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CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

AND

THE HITCHCOCK FOUNDATION

DARTMOUTH-HITCHCOCK MEDICAL CENTER

WORKSHOP ON USE OF RADIOLABELED PLATELETS FOR ASSESSMENT OF IN VIVO VIABILITY OF PLATELET PRODUCTS

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P R O C E E D I N G S

DR. ZUCK: Good morning. Good morning, and welcome to the day's symposium or workshop. I'm not quite sure what it is. I think just a workshop.

For those that don't know who I am, I'm Tom Zuck. I've been around blood banking for a few years.

And to make the introduction and start off the morning is Karen Midthun, who is the acting director of medicine, the Center for Biologics Evaluation and Research (CBER). Before joining the FDA, she was assistant professor of the Department of International Health at Johns Hopkins. She trained as a resident in internal medicine at Johns Hopkins and as a fellow in infectious disease at Johns Hopkins.

Dr. Midthun?

DR. MIDTHUN: Good morning, and welcome. On behalf of the Center for Biologics and the Food and Drug Administration, I welcome you to this workshop on this very important topic of platelets. And I would also like

to thank our co-sponsor, the Hitchcock Foundation, for their contributions in making this workshop possible and to others who have also helped to make this a reality today.

I'd like to spend a few minutes just laying some groundwork for some of the topics that we'll cover today, and then we can get on with the real work at hand.

The Center for Biologics and Research evaluates blood products for safety and efficacy. Platelet efficacy has been evaluated in part by recovery and survival of radiolabeled platelets in healthy human volunteers. And actually, almost 20 years ago, there was a symposium on radiolabeling of stored platelet concentrates here in Washington, D.C. So it's time that we revisit this.

The current design of in vivo radiolabeled platelet studies has been to compare the performance of novel platelet products to a 5-day-old licensed platelet product. A small amount of decreased performance is

acceptable due to variability in experimental results. The licensing of the novel product sets a new standard, which could be slightly lower than the previous standard.

And repeat applications of this process could potentially lead to a decline of platelet product quality over time.

What is an alternate approach to consider? Establish a "gold standard" based on performance of fresh autologous platelets in a healthy donor and compare all future platelet products to fresh autologous platelets with a standardized protocol.

Future platelet products that may test the limits of platelet performance include pathogen-reduced platelets, extended shelf-life platelets, low temperature storage conditions, additive solutions of platelet storage, and new storage containers.

So today's workshop on the use of radiolabeled platelets for assessment of in vivo viability of platelet products will review the current evaluation practices for

platelets, outline an alternative approach, present standardized study protocols, present preliminary data using the alternative approach, and elicit expert panel discussion on the appropriate standards using the alternative approach.

The goal of today's workshop is to orient the transfusion community towards a new approach for assessing the quality of platelet products through radiolabeling studies in healthy volunteers.

And the objectives include discussing the merits of the novel approach to evaluating platelet products by radiolabeling studies, discuss appropriate study protocols for comparing platelet products to the standard, and see if we can reach a consensus on establishing a minimum performance standard for platelet products in radiolabeling studies.

And without any further ado, I turn the conference over to the real work that needs to be done. So thank you very much. And again, welcome, and thank

you for coming today and helping with this very important topic.

[Applause.]

DR. ZUCK: Our first speaker today is known to all of us. It's Scott Murphy, who is chief medical officer, American Red Cross Blood Services for Penn-Jersey, an adjunct professor of medicine at the University of Pennsylvania Medical School. He is a member of many professional organizations known to us all and is a member of the Biomedical Excellence for Safer Blood Transfusion Working Party of the International Society of Blood Transfusion.

Scott?

DR. MURPHY: Thanks, Tom, very much. It is a pleasure to be here with so many familiar faces.

I'm going to be--my title is "Introduction and Historical Perspective." I will also, towards the end, have some discussion about the suggestions for a new standard.

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The gentleman that you see before you is Frank Gardner, with whom I trained in hematology in the mid ‘60s. He also stimulated my interest in platelets. He did so by when I went to him to ask, "Well, what do you think I should be interested in?" He said, "Murphy, platelets."

[Laughter.]

DR. MURPHY: So that's literally, literally what happened.

Frank and a colleague, Knut Aas, in 1958--is there a pointer here? In 1958 published a paper in the Journal of Clinical Investigation, "Survival of Blood Platelets Labeled with Chromium 51." I think this was the first discussion of labeling platelets with chromium.

A couple of comments from the paper. At the time, there really was one citrate anticoagulant, which was ACD. And Frank said that numerous microscopic aggregations of platelets that do not resuspend after centrifugation and labeling with chromium with platelets

drawn into ACD. And earlier studies have demonstrated that platelets are discrete and not clumped when sodium EDTA was used as the anticoagulant.

So these initial studies were done in plastic containers, which I'll describe, but the primary anticoagulant was ACD. And this is an example of how platelets had been labeled after centrifugation within bags. We'll talk later about the indium method in which we label the platelets in tubes.

But anyhow, this method, a unit of blood is drawn. Centrifuged slowly to make PRP. You all know this. And then spun hard to make a platelet button. Plasma is decanted, and then chromium is injected. Certainly, in my career, up to about 1992, this is the way I labeled platelets and others here today do as well.

The measurements that were made by Frank were--did not include any measurement of recovery. There was a very striking initial sequestration of EDTA platelets, a rise in the radioactivity on day 1, and then

what was taken to be the platelet survival curve was measured.

So there was no effort, as I said, to relate the actual radioactivity here to how much radioactivity had been infused, and there was no attempt to model this disappearance of radioactivity against some standard.

Dick Aster was across town at Boston City Hospital at the Thorndike, and I don't know how much interaction there was between him and Frank. But nonetheless, he came up with the idea of adding 50 percent more citrate to ACD and also tipped the balance toward citric acid so that blood pH was 6.5 after drawing it. And he was able to show then that platelets could be labeled with that primary anticoagulant.

In his hands, EDTA platelets showed the same early sequestration and then survival, but much higher recovery. Here, he was measuring recovery with the platelets labeled in citrate. And you'll notice that throughout the study, there was extensive radioactivity

in the spleen, which we'll come back to.

Quickly, Phil Cohen, working with Frank Gardner, began to study the radioactive yields of platelet concentrates derived from blood anticoagulated with EDTA and ACD. In other words, they were beginning to think about transfusion medicine and what's the best yield for the patients. They also did not use Aster's solution, but rather simply added extra ACD to the platelet-rich plasma prior to centrifugation. We still are, in most centers, acidifying at some point when we make platelet concentrates from PRP.

I think it's important to point out that all of these manipulations, both in Aster's lab and Gardner's lab, were done in the cold, and also there were certainly no rest period, as we use now, prior to platelet suspension.

I hope you can see this. This is my phylogenetic chart of people who have been interested in this field. And if I leave anybody out, please forgive

me. But in any event, here's Frank Gardner and I think helping, stimulating, whatever, Aster. And then back to Phil Cohen, who used it with Frank thereafter, that method thereafter. And I trained with Phil Cohen and Frank, of course.

Now, as I understand it, Larry Harker went to Aster and learned how he was doing things, passed it on to Sherrill Slichter, and not included is that Sherrill passed it on to Toby Simon. All these familiar names.

Meanwhile, a gentleman named Thakur in St. Louis--he's a nuclear medicine type--labeling platelets with indium in order to image thrombi, infection, and the like. Andrew Heaton joined the lab in St. Louis, and had the very good idea of using this method not for imaging, but for measuring platelet survival.

And I hope I spelled this right, Ezechowitz is the cardiologist who came to Yale from Thakur's lab, and Ed Snyder took advantage of his presence to learn the

method of indium labeling.

Andrew Heaton moved to Norfolk, where he got together with Stein Holme to produce again a very nice discovery, the combination of chromium and indium with the potential for doing the control and the test product at the same time.

Jim AuBuchon learned from Norfolk lab, and I learned how to label in tubes from AuBuchon. And meanwhile, the lab has prospered in Norfolk, even with the departure of Andrew and Stein through Elfath and Taylor, but I think with a major contribution from Pam Whitley, their chief technologist.

This is old data that I collected 20 years ago about chromium yields in patients and finding that in vivo recovery was about 70 percent, as it had been as measured by Aster. But people that had their spleens out were close to 100 percent. In people with large spleens, it was lower. This was already known, but this is what I have a slide of.

And that led to the hypothesis that in normal individuals, for every two platelets in circulation, there was one in the splenic platelet pool, and this is a very important concept as we measure recovery today.

What we have found, I think, is that even with fresh platelets there is considerable variability in the recovery that's measured with normal individuals, and that continues with storage.

And why in the normal population are the normal recoveries with fresh platelets variable? It's known that the size of the spleen, which correlates with the size of the pool, varies among normal individuals. Splenic pool varies inversely with the platelet count. The lower your platelet count among normals, the more platelets are in the spleen.

Other things that affect our measurement of recovery is that we estimate the blood volume by body surface area, which can be--the correlation is not as tight as we would like it to be. And over time, we've

learned that different labs get different results for their mean recovery. It's hard to compare yields and survivals from lab to lab.

There is similar variability--and here I used the half-life as the measure of platelet survival. And again, there's a good bit of variability within the normal population. Our experience and that of others suggested that with any given donor, they tended to produce high yields or tended to produce low yields so that you could compare control--well, here we were studying two types of agitation. You see that since the studies are paired, you can clearly show differences which would not be evident if you did not have a paired design, and the same thing is true with T 1/2.

There is an example of platelets stored for 5 days using two different plastics, and again, in people who produced high yields in one, produced high yields in another. And with a paired design, you could show statistically significant differences.

Here is yet another one when we compared 7 days of storage to 10 days of storage. This data is from Holme and Heaton. Here, for the recoveries, comparing storage in plasma with storage in an additive solution. You can see clearly that the additive solution is better. But again, the tendency for a donor to produce a high yield in both arms of the study.

So I think we have to keep in mind that you could get any number you want, depending on whether you select donors who tend to give high recoveries.

DR. SLICHTER: But do you think that means that if you would select the donor with a high platelet count, then you're likely to get a higher recovery because the platelet count correlates with the spleen size?

DR. MURPHY: Not the spleen size, but the percentage of the body's platelets that are pooled in the spleen.

DR. SLICHTER: Right. Yes.

DR. MURPHY: You have a high platelet count,

there's fewer stored in the spleen.

DR. SLICHTER: And therefore, that might be a way that you could influence the data?

DR. MURPHY: Right. Right. I think it's just another reason why the paired design is so important.

Now this is just studies we did storing platelets in the cold. Important point for me was that there was no--you weren't going to find a simple model for platelet survival which would be true for different studies. And here, the 8 hours of storage in the cold produced a curvilinear line as opposed to the straight line.

So we needed creative work to figure out how to do this, and another Murphy in Toronto built the multiple-hit model. And Lotter, et al., who I understand from Andrew Heaton were South Africans, produced a computer program in compiled BASIC for the IBM personal computer to calculate survival by the multiple-hit method.

It turns out, I believe, that all of us who are doing these studies are using the same program that was--which came to the United States to Andrew many years ago. And so, this program has been passed on from one group to another. It's called the COST--should be capitals--program.

So I think Jim AuBuchon will be telling us today if you sent around data to a group of investigators, will they all give you the same numbers with their program? But when we started using it, we found that the program gave you at least three ways to measure recovery using the highest value on day 0, extrapolation of the survival line back to day 0, or doing that plus omission of outliers, if you ask the computer to do that for you.

So we will be talking, I'm sure, about today what's the best way to handle this situation.

So the current paradigm, as we've heard, is that we've measured test and control in the same individual and at the same time with two isotopes, indium and

chromium. But what is the best control, and what do we mean by "same time?"

The typical control in 2002 was what I would call "regular old platelets." At the end of their licensed storage interval--and this would perhaps be a worst-case scenario--there's no line in the sand drawn for acceptable recovery and survival. And I would submit that that's not the way to do it because of variability from lab to lab.

There's no delineation of acceptable inferiority for test versus control, if any. And of course, the regular old platelets will vary widely from study to study in different labs. And as was mentioned in the introduction, you have the potential of creeping inferiority or a slippery slope. There should be arrows in here, which you can see faintly.

But here is the result in 2004. Another method is passed. And then the slightly lower recovery, and then you keep going like that, slightly lower recovery,

very low recovery if we continue to do what we have been doing.

So I would propose that the control should be fresh platelets. Experimental results should be expressed as percentage of control. Acceptable would be a recovery of two-thirds of fresh. Survival, one-half of fresh. And it would be acceptable, of course, to have a reduction of the experimental value beyond these if there was a significant patient benefit by the technology that was being studied.

Why am I saying there could be a more lenient standard? In practice, time to next transfusion, clinically, in thrombocytopenic patients is no more than 2 to 3 days. So I think we could be a little more generous with the mean cell life measurement.

And what do we mean by the "same time?" Do we collect and label fresh platelets on the day test is obtained so that the platelets are identical or on the day that the test is reinfused? The platelets are not

identical, but at least the donor is in a standard situation for the two survivals to go on together.

And then we have to come to a conclusion about the fresh control, what should that be? Collect a unit of whole blood in plastic container and prepare a traditional platelet concentrate for labeling. Collect 50 to 100 mLs of blood in plastic tube and process to obtain the platelets for labeling. So I think these are topics to be discussed.

So thank you all for being here and for the attention you're giving to this matter. We're still looking for changes and better ways to do things after 45 years of use of radiolabeling.

Thank you. Are we going to have questions later?

DR. ZUCK: Later.

DR. MURPHY: Later. Okay.

[Applause.]

DR. ZUCK: Thank you, Scott.

sj

The next speaker is Salim Haddad, who is a medical officer of the Division of Hematology at the Center for Biologics Evaluation and Research. He completed a two-year fellowship in the Department of Transfusion Medicine at NIH, and it's amazing the number of people on this program today that have had that experience with NIH.

So Dr. Haddad is going to speak on the current approach to evaluation of platelet products. Dr. Haddad?

DR. HADDAD: Thank you, Dr. Zuck.

Good morning. So in the next 10 to 15 minutes, I will be presenting the current approach that FDA is using to evaluate the platelet efficacy, and this is based essentially on the 1999 guidance.

So we can start by defining platelet efficacy. It is the ability of the platelets, of the transfused platelets to circulate for the expected lifespan after transfusion and for their ability to participate in

hemostatic processes to prevent or stop bleeding.

What kind of testing does FDA look for for the proper evaluation of new platelet products? Well, that depends on our level of concerns. This is the pyramid of concerns. And at the bottom, we have the minimal concerns and at the top the major concerns.

We can start at the bottom. For current storage conditions, we require in vitro studies. And as we move up to more serious concerns, such as for a new 5- to 7-day storage container or for new apheresis collection devices, then we like to see radiolabeled studies.

At the top of the pyramid, we're dealing with--usually with new methodologies that may affect basic platelet physiology with unexpected consequences on platelet performance. And falling into such a category are the platelet substitutes and also chemically treated platelets such in pathogen reduction. And for those kind of products, we like to see hemostatic clinical trials.

Now, obviously, we run into gray zones on

whether to classify a specific product in an upper or a lower zone. For example, for a minor modification to current storage conditions, that would fall probably between radiolabeled studies and in vitro studies, whereas for new storage media or extension beyond 7 days, that would probably fit between the radiolabeled studies and the hemostatic clinical trials.

For the in vitro tests, we classify them in four major categories: morphology, biochemical status, platelet activation and apoptosis, and physiologic responses. Now you have the list of those tests in your handout. And for licensing purposes, obviously, we do not require all those tests. However, what we would like to see is an assortment of tests that look at different aspects of platelet physiology.

And in each category, we have two groups. One is the core group of tests that we recommend, and the second group are the supplemental tests that we usually reserve only for those conditions that can be associated

with significant damage to the platelets or to platelet safety.

For all the in vitro tests or for most of the in vitro tests, there is no absolute minimal level performance set. So the new platelet product should be compared with the control platelets in a study that has the power to detect a 20 percent difference in value because that's what's considered clinically significant. And also the in vitro test has to be run in a serial fashion over the storage period of the product.

How does in vitro testing correlate with the in vivo viability? Poorly. Not too well. Over the years, there is no single in vitro test that has stood out as direct surrogate markers for platelet efficacy.

The tests that have been reported as correlating the best with in vivo viability are the pH, the hypotonic shock response, and the extent of shape change. Also the rate of increase in lactate production has been inversely correlated with in vivo viability.

So considering the poor correlation, why does FDA still require the in vitro testing? Number one, because they constitute a screening process to eliminate those procedures that clearly result in some outdated platelet products and also to avoid subjecting a donor to radioactivity in radiolabeling studies.

Another reason is that those in vitro studies, when used in conjunction with radiolabeling studies, with post transfusion assessment, you can get a good handle on whether the product is usable or not. Another advantage, and that's outside the regulatory process, is that in vitro tests can serve as a quality check over time of process methodology. So that if you have a product made this year, you can compare it to a product made next year without having to undergo more elaborate studies.

The next step after in vitro studies is to evaluate the platelet survival in the circulation. And this is done by in vivo radiolabeling studies, which is the surrogate marker for platelet--for hemostatic

efficacy and also is now considered the gold standard for studying in vivo platelets.

And this approach is based on the assumption that circulating and viable platelets that have demonstrated little defect in the in vitro phase of the testing can participate in the physiological mechanism that constitute the platelet clinical efficacy.

So you have a new platelet product. You radiolabel it, and then you infuse it back into the volunteer donor. And then you monitor recovery and the survival. And when you have much damage to the new platelet product, it will be clear at a much faster rate than the control platelets in the paired comparison experiment.

And this is an illustration of our current approach when, for example, we are evaluating a new 7-day apheresis platelet product compared to a 5-day established product. We have the donor. We collect a unit, or a unit is collected from this donor, and it's

stored out to day 5. And then on day 5, a sample is taken. It is radiolabeled and infused back into the donor.

Two days later, on day 7, another sample is drawn, and it is radiolabeled with the alternate isotope and reinfused back into the same donor. And then the recovery and survival of both products are tracked by the radiolabeling.

And this is the curve above is the survival curve of day 5 platelets. Where it intersects the Y-axis is the recovery, and where it intersects the X-axis is the survival. And for the 7-day platelet product, you would expect a lower recovery and survival. And we compared the two products based on the difference in the mean survival and in the difference in mean recovery and allowing for 10 to 20 percent difference.

However, as Dr. Murphy has mentioned, there are problems with the current approach, is that there is no minimum standard set for platelet quality. All we're

doing is comparing a new product to an old established product. And on repeated application of this standard to successive products, that can lead to what Dr. Murphy described as the creeping inferiority in terms of platelet quality.

And that's really--that observation has prompted the call for a new standard, and that's what this workshop is all about.

Clinical hemostatic trials, as I mentioned earlier, are reserved for those new methodologies that can carry a greater risk for platelet damage or to platelet safety. And these are randomized blinded phase III trials in thrombocytopenic patients. And the objective is to demonstrate the participation of the experimental product in actual hemostasis with the primary endpoints being the extent and significance of bleeding in patients on the experimental platelets versus those on the control platelets.

And the safety consideration should be

addressed, such as thrombogenicity and immunogenicity. And needless to say, these are large and costly trials.

The platelet substitutes are in a class of their own. These are products, either synthetic or platelet-derived, that have been explored as alternative to liquid stored platelets. And since they have been designed to mimic the hemostatic properties of in tact platelets, however, they don't circulate as well as normal platelets. So defining their efficacy in terms of in vivo radiolabeling studies poses a challenge.

And the proper approach is to define a specific aspect of platelet function that these products seeks to emulate and test the clinical benefit accordingly. And the evaluation can start with in vitro testing, and the supplemental tests that I mentioned earlier would be in order here.

Concurrently, animal tests can be conducted to define the early properties of the product in terms of circulation and in terms of hemostasis. And again, the

animal tests should explore the safety issues, such as prothrombotic potential, immunogenicity and the toxicity of the additive solutions.

Whether to conduct in vivo radiolabeling studies will depend on whether the product has shown any circulatory properties in animal studies. Otherwise, there is no requirement for them.

And in terms of human trials, phase II trials will establish the proof of concept for a hemostatic effect of such products and will give preliminary evidence on safety issues. A phase III human trial will explore those issues more in detail in the proper patient population.

So as a brief summary for the modification to the current collection, processing, and storage condition, we like to see in vitro and the radiolabeling studies. For the very novel methodologies, such as pathogen reduction, we like to see additionally hemostatic trials. And for post marketing surveillance

studies, depending on the case-by-case basis, they can be required.

And that's the end of the talk, and thank you for your attention.

[Applause.]

DR. ZUCK: Thank you, Dr. Haddad.

Our next presenter is Larry Dumont, who is currently a Ph.D. candidate in clinical sciences at the University of Colorado. He's spent 25 years at COBE Laboratories as an engineer and a manager in quality, and it's good to see you again. And Dr. Dumont is going to speak on statistical comments on the current approach.

Dr. Dumont?

MR. DUMONT: Thank you, Dr. Zuck, ladies and gentlemen. Thanks to the committee for inviting me to speak today. Thank you also that this talk is not after lunch. I really appreciate that. I usually get right after lunch.

[Laughter.]

sj

MR. DUMONT: So, hopefully, we'll all be able to stay awake if the lights don't get too low.

What I'd like to discuss today is the statistical approach to the design and analysis of platelet pharmacokinetic studies. And some would say talking about statistics is the discussion of an uncertain science. I prefer to think that we're going to explore the science of uncertainty.

Dr. Murphy told me today that when we talk about statistics, he feels like it's an Italian opera where you kind of get the general idea what's going on, but you don't know what the specifics are. So, hopefully, today there will be at least one specific thing from this talk that each of you can pick up on and take away with you. And I'll guarantee that there will be at least one specific thing that you probably don't understand.

So my objectives are to describe the key design and data analysis principles associated with these studies, including some sample size estimations. I want

to propose data analysis and reporting methods and propose acceptance criteria or ways to interpret the data. Some methods to do that, I think, that are applicable to the question on the table today.

This is to remind me that there are many, many ways to bake and slice the study design and analysis pie, and they're all probably valid. And I'm going to show you one way today that I think will be helpful for us all.

In the end, I'm going to recommend--this is where we're going--is that, number one, we should plan and perform equivalency studies. Also it can be thought of as a noninferiority type study.

Number two, perform a paired design. We've already heard some about that, with appropriate care to randomization, especially with respect to labeling.

And construct a one-sided confidence interval of the difference--this is very important--the difference between the control and test products. And to construct

the maximum acceptable difference from the data. And I'm going to show you and walk through an example of all this for you later.

But this would be--for example, the maximum acceptable recovery difference would be the control value minus, say, two-thirds of the control, or for survival, it might be the control minus half of the control. And of course, these numbers are yet to be determined.

And then we would reject the null hypothesis if the confidence interval does not overlap the maximum difference for both recovery and survival. Again, I would propose that this "and" needs to be explored a little bit. Or in other words, we would conclude that the control is equal to the test.

And finally, on sample size, you're going to see that that depends.

So what's an equivalency test? Well, the objective of the studies that we conduct, what we want to do is we want to say that test platelets are equivalent

to control platelets. Now most of us are probably familiar with a superiority/inferiority study design. And that's where the null hypothesis is stated as the mean of the test is equal to the mean of the control. And the alternative hypothesis is that these means are unequal.

And of course, these are stated with certain a priori assumptions about alpha risk, beta risk, power, and a difference beyond which we would want to detect and reject the null hypothesis. So really the whole objective of this type of study is to reject this hypothesis and accept that one.

But that's not really the question we're asking here. In an equivalency study, it's kind of the flip of that, where the null hypothesis is actually stated that the means are different, and the alternative is that the means are equivalent. And what we would like to do is reject the null hypothesis and accept the equivalency statement.

