that risk less severe, make the safety issue more valid.

I'm not sure that that can be done. I think DIC spans the range from patients that have overt problems, but there's many, many subtle aspects of DIC.

The issue on these prothrombin complex concentrates is their use in combination with aprotinin or Amicar, and that may be an issue, and that may just be something that has to be labeled and avoided.

There are new congenital predispositions for thrombosis, and you can't predict all of those, and you shouldn't screen for all of those, the Cs, the Ses, the prothrombin changes.

I think that I would -- I haven't said anything because I would echo what everybody has said here. I don't think that this is maybe at all analogous to red cells, except that you use the verb "transfuse" in both instances.

In red cells, it's real simple. You're carrying oxygen. You need some volume, and that's all there is to it, and I think in platelets, you could have negligible recovery and an effective product.

I liked what Scott Murphy said about what's the real world recovery. None of that is published in the literature, but there are platelet concentrates that get handled. They get driven across town. They get sat on the blood bank bench. They go up in the elevator. They get lost. They get retrieved, and what's the recovery of those patients or those platelets? And yet I think platelet transfusions in spite of all the limitations are generally effective.

And if you held a higher standard of 40 percent you might kick out half of the platelets that we use in our university hospital.

So I'm sensitive to the dilemma of what you say to the manufacturers and the developers, but I think the standards have to be variable, and I think they can be minimal if there are other advantages, such as safety.

DR. SANDLER: I'm Gerry Sandler. I run a blood bank and interact with surgeons every day.

One bit of advice that I would give the FDA is that it's going to be very important to work out what I'm going to call the overdose toxicity before a product is licensed. We don't want the overdose toxicity to be worked out when the surgeon tries this hemostatic adjuvant and sees a little effect and says, "Okay. I want it ordered every two hours," and then we find out what that's going to be.

All of the products today were described, but I didn't see any of them pushed to the point where what looks like a very safe drug becomes dangerous, and I can assure you that that will happen in the

clinic, and we want to know what those risks are going to be before the product is licensed.

CHAIRPERSON VOSTAL: Good point.

DR. REID: A question for Dr. Raskob. I'm Tom Reid from WRAIR.

I think your proposal to show that a platelet substitute or product be better than or equal to what we have in platelets is a pretty tall order. Why can't we just have a clinical study that would show that it's no worse than platelets?

MR. RASKOB: Yeah. Whether we talk about equivalence or non-inferiority, that's fine, yeah, but recognize if I'm a patient and you come to me explaining to me a clinical trial where you say, "We think that this new product is not worse than what we'd normally give you, but we don't know" --

DR. REID: But that's not the same as equivalence.

MR. RASKOB: No.

DR. REID: We're not asking the two to be equal. We're certainly asking that it not be any worse than.

MR. RASKOB: Sure. Not worse than a clinically important amount, but what I was getting at is I think if that's all you have and you're asking me now to say, yes, I will participate in this study, as a patient I would say, "What potential benefit may I derive from this, and am I only facing risk?"

And, therefore, I may say, no, I want the standard care. So I think what I'm trying to emphasize is to search, if we can, for

those clinical scenarios where we may have a benefit on either effectiveness or safety, a potential benefit.

CHAIRPERSON VOSTAL: Any other comments or burning questions?

(No response.)

CHAIRPERSON VOSTAL: Well, if not, I think we can bring this workshop to a close.

I'd like to thank the panel for their lively discussion and their input, and we really appreciate it here at the FDA, and thank you again, and have a good trip.

(Whereupon, at 5:15 p.m., the workshop was concluded.)