And again, this has some a priori statements of risk and a difference which is acceptable and beyond which we wouldn't want to reject the null hypothesis.

So this is a cartoon to kind of conceptualize what the idea is before we start looking at numbers. With the equivalency test, we'll want to conduct a confidence interval for the difference between the tests and the control. So what I have here on this axis is the difference between a control value and a test value. So if we had a subject with their recovery at 65 percent for the control and the test was 50 percent, the difference there, of course, is 15.

And then let's say we do this paired study on several subjects, and then we can actually estimate what the distribution of the differences looks like. And then from this estimate, we can construct an upper confidence limit, and we can compare that to a maximum difference for a specification, if you will.

In the case of the upper example, as we compare

the upper control limit to the maximum difference, the upper--or excuse me, the upper confidence limit. The upper confidence limit is less than the maximum difference. So we would reject the null hypothesis, and we would conclude the test is equal to control.

And in the bottom case, since the upper confidence limit is beyond the maximum difference or this confidence interval that we've constructed overlaps this maximum difference, we cannot reject the null hypothesis, and we can only conclude that there is inadequate evidence to make a statement of equivalency. So that's the conceptual picture of where we're going.

So why do we want to conduct paired study design? We've already heard a little bit about that, and--

DR. SLICHTER: Can I ask a question?

MR. DUMONT: Yes.

DR. SLICHTER: How do you determine the maximum difference? How do you--on the last slide, how do you

determine the--is that the maximum difference based on the data?

MR. DUMONT: Yes, and I'll show you that.

DR. SLICHTER: Okay.

MR. DUMONT: Yes. We'll get to that in two slides, three slides, four slides. Something like that. Good question.

So with a paired design, what we do is reduce residual error. And this is an example of that. Here is some data from the study we published in Transfusion, comparing plasma stored platelets for 5 days and 7 days. And if we take all the transfusions together, this is the spread of recovery that we have with an estimate of the standard deviation.

Now as we start to assign causes for this uncertainty, like how old are the platelets? Where were the platelets labeled and transfused? And then start to account for specific pairing between subjects, so if we look at the difference then from 5-day to 7-day, we get

this axis down here, which is the delta recovery. You can see that we've reduced the standard deviation actually quite substantially from what we had in the beginning. And this has a very large impact on the sample size of the study.

So we want to analyze the data. Well, how do we go about doing that? There are lots of ways to do it. Every study that I've ever observed in the literature on this topic have all used a two-stage analysis approach, where the first stage, there are some adjustments for elution, cell-bound label, baseline or red cell bound label. Fitting the data to some nonlinear model, for example, the multiple-hit model. And then an estimate of these model parameters, such as recovery and survival.

Now these estimates then are taken into a second stage, where we actually do the hypothesis test, and there is a variety of ways to do these. Typically, it's done with a paired t-test, or it can be done with a regression model.

Now there is also a more modern, sophisticated approach, where all this can actually be done in one analysis. This is complex, really requires your biostatistician expert to do this. So most people will probably opt for the first option, and we'll spend some more time on that.

So this is an example of what data might look like if we have one transfusion in one subject. This is recovery on this axis. This is time. And there are some serial samples taken after the reinfusion of the radioisotope labeled platelets. And we have an unadjusted data of recovery.

Now we'll probably hear more today about some of these steps for data adjustment. But typically, there is an adjustment for the amount of actual labeled platelets that are reinfused, and this is usually called the elution correction. And then for each of these time points, there is an adjustment for cell-bound radioisotope because we don't want to count the

radioisotope that might be up in the plasma. So that's the second correction that's made to each of these points.

And then, finally, there is a correction here at the end that's either a baseline correction or it could be labeled red cells. And Stein will probably tell us more about that later.

Well, let's assume then that we've taken these data and we've done those three corrections. We might have something that looks like this. We've got recovery versus time, and we've got the data points. These data points then need to be fit to a nonlinear model, and there are actually many nonlinear models that will fit this data just as well as any of the others.

From this model then, the parameters of recovery and survival are typically derived. Recovery is the intercept of this regression at the Y-axis, and survival is the tangent at that point to that curve, extrapolated to the X-axis.

sj

Now in the literature, sometimes this number is reported, and sometimes it's just an early count that's done--for example, a 2- or 3-hour count after the infusion. So you have to be careful when you're reading the literature which one is reported. We probably ought to decide which one we should use.

One can also derive the area under this curve, and this is perfectly analogous to drug pharmacokinetic studies you've all seen with area under the curve.

I do want to point out that with survival, here is the survival. Look where all the data is. That's kind of striking, isn't it? Survival is actually a ratio of a parameter, and because of that fact, there is more uncertainty in this estimate than there is in that estimate. And we'll see some data on that in a second.

So this is the first-stage analysis, where we come up with estimates of these parameters, and then these parameters are taken to the second-stage analysis. So this is an example where we'll calculate the

confidence interval estimate for recovery.

So, again, these are the data from the 5-day study, 5- and 7-day study. And I'm going to assume for this example that this is the control, day 5, and day 7 is the test. I'm not suggesting that day 5 platelets should be our ultimate control, but just for this example.

So for each subject, we have two recoveries for the test and control, take the difference between these two. This is going to look a lot like a paired t-test. Calculate the mean of these differences in a standard deviation of these differences.

The upper confidence limit of these differences now will be calculated with a t-type interval, where we have this mean, plus the appropriate t value, given the alpha that we select, and an estimate of the standard error so that our upper confidence limit in this example is 12.4 percent, and this was calculated at--this is a 95 percent confidence interval.

So that's a very important slide. So we took the difference, mean standard deviation, calculated upper confidence limit. So now we get to Dr. Slichter's question, how do we test that? What's our standard?

My suggestion is the acceptance limit be based on these data. These are the data we just looked at, and here is our control value. My suggestion, we calculate a critical difference, which would be a control mean right there minus, in this case, two-thirds of the control mean. And that number is yet to be determined, which would give us 21. So that means our acceptable lower limit then for recovery is 63 minus 21, or 42 percent.

Now we can do the same thing for survival, go through the same exercise. And when we do that, then we can test our hypothesis. So here's the critical difference that we just calculated for recovery, the upper confidence limit that we calculated, and I didn't show you those calculations. And then we can test the hypothesis.

Since 12.4 is less than 21 and 44 is less than 80, we can reject the null hypothesis and accept the test equals control. So there's the essence of the whole thing.

Now, of course, you can get your statistical package to do this for you, too. Mine, when I run a paired t-test, I can ask it to give me an estimate of the difference with a confidence interval.

So this confidence interval, it spits out a two-tail confidence interval. So this is 90 percent, which makes the tail up here 5 percent. So this is our upper 95 percent. And lo and behold, we get the same numbers. That's very convenient. And we can get the same conclusion as we do the hypothesis test.

Now we can also do the same thing with regression analysis, and that was actually done in that transfusion paper. That's more complex. You've got to have somebody that's trained in doing those types of analysis. And if you are one that's trained to doing

those analyses, the donor should be treated as a random effect. Centers may be treated as random or fixed effect. Since we're looking at a difference, that doesn't matter. And if you don't understand this, don't worry about it because you'll never be doing this regression.

There is an advantage to doing the regression analysis, though, that you can explore corrections for other true co-variates, such as radioisotope or maybe subject age or maybe subject platelet count or whatever you'd like to do. But again, you need to be trained and understand what you're doing when you put in these co-variates.

And then, of course, this whole thing can be done with really complex models all at once. Basically, a nonlinear mixed model would be applied, and again, we have the same assumptions about the regression. And you really need somebody that understands these. These are pretty complex.

sj

I would suggest the two stage is simpler, and I would imagine everybody in this room could do those successfully.

So now comes the question of sample size. Oh, my gosh. This is what my boss always asks me, you know? Before we know anything, it's "how many tests do we have to run?"

Well, to really figure that out, we've got to have an estimate of the variance of the difference. Well, amazingly enough, with all the studies that have been published, not very many of us ever published the variance in the differences. We always do these summary statistics. I do them, too. I don't know why we do that, but I guess the editors want them. So it's really hard to find.

So I did find an old study by Stein, where he actually put the data in the paper so I could calculate these myself. I went back to a couple of our studies, where I had the raw data so I could recalculate them.

And here is my estimates of standard deviation for recovery and survival.

Now the interesting thing about these studies here is these are essentially identical products transfused to the same subject. Now I can't interpret what that means and what this means, but these are essentially identical products. These products down here are actually--there is a test arm, a real test arm.

For this first study, we looked at routine apheresis collected products and high concentration products that were stored for different periods of time, and then this was the 5-day and 7-day. And so, we had these estimates of recovery and survival standard deviation, and I've actually used these in the example.

Now some people would say, "I don't want to take a difference. I just want to do a ratio." I want to do test divided by control. Well, for those people, I've also calculated that standard deviation from the 7-day study. And for those that don't want to do paired

studies, hopefully they're not in this room, but there is the standard deviation estimates from the 7-day study again. And that's also based on whether you do a fixed center or a random center effect.

So with the assumption that we have an alpha risk of .05 and an 80 percent power, I'm assuming for this calculation that the control mean is 65 percent. My treatment mean is 50 percent and that my lower limit is two-thirds of the control. And using the standard deviation I just showed you, I calculate I need 13 pairs to run my test.

If you're a ratio kind of person, those are the numbers for the ratios. A paired ratio study, with that standard deviation, you require 16 pairs. So you need a few more subjects.

If you're the kind of person that doesn't want to do paired studies, there is those estimates, and those numbers start to get pretty large pretty fast. I would suggest not doing ratio. I want to say some more about

that in a second.

So what's the effect of having different standards for survival than we have for recovery? Here are the numbers we just calculated for sample size estimate for recovery. And if we go over to survival, if I assume control of 180 hours, a treatment of 140, and our lower limit as either two-thirds or one-half of the control, with these standard deviations, here's a sample size estimate.

So these data would suggest that if we go with two-thirds survival as the standard, our whole sample size would be driven on survival. I'm not saying that's why you should pick two-thirds, but that's what will be the outcome. So sample size depends.

Here is an example where if we have a control of 65 percent and if our minimum acceptable number is 43 percent, as the treatment mean gets closer and closer and closer to this critical value, the sample size requirement so that we can accept that there is an

equivalency between our test and control gets very large very quickly. So if your test is down here, plan on spending a lot of money if you want to use this to demonstrate equivalence.

I would additionally recommend that you don't use ratios. And there's really two reasons for this. One is, as we just saw, there is an increase in the uncertainty. Sample sizes go up. The other reason is biostatisticians don't like ratios, and that's because the assumptions of the models may not hold. And the data that I just showed you, normality does not hold when you look at ratios.

So if you want to go that way, you better get your experts involved so that they can verify that everything is copacetic on that score.

I also suggest do not use an absolute standard. I don't think we should walk out of here today and say all recovery should be greater than 43 percent because there is just too much uncertainty as we go from center

to center and donor to donor and labeling technique to labeling technique. And that increase in the sample size will be very costly.

So, once again, I recommend that we plan and perform equivalency tests. We perform paired designs with appropriate care to randomization. That we construct confidence intervals based on the difference, and a maximum acceptable difference based on the data, the data of the experiment.

And that we would reject the null hypothesis if the confidence interval does not overlap the maximum difference. And that's for recovery "and" survival, not "or." And then sample size, that depends.

I would suggest today that we need a couple of things from the panel. One, we need concurrence, some concurrence that we should do equivalency testing, planning and testing. We should do paired designs with appropriate care to randomization, and that really as far as fitting the data, as long as we have a good model, it

shouldn't matter which specific one we use, whether it's the multiple-hit that's in COST or whether it's the multiple-hit that's written into SAS or S-plus or SPSS. That doesn't matter.

Or all kinds of other pharmacokinetic models would fit the data just as well. But we should describe the data.

I think we do need a statement and an answer on if we need to have both recovery and survival paths. That's critical in interpretation. We need to know what the acceptable difference is. Is it going to be two-thirds of the recovery? Is it going to be two-thirds of survival, half of survival? I don't know.

Alpha risk, I would suggest that that's a regulator call. FDA should tell us what that number is. Beta risk or the power associated with the study, I think that's up to the sponsor. How much money do you want to spend to give yourself a high probability of demonstrating that your product is equivalent to control?

That's essentially what the question would be.

There are some other points that we'll hear more about--data adjustment, what's appropriate? What specifically do we call control? And what parameters do we evaluate? Is it just recovery and survival? Do we also want to look at area under the curve? I'm sure we'll hear more about that today.

Thank you very much.

[Applause.]

DR. ZUCK: Thank you, Larry.

The next speaker is well known in these areas. When you review the literature on this topic, along with Scott and a couple of others, it's amazing how much Jim has actually written.

He served at the National Red Cross Headquarters for years and was named chair of pathology--not concurrently, I might add--at Dartmouth. And it's a great pleasure he and I share, an honor of which I think we're both very proud of, we're both members or fellows

of the Royal College in Scotland.

Oh, he's going to talk about proposed criteria for radiolabeled platelet studies. Jim?

DR. AUBUCHON: Thank you very much, Tom. Very kind of you.

As several of us began talking about, thinking about the potential for adopting the proposal that Scott had developed some months ago for wider use, a number of questions came to mind, and I began jotting some of these down. And that became the basis for this presentation this morning.

I appreciate the assistance of a number of colleagues, particularly in BEST and particularly Dr. Slichter, for adding some additional thoughts here. And I hope the panel will find these comments useful. I will warn you that the next few minutes have only questions. I have no answers, at least at this point.

I will offer, however, Sloan's law. And I think this is appropriate here. And hopefully, today we will

be able to leave here with a model that is, indeed, so attractive that we can feel good about adopting it and deal with some of the issues that are at the moment troublesome or have no specific answers. Because, indeed, the devil is in the details, and my name at the moment is Lucifer.

[Laughter.]

DR. AUBUCHON: The concept which has been proposed, and which in our laboratory we refer to as "Murphy's law," is that one of the primary means of assessing the efficacy of a new means of handling platelets would be autologous radiolabeled recovery and survival studies comparing fresh to the test. So the fresh becomes the standard, as you've heard, and then the test involves some type of collection, treatment, or storage alteration that then is reinfused back into the same subject in a comparative manner. So each person serves as their own control.

Here are some of the details. I will walk

through and pose some questions for discussion and questions for the panel hopefully to comment on later on.

The first is where does this comparative standard come from? Does the standard need to come from the same subject? Could the comparison be against an absolute criterion? And you heard Larry speak about that just a minute ago.

But we do have an absolute criterion that's used for red cell transfusion, and this has been around for many decades, where it's the expectation that at the end of the storage period, reinfusion of radiolabeled red cells will allow recovery of at least 75 percent of them 24 hours later. We could adopt a similar approach with platelets, whether it's 43 percent or some other number.

If we were to use an absolute standard, it would mirror what we do with red cells. And even if the study were intended only to look at the control arm, as it were, if we found a subject that gave unusually poor results, as certainly can happen with both red cells and

platelets, we could follow that up with a fresh study to validate whether it's just this particular subject's platelets that don't work well or whether the problem is really with the test system.

However, if we were to use this approach, it would require the calculation of an acceptable performance standard. Is it 43 percent, or exactly what is it? Because it would be carved in stone.

Moreover, it would require labs to verify their methods. Even if we were to all adopt exactly the same labeling method, there are some differences, whether it's altitude or humidity or whatever. But there are always some slight differences between procedures. And the lab would need to verify that they got the same result that someone else got.

How often would they have to do that? Just when they first start up doing these kind of studies? Periodically? Once a year? At the beginning of every study? For each sponsor? For each new approach? That

question would become problematic. It might require establishing some type of performance standards for laboratories. A new means of accreditation. I don't think many of us would be looking forward to that.

Also this approach would not account for any procedural "drift" over time. The alternative would be using the subject as his or her own control, which accommodates subject individualities, as you saw from Scott's data earlier. It would allow compensation for some lab variability in the way of one particular lab happened to do the testing. It would allow for accounting for drift in procedures over time. And it would probably reduce or limit the number of observations or radioactive exposures that would be needed, particularly using a paired study.

However, it does create some logistic difficulties. It requires more of the subjects, more needle sticks, more visits back to the lab. And if you are going to have a paired study, an expectation of a

paired study in each and every experiment, you will then obviously require at least two sets of radioactivity exposures rather than just potentially a single one if you had an absolute standard.

Another question is where do these fresh platelets come from? Should they come from an aliquot of the unit that is then going to be stored or treated in some manner as the test and therefore collected right from the unit? Or should it come as a separately collected specimen, either immediately after the collection of the test platelets or at some time later on, such as at the end of the storage period?

Using a separate collection prevents damage at the collection of the test unit from creating a lower standard. For example, if the device under consideration was, say, an apheresis device that somehow damaged platelets during collection, and then a sample of those damaged platelets were taken as the fresh platelets to set the standard, well, the standard would become very

low. And perhaps the bar then would be very easy for these damaged platelets after storage to meet. So this would potentially be a problem.

However, if you were to collect the platelets in a way at the end of storage via another method that damaged them, you would also create a lower standard. If you only had one means of collection that was used throughout the entire experiment, you would have less variability in that.

However, there are some problems with using the actual unit as the source of the fresh platelets. It relates to the way in which the platelets are collected, the mean age of platelets at the time of collection. Collecting them separately would require separate phlebotomy obviously and potentially a new technique, also more costly, and may introduce its own artifacts. And Ed Snyder will be talking this afternoon about a standard way of collecting fresh platelets at a separate collection.

sj

The aliquot from the unit, as I said, allows some positives and some minuses. If you collect from the unit itself, you're going to get an accurate representation of the platelets that are actually in that bag. Some apheresis techniques may be less injurious and may be less expensive and a simpler way to go.

However, with each study, there will be a different standard for fresh platelets. So if the fresh platelets are collected on a Gambro instrument versus a Haemonetics instrument versus a Baxter instrument, the fresh platelets may not all be the same. And the FDA may have difficulty in interpreting the study as a result.

If one collects an aliquot from the unit, that would imply that it would be reinfused on day 0 or the morning of day 1, shortly after collection. And there would be some variability that would be created potentially between the status of the subject on day 0, when the platelets are reinfused, versus on day 5, day 7, day 10, whenever the test platelets were reinfused. So

this is an issue that would also need to be considered.

If the fresh platelets are collected separately, how should they be collected? Should it be collection of a whole blood unit? That is, separate from whatever collection was done to generate the unit that was then treated or stored, should the fresh platelets be prepared via whole blood collection? We certainly have a lot of experience doing that millions of times a year.

However, this creates an alteration in the subject blood volume. And since blood volume is estimated based on height and weight, a change in the blood volume could potentially distort the results.

If the collection that was used initially is an apheresis collection and then a whole blood unit is collected on top of it, this is quite a significant change in blood volume. There is already a significant change in blood volume from an apheresis collection.

If the hematocrit of the subject is altered, the platelet kinetics may change. And Bob Valeri has

published some work on this as well. We don't have a good feeling for exactly what this means in normal subjects, but we have a hint that it may mean something.

And because of these factors, if you were to collect a whole blood unit, one might have to reinfuse the red cells and/or the plasma or some plasma substitute, albumin, for example, back into the subject in order to recalibrate their blood volume before moving onto the radiolabeled survivals.

And I've heard from colleagues in the United Kingdom that if one wanted to reinfuse albumin to re-equilibrate or to re-establish blood volume back to some baseline level, the study would not be acceptable because there are concerns about infusion of plasma product in that country.

And if you did collect a whole unit of blood, how would you create the platelets? It would probably have to be stated what method would be used, whether it would be the PRP method or the buffy coat method.

If instead of collecting the whole blood unit, one collected a small volume, just enough to collect enough platelets to be labeled and reinfused, you would be able to standardize the entire process of creating a fresh platelet. You wouldn't be depending on the unit that was being collected, an apheresis unit, for example.

You'd be able to collect in a routine, repetitive manner that was not dependent on any one manufacturer or the continued availability of any one instrument in order to establish what was a fresh platelet. The amount of blood volume disruption would be minimal, and you wouldn't have to reinfuse any red cells that had been taken out.

However, this would require standardization of this technique. There certainly are already standards in the literature. And as you'll see later today, I think this can, indeed, be standardized quite readily.

If the fresh platelets are not an aliquot of the unit, then how should they be collected? Could that

question be answered by apheresis? Some instruments cause very little platelet damage, but then you get into a manufacturer-specific protocol and potential for obsolescence. And also we're looking at blood volume changes again.

Should the fresh platelets be reinfused at the--around the time of collection of the test unit--that is, day 0 or day 1? Or should they be collected and reinfused on the last day of storage or whenever the stored platelets are tested?

If you were to collect the fresh platelets on the day of collection of the test unit and then reinfuse them right away, it would allow the platelets that are being used to create the standard to reflect the platelets that are being stored. With the collection of an apheresis platelet, up to a third of a normal subject's platelets have been removed from them.

As a result, thrombopoietin levels would increase, and over the next few days, the marrow would

begin to create more platelets. That would mean that on day 5 or day 6 or day 7, for example, the mean age of the platelets that are circulating in the subject may be less than the mean age at the time that the test unit was collected.

Those younger platelets, on day 5 or day 7 or day 10, may survive better after radiolabeling and therefore create an artificially high standard for the test platelet to overcome. If so, by collecting the fresh platelets on day 0 gets around that problem and allows the fresh platelets to really represent the same population of platelets that ended up in the storage bag.

If you were to collect the fresh platelets on day 0 and reinfuse them at that point, and if the storage arm were long enough, you could potentially use the same label, same radiolabel for both arms of the study. As you'll see later today, chromium and indium appear to be--appear to give the same results. But if you could use the same label, that would be--would remove that

potential source of variability.

There is a reported intra-subject reproducibility that is very good over time. Although separating the time of reinfusion of fresh and stored platelets may introduce one more variable. But in most laboratories, there is relatively little over a short amount of time.

However, if you reinfuse fresh platelets on day 0, we're required two sets of post infusion samplings, and the subject will look very much like a pin cushion by the time that you're done.

Some collection and processing techniques may cause the fresh platelets to then have to be reinfused on day 1. Is that acceptable? Is that still a fresh platelet?

And the collection technique, of course, may have altered the subject's blood volume. One takes 300 or 400 mLs out of a subject at the time of an apheresis platelet collection, what should be done to make sure

that their blood volume is as being calculated? And does that alter the projected recovery of fresh platelets when they're reinfused shortly after an apheresis collection?

On the other hand, if you reinfused on the same day as test platelets, that is you collected the fresh platelets at the end of the storage period and reinfused them at that point, you could reduce the variability in the subject status at the time of reinfusion because the fresh and the test platelets would be reinfused at the same time.

However, as I said, this may lead to the fresh platelets being of a younger population, and of course, this approach requires a double label technique, which certainly can be done and many people have had success with. But it is an additional complication.

Another question that has been raised is that should test platelets be reinfused on the last day of intended storage or the day after the last day of storage for which the manufacturer is seeking licensure? The

current method is to reinfuse on the last day of storage. Day 5 platelets are tested with radiolabeled studies on day 5.

This would--if we were to adopt this for the future, this would allow us to compare future studies with previous studies. However, it may overestimate the efficacy of platelets that have been stored for 12 to 16 hours afterwards. Most laboratories I think would begin their labeling process probably in the morning of day 5, and yet those day 5 platelets after licensure could be transfused as long as up until midnight of that night.

If, however, one were to reinfuse on the day after the last day--that is, on day 6 for a day 5 platelet--this would provide assurance of functionality all the way to and even beyond the intended out date of that platelet product. However, it would require us to reassess the approved systems already in the marketplace for comparative purposes. That is, we've been talking about comparison difference between fresh and control of

two-thirds.

As you'll see later today, that seems to work for recovery. But would it work if we looked at day 6 rather than day 5 for current day 5 system platelets?

And indeed, the system could be manipulated. And that is if a manufacturer were worried about whether or not their component would actually make it to day 6 but seemed to do okay on day 5, based on in vitro data, for example, the manufacturer could specify that the platelets--test platelets be collected late in the afternoon of day 0 and then require the laboratory to get up at the crack of dawn and label very early in the morning of day 6. And so, the end result really would not be much different than what we have currently.

There are many technical details of platelet radiolabeling that I'll just mention here briefly. Many of these have more or less been standardized through the--I was going to say ages, but we haven't been doing it for that long--through the last several decades. But

ultimately, I think if the agency is going to speak more specifically on how radiolabeled studies are to be performed and interpreted, these issues need to be set down in some definitive way, which may be either through guideline from the FDA or maybe through some collaborative group publishing a new proposal for standardized methods.

For example, under radioactivity dosage, what dose should be injected? Should there be a minimum number of counts required at a certain point in the survival curve? Should all laboratories be required to use a high-efficiency counter in order to limit the amount of radioactivity that has to be infused? Should some threshold over background be required for the dosage that's given?

Should the number of platelets that are labeled be specified? This is not so much a problem for the test article because platelet concentrates, whether apheresis or whole blood derived, generally have around the same

concentration. But if you're collecting fresh platelets, you may end up with different numbers of platelets. And so, what number of microcuries need to be put on what number of platelets? Does that need to be standardized?

Should the labeling environment be standardized? What should platelets be suspended in at the time that they're actually labeled? In what should the platelets be labeled? Should it be a plastic bag, or should it be a conical tube?

When should the subject be sampled after reinfusion? Usually within the first couple hours, several times is standard. And then often what you see in the literature is daily for 10 days. Does that really mean daily for 10 days? How do you accommodate weekends and holidays?

And are all these samples really necessary? Do we really need to go out to a week and a half to get an accurate picture of the survival curve when, as Larry just showed, it's really the first few points that

determine both the recovery and the survival?

How should that recovery be determined? As Larry noted, it might be the highest of the first several points on the curve. It might be specified as the 1-hour or the 3-hour sample or some specific point or back extrapolation from the survival curve. And no matter how you do this, you will find situations where it doesn't quite seem to make sense.

How should the subject's blood volume be estimated? Based on height, weight, and gender, and which formula should be used? Or should it be estimated directly through, for example, infusion of fresh radiolabeled autologous red cells? Is that necessary?

How should the recovery curve be calculated and stated? Usually, as the numerical expected lifespan, but using which mathematical model and using which computer software? As we'll see later today, those questions are not as large as one might think.

Is there any value to calculating the area under

the curve? Does that lead to excessive reliance on survival, or does it allow us to look at something that we really haven't been looking at up to this point?

Could we approximate it linearly just by taking recovery and the survival and multiplying together and multiplying by half? The curves are not linear, but that might be an estimation. Or should we use the COST program to generate the area under the curve, which it can do?

What corrections should be included in the calculation? As Larry noted, there are three which are usually considered. First is correcting for the amount of radioactive material, which is not actually on the platelets at the time of reinfusion. If the radiolabel is already in the plasma by the time you squirt it into the subject, that cannot possibly relate to the survival of those platelets after infusion and therefore could, in my opinion, legitimately be excluded from the calculation.

There needs to be, in my opinion, a correction for the radioactivity in the plasma of each sample because what appears there is obviously not in the platelet and is not helping the subject maintain hemostasis. And then the question of correction for red cell labeling has come up and is a point of some debate, particularly when speaking of apheresis platelets because apheresis platelets have very few platelets in them.

But the kind of curve that Larry showed is a stylized curve where it ends up above the baseline, does not go to zero, needs some correction to zero it out. Maybe due to red cells, maybe due to something else. But it would appear to be necessary in order to achieve accuracy.

However, should this only be done with chromium, or does it need to be done with both chromium and indium? Does indium, as it elutes from platelets as just ionic indium, have the capability to label other cells?

So there are many questions here that have been

posed. Let me just give you a couple sample protocols to pull a couple of these ideas together and indicate the ways in which some of these issues might be put together as an actual protocol.

For example, if one were to create an absolute standard, one might say that the maximum platelet recovery that we could anticipate in a normal individual with a normal size spleen would be 70 percent times 67 percent is 47 percent. Nine-day maximum survival time times 50 percent, if that's the ratio that's going to be used, would be four and a half days.

That becomes the gold standard equivalent to the 75 percent, 24-hour recovery for red cells. A laboratory would validate its procedure when it gets into this realm of experimentation and would repeat that periodically, and then would perform a clinical trial and compare the results of the clinical trial with sort of a single arm trial with all of the problems associated with that. But compare the results of that to the absolute standard.

sj

That's exactly what's done today with red cells. If any unexpected results were encountered, the study might be expanded to look at fresh platelets from either the subjects that gave poor results or all of the subjects after enough time so that the lack of randomization would not be a problem. And hopefully, that would explain any discrepancies or inability to meet the standard. If not, one might have to start over again.

Another approach would be to use, as the comparative standard, a sample from the unit. So a unit of blood, a unit of platelets would be collected via some type of approved device. After a defined holding or resting period, a sample might be taken from the unit and infused as the fresh platelets on day 0 or within 24 hours of collection.

The test unit would be carried on in storage or treated in whatever way it was going to be handled. And then at the end of storage, either on day X or day X+1,

it would be reinfused as the test arm, and there would be a comparison between the fresh and the test platelets. That has problems as well as advantages.

Another approach would be to collect a comparative sample on the day of collection, but separately from the unit, where the test unit would be collected in whatever method was being examined. The fresh sample would be collected separately. It would be reinfused within 24 hours. The test unit would be held and then reinfused at a later time.

Another approach would be where the comparative standard would be collected and reinfused on the day of reinfusion of the test platelets. The same concept as in the previous slide, but now moving the collection of the fresh down to the last day of storage or the day after, depending on whenever that was going to be.

And possibly different approaches could be used with different types of test platelets. For example, with apheresis where the standard would be collected and

reinfused on the day of reinfusion of the test platelets, the same concept as in the previous slide, but now moving to the collection of the fresh down to the last day of storage or the day after, depending on whenever that was going to be.

And possibly different approaches could be used with different types of test platelets. For example, with apheresis platelets, we run the problem of altering the subject's blood volume, but also in creating a thrombopoietic drive that at the end of the storage period of the test platelet may yield a platelet population with a younger mean age.

So with apheresis platelets under study, one might collect the fresh sample on day 1--for example, the day after apheresis--in order to allow equilibration of blood volume, but before the new platelets would begin to emerge from the marrow, and reinfuse that as the fresh platelet early on in the storage period and compare that with reinfusion a few days later with the test or stored

platelets.

For whole blood drive platelets, however, there is less of an issue of either a thrombopoietic drive creating younger platelets or alteration of blood volume. And so, because of the lack of issue about platelet age in particular, we might be able to compare the fresh sample drawn at the end of storage.

So there are many different issues to be addressed. I think we will see some data today on some of these issues. I don't think we'll see data on all of these issues, but I hope that we'll be able to reach consensus on a number of the important ones.

Thank you very much.

[Applause.]

DR. ZUCK: Thank you, Dr. AuBuchon.

We have about 20 minutes that we can discuss these papers presented thus far today. For those that wish to make a comment, there's a microphone in front of you, and there's a round button in front of the

microphone. If you push that button, a red light goes on, and you're on.

We can start with anybody that wishes to start.

DR. ELFATH: Dean Elfath from Baxter Healthcare. I just wanted to ask the people who have practical experience if the creeping, you know, decline in quality due to comparing different tests have been shown to be true historically? Or the creeping inferiority, trying to prevent creeping inferiority, I think, is a good concept. I just wanted to see if that has been shown by the data in the different testing sites.

DR. ZUCK: Did the speakers understand that?

DR. ELFATH: The question is to Dr. Jim AuBuchon, Dr. Sherrill Slichter, Dr. Harry Taylor. Have they seen creeping inferiority in the different studies that they have done over the years? Is that proven by looking at the data?

DR. MURPHY: Yes.

DR. ZUCK: That's the short answer.

DR. SLICHTER: No.

DR. ELFATH: That's another short answer.

DR. MURPHY: I think I showed a couple slides today comparing day 0 to day 7, and there's clearly a creeping down of the recoveries and survivals with time, just with storage of regular old platelets.

I think we know that.

DR. ELFATH: Maybe, Dr. Slichter, have you seen over the years when you do studies that actually the recoveries and survivals of different products have declined over the years because they are being compared to previous studies, or the quality has shown some improvement?

DR. SLICHTER: Well, I--I mean, I'll talk this afternoon and show you our current data. But I think if anything, the products that you people are providing us--and I don't know what it is--are actually getting better. So I think we have better quality products.

I remember when we first started to discuss

extended storage data with Dr. Vostal. He got me up at a meeting because I was quite concerned, in fact, that, you know, there was a progressive loss of viability with the platelets during storage. And so, I was quite concerned about extending the storage time.

But if anything, I mean, I have an N of 1. I always like to talk about Ns of 1. But I have an N of 1 where the recovery after 21 days of storage in plasma life, the recovery of the platelets is 44 percent. Now that's pretty astonishing.

And I can share with you data, that our statistician has looked at our data and basically has said that in terms of recovery, the recovery of the stored platelets is directly related to the recovery of the fresh platelets from that donor but does not vary by machine, apheresis collection, does not vary by radiolabel use, does not vary by storage time. So basically the recovery remains flat.

The survival goes down by about--after you get

to about 7 days, it starts to go down. And my 21-day platelets only live 2.8 days. But again, as Scott has said, you know, we're talking about patients who allow the platelets to survive only maybe 2 or 3 days.

So I'm convinced--I'm, I guess, astonished that platelets are much hardier than I ever would have predicted. I would never have predicted that we can store them as long as we apparently can store them. And I think, if anything, the apheresis machines, the storage bags, I mean, I don't know what you people are doing, but you're doing something that's very nice for the platelets, and we appreciate it.

[Laughter.]

DR. ZUCK: Dr. AuBuchon?

DR. AUBUCHON: I agree with Sherrill that we are seeing collection and storage methods, bags, that are less injurious to platelets than what we had two decades ago.

DR. SLICHTER: Yes.

DR. AUBUCHON: And indeed, if we look at the data that we were able to generate with Norfolk in the protocol that Larry was discussing earlier, looking at 7-day platelets, the 7-day platelets we were studying 2 years ago did much better in recovery and survival than the 7-day platelets from two decades before that the FDA had licensed for 7-day storage at that time.

That could be a difference in the radiolabeling techniques. That's true. But if you just look at recovery and survival, looked like we were doing at least as well, if not better, than two decades before.

However, there was clearly a difference between day 5 platelets and day 7 platelets. And as Salim mentioned earlier, the agency accepts 10 to 20 percent difference from the test to the control, when the control is regular old platelets as defined by Scott. So right there is a potential for inferiority, and the slippery slope has begun.

We could--if 7-day platelets were licensed, I

could probably take the same methodology and go out to 8 days or 9 days, and it wouldn't be different by more than 10 or 15 percent. Would the agency then be required to license that? And then I could go from, you know, 10 days to 12 days. And we could go on and on until there was no recovery and no survival, but it was not statistically different from the previously licensed approach.

DR. ZUCK: Yes?

DR. AEBERSOLD: Paul Aebersold, FDA. The 5-day, 7-day question is very interesting because we should remember that the kind of statistical comparisons we're talking about are noninferiority. That doesn't prove their equivalence. You can be different and still be noninferior. As a matter of fact, depending upon the parameters, you could be statistically significantly different and still be noninferiority, depending upon the delta that you pick.

So 5-day platelets are not--we're hearing that

7-day platelets have lower recovery and lower survival. Yet you can do a study that says that they're noninferiority. That they're not inferior. That doesn't mean they're equivalent. And it doesn't mean if you had perfect knowledge--perfect knowledge, i.e., you know, a big bleeding study with 100,000 patients per arm that will never be done. If you had that perfect knowledge, 7-day platelets might not be as good.

So when you pick a number like.667 on recovery or .5 on survival, you're making an assumption that the differences are either too small to worry about or that you're not going to think about it, one of the two.

DR. ZUCK: Just an uninvited comment from the chair. If you think back--I'm looking at this corner of the room. We started making platelets in the early ‘70S, and you think of how we did it, it's just almost unbelievable that we got anything because we were very careless. We did everything in the cold. They aggregated. We didn't have the right anticoagulant.

We didn't handle them in a manner to let them--we didn't do any of the things that are so common to everybody. And not all of you may remember those days, but it's amazing that we got any hemostasis in patients ever. At least that's what I think compared to what we see now.

Any other questions? Discussions? Point to be made? Yes?

DR. LEITMAN: This may come up in a future presentation, but are there direct data saying donor comparison of chromium compared to indium comparing the labeling technique directly? There are?

DR. ZUCK: Jim, you want to answer that?

DR. AUBUCHON: Yes. You'll see data this afternoon, Susan.

DR. LEITMAN: Okay.

DR. ZUCK: Yes? Jaro?

DR. VOSTAL: Jaro Vostal, FDA. This is a question for Scott. You proposed that you have 66

percent for recovery and 50 percent for survival in the new standard. The way I understand it, the reason for doing a shorter lifespan number is that because these platelets are going to be used in thrombocytopenic patients, and they have a shorter survival time.

But my question is these studies are going to be done in healthy donors. Should we accept this decreased criteria for survival in healthy donors? Or should we expect in a healthy donor the recovery and survival should have the same type of recovery number, or same type of percentage?

DR. MURPHY: Yes. To me, it's logical that you don't have to retain capacity to circulate because in practice, they never live that long. And yet when you're transfusing a patient who's bleeding, you want to get the maximum in vivo recovery. So that's why, in my proposal, I made a distinction.

I'm very interested in making sure that I get all the stuff that Larry taught about ratios not being

the most useful way to do it. But nonetheless, I think that--I think you can be easier on the survival than on the recovery. And the way I developed the idea, the idea stays the same and you choose the percentage--you can choose a different percentage, but the basic philosophy stays the same.

DR. VOSTAL: Just a follow up question. If you see a difference in healthy donors in survivals, would that be exaggerated if you transfuse it to thrombocytopenic patients?

DR. MURPHY: I guess we don't have any data about that. In other words, you can hypothesize, I suppose, that the fact that it's a transfusion in patients that shortens the survival. And then there's an additive, and then they're shortening the survival in normal volunteers. If those two things are additive, that could be important for the--perhaps it could be important for the patient.

DR. SLICHTER: I would maybe like to add a

comment as well. Your question really relates to the fact that if the survival is only half in a normal volunteer, is it going to be--is it going to be the same in a thrombocytopenic patient, or is it going to be even less than that? And so, have you further compromised the transfusion product?

And as Scott says, we don't really have the data to substantiate that. But I'll show data in my presentation this afternoon that basically there is a direct relationship between platelet count and platelet survival in thrombocytopenic patients.

And as Scott has mentioned, all of the studies--and there now have been several very large transfusion studies in thrombocytopenic patients given for a variety of reasons--the recent trigger trials, the Cerus Baxter pathogen and activation study, the TRAP trial to look at alloimmunization. And basically, since we now use a trigger of 10,000 or even 20,000, the longest interval between transfusions averages about

somewhere in the 2-day range.

So I don't know of any data to suggest that if we, you know, had a, say, in normal volunteers, if we had an average 4- or 5-day survival of the platelets, in my opinion, that would allow the patient to have those platelets survive as long as they're going to survive. That's really based on his or her platelet count at the time following transfusion.

So I am in favor, basically, of Scott's proposal that the standard for the survival be 50 percent and the recovery be 66 percent. And I don't know of any evidence that we are really compromising the care of the patient by allowing a differential effect of the standard for recovery and survival.

DR. ZUCK: You wanted to say something?

DR. HEATON: Andrew Heaton, Chiron. I'd like to make a couple of comments.

The rate at which platelets are used physiologically is dependent on the endothelial

requirement, which is relatively fixed. In fact, Sherrill, you did the work with Hanson. So if you have enough platelets to meet the endothelial requirement, you'll service the clinical requirements of the patient.

The key issue is that how many more than that do you need in order to provide for longevity decreases over time? So the more you reduce the dose, the more you will increase the endothelial use, and therefore, the more nonlinear your decay scheme becomes. And it requires you to slightly increase the frequency of transfusion.

So recovery is probably less of a priority for the average patient being transfused 2 days who's thrombocytopenic because they've got a very high ratio of endothelial use to senescence. So to answer Jaro's implied question, providing that the survival is longer than 3 to 4 days and you transfuse every 2 days, you, in fact, ought to titrate your dose to the lowest possible dose.

DR. ZUCK: Larry?

sj

MR. DUMONT: Well, if you remember the plot I put up there, where survival was, it's away from all the data. And the modeling was actually first developed for survival in patients for in vivo labeling, and then it was expanded to cohort labeling of patient platelets to evaluate clinical conditions.

And the survival is really trying to estimate the survival of the freshest platelet in a cohort. And I struggle with understanding what that really means in our situation and how to relate that in a physiological sense to thrombocytopenic patients. I don't have the answer to this.

But, in fact, maybe our survival number that we get is just a parameter of the model and it has very little meaning physiologically, and maybe we ought to think about area under the curve, that that would be closer to having a physiological meaning.

So we've got to be careful between the analysis methods and just mathematical approaches, which are

helpful and useful and good for comparisons, and extrapolating that to physiological situation because I don't think we understand that leap. So just a comment on that.

DR. ZUCK: Yes?

MR. : Have you run the sample sizes on area under the curve? I mean, would you have that same difference that you put up sample sizes for .67 on survival versus .5. Have you done a similar thing for area under the curve?

MR. DUMONT: No, I didn't do those. I ran out of time.

MR. : It would be interesting.

MR. DUMONT: Yes.

DR. ZUCK: Any other comments or questions? It's very close to 10:00.

DR. SLICHTER: I'd like to ask--

DR. ZUCK: I'm sorry. Didn't see your light on, Sherrill.

DR. SLICHTER: Go ahead.

MR. : It would seem that area under the curve would be a fairly precise measurement to me and not something that would be extrapolated from two or three points fairly far away with an uncertain slope. So it would seem to me like that would be something that would have some certainty to it. And it is easier to conceptualize in terms of the circulation of the platelets with time in a patient.

DR. ZUCK: You have another comment, Sherrill?

DR. SLICHTER: Yes. A comment for Larry. I'm not a biostatistician, don't pretend to be, try very hard to understand when statistical issues are discussed. But I've been in a fair number of clinical trials recently where good biostatisticians have been involved in analyzing the data, and I think uniformly I've never heard a biostatistician who hasn't been unhappy with ratio measures, first of all.

And secondly that doing regression analysis

really allows you to look at a variety of parameters that may be affecting the outcome of the measurements that you're trying to make and therefore really allows you to look at how things are influencing your results.

So Larry gave a very--I thought a very nice presentation this morning on looking at the difference between the fresh and the stored data as a statistical method of analysis. But even in his discussion, he suggested that the regression analysis was more powerful, although not available to everyone in this room. But everyone in this room, and certainly the companies who are interested in helping us provide stored platelets, we can get statistical help.

So my question really is why would we not want to use an analysis of the data based on regression analysis rather than some simpler techniques if it really is more powerful and provides us with more data and simply means that we have to smile at a biostatistician to get them to help us with the analysis? I'm smiling at

Larry, in case.

MR. DUMONT: That was a long question. My thought on that is that both methods are valid and both methods should be allowable. In fact, if you go through--instead of doing the area under the curve calculations, I actually did lots of comparisons between regressions and t-tests. And if you make some right choices in your regression model, you get exactly the same numbers.

And personally, I do the regressions. But other people don't do that for different reasons, and I think kind of the paired t-test approach is perfectly valid, should be acceptable.

The other thing that I've seen is that one has to be careful of is just putting too many co-variates in your modeling when you do regression, and then you can start to run into all kinds of other problems and misinterpretation of data. So that's one extreme. So if you've got a good biostatistician, do the regression. I

like that.

DR. ZUCK: With that, on behalf of the attendees, we want to thank the presenters this morning. We'd like to see you in exactly 15 minutes so that we don't fall behind for the day.

[Recess.]

DR. ZUCK: Okay. Let's get started, please.

There are two handouts which didn't make it in your bundle that was provided by the FDA, et cetera. One is Vostal's handouts, and the others are Ed Snyder's. Those can be picked up on the table beyond the coffee. There is plenty of copies for everyone here.

All right. To pick up, the next talk is on chromium/double label approach by Andy Heaton. Andy Heaton, again, many of the speakers are known to most of us. He served--he is currently chief medical officer of Chiron Blood Testing and is responsible for their clinical and scientific affairs.

Andy served as executive vice president of Blood

Systems, and following its merger, he was at Irwin Memorial Blood Bank, vice president and CEO. He currently serves on the Advisory Committee for Blood Safety Availability to the Assistant Secretary of Health.

Andy, without further ado?

DR. HEATON: Good morning. Thank you very much for the opportunity to be here and to talk a little bit about the development of radioisotopic labeling techniques.

First, I'd like to recognize my great team from Norfolk. It's been a very consistent team, Pam Whitley and Stein Holme, who worked with me on the work. And subsequent directors in Norfolk, Joe Sweeney, Dean Elfath, and now Harry Taylor, who make up the Norfolk mafia.

I'm going to walk you through today the development of the double label technique, the indium technique, and also a number of studies that best exemplify the application of the double label technique.

sj

So I plan to review the principles, the physics, the process, the improvements, statistics, and some of the results.

The objective of any tracer study is to have internal or external quantitation of platelet kinetics. You'd like a radionuclide that is readily detectable. You'd like preferably a physiologic element. You certainly don't want the tracer to be toxic to the cell or to the patient. You don't want it to perturb the study, to affect the survival of the platelets under study.

The key issue is you'd like minimum reutilization and elution. You'd like an ease of administration and sampling. Obviously, you'd wish to have selective tracer uptake because it makes the labeling process much easier. And finally, you'd like to have homogeneous distribution/cellular distribution of the isotope of interest.

Well, as we look at radionuclide studies, these

are dilution studies in principle. There's a dose. There's a donor. There's a defined distribution and the sampling. In terms of the representative dose, I'll talk a little about the acquisition of a representative aliquot and how you can use the technique to reduce your process loss, ensure consistent tracer uptake, and we'll talk a little bit about damage and elution.

Now in terms of the donor, obviously, the donors, some of them have variable cell quality. Platelet recovery is not the same for every donor. And of course, the donor's turnover may change during the course of the study. You can eliminate this variable by doing simultaneous paired concurrent studies.

You do have a defined volume of distribution, which you can either measure with another isotope or you can estimate it, and the current characteristic most of us who perform studies use a nomogram. Which although it's inaccurate, and Stein will show you some numbers to show just how inaccurate that nomogram is, if you do a

paired simultaneous concurrent study, you, in effect, factor out that inaccuracy, and you're left with the variability only up here in the labeling portion.

And finally, with any isotope study, you have to be very careful with your samples, that you have constant volume, that you sample across the period of study, that you don't frontload your study, and that you do very accurate counting.

Now to move off the principles then and look at the practice, the method that we will describe uses either 43 mL of whole blood, ACD whole blood, or 10 to 20 mL of platelet concentrate. It involves tube processing in order to maximize the platelet recovery and minimize plasma carryover. It uses very high uptake, 60 percent of indium, slightly less in terms of chromium. And it's been customized to reduce it to one soft spin, two hard spins in order to minimize the perturbation of the study.

In terms of the counting, a key issue here relates to some of the atomic physics and the dilution

characteristics. You dilute hot isotope in a cold donor about 1 in 5,000. So when one performs one's standards and counts, it's very important to use a number of standards because the isotope doesn't distribute completely linearly. You need to average your standards. And you need to dilute down to the area of counting because you can't always presume that your gamma counter is entirely linear.

You certainly need to have weighed samples. One tends to think that you can prepare it accurately. But again, it's surprising how frequently there's variation in sample preparing.

And finally, you have to be very careful about your counting to a defined error, and you have to ensure that you have enough isotope that when you count the sample you get an accurate and representative count.

Let's talk a little bit about the atomic physics and the differences between the isotopes and their implications. Indium is a high-energy isotope. Has two

photo peaks, 172 and 247 KeV, which is very well suited to sodium iodide detection. Decays by internal capture, and you get a very high emission, 90, 94 percent of both these photo peaks.

Chromium has a higher energy of emission, 320 KeV and only a 9 percent emission. And as a result of the high KeV, you don't get as high quantum energy detection in your sodium iodide crystal.

In terms of selective uptake, indium binds to oxygen very nicely. It's lipid soluble. And platelets bind somewhat more than white cells, but the indium oxine binds a lot more to these cells than it does to red cells.

Whereas in the case of chromium, chromium, which is hexavalent sodium chromate, binds very nicely to hemoglobin much more than it does to platelets and white cells. And therefore, if you're using chromium, you must have a very pure sample preparation.

Toxicity. Well, indium is a little bit toxic to

cells. But if you control the volumes, as I will describe later, you can get very good labeling and no evidence of toxicity. And in the case of chromium, the specific activity of the radioisotope or radionuclide, chromium is so good that you, in fact, will never exceed the toxicity limits.

In terms of detection, you get about 40 to 50 times more counts off oxine than you do off chromium, and one of the problems associated with this is that if you use a standard detection crystal, you'll get only about 3 percent detection efficiency. And as I'll tell you later, you need to use a high-efficiency counter.

Once you've labeled the cells, for red cells, indium elutes quite fast, about 8 percent per day. Whereas with chromium, it elutes very slowly off red cells. Usually about 2 percent in the first few days, 1 percent per day thereafter. In the case of platelets, indium elutes at about 11 percent in the first day, and chromium about 6 percent comes off usually immediately

following the label and not in the patient's circulation following infusion.

What are the physical implications of the tracer characteristics? Well, indium is physically very similar to iron, and transferrin has one of the highest affinities of any binding protein. So if there is any transferrin around, it seizes the indium off the oxine, and you get poor labels. So you have to be very careful with indium to wash out all your transferrin.

In the case of chromium, its uptake is relatively low. So you need to have a very high platelet count, and then you have to be quite careful that your concentration of chromium doesn't exceed the toxic level. We did a series of studies years ago, looking at the effects of chromium on glutathione reductase, and it's quite sensitive to levels of chromium.

In terms of plasma clearance, indium is cleared quite quickly--8 hours, T 1/2 of 8 to 10 hours. Whereas with chromium, at first it's cleared from the plasma very

quickly and excreted about 3 percent per day in the urine. Indium is lipophilic when it's bound with oxine. But it's not lipophilic without the oxine. So once the oxine is gone, indium does not relabel cells, and it's almost impossible to show any significant reuptake.

In the case of chromium is slightly different. The chromium is taken up as sodium, hexavalent sodium chromate, gets oxidized to chromic and chromic is not taken up by red cells. So you don't have very much reutilization.

In terms of cell uptake, the cells--pretty well all the cells are equivalent. Most of the indium is in the cytosol, or in the plasma. Whereas in the case of chromium, a fair amount is ATP associated, and there were some early concerns that this might be associated with energy dependent uptake. But that's not proven a problem in practice.

Indium has a 2.8 day half-life, which is ideal for imaging. The trouble is that if you don't count it

rapidly, you'll get decay. And therefore, you need to count your sample soon after the study is over, and you have to do elapsed time correction. Chromium has a 28-day half-life, which allows you to do delayed counting if you wish. But its photon emission is such that you can't image with it.

So in terms of technology, with indium, you need to correct the count times, and in terms of technology, the key outcome of chromium is that you should use a three-inch sodium iodide crystal detector because that doubles your quantum energy of detection.

So to summarize then some of the atomic physics, low photon yield mandates high energy counters. The photon scatter requires indium sum peak counting, which is fine. You can count the chromium directly, or you can wait 28 days and let all the indium decay out and recount it. Your low counts, the 10 to 15 microcuries that we like to use do require quite a long count times. You certainly should use elapsed time count correction.

sj

And then a couple of technical points. You've got to watch out in that your indium and your chromium will elute in the sample as much as they do in vivo. So once you've collected your samples, you need to rapidly separate it and count it, lest you introduce another variable into your outcome.

And finally, with gamma counters, whilst they're pretty good in linearity, you cannot presume that they're totally linear. And therefore, you must be careful to dilute your standards to approximately the same level that your samples would be.

Going to move on then and talk about the development of the double label technique. And in fact, we devoted a large amount of my research life to developing different isotope techniques. Our goal here is to develop an indium and chromium platelet technique for consistent results. And I'm going to show you three sets of studies focused on in vivo and in vitro elution first, the labeling effects on platelet function, assess

indium and chromium red selectivity, and then finally to develop corrections to support generation of equivalent outcomes.

One of the variables we wanted to freeze was to have similar methods of extracting the platelets out of the blood. So we opted to go for a tube technique with similar electrolytes simply to maintain comparability. We wanted to develop a simultaneous indium and chromium procedure, and we wanted to eliminate all the sampling variables.

So this study involves or this method involves the mixing of your injectate, the creation of mixed standards, and counting your samples in the same--counting for chromium and indium in the same samples at the same time. And I'll show you later that dramatically reduces your sample error.

The technique, which is--this is the current technique. It has drifted a little bit over time, but it's been pretty consistent. 43 mL of whole blood or

some PC, soft centrifugation to pull down your red cells, some extra acidification for the fresh blood to stop the platelets from clumping. Put these in two conical centrifuge tubes. Give them their first hard spin. This allows you to remove pretty well all of the plasma in a single spin.

Resuspend in a fairly small volume about 2 to 3 mL of ACD saline. Incubate 22 degrees centigrade for 20 minutes. And then at the end of it, you add some ACD saline and some plasma to scavenge out the poorly bound indium. Mix it up, do your second spin. Finally, resuspend in 6 mLs of autologous plasma and, most importantly, infuse within 1 to 2 hours before there's been significant change to your injectate.

Now report three lots of studies that we looked at. First, elution studies. Second, red cell elution studies or red cell correction studies. And finally, some validation studies.

The first series of studies involved 63 paired

in vivo, in vitro studies, and we looked at the elution in the injectate. And we looked at the elution in the injectate sitting on the benchtop at 22 degrees centigrade, but also in injectate diluted at 37 degrees centigrade, 100 microliters of injectate, and 10 mL of EDTA whole blood.

And we looked at the effect of increasing the temperature and diluting the injectate on elution. And finally, we developed a processing method, which I will show you in a minute, which allows for an elution correction.

So in series A studies, we got very good label uptake. 72 plus or minus 8 percent. We actually got very good chromium uptake as well. 42 plus or minus 10 percent. In these early studies, we did lose about 35 percent of the platelets during the processing, which was higher than we later experienced, but it was similar between the two techniques.

If you look at the elution then of the isotopes

in the plasma, you get somewhere between 5 and 6 percent of the indium if you just inject it into the individual, the donor, and then do your splits and you calculate the amount of indium present in the plasma. Somewhere around 5 percent is in the plasma. Notice how it stays relatively stable, so there's clearly some ongoing elution here during these first few hours.

In the case of chromium, it was about 3 percent in the plasma, and it came down quite quickly as a result of the chromium being cleared from the circulation. When you compared this with the neat injectate, there was about 3 percent in the neat injectate, 6 percent in the chromium, almost certainly just carryover. But once you diluted the injectate, 100 microliters and 10 mLs of EDTA whole blood, your elution went up quite significantly. And the in vivo and in vitro correlation was really very good between 1, 2, or 3 hours, best correlation at 3 hours.

In the case of chromium, you've also got an

increase in the dilution of injectate in whole blood, and it did correlate with the 5-minute plasma injectate, the plasma chromium, but it did not correlate very well after that, implying that the bulk of the chromium here is, in fact, poorly bound chromium and quite quickly cleared from the circulation.

This slide, in fact, summarizes that the relationship between the in vivo plasma activity, ranging from 2 to 10 percent, the in vitro elution, and it shows an excellent correlation, .82, between the 3-hour post infusion in vitro elution and the in vivo elution.

So from this, we concluded that we should correct the counts that were injected by reducing it for the elution fraction because there was such a good correlation between the two.

The second issue we looked at was the effect of red cells. So we soon noticed that when you did both chromium and indium together, you got slightly higher, consistently higher post transfusion recoveries using

your chromium product. And this was unrelated to the post transfusion recovery. It wasn't a recovery-related loss of chromium or increase.

And then we later then began to look at the--using density gradients, we began to look at the amount of red cell activity. And in fact, in our earlier techniques, we used the 10-day chromium red cell activity, expressed it as a percent of the total injectate, and we registered about 7 percent of your red cells at the end of 10 days, where of your chromium was red cell associated at the end of 10 days.

If you did the most simple correction, which is simply to deduct that from all your recoveries, you, in fact, get an almost identical decay scheme through the use of back correction. It's interesting, later we went back and did density gradient studies. We found about 11 percent of the original injectate with red cell chromium, and it came down at about 1 percent per day over the period of time. And in fact, you could calculate your

recoveries. We, in fact, recovered 101 percent of our injected chromium.

In the case of indium, this comes down much faster because indium elutes off the red cells. And 10 days, you only had about 2 percent of your indium activity was red cell associated.

So we then did another series of studies, in this case using density gradients. We looked at density gradient associated indium and chromium. We then performed paired studies. We were very careful about the counting, and we corrected our standards for both elution and red cells. So we calculated the injectate for elution and the samples for red cells, and we corrected for plasma activity.

When you did this, you'll see that its storage duration, zero days, you got very, very similar post transfusion recoveries. And as your post transfusion recoveries came down, you maintained excellent correlation in your post transfusion recovery and,

indeed, in your post transfusion survival.

This was over a very wide range of recoveries. The lowest recovery was 7 percent. The highest recovery was 81 percent. And the shortest survival was 5 hours, and the highest recovery was 230 hours. So if you used both the elution and the red cell correction, you got extremely consistent results over a very wide range of post transfusion recoveries and storage.

Finally, we did a third validation study. In this case with all the corrections, and we did a 5-day, 22 degree centigrade, stored platelets, double manual apheresis. Stored one with chromium, one with indium, performed the studies using density gradient centrifugation. And you can see that you got almost identical recoveries, extremely close survival, and the integral area under the curve was also very close.

To respond to a question that Larry raised earlier, the mean percent difference here was--the mean difference was 4 percent of all the samples separated

individually, and the standard deviation of the difference was 2.8 percent. So it turned out to be an extraordinarily reproducible technique.

I want to switch my comments and talk a little bit about recoveries and survivals and what we are measuring, to talk a little bit about some of the physiology. The current statistical approach to the evaluation of platelets is based on Edmund Murphy of Canada's original presumption, and he developed the multiple-hit concept.

And his concept was he presumed, from a statistical basis, that he should calculate the survival of every new platelet as it dropped off the mega-carrier sites. And so, he calculated the numerical expected lifespan using the multiple-hit program.

In practice, as the platelets drop off the mega-carrier site, they really have two choices. They either die of old age, which is a linear decay, or else they're removed. And they're either removed by the

endothelium or they're removed presumably because some physiologic receptor has been activated, maybe this GP1b cold receptor. And they're removed on a random single-hit exponential basis.

So the larger, the higher your platelet count and the more normal you are, the more likely the decay scheme is to be relatively straight, and the more abnormal the platelets are and the more random they are, the more they will be curvilinear in decay.

The current multiple-hit analysis, in effect, calculates survival as a numerical expected lifespan being a tangent to the first portion of the curve. So it's heavily dependent on early samples in the curve. And later, and Stein will give you more information later, we came to view this as probably not the world's best way to calculate post transfusion recovery.

As a practical matter, we also noticed that the 3-hour sample has a tendency to be below the regression curve. So if you run a regression curve back, you'll

nearly always find it gives you a higher recovery than you would get if you used your first 3-hour sample. That appears to be associated with an element of platelet damage during the labeling process or during the storage process and is associated with some recovery and return to the circulation of the platelets.

So the first key issue is what we call survival or multiple-hit survival is, in fact, a synthetic number. It's the numerical expected lifespan, and it's the tangent to the curve from the first points in the curve. However, what the patient actually benefits from is not the survival or the recovery. They benefit from the total mass of platelets underneath this curve or the integral availability.

There is an alternative way of looking at this, which is looking at the average lifespan of the platelets remaining, and Stein will tell you much more about that. But in our results, you can always see reported the post transfusion recovery extrapolated back, the numerical

expected lifespan, and then the area under the curve.

Once we had finished doing the double label studies, we went back then and performed power analysis to look at the sample size that you would need to detect differences in either the recovery or the survival. So if your detection goal was to pick up a 10 percent difference in your post transfusion recovery, if you did concurrent studies, you only needed a sample size of 5 to have an 80 percent power with an alpha of .05 of detecting a 10 percent difference in the mean.

Whereas if you did them separately, and I'll show you the reason for that, you need a somewhat larger sample size to achieve the same level of statistical power. And you can see that as you, of course, decrease your goal of detecting differences, of course, your sample size increases quite significantly.

Now I'm going to report on a series of studies where we looked at the application of this technique under different circumstances. Each of these is slightly

different so it's important for me to explain the detail of each of these studies.

The first study was an indium-only study, where we looked at indium on 35 time-separated paired indium kinetic studies. We were comparing platelet solution and CPDA-1, and we were looking to look at the effect on post transfusion recovery, the numerical expected lifespan, and the area under the curve associated with platelet storage. We wanted to see what the effect of storage was on the platelet decay kinetics.

Well, as you might expect, the post transfusion recoveries, you went from half a day to 5 days to 7 days to 14 days. Came down pretty dramatically. As did your survival and ours. Survival in this case being calculated as the numerical expected lifespan.

We also performed statistical analysis on the decay scheme. And if the decay was perfectly linear, you would have a shape factor of 0. And if the decay scheme was perfectly exponential, you would have a shape factor

of 1.0. So this reports the degree to which the decay scheme became curvilinear or as a result of random removal, as a result of either damage to the platelets or to selective uptake by the endothelium.

And in fact, you can plot the survival here. This is the gamma numerical expected lifespan against the shape factor, and you can see that as the platelets deteriorate in quality, their decay scheme becomes increasingly curvilinear. So this suggests that once you damage platelets due to storage, you cause some event that allows rapid single-hit random removal.

Second series of studies then were focused on the use of indium and chromium in a double label design, and we were looking to see whether we would be better to do the indium studies and the chromium studies on the day of infusion or whether they should be time separated. So these were paired in vivo chromium studies performed 28 days apart, and the platelets were randomly processed into buffy coat platelets or PRP platelets.

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Now after 5 days at 22 degrees C storage, chromium in vivo studies were performed. So these are time-separated chromium studies, 28 days apart. However, on each day where we did the chromium study, we also infused fresh indium 111 platelets and did both test and control outcomes, and we expressed the stored chromium values as a percent of the fresh indium values. So this will give you a direct numerical representation of what Scott Murphy has been proposing for the new standard.

Well, here's our BC-PC stored platelets up here--53 percent, plus or minus 8, PRP PC 49, plus or minus 10. Relatively close to what's been reported in the 5-day stored platelets. There was some variation, though, we noticed in the fresh indium platelets. But when you calculate your stored platelets as a percentage of your indium, you're up over 80 percent, which easily exceeds the 66 percent that Scott has proposed.

In terms of platelet survival, much the same. Got very comparable results with your numerical expected

lifespan, very comparable results with your fresh, and then when you calculated it out, you came in at between 77 and 79 percent of your post transfusion recovery.

But from our perspective, much more interesting was plotting the percentage of indium fresh platelet recovery against the percentage of stored chromium recovery. First thing we noticed was that the fresh versus stored had a very good correlation. Oddly enough, a much better correlation than the same fresh platelets when it reinfused into the same donor 28 days apart. The correlation there was only .41. This implied to us that either there was physiologic variation in platelet survival, or alternatively, there were other variables during the labeling that made simultaneous concurrent much more powerful than time-separated paired studies.

Interesting, the slope was very close to unity. So this implies that assuming that you use fresh indium as your standard, chromium gives you very nice representation all the way down from high recoveries to

low recoveries. It gives you extremely consistent correlation.

And finally, your intercept is the pooled effect of your storage. And in practice, this was a continuous relationship with buffy coat and PRP PC, and it shows that 22 centigrade 5-day storage costs you an absolute of -9.4 percent of your recovery. So the use then of fresh indium, storage chromium gives you very good correlation. It implies that you want to do the study simultaneously and not time separately, and it also implies a significant amount of biologic variation in normal donor platelet survival.

Moving to another study, where again we were looking at validating the double label technique, and we were also looking, in this case, for very small differences in platelet quality. So what we were looking for here was the effect of reducing the amount of plasma that was on a random donor platelet concentrate to determine at what level you began to cause a decrease in

your platelet quality.

So we did double manual apheresis into standard and reduced volume PC, 20 unit with crossover--a randomized crossover study, indium or chromium simultaneous infusion, and we did red cell and elution corrections. Well, you could separate this group into two subsets, the tests. First, those that are greater than 35 mL and those below 35 mL.

But here's the comparability between indium and chromium greater than 35 mL. You got 99 percent with only about a 6 percent spread of around your mean, which is extraordinarily precise. And if you look at the integral, your gain got 99 percent, and again, a very, very narrow spread about your mean, which suggested to us that we had, indeed, developed a technique that gave you remarkably reproducible results.

But of even more value is if you select out this subgroup of 30 to 34 mL PC, you could detect a very small decrease in post transfusion recovery, but you could show

that significant increase at a very, very high P value level. So when you applied then the double label technique, we were able to validate the concurrence under normal circumstances and then detect extremely small differences when you were looking for just minor deviations in platelet quality.

Now I'm going to move to another series of studies we did using the same technique, in this case, looking at the functionality of stored platelets. And in this case, we collected platelets, stored them for 5 days at 22 degrees centigrade, and then on the fifth day took a fresh sample from the donor, labeled it with indium, mixed it up with the stored chromium platelets, transfused it into the recipient.

However, on this occasion, we took quite large blood samples--15 mL, 20 mL samples--made platelet rich plasma, added an aggregating agent, and then filtered them through a column with cotton woolen to remove the aggregates.

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We then collected the eluate in series, and we counted the cells that made it through and calculated the percentage that aggregated and didn't aggregate. We calculated the number of fresh indium that hung up in the filter and came through, and then the same for the chromium. We used three aggregating agents, epinephrine, ADP, and ristocetin. And our goal here was to show what the quality of the stored platelets was and how quickly it recovered in the circulation following transfusion.

Practice what we found, and you're looking here at this case, at the ADP aggregation expressed the chromium, expressed as a percentage of the indium. On day 5, you have about 2 percent aggregation to ADP and about 10 percent aggregation to epinephrine.

If you incubate those platelets in plasma and infuse them, it goes up to about 30 percent. And then when you transfuse them, you can see here we did 1-hour sample and 3-hour sample, your chromium stored platelets took about 3 hours to get up to about 70 to 80 percent

post transfusion recovery of the aggregability. By 24 hours, they're up to 90 percent, and by 72 hours, they were basically--the stored chromium were equivalent to the original indium platelets.

Of some interest, however, is that the stored platelets maintained that aggregability comparable to the indium platelets for 5 days. So it showed that the platelets were storage damaged. They recovered in the circulation, and you could hold them in the circulation for 5 days, and they maintained their performance, their in vivo aggregability.

We also looked at the indium, the control indium platelets. We found that the indium aggregation agreed very nicely with the numerical aggregation and that over a 5-day period, you could demonstrate almost no variability in indium platelet aggregability. This implies that platelets' capacity to aggregate doesn't change in circulation over their lifespan. And from our perspective, it also shows that you didn't damage the

platelets during the course of your indium or your chromium labeling because they were able to recover their aggregability in circulation and demonstrate it consistently for a 5-day period.

So I hope then that I've been able to walk you through the development of the double label method. I don't find the labeling issues relative to selective tracer uptake. Talk about the technique issues relative to differential radionuclide counting, the importance of sampling and dilutions and the atomic physics of the crystal that you use to count.

Talk about the procedural issues relative to the result acquisition and interpretation, and then I've looked at some of the physiologic observations that this double label technique allowed us to make. We've looked at the storage-associated loss of in vivo efficacy and the capacity to recover.

I didn't show you the storage damage sites. It's the liver, but I didn't have time to show you that.

And I certainly have given you some hint of the level of chronological variation in platelet turnover. And I hope that we've proposed a study model to allow accurate kinetic analysis, provided some insight into functional platelet recovery, and in some other studies, which I actually haven't reported here, we've suggested a driver to platelet senescence.

Thank you.

[Applause.]

DR. ZUCK: Thank you, Andy.

The last presentation for the morning session is by Dr. Stein Holme, vice president of scientific and laboratory services of Pall Corporation. And he holds, interesting enough, five patents on blood additive solutions.

Dr. Holme? The title of his paper is "Indium/Data Interpretation."

DR. HOLME: First, I'd like to thank the FDA for the invitation and also for the opportunity to meet all

friends and colleagues here.

My talk is about some of the issues that have already been raised by the previous speaker regarding the accuracy and the interpretation of platelet viability measurements by radiolabeling studies. I'd also like to mention that the studies that I'm going to present in my talk were done at the American Red Cross Research Department in Norfolk.

This slide shows several issues that are related to the accuracy and interpretation of viability measurement. I'm not going to go over all this issue, but focus on three in particular. First, mainly what was addressed by Scott Murphy, the donor variability and percent recovery. As shown by Scott Murphy's slide, there are enormous variability in percent recovery. And what I would like to show that a major source for this variability is the inaccurate estimation of blood volume using the formula for body surface area.

I will also go into and talk about the

importance of the labeling method and in particular the importance of label a representative platelet population from the product that you're going to evaluate.

And finally, I'm going to talk about the data processing and interpretation, which data point you may want to include in our analysis, what kind of a mathematical model that we should use, how they fit to the raw data, importance of having meaningful parameters, and also finally talk about some mathematical models that will compare the test product to fresh platelets.

First, the variability in percent recovery. This figure shows recovery of a typical 5-day stored platelet product, and you can see it ranges from about 30 percent almost to 70 percent. And the question is then does the platelet in the plate product that has 30 percent recovery differ from that of a 70 percent--product that has 70 percent recovery in terms of viable platelet?

And apparently, that is not the case. Because

what we're seeing here is that the recovery of the stored platelets correlate strongly with the recovery you can obtain by labeling fresh platelet from the same donor. And by doing a regression analysis on the variability in percent recovery with 5-day storage, you can find that 79 percent of the variability is related to the recovery of fresh platelet from the donor and only should be 21 percent is related to product viability during 5 days of storage.

And this has some implication. Since only 20 percent is related to product viability, it's not surprising that we see poor correlation between in vitro parameters and in vivo recovery because recovery by itself is a poor predictor of platelet viability.

What's the cause of our ability or source of our ability in recovery? This is shown by the equation for percent recovery. We see that blood volume plays a major factor. And if blood volume is inaccurately estimated, of course, recovery will also be inaccurately estimated.

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If blood volume is overestimated 50 percent, so will the recovery be overestimated 50 percent. Commonly, the estimation of blood volume in platelet labeling studies are done by formulas for body surface area, such as an Nadler formula.

This figure here shows the Nadler-estimated blood volume based on surface body area versus the actual measured blood volume using radiolabeling technique, in this case, techniques found to be a very accurate method to be used to calculate the red cell mass as well as blood volume. And you can see here that this is a line of identity that Nadler-estimated blood volume is overestimating the actual blood volume and also that there are relatively poor correspondence between the estimated and the measured blood volume. And this is the result from a large number of studies that have been conducted in American Red Cross in Norfolk for several years.

Again, this shows the ratio of estimated versus

measured blood volume. You can see it can range from about 75 percent to about 150 percent. And that means that in many, many cases here, we have a large overestimation of blood volume by the Nadler's method.

So in summary regarding the donor variability in percent recovery, in showing that the major source in the variability in percent recovery of 5-day stored product is related to inaccurate estimation of the donor's blood volume and relatively to the viability of the platelet product after storage.

So that means that the determined percent recovery is not by itself an accurate measurement of platelet viability of a 5-day stored product. So saying as many--all the people have been saying before me that paired studies (test versus control product from the same donor) is thus preferred for determination of a potential change in the platelet viability of a test product.

I will now go into the second issue, which is labeling a representative platelet population of the test

product. With radiolabeling studies, it's based on the assumption that platelets in the product are uniformly labeled in terms of viability. That means after infusion a certain decrease in the radioactivity represents a similar decrease in the number of injected platelets from circulation.

And this slide is a few examples that I'm not going to go over this, but focused on some studies we did in Norfolk about 10 years ago, where we looked at platelet subpopulation from freshly collected blood. And this is an important issue since it has been proposed to use platelet from freshly collected, freshly prepared blood as the standard to which the test product should be compared to.

The concern here when using freshly prepared platelet is that the percentage of platelets that are recovered for labeling during the preparations potentially could affect the outcome, the viability of the platelet. So if, let's say, you recovered 40 percent

platelets from the whole blood to be used for labeling versus recovering 80 percent of the platelets for labeling, will they have the same outcome in terms of viability, percent recovery, and survival?

In other words, will there be any difference between subpopulation for freshly collected blood that has been separated by centrifugation in terms of viability? To look into this, we had subpopulation of PRP. This was using a standard centrifugation technique with random donor whole blood units, and what we did was to take the remaining sedimented platelet subpopulation and separated those by additional processing, by adding plasma and then repeated centrifugation.

And in vivo studies were then conducted to determine the viability of these two platelet subpopulations using simultaneous labeling and infusion with 111 indium and 51 chromium.

This slide shows what we recovered in terms of platelet yield with from whole blood. For the

supernatant PRP platelet, we recovered about 60 to 70 percent. For the sedimented larger, heavier platelet, they recovered about 30, 20 percent. In some cases, as much as 40 percent. So there was quite a variation.

As expected, in terms of the mean platelet size, the sedimented platelets were much larger. Averaged about 8 cubic micron versus the supernatant platelet, the smaller platelet had a size about a little bit more than 6.5 cubic micron.

In terms of percent recovery and viability, did they differ? I expected to see a difference in terms of recovery and survival. They didn't see anything, and that was quite surprising. This slide shows the recovery of the supernatant PRP platelet and how closely that correlates with the sedimented heavier, larger platelets from the same donor.

And there were absolutely no significant difference. As you can see here, there is a large variability in recovery. The recovery is about 73, 75

percent for these fresh platelets.

And again, the survival did not show any significant difference either. For the mean sedimented platelet, it was 193 hours with the numerical expected lifespan, and for the supernatant platelet, it was similar, 192.

So the conclusion, using then the freshly collected whole blood, we did not see any difference in terms of platelet subpopulation, which was separated by size or actually separated by centrifugation in terms of any significant differences in recovery and survival.

And that means that when we're preparing using the new standard, using fresh platelets as standard, you should maybe there's not so much concern about getting a representative population because it appears that if you get 20 percent or 30 percent of the platelets recovered by preparing platelet from whole blood, you get very much the same results as you would get if you had 80 percent of the platelets recovered for doing the labeling. And

again, we didn't see any statistical significant difference between results obtained using 111 indium versus 51 chromium.

The final topic I'm going to talk about is about data processing and interpretation. Of course, the objective of mathematical modeling of the raw data is to reduce the data to a few accurate and meaningful parameters that can be used to evaluate platelet viability of a product. And the method that is used in this regard is the so-called least sum of squares, where the difference between the observed values and the model predictions squared is minimized by using iterative methods.

Which points should be included in the data analysis? That was also an issue somewhere raised by Jim AuBuchon earlier today, and I will give you some illustrative examples of the effect of excluding or including certain data points.

This is a printout of the COST program that has

been talked about earlier today. In this case, the data were analyzed by weighted mean function. We used a 5-day stored platelet product that was radiolabeled, and data points were taken at 1 hour, 2 hour, 3 hour, and then daily for up to 8 days.

The S.T. here is the numerical expected lifespan with the standard deviation. Percent standard deviation here is--actually, it's not a standard deviation at all, the numerical expected lifespan, but is the residual sum of square in percentage of the recovery. So it's a sign of how well the data fit to the--the raw data fit to the survival curve. Percent recovery closely intersects with the Y-axis.

By including all the data points--1 hour, 2 hour, 3 hour--we see that there is a poor fit of the data to the weighted mean function. And that is apparently because there is a bias. We have so many points early on here, they're not evenly spaced, and that's why there is a bias towards the initial points.

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But only using the 3-hour points and then daily for the remaining time, there is an improved fit as shown by the percent standard deviation reduced to about 7 percent, compared to the 18 percent. And there also actually is a somewhat higher recovery.

And finally, by using the 24 hours measurement, we see an excellent fit. Percent standard deviation in this case has decreased substantially, but we can also see that the recovery has increased, and the survival have decreased. So it's very critical when we do this type of analysis to be clear that the data points that are included or excluded could have a major impact on the parameters that are measured.

Models that have mathematical or function that we use in platelet survivals are listed here. We have the linear, exponential. Andrew Heaton mentioned the multiple-hit kind of function. We have the weighted mean, Meuleman, and Dornhorst. And as mentioned earlier by Scott Murphy, the requirement for these functions that

it must be able to fit a wide variety of typical survival curves for platelets stored and processed under various conditions. And the goodness of the fit, of course, can be determined by the residual sum of squares.

And by analyzing--when I was with American Red Cross in Norfolk, we looked at these various functions. And overall, as far as I remember, the Meuleman was the function that showed the best fit in terms of having the least lowest residual sum of squares. Of course, linear and exponential cannot be used. They are not longer used because they have a very poor fit.

What are the parameters that are used to measure or determine platelet survival? I've listed here three parameters that are typically used. The numerical expected lifespan, which, of course, is the initial tangent of the survival curve where it hit the X-axis so we get the time measurement.

We have the mean residual lifespan, which is the area below the survival curve divided by percent

recovery. And we have the T 1/2 is the time after infusion at which 50 percent initial recovery remains.

And it's important that it's distinguished that you have different parameters for survivals. I've seen in some papers that there is used interchangeable the T 1/2 with, for instance, with the numerical expected lifespan.

What does it mean, the numerical expected lifespan? Actually, it's the birth cohort lifespan of platelets newly released from the bone marrow. And it's been used in the estimation of platelet survivals in thrombocytopenic patients to determine the platelet turnover rate and events in the circulation, such as senescence versus random destruction.) And the question is, is it meaningful in the estimation of survival in the platelet product?

Here is shown an example of what the numerical expected lifespan is about. We have here 11 subpopulation of platelets, and what it is, is the entire

lifespan of the platelet from it was born until it has died in circulation. So the numerical expected lifespan is the average of these 11 subpopulations.

And again, as mentioned, it is determined by taking the tangent to the survival curve. And for fresh platelets, in this example, the numerical expected lifespan is around 8.5 days.

Residual lifespan. The mean residual lifespan in circulation--the definition is the mean residual lifespan in circulation of the labeled and infused platelet population--we're only talking about the platelet product itself, which is what we call that sample population or cross-sectional population.)

And potentially, this is a more meaningful parameter. It shows here what is meant by the cross-sectional population. Let's see at the time key here, this is when we take the sample or the platelet product and do the radiolabeling. And the residual lifespan is the remaining--average of the remaining

lifespan in circulation after infusion.

Also notice here that it's the cross-sectional, the sample population is different from the birth cohort population. There is a bias with the cross-sectional population that you can lose platelets that have short survivals. So this means that when we talk about numerical expected lifespan, this is more a hypothetical population that not really exist that we're trying to estimate the in vivo survival for.

And here, this is how the residual mean lifespan would be calculated. It's the area below the survival curve and divided by the recovery. So in this case, it's 5.5 days for fresh platelets. That means that the infused platelets live on the average 5.5 days after infusion. And potentially, this is a more meaningful parameter to be used when we're dealing with the validation of platelet product.

How does these parameters compare to each other? I've shown you the results of recent studies where 5-day

stored platelet product was compared to 7 days storage, and this was with random donor platelets using CLX bags. And here on the X-axis, we have the numerical expected lifespan, and here we have the residual lifespan. And overall, it was--in this case, it was a good correspondence between those two parameters.

Here we have numerical lifespan versus T 1/2 days. So that's a time where the 50 percent of radioactivity remains. And again, this was calculated by using the weighted mean function. It's not graphed, but actually calculated by mathematical model. And again, you see there's a good correspondence between the numerical expected lifespan and the T 1/2.

So in this case, is there anything to gain by using different survival parameters? If you, looking at the summary, the summary table of the study, we had 24 pairs for 5 days storage. Again, was a double labeled study, and these are the parameters that had been calculated using the weighted mean function.

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Numerical expected lifespan for 5 days was 5.3 versus for 7 days, 4.4. And it was a statistically significance difference. Residual lifespan, 3.6 versus 3.2, statistical significance quite a difference. Again, T 1/2 was 3.3 versus 2.9, statistically significant. So it didn't really matter in this case which survival parameter that was used to determine the survival.

Again, a little more comments about mathematical models. We could potentially obtain more information about the quality or the viability of a test product by comparing test platelet to that of fresh control platelets by using certain mathematical models. And this can then give us more indication about the nature of the lesion or damage that potentially can occur with the test product.

We, for instance, can look at the percent recovery due to aging, straight aging versus due to random destruction. We can also look at using the residual lifespan and see what is caused by aging versus

again random damage to the platelet.

And I've given some examples in the next few slides. With platelets stored for 5 days using--with standard product, the change or the difference in that curve between fresh platelet and stored platelets indicates that the loss in recovery as well as decrease in survival is basically aging related. In terms of mean residual lifespan, it decreases from 5.5 days to about 3.5 days. And that can be explained basically by an aging process.

On the other hand, if you're dealing with cold stored platelets, which is typical that the initial recovery is very much the same as for fresh platelets, but it's a very short and exponential curve. This type of behavior can be explained that there is a 50 percent reduction in residual lifespan for each platelet. So for each cold stored that we're dealing with, each of them will have a 50 percent reduction lifespan, and this is typical for this type of conditions.

And finally, this is typical survival curve for frozen, cryopreserved platelets. In this case, because of the freezing process, about 44 percent of the platelets were lost or damaged totally. So they didn't survive at all. However, those platelets that survived had a normal lifespan similar to what you have with fresh platelets, and this is a typical survival curve for this kind of conditions.

So in summary, I have also listed some proposed steps here to maybe ensure accuracy of viability measurement by using radiolabeling studies. One, you know, that we talked about is the donor variability in terms of recovery, and that it was caused by an inaccurate overestimated blood volume based on current formulas of body surface area. It actually doesn't really matter what kind of formula is used, if you use Hurley or Dubois, you very much get an overestimation as well as inaccurate volume for mean blood volume.

In terms of improving this, you could use a

better formula for calculation for blood volume. And again, probably what is preferred is doing paired studies.

In terms of labeling, it's critical, although we saw that with fresh platelets there didn't seem to be an issue to ensure uniform labeling of a representative population of platelet product to be evaluated. Important in this respect I feel to make sure that the platelet loss during labeling in test versus control is not very much different, that the platelet size distribution pre- and post labeling is not going to change significantly so that during the labeling process you have lost a certain population of platelet. And also important to determine uptake and elution potentially in various subpopulations.

And finally, regarding data points to be included in the mathematical modeling, I think the more the better in order to get--in order to be more precise about the measurement. And it needs to be evenly spaced.

Clustered may cause biased result, as was shown in the example I gave. And I think it's also important to eliminate contribution of labeled red cells, in particular when you're dealing with chromium.

Regarding the mathematical models and parameters that should be used, it should be based on goodness of fit by the residual sum of squares. It should be quite robust, and I think also that it should be informative about the nature of potential lesion or potential also improvement of a new product.

Thank you.

[Applause.]

DR. ZUCK: Thank you, Dr. Holme.

The last few papers are open for discussion, comment. Red lights on the button.

DR. MOROFF: I have a question for Stein Holme. Can you compare the weighted mean versus the multiple-hit? You are using the weighted mean for many of your calculations.

DR. HOLME: Yes, I was using in this case the weighted mean function. Yes, that's correct.

DR. MOROFF: Could you compare it to the multiple-hit? Because we've been hearing also a lot this morning about the multiple-hit procedure. Do you get similar numbers?

DR. HOLME: Could you repeat the question again, Gary?

DR. MOROFF: Stein, could you compare--you've used the weighted mean--

DR. HOLME: Yes. It doesn't really make much difference which mathematical model you are using, in my view, so long as you are not using linear and exponential function. There's very little difference in terms of what we get in percent recovery and survival by using different mathematical models, as long as it's shown to have a very good fit to the raw data.

But all of us need to look by looking at the residual sum of squares how well the data fit to the

different mathematical models you're going to use. I think this is important point to make.

MR. : I had a question about the red cell correction, the day 10 correction, and just a point of clarification. You mentioned that very specifically in the case of chromium labeling and using that subtraction, is that used also in the case of indium labeling?

DR. HEATON: Yes. It's much less necessary because your 10-day red cell indium activity is around 1 to 2 percent. So if you're being--if you want a really precise measurement, you would do it. In practice, it makes a negligible difference.

DR. ZUCK: I'm sorry?

MR. : Question for Dr. Heaton. Andy, one of the potential negatives about the simultaneous labeling of fresh platelets with indium and stored platelets with chromium at the end of the storage period is if your fresh platelets may represent a younger

population, especially if the stored platelets are a large apheresis collection. Do you see that as a potential problem?

DR. HEATON: The studies I reported ruled on random donor platelet concentrates, so they were not apheresis concentrates. And Sherrill reported and I believe we saw at the end of my period some slight order effect if you got into pheresis platelets. So if you take a whole pheresis unit, you probably do get a bump in your thrombopoietin and a slight change.

The only way you can deal with that, obviously, is to either space it apart or--it's not an isotope effect, so you can do a crossover between chromium and indium, and you'll get the same outcome. But there is a timing effect. So there's no way you can avoid that with pheresis because if you, you know, if you collect pheresis and store for 10 days, you've got time for your thrombopoietin to cut in, and you're going to begin to see an order effect.

So if you're going to go for pheresis platelets, you probably would need to do your indium ones immediately or your fresh platelets immediately. The difficulty is that you pay a price for that. Because if you time separate them, now you've got separate standards. You've got double set of sampling. So you've got other errors that will creep in as a result of doing time-separated isotope studies.

So from an isotope perspective, you're much better by doing simultaneous concurrent. But to eliminate the thrombopoietin effect, you'd have to think about time-separated studies.

DR. SLICHTER: We did a study a long time ago looking at collection of platelets on different machines in the same donor. And those studies were separated by 2 weeks, which we now understand is probably the optimal time if you're going to give a thrombopoietin stimulation to get a young population of platelets.

And when we looked at the data, you know, there

were effects of machines. So it got a little hard to sort out. But basically, in absolute numbers, the recovery was about 10 percent higher for 5-day stored platelets if it was the second collection, compared to the first. So the second collection, for example, would have had a recovery of, say, 50 percent, and the first one would have had 40 percent.

So it's not a trivial issue. It did not change the survival of the platelets but did change the recovery by, you know, a nontrivial--I mean not an inordinate, but a nontrivial amount.

DR. HEATON: The only other way to correct for this is that you could ignore the fresh platelets. And so, you collect your pheresis platelets and a random donor platelet on day 1 and then infuse both your chromium and your indium platelets on day 5, 10, or whatever day you do. You're not getting it paired then against the donor's fresh platelets. You're getting it paired against a reference method if you do that.

DR. ZUCK: Excuse me. Any other questions, comments? It's--yes?

MR. DUMONT: One comment. Just a note on the residual sum of squares that Stein mentioned. While a lot of us think about doing regression with least squares analysis, actually a lot of the new methods for doing that are maximum likelihood regressions, which are slightly different. And your biostatistician could explain all those details to you.

So maximum likelihoods and also a reasonable way to approach that.

DR. HEATON: One point I'd make about statistical analysis. It's very easy to spend a lot of time dancing on the head of a pin with statistical analysis. The truth is you get very comparable results whichever method of statistical analysis you use.

What's of much greater importance is the physiologic basis of what you're interested in, and the physiologic basis of what you're interested in is what is

the average survival of the transfused platelet in the recipient, the mean residual survival? That's what the patient wants. They want a functioning platelet, and they want it to survive between the time you transfuse them now and when you next see them.

So it really doesn't make a whole lot of difference which statistical method you use. You need to focus on the key physiology. And that's the residual mean survival.

DR. ZUCK: Okay. I'm not seeing any red lights or anybody with their hands up. There is, as I understand it, a cafeteria a floor below us. There is a snack bar directly behind us. And there's a building, the next building over has apparently a somewhat more complete cafeteria.

We are finishing a few minutes early. I would greatly appreciate it, and I think all of us would, if we could get a good start at 12:45 exactly. Thank you so much for the speakers this morning and all the

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participants.

[Recess.]

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A F T E R N O O N P R O C E E D I N G S

[12:54 p.m.]

DR. ZUCK: The continuation is the second presentation of our morning presenter, Jim AuBuchon. And his topic is entitled "Data Presentation--dash."

DR. AUBUCHON: Thank you, Tom.

I would like to share some data with the group about some issues that we've recently attempted to address related to platelet radiolabeling studies such as what we've been discussing. Some of these data have been published, and I'm presenting them just for the sake of completeness for the transcript, and then we'll get into some other areas that have not previously seen the light of day.

I would like to first talk about our experience with Murphy's law, validating its applicability at 5 days and at 7 days, a brief look at comparing different labeling protocols, and comparison of different calculation techniques.

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When Scott first proposed comparing stored versus fresh platelets, it struck a very resonant chord with me. I thought that this made great sense, and we dashed off to the laboratory to see if it would actually work, to see what we would get. When we first began this work, we collected platelets on an apheresis instrument and took an aliquot, labeled it and reinfused it autologously within 24 hours, and then stored those platelets out to 5 days, used the other radiolabeled indium or chromium and reinfused.

So this is taking the approach of taking an aliquot early on in the storage period from the unit. The technique that we used of radiolabeling was the one that Andy Heaton and Stein Holme taught us in. I am I guess proud to claim that Andy Heaton apparently is now my father, according to Scott Murphy's genealogy. Although with red cells, Rick Davey also says that he's my father. So now I have two fathers. I'm not exactly sure how you do that.

[Laughter.]

DR. AUBUCHON: The technique is one where 20 mLs of the component taken out, ACD is added, and the nonplatelet cellular portion is spun out before taking the platelet supernatant and spinning it to a pellet, bringing it up in acidified ACD.

And that acidified platelet concentrate then has added to it either sodium chromate or indium oxine for a 20-minute room temperature incubation before ACD saline is added in order to bind any unbound label, particularly indium. It's spun again, and autologous platelet poor plasma is added before 10 to 15 microcuries are injected into the subject.

Using this labeling technique, fresh apheresis platelets had a recovery of 75 percent and survival of 7.5 days. Using the multiple-hit model, with an N of 11 subjects, the day 5 absolute recovery was 58 percent. And using what I now understand is an oversimplified ratio method--thank you, Larry--the day 5 result

expressed as a percent of the fresh result was 78 percent for recovery or 92 percent for survival.

So that was a simplified form, and we felt good, and it was accepted for publication. However, we now understand that that wasn't the best way to do it. And I appreciate Larry sharing with me last week a previous issue, draft of his slides. And so, we understood that rather than looking at the means of these two sets of data points, we should look at the difference between each one of the points.

And after a quick phone call with Larry, he explained how we were supposed to do this, and I actually tried it. And amazingly, it worked. So we looked at the difference between each one of these points, generated the upper confidence--upper limit of the confidence interval, and compared that to what was an acceptable difference.

And the upper limit that we found was 21 percent. 74.7 percent, remember, was the observed mean

recovery of fresh platelets. Using a .667 multiplier, the target then came to be 50.0 percent in this experiment. So the maximum acceptable difference is this minus this, or 24.7 percent.

And of course, that is less than the upper bound of the confidence interval. That looked good. We did the same thing for survival, and I haven't shown that here. But the survival was similarly acceptable. And applying the "and" criterion, we were then able to accept that Murphy's law worked at 5 days with these apheresis platelets.

We then moved to 7-day platelets, initially using exactly the same protocol. That is, collecting by apheresis, reinfusing an aliquot within 24 hours, holding out now to 7 days, chucking only pH. We didn't do many in vitro studies in infusing with the other radiolabel.

We only did this on four subjects because we were hearing increasing information from others, thoughts from others that a separately collected fresh aliquot

would be a better way to go. So this only had an N of 4 before we switched.

And a larger study, an N of 11, where we'd held the apheresis unit out to 7 days and on day 7 collected a fresh portion of whole blood and labeled that as our standard. The fresh blood was collected, 43 mLs was collected and 7 mLs of ACD-A into a syringe. This was allowed to rest for 1 or 2 hours before being spun to produce PRP.

The PRP then had ACD-A added to it. It was spun again to remove the nonplatelet cellular portions generating a supernatant with platelets. This was hard spun then to generate a platelet pellet and platelet poor plasma. The platelet pellet was brought up in ACD-A saline, and both the pellet and the platelet poor plasma were used in the labeling procedure as shown before.

This time, the fresh platelets handled in this manual means had a recovery of 61 percent. The day 7 recovery was 52.9 percent. So applying just the simple

approach, 89 percent comparison between day 7 and fresh, and using the more rigorous approach, as Larry showed this morning, both recovery and the survival did pass.

However, we were concerned because the previous study that had used apheresis platelets for fresh and this study, which had used manual platelets for fresh, yielded different, very different recoveries. And had we used a manual technique that was somehow injurious to platelets?

You can see that the day 7 recovery is a little bit less than day 5 recovery, and that would be expected with additional days of storage. But there's a marked difference between the two fresh studies. Why was that, and had we not chosen the right manual preparation technique?

Several of our subjects had been involved in multiple arms of the study, and we were able to compare their fresh platelet recoveries handled by different means. So you see here in the blue squares, the fresh

apheresis from the first study, where we're looking at day 5 platelets, and the purple squares looking at day 7. But this is the fresh result.

We can compare that some subjects also had their fresh platelets handled via a manual method with a 4-hour hold. We also later went to a 2-hour hold. You can see the various comparisons here. There was no difference between the 2-hour hold and the 4-hour hold, and we have since gone to just a 2-hour hold. But it did appear that there were some differences between the apheresis platelets and the manual platelets.

If you look at the actual means, indeed, there were--and over time, it seemed like we were going down in these means. There were certainly some individuals where the apheresis was markedly better. But there were others where the apheresis was really the same as the fresh.

We ultimately ascribed the differences to just the tyranny of small numbers. There were very few subjects involved in these studies, and we just happened

to pick a few people that gave us better results with apheresis platelets than manual platelets.

So our conclusions from these initial validation studies was that a comparative approach, as Scott Murphy had proposed, was indeed feasible, that meaningful comparisons between fresh and stored platelets were possible, that clearly there are many technical details which are very important, and that sufficient sample size is also very important. And using the calculation technique that Larry has now proposed, I think we can get around some of the sample size problems.

None of these studies were sized to be appropriate for submission to the FDA for licensure or anything such as that. These were just preliminary studies to see if the concept was worth investigating further.

As was mentioned earlier, the most commonly used approach for determining recovery and survival is using the COST program from a researcher in South Africa. And

we were concerned--I had a discussion of this topic at the BEST collaborative back in February--that possibly not everyone's program was turning out the same number. Although we all had a bona fide copy we felt, all of these copies have had to be modified in some way to run on different systems.

The program is a bit fluky, and you can't just load it on and expect it to work like another program that Bill Gates could provide you. This does require a little bit of manipulation in order to make it work in different systems. So we weren't absolutely certain that we were all getting the same numbers with the same data.

Through the BEST collaborative, I was able to enlist seven laboratories in analyzing data sets that we had culled randomly, more or less from studies we had performed in the past. We provided the data and just asked each laboratory to insert the data into their own COST program and tell us what they got. This was not any attempt to see who did radio elution this way or who

counted for this or that. The instructions were just put these numbers in your program and tell us what it spit back out.

The folks at Navigant Technologies were able to run this both on their COST program and in an SAS program that they had modified to do similar technique. So their data actually appear here twice, once in COST and once in the SAS program.

Shown here are the survival calculations in days using an exponential model. And I didn't calculate means of standard deviation because I think a quick review of the slide will show that everyone got the same answer, which was very gratifying. Weighted mean model for survivals, again all the same answers. Multiple-hit models, a trivial difference in lab E with data set 2. And recovery calculations only had the multiple-hit modeled here, but again, all the same answers with just a trivial difference in laboratory B on one of the data sets.

So it does, indeed, appear that our COST program, at least amongst these laboratories, are all turning out exactly the same information, and that's good news.

We then moved to address several other issues. This was spurred on by some concern that indium and chromium may not be yielding the same recovery and survival, particularly the same survival calculations, when platelets have been stored for longer periods of time.

The work that Andrew Heaton and Stein Holme did a decade ago worked primarily with 5-day platelets, although they did do some work a little bit beyond that. But it was mostly with 5-day platelets. And now we're looking at 7-day platelets or maybe even longer platelet storage that I think Dr. Slichter will be telling you about momentarily.

So we were concerned that the use of radiolabels, particularly chromium versus indium, beyond

7 days had not been adequately documented. So we constructed a study whereby 12 normal subjects donated a single apheresis unit. This unit was held until day 8, and then two aliquots were pulled simultaneously and labeled simultaneously with indium and chromium injected simultaneously to see the difference.

With these data, also we were able to begin looking at the effect of different calculation approaches. What if you included this correction, but not another correction? What if you only used early data points versus later data points?

This is still a work in progress. The last bit of data that I'll be showing you today I received Friday at 4:00. We're still working on this, but hopefully, we'll have something that you will find useful.

To begin with here, here are the recovery comparisons in these subjects, the apheresis platelets on day 8 comparing indium and chromium. And you can see that in general, the two results are entirely analogous,

with the exception of Subject 651, who seemed to have much higher indium recovery than chromium recovery. But other than that one subject, the two results were very, very similar.

But let's look at some of the different ways of manipulating the data. There are three different kinds of corrections that you heard about this morning that can be used in these calculations. That is correcting for loss of the radiolabel from the platelets prior to injection of the platelets. That's usually called the elution correction.

There is the correction for the amount of radioactivity in the plasma of each sample. That radioactivity could be left over from the injectate, where the radioactivity was in the plasma to begin with or lost from platelets either from activation or demise of those platelets.

And then the last correction has to do with correcting out for any red cell radiolabeling that may

have occurred and that may have raised the baseline essentially throughout the entire time period of samples.

Looking at using all of these corrections versus none of the corrections, as shown here, is the percent difference in recovery, the absolute percent difference in recovery for the indium label in this study. And you see that for some subjects, the change was quite small from a low, really, of 1 percent up to 20 percent.

Looking at chromium corrections, they are generally similar, but not always. And you can see there was quite a substantial reduction in the recovery for the second subject, and that related to red cell labeling apparently. It was a fairly large deduction from the day 10 sample.

So there are differences that occur if you use these corrections or if you don't use these corrections. You can see it's labeled out here, indeed, for the red cell labeling with chromium. It has very little effect on the survival curve, however, and you see the

difference for correcting for red cell labeling with indium or not correcting. Again, no affect on the survival.

We also looked at how many data points should be included in the COST program data entry. It's been the technique that we have learned from the folks in Norfolk to take samples out to day 10. The day 10 sample is actually used to correct for the red cell labeling, whereas the samples out to day 9 actually get used for determination of the survival curve.

Other laboratories only go out to 4 days or 5 days. Does that make any difference? I haven't shown all the data here, but in the left-hand part of the curve, looking at just recovery for the first half dozen individuals involved in the study, what you see here is the absolute difference in percentage points for recovery using all points versus using only those points out to 100 hours.

And the difference at most was a -.7 percentage

points. So it was really quite minor, did not make much of a difference. Stein pointed out earlier this morning in his talk, it's probably more important to make sure the points are evenly spaced than exactly how many of them you have.

Survival, the trend seems to go a little bit the other way. The absolute difference here is shown in days. But even with that, the largest difference was .6 days, not a huge difference. So although it would appear that the number of points on the curve does have an effect, the effect is relatively minor.

We've tried to look at this for a number of different parameters. I'll just give you my general feeling here as to how much of a difference it can make. For example, whether you correct for elution of the radiolabel prior to injection, that may be a difference of up to 30 percent, but it's usually not anywhere near that. For day 7 platelet, probably less than 10 percent.

The amount of radiolabel in any one sample's

plasma, again, could be a difference of up to 30 percent. It tends to be greater later on in the survival curve, but usually less than that.

Presence of radiolabeled red cells, more of a problem with chromium than with indium radiolabeling. It can be up to on the order of 10 percent. Usually, it's just a few percent or, as you saw from Andrew Heaton's data, maybe 5 to 6 percent for chromium.

The length of the sampling period, really a very small difference. And which mathematical model you use for a determination of recovery and survival could, in some rare occasions, be quite large. But as Stein nicely noted, it really is usually a very small difference.

I think it is important, however, that if we move toward a defined method of evaluating new platelet preparations with a radiolabeled technique, it will be important that we standardize these issues. None of them, in and of themselves, make a huge difference, but we should all decide how we're going to do this and then

do it in a standardized fashion.

So to actually return to the study where some of these data came from, what happens if you look at chromium versus indium on day 8? Correcting for elution of the radiolabel, correcting for plasma radioactivity in each sample, correcting for red cell labeling on day 10, taking samples out to 9 days to include in the survival curve, using a multiple-hit model on the COST program, and extrapolating T-zero recovery from the curve?

The answer is chromium and indium are exactly identical. You can see here for both recovery and survival, you get equivalent numbers. There is no statistical difference between them. And this study, although small, did have a power to detect a 6 percent difference in recovery and .8-day difference in survival.

So from our recent experience in radiolabeling studies, we feel that comparing stored platelets to a fresh sample is a very reasonable means of assessing platelet recovery and survival, and the parameters as

suggested by Scott Murphy--that is, 67 percent recovery and 50 percent survival--can be met by currently approved techniques of platelet collection and storage and also would appear to be applicable for 7-day storage, which the agency appears to be feeling we already have reasonably good data for at least a couple different manufacturers on 7-day storage. So it seems to fit within both of those parameters.

The importance of having a sufficient sample size to preclude small samples from skewing the results I think is evident. It's always evident. Manual collection of fresh platelets is feasible and desirable, and Ed Snyder will be talking a little bit more about this later on.

That radiolabeling with chromium and indium provides similar results, at least to 8 days of storage, and I'd love to have a platelet preparation that we could test that out even further. And that mathematical manipulations of the results should be standardized.

I would only offer one other comment in closing. And I'm not here trying to hold out my hand, but I'm holding out my hand. And that the studies that we've been doing and that Ed will be talking to you about and Sherrill will be talking to you about are expensive studies to do. Anyone from the manufacturers who have supported us in doing clinical trials know that it's expensive.

But we burned through about $100,000 in the last four months doing these kinds of studies. And that was without any support from any manufacturer.

Trying to get federal support for these kinds of studies through our one mechanism is obviously going to be a futile attempt. This is not something that NHLBI is likely to look favorably on. So the source of funding for laboratories like ours and others to proceed, to try to answer some of the questions that will undoubtedly be outstanding at the end of the day, is not entirely clear and that we would hope that if this group or if the

agency feels there are still questions to be answered, that there will be funding that will be able to be found so that all of the laboratories that are interested in this will be able to address those questions.

Thank you very much.

[Applause.]

DR. ZUCK: Thank you, Jim.

The next presentation is by Sherrill Slichter. Again, a person, researcher known to all of us very well. And her work in platelets is legendary.

The biography that I was given is about 11 lines. It ought to be three pages. But we all know that she's been a lifetime researcher at Puget Sound Blood Center. Her contributions have been enormous, and it's a privilege to be able to introduce her.

And her topic is very similar to the other topic that previously was given. It's "Data Presentation" without a dash.

DR. SLICHTER: Well, thanks very much. It's a

pleasure to be here. I actually remember a bus trip with Tom Zuck, when he was--had some responsible position at the FDA, when they had just shortened the dating period of platelets from 7 to 5 days because of bacterial contamination.

And I got Tom on the inside of the aisle in the bus, where he couldn't move while we were on this little expedition, and I said to him, "Tom, was it just because of bacteria, or was there any concern about the quality of the platelets that also was a factor in your short dating?"

And he then basically didn't have the buzz word of downward creep. But I think he had some concerns about that as a particular issue. So I'm going to give you a presentation of some data that we have been doing on stored platelets. If you think you're going to be smarter or I'm going to clarify anything from this talk, you probably should get up and leave now. As has already been pointed out, this is after lunch.

So anyway, let me--what I'm going to share with you today is some observations that we've done that hopefully will be able to kind of convince you that extended storage of platelets is, in fact, possible, much to, in fact, my surprise. A lot of the data similar to Jim's. I don't have big numbers, but I think overall the data would support that particular conclusion that extended storage is possible.

I'm beeping, not moving. Oh, went the wrong way. Okay.

To just start the discussion, I want to go through a little bit with you one of the points that we've already discussed somewhat today, and that's the issue about recovery and survival measurements in thrombocytopenic patients versus, in fact, normal volunteers. And what we've already heard in some detail is that the recovery of platelets in circulation if you're thrombocytopenic is really pretty close to what you would expect if your platelet count is, in fact,

normal. But the survival, in fact, is different.

And what we determined in the study that we did many years ago was that platelets are basically lost from circulation by two mechanisms. One is senescence, and the other, importantly in thrombocytopenic patients, is apparently a random loss of platelets in probably an endothelial supportive function, which amounts to about 7,000 platelets per microliter per day.

Now if your platelet count is 250,000, that 7,000 you can't observe. But if your platelet count is only, say, 30,000, that's basically a large fraction of your platelets which are lost randomly, and that directly affects your platelet survival. So that at platelet counts greater than 100,000, basically your survival in normal individuals is somewhere between 9 and 10 days. At platelet counts of less than 100,000, your survival is a direct function and directly related to your platelet count.

So I, at least as I alluded to earlier today, am

in support of Dr. Murphy's position that we ought to have a higher standard for the expectation of recovery of platelets than maybe is necessary for survival because we only have to have platelets that are going to survive as long as the patient is going to allow them to survive. And in most thrombocytopenic patients, as has already been discussed, that's on average somewhat a little more than 2 days.

And so, as long as we have platelets that are able to do that, in my opinion, they ought to be effective and adequate for the support of thrombocytopenic patients.

Now the proposed FDA guidelines are that the values ought to be 66 percent for both recovery and survival. As you've heard, Scott has made an alternate suggestion that recovery ought to be 66, but survival only 50 percent.

Now I'm going to show you some data on the factors that affect platelet storage results. We've

already heard a discussion about the donor, and I will show you some data that looks very similar to data that you've already seen in the sense that each donor seems to have recoveries and survivals that are unique to that donor, and that influences the data that you will see when you store the platelets.

In our studies, we looked at two different apheresis machines, either the COBE spectra or the Haemonetics MCS Plus machines. And so, I'm going to show you that in some circumstances, the machines don't make a difference. In other circumstances, they do.

In addition, we've looked at the storage medium being either plasma, which is our standard method of storing platelets, or in plasmalyte. Plasmalyte is a licensed electrolyte solution in the U.S. which I'll show you data that, in fact, platelets do better in plasmalyte than they do in plasma. And it's always been interesting to me that although we have looked for many years for additive solutions that will improve red cells, we've

never taken a similar approach to see if there is something better that we can store platelets in rather than plasma.

And in contrast to what Jim has just showed you, and also Andy and Stein, the radiolabeling method that we used to label platelets, which is basically the old method in bag labeling that Scott alluded to you this morning, we've continued to use that method of radiolabeling, and we do get differences in the survival of stored platelets with indium, shorter with indium than with chromium, and I will share that data with you.

In addition, the one factor that I didn't put on here is obviously the storage results in some circumstances may be based on the storage duration.

Now the data that I'm going to share with you is drawn from the apheresis studies that we've been involved in. We used either of two machines, as I've said. One bag of the--what we did is one bag of the apheresis platelets. So we did a collection of apheresis platelets

and stored them in two different bags, so that one bag we considered to be the control.

Early on, we were doing comparison of the current standard, which is 5-day stored platelets, compared to extended stored. More recently because of the interest in the FDA having a fresh standard, we've collected on day 0, and then we have radiolabeled on day 1.

So these fresh collections that I will discuss with you are, for the convenience of the laboratory, basically transfused within 24 hours of collection, which is basically as soon as we can get a product out of the blood center anyway. And then the platelets in the experimental test bag were either stored in plasma or in plasmalyte. We could then store these two products for the same or a different number of days.

We then rotated the label on the two products between indium and chromium. We reinfused the autologous radiolabeled platelets and did post transfusion samples.

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I would tell you that we used the Nadler method for determining blood volume, which has already been suggested as being not appropriate. We also only collect samples for--we collect a 1-hour sample, and then we collect daily samples for 4 to 5 days post infusion. We do not collect long-term samples.

And the reason for that is because, as Jim has indicated, and Stein and Andy, that the calculations are really based on the tangent to the initial slope of the disappearance curve. And so, we don't see any reason for collecting long-term samples. In addition, none of these data were corrected for elution or red cell residual presence or any of the calculations that have been suggested by some of the prior speakers.

Now, this now shows you these are the plasma stored platelets, collected and transfused either within 24 hours of collection or stored for 5, 7, or up to 8 days. And what you can see here is that at least in our hands with our radiolabeling technique, the data on these

figure slides is all going to be presented as the average plus or minus one standard error.

And so, what my friendly biostatistician has told me is that if the error bars do not overlap, this is probably a statistically significant difference. So we tend to get a bit higher recoveries with indium as a label than with chromium. But for the stored data, we basically get the same answer.

In contrast, if you look at the survival data, the survival data with indium or chromium on the fresh platelets is the same. But even with 5 days of storage, we start to see statistically significant difference between the data with chromium and the data with indium. And so, what you can see here is that even with 8 days of storage, if chromium is used as a label, this is basically a straight line. With indium, there tends to be a difference, and this difference between indium and chromium is as much as 2 days different in the survival.

I've shown this data before to Andy Heaton, who

looked at the data and said to me, "Well, Sherrill, you're radiolabeling red cells with your chromium, and that's why you're getting these apparent long survivals with chromium and not with indium."

Well, we then started to look at the samples any way we could. We basically do a soft spin of the platelets once they're radiolabeled to remove any residual radiolabeled red cells. So with either indium or chromium, we basically have almost no activity on red cells.

But what we did note was that although the recovery of the indium labeled platelets is basically the same as with the chromium except for the fresh, so that the label binds by the technique that we have. When we looked at the indium activity in the plasma versus the chromium activity in the plasma, what we found, in fact, was that the indium was eluding from the cells after they were transfused and were in circulation.

So what I'm going to concentrate the data on in

the rest of this presentation is basically on the chromium stored data, and we'll use some indium data if I'm talking about fresh samples.

So then looking at a comparison between the Haemonetics versus the COBE machine. So the blue is COBE. The red or pink is Haemonetics. Again, the data is the average plus or minus one standard error. Then when you get out to 8 days, this is then the percentage of the respective fresh chromium data. So that because the Haemonetic--I'm sorry, the COBE machine tends to give a higher initial fresh recovery, when you do a ratio between the fresh and the stored, although it meets the criteria for recovery being greater than 66 percent, because the Haemonetics has a lower initial recovery, it's at 87 percent.

But if you look at the actual data, these two are basically the same number, which I think gives some validity to two things. One is that you have to be careful what your fresh standard is that you're

comparing. And secondly that a ratio measure, as we've already discussed, may give some data that really isn't helpful.

Because my bias--and the data would support, although the numbers are not big since half of our studies were indium and half were chromium. So although our numbers were bigger, I'm only presenting the chromium data, that basically this number is the same.

And this now is the survival data. There is not as much difference between survival fresh, but there is some difference. And so, again, it looks as though by the ratio measure that you're better of with the Haemonetics platelets than with the COBE platelets. And because these error bars don't overlap, there may be a difference at 8 days of storage between what you get with COBE and the Haemonetics machine. But basically, you get the same answer with either machine.

We did do five 9-day studies, and we stopped because two studies--one COBE and one Haemonetics--had

pHs that were 6.2 or less and very poor recoveries and survival. So at least based on this preliminary data, we think we can meet FDA or Murphy's law with either machine for 8 days of storage.

And what I've shown here is now just the accumulated numbers so you could look at the actual data. So that for less than or equal to 1 day of data, this is Haemonetics data, 42 observations. COBE, 13. This is the actual recovery data. And what you can see is there is really not much difference in this data.

At 8 days of storage, we've got recoveries of 50-some percent. That, I think, fits with what Jim had. And Jim used the COBE machine, and he was getting 5-day data at 8 days of storage. And as I've said, we may get a bit better data on the Haemonetics with 8-day data in terms of survival, but obviously not in recovery. And again, these are, I think, astonishingly good numbers.

In response to Dean Elfath this morning, I told him that I thought that somehow the manufacturers were

providing us with better machines or better bags or something that was better that was giving us these results.

And this now just shows the direct comparison of fresh versus stored viability, and we have five of these studies where we've actually done fresh compared to stored in the same normal volunteer. So this is fresh and stored percentage of both to just let you look at the actual numbers. Overall, we've now got 10 observations, and our recoveries are 71 percent of fresh, and survivals are 88 percent of fresh.

Now I'm going to talk to you now about storage in plasmalyte and would just say one thing. This is the composition of plasmalyte. Again, Scott Murphy has kind of been our guru in terms of platelet storage for a long period of time. And based on calculations and estimations which he had done, he basically has postulated that you need about 2 millimolar of acetate per day of platelet storage.

And so, if you divide this number by 2, which I can even do in my head, you get about 13 or 14 days. And that's about how long we think we can store platelets in plasmalyte. And in plasmalyte, acetate is used as the source of energy metabolism rather than glucose because by 8 or 9 days of storage, there is no glucose left.

All of these studies were done with a concentration of about 80 plasmalyte and 20 percent plasma. And again, we did the studies on the two machines. We started off with 5 days of storage, and as I've mentioned, we've gone up to 14 days of storage. And you can see that with the two machines, we get about the same data for 5 and 7 days of storage. By 8 days of storage on the COBE machine, we are not meeting our criteria of 66 percent of fresh, but we are able to meet it with the Haemonetics machine.

Now let me explain that in order to do these plasmalyte studies, you can concentrate the platelets during the collection on the COBE spectrum machine so

that we concentrated the platelets, resuspended them in plasmalyte, and got this data. In contrast, you can't concentrate the platelets during collection on the Haemonetics machine. So we had a Haemonetics engineer who came out and gerry-rigged the machine so that we could elutriate with the plasmalyte instead of using plasma.

So the first half of the donation, the control donation, we collected on the Haemonetics machine in a standard way and on the COBE machine in the standard way. The second half of the collection, we concentrated the COBE platelets, resuspended in plasmalyte, and with the Haemonetics platelets, we elutriated.

And so, we got a significant difference between the two methods. And what I would share with you is that I think the fact that we concentrated the platelets, in other words, we started to put them in a more abnormal kind of environment, that then resulted in these differences between the results when the platelets were

stored for an extended period of time. And so, with the plasmalyte collection, we can't even get 8 days with the COBE machine, which we could get with a standard plasma collection.

This is then the survival data. Again, the Haemonetics data, COBE data, showing a difference between the results with the two machines. And then this is, again, the actual data for 5, 7, 8, 9, da-da, da-da, da-da. And what you can see is what you saw on the graphs that by 8 days, we're starting to see substantial decreases in the recovery of the COBE platelets, but not in the Haemonetics collected platelets. And again, after 14 days of storage, we've got basically 44 percent recoveries and 5.2 day survivals.

Now we did do some additional studies with the COBE platelets at 13 days of storage, and what we did here was to try and determine whether the difference between the two machines was in the method of collection or, alternatively, whether it was a difference in the

storage bag. So that we collected COBE platelets but took off the COBE storage bag, sterile docked on a Haemonetics bag and then looked at the data.

And what you can see is that we--none of these answers are probably statistically significantly different. So it does not appear to be a bag-related problem but, rather, a collection-related problem.

And now here is the direct comparison of fresh versus stored for Haemonetics 13, Haemonetics 14, and this is percentage of fresh which, for 13 days, looks good. For 14 days, we may be starting to have a problem with recovery, but not survival. But I would encourage you to understand that the numbers are small, and part of this data is a direct effect of this one donor who had very poor storage data.

What I'm showing you here now is only Haemonetics collections stored in either plasmalyte, the pink, or plasma, the blue, and basically just showing that you get basically the same answer for the storage

duration in plasma or plasmalyte. This is the survival data. And so, we can store out longer in plasmalyte than plasma. But up until the time when we can no longer store in plasma, we get basically the same data.

Now this is a slide similar to what you've already seen. What it is, is simply the paired data for all 48 observations in which we had paired data, 1 day versus extended stored in either plasma or plasmalyte. So the indium was used as the fresh for these studies. So this is indium fresh platelet recovery. Chromium fresh platelet. Chromium stored recovery results.

And what you can see is what's already been shown to you, and that is the relationship between the fresh and stored recovery is highly statistically significant. And at least my biostatistician, who's analyzed this data by regression analysis, has said that the only factor which really affects stored recovery is the donor's fresh recovery.

So that the isotope used for labeling doesn't

affect it. The storage duration doesn't affect it. I've already told you we have an N of 1, where we've got 44 percent recovery at 21 days. And the machine doesn't affect it. So the only thing that affects it is, in fact, the donor themselves.

This is now adjusted stored platelet recovery. The chromium data versus the indium fresh data. Again, there is a statistically significant correlation, but it's not nearly as good as the fresh. And for the stored data, there is an effect of days stored so that between 7 and 14 days, you lose about .5 days in survival.

There is also, as I've mentioned, about a 2-day effect of indium being lower than chromium, and also you get an effect of the apheresis machine where for extended storage you get a bit better data with Haemonetics than with chromium when we looked at just the plasma data.

So in summary, these are the preliminary conclusions. Platelets can be stored for at least 8 days and still meet proposed FDA guidelines. Platelets

collected with either machine give comparable results for 8 days of storage. Plasmalyte storage you need to use a Haemonetics machine that you can elutriate the platelets, and Haemonetics platelets stored in plasmalyte may be able to meet FDA guidelines, I think, clearly for 13 days and possibly for 14 days as well.

Thank you very much.

[Applause.]

DR. ZUCK: Thank you, Sherrill.

The third presentation on this protocol design is by a person known, again, to us all, Ed Snyder. And Ed graduated from New York Medical College, Montefiore residency. He is currently a member of the National Marrow Donor board of directors, and he's an associate editor of Transfusion and has been a colleague and respected one by all of us for many years.

Ed?

DR. SNYDER: Thank you very much.

What I am going to talk to you about today is

what I call the front-end studies. In discussion with the group, everyone was doing studies of fresh product, meaning fresh collected, either apheresis or Sherrill had used bag studies.

What we were going to do--if this was going to work, someone needed to verify that indium and chromium could both be used to label platelets collected in a tube, because you really can't collect a whole unit of blood and not affect the blood volume, and see what those studies showed. So that's what I call the front-end studies, which we do, which we did. And I'm going to present that data to you.

This is just the conflict of interest statement that I always show because I do studies for many companies, as do most of the other speakers here.

So the purpose of this study--I guess I'll look at here--was to validate a dual platelet radiolabeling protocol using chromium and indium to radiolabel fresh autologous platelets en-tube, which I use as my little

French attribute there. I guess I must have had encrusted salmon sometime prior to writing this slide.

Based on the protocols from Jim AuBuchon, Jim provided us with a general approach, and we also used our approach for indium labeling, which we used many years ago when we did studies for the Cerus S59 trials, going back almost about 10 years, I guess. So that the purpose of this was to determine whether you could use 51 chromium and indium in a tube to label and see what the efficiencies were. We were--doom and gloom was abounding that you wouldn't have enough platelets, couldn't do this.

To determine in vivo recovery, in vivo survival, validate that sampling out to day 7 was adequate versus sampling out to day 10. Now that doesn't mean storing the platelets. It means these were fresh platelets that were infused within an hour or two of collection, and then we sampled the donor out to 10 days, which I'll explain more later.

We also needed to determine the percent of the control value that could be used as acceptable for test recovery and survival, which is what everyone's been talking about, and then we analyzed it with the same COST program, although there were some differences that I will bring up.

So donor processing, and I'm going to go through this step by step. Because in view of what everyone has said, there are certain things as I was listening, I was cheering and saying, "Oh, yay, we did that." And then there were times when I was crying because, "Oh, my God, we didn't do that."

So I'm putting all this up because no one else, I believe, has ever published any data looking at this. And again, as Jim has said, the tyranny of small numbers abound, and we are looking to have this verified and validated by other laboratories.

So we got IRB and radiation safety approval. We recruited volunteer donors, had all the usual

TTD/pregnancy testing. The unknowns we were looking at was the quantity of platelets that were needed. How many platelets--would we get enough from a single collection in a tube?

The volume of blood that was necessary to be drawn. The labeling efficiencies, the chromium elution, the equivalency of indium with chromium en-tube labeling, recovery and survival characteristics with known low labeling efficiencies, which we assumed we would get with chromium. And also I will discuss our window settings, crystal size, counting time, and sampling days.

So for whole blood processing, we used 1 19-gauge needle. We collected in polypropylene syringes 7 mL of ACD-A was used to collect 43 mL of venous blood. So we collected a total of 100 to 125 mL of blood. That's the maximum we allowed ourselves because much beyond that, you were really looking getting close to a volume that we thought would be too high.

So we generally collected 100 to 125 mL. There

was a 7 mL ACD to 43 mL ratio, which is what we used for citrate, for indium labeling, and we applied that to chromium as well. Again, for right or wrong, this is what we did.

The contents were transferred to a 50 mL conical tube, left undisturbed at room temperature for an hour to allow sedimentation so we could get rid of a lot of red cells. We used a soft spin in a conical tube at 200 G for 15 minutes in a swinging bucket at room temperature to get red cell poor, platelet rich plasma.

We then removed the PRP with a spinal needle, and we were allowed to spin again in order to remove more red cells as needed, which was done by I. Obviously, avoid aspirating red cells. We then added a volume of sterile saline equal to 15 percent of the PRP volume, mixed by inversion, and we split it.

We gave a little more, 60 percent, for the chromium to try to hedge our bets to hope that we could get enough platelets to actually get a label that we

could detect. And we knew 40 percent would be all right for indium because labeling efficiencies were so much higher.

To prepare the PRP, we centrifuged it at 2,000 G for 15 minutes with the brake off for both labels. And we used the same technique for both isotopes in order to ensure at least consistency, so we didn't have to switch back and forth and make things nuts. We thought if we're going to do it, let's try it the way it would be easiest if it worked, and then we can modify it going forward, as they say.

We removed the PRPs completely as possible because of the concerns Andy mentioned about transferrin and so forth with indium certainly, and we resuspended the pellet with 3 mL of ACD-A in this same polypropylene tube.

For the labeling, we added 100 microcuries of indium oxine to 4 mL of the ACD-A saline in 4 mL to the washed pellet, which is what our standard procedure is.

sj

Gently resuspended, incubated for 25 minutes, and then mixed gently at 10 minutes.

For chromium, we started off with 200 mikes. Two hundred microcuries of chromium is very expensive, but we felt we needed to really give it a big slug because we weren't expecting much label. So we used 200 microcuries of chromium, gently resuspended the pellet, incubated for 25 minutes, having the same time for the two isotopes, and mixed gently at 10 minutes.

After incubation, we added a half a mL of autologous platelet poor plasma and 3.5 mL of ACD saline. We centrifuged at 2,000 G for 10 minutes, removed the supernatants, saved in a separate tube, and determined the activity of the supernatant in a dose calibrator.

For labeling efficiency, and these were some of the differences up here, here and other places, we gently resuspended the platelet pellet in 6 mL of autologous platelet poor plasma, and we determined the exact activity of indium or chromium using a dose calibrator.

And this is the labeling efficiency--and by the way, all of this work was done by Laurene Baril, who is here and will be glad to answer any difficult questions I can't answer, as well as Tammy Corda. They're the ones that actually did the hands-on labeling.

The labeling efficiency is as you see, and then we--the plan was to aspirate a volume of labeled concentrate containing up to 40 mikes of indium or chromium and to a 3 to 10 mL plastic syringe. We knew we wouldn't have trouble getting this with indium because we label indium all the time without a problem. We weren't sure we were going to get up to 40 mikes with chromium.

This is our labeling efficiencies, and you can see this is an N of 9. We did three in vitro studies, which was just to look at chromium labeling without any injections. And then we didn't use indium for that, just chromium, because we knew we could label indium well. We wanted to see if there was any point in labeling with chromium. And there was. We got about a 16 percent

labeling efficiency.

And then we did six paired indium/chromium. So that's why there's nine for chromium and six for indium. Nine for chromium, six for indium. So, and our labeling efficiency was about 16 or so percent for chromium and about 70 to 80 percent for indium, which is about what we see with indium.

For standards, we did a 1 to 250 dilution of chromium and indium by adding exactly .1 mL. Now Andy said you have to weigh everything, and that's one of the reasons I was crying when I heard this. Because we've--actually, for red cells, we do weigh. But for platelets, we've always done volume. So this may be a source of some error and some concern. And we'll need to discuss that.

But we measured .1 mL into a 250 mL volumetric flask and QS'd with water. And then we transferred 2 mL aliquots to each of three counting vials for each isotope. Obviously, you have separate standards for the

chromium, indium. And for the eluate, we incubated the remaining injectate in autologous plasma for 2 hours, which was within the timeframe, I believe, that was mentioned. It had to be within 3, I believe--I see Andy's head shaking there--from the time when the injectate was prepared.

After 2 hours, we mixed the sample, transferred a mL to another polypropylene microcentrifuge tube, and centrifuged at 10,000 for a couple of minutes. And we prepared two elution samples as shown there, transferred 100 mikes to the counting vial. We added 1.9 mL of water again. This was all by volume, and no weighing, to bring it to a volume of 2.

We prepared two background tubes, and then when we counted, we used two background samples, two elution supernatant samples and three platelet standards. And we were suggested three standards were necessary because of the maldistribution, so I felt good about that, that we had been doing things in a reasonable way. And we used a

gamma well counter--more on later.

So our elution results are seen here. We have about a 5 percent elution for chromium and about a 1-point-something percent elution for indium with the N of 6.

The elution calculation is as you see here. This calculation was dating back to work we had done, again, with Cerus way back. And there is--the value that you see here, there is a correction factor in the denominator of 1.125, which unfortunately has been lost in the dim time as to exactly where that came from. It is probably a correction factor for the volume of the standards, but I have yet to ascertain that.

This is what we have used for all of the studies that we've been doing with our COST program and so forth. So we were doing everything the way we had been doing it, and we did this for the indium as well as the chromium. So that correction factor is in there.

Unfortunately, when I spoke to everyone else, no

one had a clue what that was. No one was using that similar technique. So that may be something, we can always reanalyze our data. We have all the counts. It's just a matter of recalculating. So we will do that.

Sample injection, performed a venipuncture using a 19-gauge butterfly and a stopcock. I mention this not because it's not obvious, but things happen. And we collected two purple top CBC tubes as baseline, and we ensured vein patency, which doesn't always happen. And as you will see, in one of the veins, it didn't happen.

We infused indium. Generally, we infused indium first because it absorbs to surfaces, and we didn't want to contaminate the lines by having it wait around. So we usually injected indium first, although there were just a matter of minutes between the two injections. They were sequential. although they were not occurring at exactly--they were not, what's the word, concurrent. They were sequential, but they were as close to concurrently sequential as you can get.

sj

The tubing was flushed. A second syringe with chromium was infused, and then the tubing was flushed again, and residual radioactivity in the syringes was measured and accounted for in the calculations.

The amount injected is interesting. The chromium, this is mL. So we injected about 6 mL of chromium labeled red cells--platelets rather. Sorry. And a lower amount of indium. But the amount of microcuries injected was about 20 to 23 for chromium and about 35 or so. We inject up to 40 for indium.

And the reason--one of the reasons we inject up to 40 is we are one of those archaic places that has a 2-by-1 inch crystal. So--but that's what we've been using and actually as of about a week ago, the three-inch crystal arrived. So I'm glad it arrived, but it was too late for these studies.

So you inject more chromium than you do indium, but you have a much higher amount. So this could obviously be lowered to 20, 25, but it still is within

the levels that our IRB and Radiation Safety Committee has approved.

So the total platelets injected, the number of platelets in the injectate was about 1.5 billion for chromium, a little more than that, 1.6 billion. And for indium was about 1.1 billion, something like that. So we got enough platelets. And as you'll see in the data that I show you, that was good to see because not everyone who walks in is going to have a platelet count of 560,000.

So for sample collection, we collected two purple tops at 1 and 3 hours post infusion, and at 24 hours, and then every day from days 2 to 7, not counting Sunday. We gave people Sunday off. And then we counted again, and we sampled again on day 10.

So the patient was injected on day 0 and then 1, 2, 3 hours later that same day, the next day, and then daily, except for Sunday. And also we didn't collect on days 8, 9, and we collected again on 10, and that was the end. We wanted to see if you needed to sample out to day

10, or if sampling up to day 7 would be sufficient.

For processing, we drew two 10 mL purple tops. We aliquoted two 2 mL whole blood samples for counting, and then we gave a hard spin and collected two 2 mL plasma samples for counting and stored them at room temperature. So it was--the geometry was a 2 mL size and equal geometry.

For counting, we used a Wallac/Perkin Elmer 1470, which is a two-inch crystal, sodium iodide. We've been using that forever.

The windows were set to count indium and chromium simultaneously. We used a 5-minute count. I think that may be too small. I thought it may have been too small, but we had 54 tubes to count. We had six patients we were counting or three sometimes on one day. It came to like 19 to 25 hours of counting. It was a huge amount of counting. So if we had gone to 8 minutes or 10 minutes, so we did it at 5 and said let's see what we get.

The window settings were 165 to 215 KeV, which was 171 and 247 for indium with a sum peak at 419. And the chromium windows were set at 295 to 340. The counter software does adjust for decay in background, and only the counts in the selected range were there.

Now the efficiency of labeling was low. It's a two-inch crystal. You don't have a very large amount, as usually the amount of microcuries injected for chromium that we wanted, we were counting for 5 minutes. We didn't really think we were going to get much of anything out of this.

Let me walk you through this because there was no way to do this in big numbers here. So here we have the first three--can you hear me? The first three were the in vitro. So we had platelet counts in our patients of about 300,000 to 218, which is not unreasonable. You might have some people with 125,000 platelet count. That may be something to consider. Do we want to do this only on people that have high platelet counts, or what would

be considered, at least above 200,000?

The starting microcuries of chromium we used, as I mentioned, was about 200, and we didn't use indium. And the labeling efficiencies were 16 and 14 percent, lower than Sherrill and others have reported for a bag, but respectable.

And then we have the results for the one, two, three, four, five, six people that we did. And what we see is that we had platelet counts ranging from about 398 down to about 199--I think that's 199--for a mean of 289, and that's for all nine. The starting microcuries was 200, as I mentioned. It was 116 microcuries for the indium, which I mentioned as well.

The percent labeling efficiency averaged 15 percent for chromium. For indium, it was 76.2. For the mLs injected of chromium, it was 5.5 mLs. I showed you that. And 20.2 for microcuries of chromium. So 5.5 mLs of chromium to inject 20.2, and we injected as much as we could to get that up as close as we could to 40, so that

was all there was, or all she wrote, as they say.

3.4 mL is what we injected of indium, and we were 35 microcuries of indium injected. The chromium elution was 4.9, indium elution 1.1 mean, and then the chromium and the total platelet--for the total platelets injected was 1.6 billion versus 1.01 billion for the total indium.

So those were the--that's the in vitro data that we had. So what were the results? Well, looking at these six, let's do the means first, and then we'll go up. The mean for those counted out to day 7--there was a predominance of women in the study, admittedly. For day 7, the percent recovery was 50.83 for chromium and 53.6 for indium. And the survival for day 7 was 223 hours and 214 hours.

For the day 10, meaning we counted additional day 10, we put another point into the COST program, was 51 and 54, with 231 for chromium survival and 217. So I looked at this, and 58.83 versus 51, and 53.9 for day 7

versus 54. So our initial thoughts were that there was really no need to collect a day 10 sample. Collecting out to 7 days appeared to be sufficient. Small numbers, at least this is what we're postulating now, 223 versus 231 and 214 versus 217.

When you look at the data, you see 51 and 63, 69, 68, 68, 60. And here is the one that I was concerned about, volunteer G complained that when they injected the sample, although they checked the patency with the saline, when they went to inject the sample, there was burning, and she thought there was a bleb--she's a nurse--and she thought they infiltrated.

As a result--and when we looked at the results, 26 and 27, with the normal essentially survival, I agreed that there was a possibility. And so, I show the data with that in there and with that one backed out. Despite that, there is also a lower one here, 46 percent versus 37, which is also low. But this volunteer, when questioned, said there was absolutely no infiltration.

Everything was fine. She had no marks or any blebs or anything.

So it appears that--and we've been discussing over and over again--donor variability, which to me just reinforces the need for pooled random donor platelets versus continued reliance on single donor. But I digress, and this is not the forum to discuss that, thank you very much.

[Laughter.]

DR. SNYDER: So anyway, these were the results, and I was a bit concerned about this. I was concerned enough to call Jim and ask him about this. And let me show you what else--some other things, and I'll tell you what we talked about.

So chromium on day 7 was about 50, indium was about 55, 53. Chromium on day 10 was almost identical, and the indiums were identical to day 7 and day 10. So if anything, I think we've shown you don't really need to make another pin cushion out of someone on day 10, I

think. But you do need probably to go up to 7, and we didn't look at lower.

And then the survival in hours was about 220. The survival seemed quite good and were about 9- to 10-day survivals.

So this was the one I was concerned about. So let me take that value out, knowing full well we're doing that, and now we get a mean of 55.8 versus 58.8 for the--in chromium and indium, the same--almost essentially the same recovery--survival, rather, 221, 214, and the results were again 55.8, 56, 58, 59, and the recoveries were similar.

So when I called Jim up to ask him about this, he had shown me a slide that he didn't show you, but--I'm sorry. He did show it, and he made--I'm sorry. He didn't show it, he made a point of stressing it. That for the apheresis platelets, he had a recovery of 74.7 and a survival of 7.5 days. But for the manual fresh, he had a recovery of 61 with a survival of 8.9.

So if you look at 61, that's quite close to what we have here, and the survival here is just about, it's 9 days. So we basically duplicated what Jim had shown with the manual technique, that you seem to get a lower recovery, but a longer survival than you do with the apheresis product, where there was a 74 percent recovery, but a 7.5 day survival. Why that is, I'm not sure. But I was at least pleased that we were in the ball park because I was expecting this to be 80 or 90 percent, and it wasn't.

So if you start multiplying two-thirds times 58, you get 39 or 40, which is still a little on the low side. Again, these numbers are small. We had a donor here that had a lower number. If you add just the three, pick out the really good ones, you're up in the mid 60s. So, obviously, you can't do that. I was just doodling. But the survival seemed to be fine that we got.

And I think for the first go-around, at least with the lower labeling efficiency and our first shot at

this, I think the results--I was buoyed by the fact that we got similar results to what they got at the Hitchcock. And again, the recovery with the 5 is a little higher, but the ratios are all still the same, although we don't use ratios anymore. And the survivals, 220 hours.

So, in summary, the use of en-tube radiolabeling with indium or chromium is feasible, even for low labeling efficiencies and with a two-inch crystal. The three-inch crystal counted somewhat differently, maybe for a longer period of time. I don't think you'd see much difference, quite frankly. I think these results are--it may be somewhat asymptotic. You may get another percent or two. I'm not sure.

Then I would ask Andy or others to comment on whether increased efficiency would give you different results. Probably the rate, they would stay similar to each other, but they may all go up.

The labeling efficiency, is it independent of platelet count and technique? It didn't look that way.

sj

We had some, if you go back--which I will, without getting confusion here. Here is a labeling efficiency of 6 with a platelet count of 200,000, 199. Here is somebody with 205 with a labeling efficiency of 10. Here's 253, a labeling efficiency for chromium of 11. Here is one with 415,000 platelets, bless her heart, and she had 22 percent.

So small numbers, but it doesn't appear as if labeling efficiency, per se, is related just to the count. It may be to factors related to the individual. I'm sorry for the confusion.

The volunteer donors with low normal platelet counts may not prove as problematic as I thought they were. I don't know. We need more data for that.

There is a very high wastage associated with chromium. I was concerned that Andy had commented about toxicity from chromium biologically affecting glutathione. That's something that he may want to address. We did not look at that. We were too busy

crying over the cost of the chromium.

Sampling for 10 days post injection we believe was equivalent to 7 days, and I think we might be able to have that agreed upon perhaps. And additional data are needed to determine the percent of control value and also multicenter studies. These are supposed to be done by Pam Whitley. We're going to try to get Sherrill and possibly Jim to do some that is predicated on Jim's eloquent plea for some degree of financial remuneration, which would be appreciated.

Thank you very much.

[Applause.]

DR. ZUCK: Thank you, Ed.

We have a few minutes, and I'd like to propose two things. First, the next session has been opened for public comments. And as the moderator, I would greatly appreciate it if people who wish to make public comments would put their name and their affiliation and maybe a statement--or not a statement, a phrase of what they want

to talk about. And during the break, I can arrange them in some kind of rational order.

I would greatly appreciate that, and it would let us predict somewhat more accurately how to manage the rest of the afternoon. We do have some time, and we're a little ahead of schedule. So for some reason, there was no discussion of these three papers allowed for. I'd like to open it for questions and discussion now. Yes?

MR. DUMONT: I've got a question for Ed. Maybe I missed it, but when you did your calculations, did you do adjustments for elution and adjustment for cell-bound fraction and baseline?

DR. SNYDER: Yes. Yes, we did. And putting it into the COST program, we--that's all put in.

DR. ZUCK: Yes, Jim?

DR. AUBUCHON: This morning, Stein, you mentioned that it may be more reasonable for us to look at mean residual lifespan rather than numerical expected lifespan. That implies determining the area underneath

the survival curve, and the points between 7 and 9 days would add some to that area underneath the curve. Obviously, to both the control and the test equivalently you would use the same number of points.

But do you see any concerns about only taking samples to 7 days for the survival curve if we turn our attention to mean residual lifespan?

DR. HOLME: What one of the advantages of using the mean residual lifespan is that it could be used as a combined measurement of the survival as well as the percent recovery and in particular when you compare it to the fresh platelet from the same donor. Because as I said, what it means, mean residual lifespan means the average lifespan of the platelets that were infused.

So knowing the mean residual lifespan of freshly collected platelets and the mean residual lifespan of the stored or the test product, then we can see, okay, if fresh platelets has a mean residual lifespan of 5.5 days and the stored has a mean residual lifespan of 3.5 days,

then I know that a combined percent recovery and survival have decreased in terms of 2 days.

It's more that you're getting a combination of both the recovery measurement as well as the survival measurement. You will know in the recovery measurement how many nonviable platelets were infused, and by doing the survival measurement, you get an estimation of how long do the remaining platelets circulate. But the advantage, like I said, with the residual lifespan, you can get that combination of both those two measurements.

By itself, if you're looking at the residual lifespan, just looking at the--as survival parameters, then as is shown compared in 5 days versus 7 days, by itself as a survival parameter, it doesn't seem to get more information about the survival than using the numerical expected lifespan or the T 1/2.

On the other hand, I'd like to comment on the issue about red cell labeling. At least with random donor platelet, I've seen that there is quite a high risk

of getting the product contaminated with red cells. So even if you carefully try to reduce the amount of red cells by soft centrifugation before you started labeling, at least been my experience when I was working in Norfolk, that it was quite difficult to get rid of all the red cells.

And it was clear that you could see the activity of the red cells by prolonging the sampling size, sampling time beyond 7 days going up to 9 days, 8--I mean, 8 days, 9 days, and so forth because it was characterized, as Andrew Heaton was showing, that the survival didn't decrease during prolonged time of injection after the infusion. If you took sampling up to 7 days, 8 days, 9 days, and 10 days, and so forth, the activity didn't go down.

So whatever we would choose to use, I think it's important, especially if you are at risk of seeing that there is contamination of red cells in the project to have labeled, that you need to follow it longer than 7

days to make sure that there is not radioactivity associated with the red cells.

It was a long answer, Jim. Did it--any more questions?

DR. HEATON: Yes. I've got a few comments. I think first I'd like to comment on Sherrill's observation about the indium platelets and the recovery and survival.

You know, one issue, Sherrill, I noticed in your indiums that you got slightly higher immediate post transfusion recovery. I take it you didn't correct for elution in those. Because my guess is that with a higher immediate indium recovery and a shorter survival, you've probably got elution going on of the indium platelets, which have been labeled in a bag.

And one of the reasons you get higher elution is in the bag it's very hard to get all the plasma out, and you get plasma sticking to the walls of the bag. So the tendency usually with bag-labeled indium platelets is you get more elution, which would give you a higher recovery,

and it would give you shorter survival if you didn't correct for it.

So my question for you is did you correct for indium elution in those studies?

DR. SLICHTER: No.

DR. HEATON: Okay, and that's--so my guess would be that you had a little bit of plasma carryover, just enough to give you the elution?

DR. SLICHTER: Yes. We tend to get--when we do labeling, we tend to get exactly what you've said. Higher indium recovery, shorter survivals than we get with chromium.

But I think it still doesn't explain, Andy, the fact that with the fresh, we don't get elution of the label with indium. I mean, we get the same survival with chromium and indium for fresh platelets, and we only start to see really the disparity, if you will, between the survival with chromium and indium if we have stored platelets.

So somehow, you know, we're doing exactly the same thing in terms of the labeling procedure and the calculation of the data whether we do fresh or stored, but we're clearly getting loss of the label on stored platelets with the technique of labeling that we have used that obviously Jim doesn't see.

DR. HEATON: And did you only see the elution with stored platelets with acetate in it, or did you see it with plasma stored platelets?

DR. SLICHTER: We didn't--we didn't see it with plasma stored platelets. Let me see if that's right. No, that's not--that's not right. I think we saw it with both plasma and with plasmalyte. So we saw it with both, Andy.

DR. HEATON: Okay. Switching to Ed's presentation, I've a couple of observations. One is the chromium, you notice that you got slightly better label with chromium with higher counts. And there's no doubt with chromium, it doesn't label platelets as efficiently.

So that if you increase the counts, if you got more counts per mL, you'd definitely get better chromium uptake.

And I think the bigger the sample you collected and the more platelets you had, you'd find that your chromium label would go up. And you saw that to a lesser extent, and your range was 11, I think, to 23 percent. But it was related to the platelet counts that you collected.

On the issue of the three-inch crystal, three-inch crystal makes biggest difference in that it improves your count efficiency. So if you're going to count after 10 days, you've had quite a lot of decay echo, and it doubles your quantum efficiency yield of your crystal if you have a big count.

Now the value of that--you can technically, with a smaller crystal, just count longer. But the trouble is your background is linearly related to time. So, and your efficiency of counting is undermined by your net

counts minus your background. So it's very attractive to have a bigger crystal device because you get your counts quicker, and therefore, you have less erosion of accuracy because you don't have such high backgrounds.

But nevertheless, I don't believe it would make an enormous amount of difference, given the number of counts I suspect you probably would have got.

And then, Jim, I had a question for you related to the pheresis platelets. One of your studies, you did day 1. You did your pheresis, and then I think the next day or around 24 hours, you did your post transfusion recovery. And then you compared it with the test platelets 5 days or 7 days. Why did you choose day 1 rather than doing it on the day of collection?

DR. AUBUCHON: In order to maintain the employment of my technologist.

[Laughter.]

DR. AUBUCHON: We always reinfused the fresh apheresis platelets within 20 hours. But unless we could

get the subject to come in first thing in the morning, unless we could get an apheresis collection performed first thing in the morning, there just wasn't enough time to complete a hold period, and then label it and get it back in, and get the samples that we needed on that day of reinfusion in the same day. It was logistically simpler to collect it one day and then first thing the next morning reinfuse it.

As Sherrill noted or Ed noted, it's impossible to get a platelet out the door in less than 24 hours nowadays, even with rapid mat testing. So that probably represents--anything less than 24 hours probably represents the best that we can do. It may not represent the absolute best that could be achieved with a platelet, however.

DR. ZUCK: Okay.

DR. HEATON: You know, certainly, if you do paired studies, you get much better results. If you do simultaneous and concurrent infusions and even a 24-hour

hold, you can see some effects. It's amazing how quick you can see it.

The other observation I wanted to make on Jim's studies relates to this issue that we've seen a couple of times now with pheresis platelets having slightly higher post transfusion recoveries than whole blood platelets.

And one should think back to the method of apheresis collection. The new leukodepletion apheresis collections involve two elements. One is density gradient centrifugation, but they all have an elutriation element now. So they're floating the platelets out from the white cells.

Now that has the effect that it does select the slightly younger platelets because they're larger and slightly less dense. And so, in an elutriation environment, you may well get differences in your subpopulation.

We didn't see it with random donor, when Stein showed you the results with the internatant platelets and

the supernatant platelets. But with apheresis, we have elutriation, you may be subsetting a population that is selectively better.

DR. ZUCK: Okay. That will bring to a close this session. We'll take a 20-minute break. Be back here at 25 minutes of 3:00 to take up the very optimistic title "Issue Consensus."

For those who would like to have the microphone during the open comment period, again, please give me your name, your topic, and the institution you represent. I'll be kind of floating around the hallway.

[Recess.]

DR. ZUCK: The public comments issues or section of today's program was essentially to let manufacturers or representatives of manufacturers present data or concepts if they wish to. I got no--I got no notes from anyone that they wanted to present, nor did the staff of the FDA or Heart/Lung.

So what we'll do is directly go to the panel.

sj

We should be done a little early. People have tight airplanes. That should be a relief. And go directly to the panel, and if they could come up and take their places?

Paul Ness, director of transfusion medicine at Johns Hopkins, and I believe he's the editor of Transfusion now. Toby Simon we've already met. Chief medical officer at TriCore Reference Laboratories, clinical professor of pathology at University of New Mexico School of Medicine.

Gary Moroff is eight in my paragraphs. Not that I don't know these people, except I want to get it right. And Gary is currently director of blood development in the blood cell therapy development, American Red Cross in the Holland Laboratories. He is on the editorial board of Transfusion, involved with transfusion medicine for as long as most of us can remember.

Susan Leitman is deputy director of Department of Transfusion Medicine at the clinical center, NIH, and

she is former member of the FDA Blood Products Advisory Committee. Did I get everybody?

Didn't forget anybody? Did forget someone. Who did I forget? Oh, everybody knows Rick Davey.

[Laughter.]

DR. ZUCK: Okay. I've had several suggestions of how to do this. And after I introduce Rick Davey, who is chief medical officer, vice president of medical affairs of the New York Blood Center, and had a long history with the Red Cross and is a distinguished member of our profession.

We had several suggestions of how to go about doing this, this session. I thought that the way I'd first approach it is to open it up for anyone that had questions or issues they wanted to raise with the panel. If none is urgent, then we have previously received from Jim AuBuchon an outline of questions that might be worthy of addressing, and we can walk through some of those questions with the panel and see what evolves.

I think we probably ought to say at the outset, I think when I first saw the agenda and I was asked to be involved, I really kind of wondered whether this was a consensus conference in some different uniform or some way to develop a consensus on licensure or licensure approval or how the agency should approach things.

As we've listened today, I don't think that's where we are. I know--as I understand, the manufacturers would like very much to have some guidance as to what studies to do to get licensure for product, et cetera, et cetera. And whether this is the forum to give them that guidance, I don't know. It may come out of this.

But I think that the questions to be addressed are more or less intellectual, although they have a practical outcome in the results of how manufacturers develop and/or seek the license of approved products.

Okay. Does anybody in the audience want to start with a question, statement? I thought I saw a hand go up? No, just--there is a hand up. Our friend from

the Navy.

MS. GILSTAD: Hi. Colleen Gilstad. I'm here from the Navy Blood Program Office.

And my question is that has the bleeding time been--or could there possibly be some reconsideration of using bleeding times as a test for platelet efficacy as opposed to recovery of radiolabeled platelets? Which supposedly somebody could take little, you know, latex balls and radiolabel them and find that they recover--you know, you can get a good recovery.

I guess the concern would be if people want to extend the shelf life of platelets, what guarantee is there to trauma surgeons that these platelets will stop bleeding related to thrombocytopenia or platelet dysfunction?

And although bleeding studies have been shown not to be predictive, that preoperative bleeding times are not predictive of operative bleeding in clinical medicine, I don't think that--I mean, you know, if you

look at studies that have been done, it's a good lab test for this type of indication in the controlled environment.

DR. ZUCK: Paul?

DR. NESS: Well, I'm not sure that the bleeding time is the best test that we would want to do to measure interoperative hemostasis because it, first of all, takes so long. And you already quoted the topic of, you know, that it doesn't really predict preoperative bleeding very well. There may be other things that are coming along, such as PFA and other kinds of tests that may be more--better able to predict whether a platelet infusion will have some immediate hemostatic effect.

But I would like to support your question because that's been the under-riding issue that I've had as I've sat through here, wondering just as well that, I mean, we in our deliberations here, I think, are thinking mostly of chronic thrombocytopenic patients who get platelets every couple of days until their bone marrow

recovers. And at least in many hospitals, 20, 30 percent of those platelets are going to operative cases where you really do need quicker hemostasis.

I think Andy showed us some work early on that implied that the platelets that he studied using testing that, you know, stored at 22 degrees had a delay in terms of function. And we all know the old literature, which implies that 4 degree platelets may have quicker hemostatic response, and sometimes you can even use just platelet membranes to get better hemostatic response.

So I really think it is--remains a big question for the agency in the field as to say, you know, admitting that measuring recovery and survival of platelets is important, but it may not be the only thing we want to consider, and we really do need to consider some mechanism of hemostatic measurements.

DR. ZUCK: Toby?

DR. DAVEY: I would agree with what Paul says. But I do commend the agency for trying to be kind of

circumspect about this with their triangle of increasing criteria where minimal, moderate, and the very most difficult criteria being hemostatic effectiveness for like platelet substitutes or major membrane modifications.

We do have to, I think, look for some middle ground. And while the platinum standard may be hemostatic effectiveness, I'm happy with a gold standard of radiolabeling studies, especially when we're looking at essentially changing parameters of things we already do--storing platelets. So I'm happy with radiolabeling.

DR. MOROFF: The radiolabeling studies are a step forward. If they're successful, then in a lot of cases, there should be some hemostatic effectiveness study. That's what you're saying, Rick. I think that's what--the industry has done for the last 20, 25 years. This is not the only criteria that is used to judge the suitability of a product or a new product.

DR. ZUCK: Toby?

sj

DR. SIMON: Well, to address the bleeding time specifically. It was used for a while as an indicator for approval of platelets, and there are two problems. To use it in normal volunteers, one has to create some kind of artificial situation. It was the aspirinated situation that was used, where the donor was given aspirin, and then the ability of the platelets to correct that, which was questionable in terms of clinical efficacy or a relationship to clinical situations.

And in patients when it was used, the problem is that there are so many drugs and other aspects of the patient's environment that affect the bleeding time that it really was not a useful test in terms of getting at the effectiveness of a new platelet concentrate or new apheresis platelet.

So I think the bleeding time specifically has a lot of problems connected with it, which would make it difficult to use. But I do agree that the thrust of your question is very important, and we should keep our mind

open and try to look for other ways to measure hemostatic effectiveness, which is what we're trying to achieve. And I'm not sure whether we have any good candidates on the horizon or not.

In our laboratory, we have stopped doing bleeding times completely and have replaced it with the PFA as an indicator of platelet function. But whether that's applicable in this situation, I think, remains to be seen.

DR. ZUCK: Yes?

DR. LEITMAN: I agree with all that's been said. You know, it's taken us 20 years to convince the surgeons that they don't have to ask for fresh whole blood in the OR. So I don't want to suggest that the data here suggest that there is a significant lessening of hemostatic effect in platelets that are stored for longer than 5 days because it will start an epidemic of requests for fresher platelets.

And it is really--there's no data that we need

to do that.

DR. SLICHTER: Tom, could--having probably done more bleeding times than anybody else in the room, a couple of thoughts. One is, you know, obviously, the end point of the platelet transfusion is to provide hemostasis. I mean, I think we all agree with that.

I am personally not aware of a situation where if they're viable, they're not functional. So when we did the studies, for example, with 4 degrees and 22 degrees, you know, we had, as was pointed out this morning by Scott, the 4 degrees stored platelets gave an immediate good recovery, but a very short survival.

And if you infuse 4 degree stored platelets, you may have gotten some immediate hemostasis, but that shortly disappeared. So I'm not as concerned as the speaker in the audience about making sure that every product we have, we have hemostatic efficacy to document.

I don't disagree with the studies that have suggested that the bleeding time doesn't predict post

operative bleeding. But in every situation where we've really looked at thrombocytopenic patients and transfused platelets, we have seen a relationship between post transfusion platelet count and correction of bleeding time.

So I think and I know for a fact that when Cerus Baxter started to look at their pathogen and activated platelets, we did a bleeding time platelet count study, which showed that there was similar efficacy in terms of correction of bleeding time for the post transfusion platelet count for the treated versus control platelets. So that we did treated and control platelets in the same patient, and that study was done before the large phase III clinical trial that looked at hemostasis.

So I think there still is a role for doing bleeding time measurements in thrombocytopenic patients as an initial screen for hemostasis, if that's felt to be required for that particular product.

DR. ZUCK: Excuse me. Could I ask a question?

Just take the moderator's privilege. How many people who work in blood centers, hospitals, whatever, routinely notate before they release a unit of platelet whether or not it swirls?

[No response.]

DR. ZUCK: I find that fascinating. Pardon? I find that fascinating.

As one reviews--as one reviews some of the literature, which I did preparing for this, one is struck by the presence of swirls as an indicator by some investigators. I'm not saying this is the solution to everybody's problem. I just was curious because Bertolini's paper stuck out so much.

And that those that had a positive swirl were hemostatic and had a good CCI, and those that did not did not. And the difference is extraordinary--22 percent to 1.6 percent of functioning with swirl, nonfunctioning without. And so, I think it's fascinating that--now we're not maybe even using some of the tools we have, and

bleeding times is so very, very difficult.

Having injected my own stuff in there. Sorry about that. But I thought it was of great interest. Do we have any other questions or issues? Yes, Jaro?

DR. VOSTAL: Just one more question to follow up on the usefulness of radiolabeling studies. We have a pretty good comfort level using that to assess 5-day and 7-day platelets. But when we start pushing storage time out to 14 days or beyond that, 28 days or such, you know, I wonder is there any time where we're going to disassociate hemostasis from viability?

Where you could have a circulating platelet, but you wouldn't know if it's actually functional. And is there--maybe you could give us some guidance about when we should start to worry about that.

DR. ZUCK: Anybody want to tackle it? Why not?

DR. MOROFF: I don't think there's any data really, Jaro, to address your point. And so, I think there needs to be some studies to look at hemostatic

effectiveness with longer storage times. I think it's simple as that in some fashion.

DR. DAVEY: Yes, I would agree, Jaro. I mean, if we're talking about 28-day platelets, I think that probably fits in the top of your pyramid. I would think it's that radical enough of a change. But where you draw the line, I think, is problematic.

DR. ZUCK: Yes?

DR. FITZPATRICK: Mike Fitzpatrick from America's Blood Centers. I've got two questions.

One, given the comfort that everyone has with isotopic labeling, back in the late ‘70s, mid ‘80s, there was a move to move away from isotopic labeling to fluorochromes, cytochromes, things that could be used with slow cytometry instead of isotopes. And there is no mention of that. So I just wondered if the group has given up on that because of the comfort level with isotopes? That's question number one.

And then question number two is the early

literature also indicated that the removal of platelets in the first hour is not necessarily linear and that time incremental measurements within the first hour would show that extrapolating back to time 0 from a count taken at time 1 hour is not necessarily linear also, and if there is a need to address that?

DR. ZUCK: Jim?

DR. AUBUCHON: I don't have any experience using nonisotopic labeling methods with either red cells or platelets. I know some labs have looked at it for red cells. I think you're correct that there is not a great deal of fear of using radioisotopic methods amongst the researchers, nor apparently at least amongst the subjects in my area. We have no great difficulty recruiting individuals to participate.

The amount of radiation exposure is really quite minimal to the point that the Radiation Safety Committee, when they have to review our protocols, has trouble breaking a sweat worrying about infusing 10 microcuries,

when other people are proposing infusing 5 millicuries for some other study.

The--I'm blocking on the second issue.

DR. ZUCK: I'm sorry?

DR. AUBUCHON: What was the second?

DR. FITZPATRICK: The linear aspect?

DR. AUBUCHON: Oh, linear aspect. Certainly with--thank you. Certainly with red cell studies, we have a lot of experience comparing single label recovery versus double label recovery as we call it. Single label recovery, where we back extrapolate from the points in the first half hour back to time zero, versus a double label recovery, where the time zero point is set based on the blood volume determined by a technician labeling of autologous fresh red cells.

And usually those two give almost exactly the same numbers. Occasionally, the recovery is slightly different, and inexplicably, sometimes the single label is a percentage point or two below the double label,

which is the opposite way from the theory. But they're really just about the same.

With platelet studies, however, it's a crap shoot as to what the curve is going to look like in the first hour. More often than not, there appears to be some sequestration for an hour or two, and there is an appearance of platelets, continued increasing appearance of platelets in circulation up until the 1-hour, 2-hour, 3-hour point.

So back extrapolation from the first few points on the platelet survival curve just won't work. I would think that the two options are either picking the highest point on the curve or back extrapolating using whatever mathematical model was selected from the COST program.

DR. ZUCK: Yes?

DR. SIMON: I wanted to use that question as kind of a segue and also some of the other comments made about our satisfaction with radiolabeled platelets over the years perhaps as a segue into where we're going. And

I think my understanding is that the FDA is concerned enough about the standards not only to have this conference, but possibly to be holding up approvals or holding up people starting protocols until we see where we want to go.

And given the fact that our protocols have performed reasonably well over the years, not to say that there shouldn't be a move towards an improvement, if the changes are not so radical, as you were suggesting to go to 28 days or 14 days, but are within closer to the realm of what's already been approved, I would hope that studies could go on in the way that they've been conducted in the past while we were perhaps working out a new protocol.

Because I was trying to think during the conference if there had been an instance that I knew of where a product had been approved based on radiolabeled platelet studies and had found in practice not to perform. And I really couldn't think of one. The only

case I knew was a product, a bag that didn't hold pH well. But it had nothing to do with the radiolabeled studies.

So it seems to me that the kinds of studies we've been doing, with the imperfections that we've noted, have served us fairly well, and I would hope that all progress toward new approvals wouldn't be stopped until we took this further step towards an improved protocol.

DR. HEATON: I've got a couple of comments that I'd like to make. First, on the issue of the immediate post transfusion recovery of stored platelets. We did a series of imaging studies as well as some post transfusion recovery studies. And in fact, if you look at Stein's slides 18 through 21, you'll see that not uncommonly immediately following transfusion, the platelets disappear for a while, and there's a gradual rise in the first few hours following transfusion as these platelets return to the circulation.

If you image those platelets and draw out selective areas of uptake, you can actually see the platelets wash into the spleen and then gradually come out of the spleen. So you can plot the recovery of platelets in the circulation. So you do have to think quite carefully which is your first point. And if you're not very careful, you'll drag your recoveries down if you overbias your decay plot to the first few hours following infusion.

So if we're going to go for a standardized protocol, I do believe an appropriate issue for follow-up study would be to look at the validity of those early data points.

And then second issue that Toby just talked to, I think radioisotope studies are really very important, indeed. The rest of the world has almost completely switched to buffy coat platelets. As far as I know, Europe switched to buffy coat platelets before the single first isotope study was performed. Keegan and I did

studies in 1987, I think, and we were one of only two studies that were done on a brand-new product, which was already being used to service 60 percent of the Dutch market at that time.

Now I just don't think we should allow new products to be introduced without definitive proof of efficacy. And the isotope efficacy has proven to be very robust in practice. We've not heard of toxicity or radiotoxicity, and it's proven to give us remarkably viable results that relate very well clinically.

So I'm a very strong supporter of having a defined standard and having a defined method.

DR. ZUCK: Jim AuBuchon prepared some questions that he thought it might be interesting to hear the panel's opinion on. So with Jim's permission, I'm going to read some of those and see if they prompt the panel's thoughts on issues.

Not his first and necessarily his most important issue, but one that's fairly discrete. Should the fresh

platelets of an aliquot of a unit taken from it shortly after collection, or should they be collected separately in doing comparative studies, storage studies?

This is the second question on page 2. You all have these, don't you? Anybody want to tackle that?

DR. DAVEY: Sure. I'll give it a first crack. I think this is maybe one of the easier ones for us to tackle.

DR. ZUCK: That's why I picked it.

DR. DAVEY: Everything I've heard from the experts that have presented today seem to indicate that, number one, having the subjects be their own control is critical. You really need to eliminate intersubject variabilities. That's key. And Scott's proposal also manages the very key problem, which he brought to our attention, of drift toward mediocrity.

So I think it's, in my view, a pretty clear recommendation that we should not use an absolute standard but, indeed, should use results of fresh

platelets from the same subject. I think we should be pretty clear on that one, personally.

DR. MOROFF: I agree with the concept of using a sample, a fresh platelet sample for a paired study, as we've been talking about today. I think that sample should be separately drawn. It should not be from the stored unit. It should be drawn at the time that--and we'll get into this later--but at the time of infusion of the stored sample. But it should be a separate sample, prepared like Ed Snyder was talking about.

DR. SLICHTER: Can I--

DR. ZUCK: Sure.

DR. SLICHTER: --comment? I mean, one of the more--I mean, I think all of us probably agree with what the FDA in concept is trying to do. They're trying to prevent this downward creep in the quality of the product by comparing 3-day to 5-day, then 5 days to 7 days, and 7 days--so we all agree with that.

The thing that I found disturbing today in the

data presentations was the fact that when--and the FDA has suggested, and I think not unreasonably so, that maybe the fresh should not be the fresh collected by the process that you're trying to evaluate. In other words, you have new apheresis machine, da-da, da-da, da-da. But then we have two different investigators, Jim AuBuchon and Ed Snyder, who have basically told us that if they collect an aliquot of whole blood, that the recovery, instead of being in the 70 percent range, which is where both Jim and I are getting our fresh recovery data from an apheresis machine, it's in the 60 percent range. And instead of having 7.5 day fresh survivals, which Jim and I are basically getting with apheresis platelets, they're getting 9 and 10 day.

So now we're talking about a comparison of a fresh product which doesn't give us the same answer. So if we start then thinking about a comparison between the fresh and the stored, we don't--for whatever reason, we don't have the same product. And in thinking back about

the old studies that we used to do when we were doing normal volunteers or even patient studies, the data that I showed you on that first slide, we got recoveries of about 60, in the 60 percent range, when we radiolabeled a unit of whole blood, PRP, which is what we did, and survivals that were 9, 9.5 days.

So I think we've got, to my mind, a real problem trying to establish a relative fresh-to-stored control when we get different answers for the fresh compared to what we get with the fresh collected by a different technique. And we discussed a little bit at the break why that was occurring to us. And I don't know that any of us understand that. But that makes for me a problem and makes me wonder whether, you know, the issue about whether we should establish some absolute criteria that the product should have to meet rather--I mean, I think clearly the data that I showed and that others have shown is that there is a difference between normal volunteers.

I think Larry has clearly shown us that if we

establish some kind of an absolute standard, the N is going to have to be bigger and substantially bigger than if we use each normal volunteer as his own control. But all of his data has been predicated on 5 to 7, or 1 to 7, or 1 to whatever, where the 1 and where the fresh and the stored were, in fact, collected by the same procedure or the same process.

And so, if we're now talking about using a different procedure for collecting the fresh compared to the stored, and if the data doesn't--isn't the same, how do we deal with that, I guess, is the issue?

DR. SIMON: Let me ask, Sherrill, so are you speaking against--are you speaking more or less for what I would call some modification of the status quo, where we compare to a set standard and expect the FDA to evaluate based on that? Or are you trying to seek a--or you want to try to use the individual as his or her own control?

DR. SLICHTER: Well, I'm--what I'm disturbed by,

Toby, is that I did not anticipate that both Jim and Ed would get for the "fresh control" that we may be interested in using a substantially different answer for the fresh control made from an aliquot of platelets, that that would be a substantially different answer than what we get with fresh apheresis platelets.

DR. SIMON: Or with the ones you did in the past, which were from a whole blood unit.

DR. SLICHTER: Well, and the whole blood unit data that we got, Toby, looks very similar to the aliquot data that Jim and Ed have been--have just presented.

MR. : I have a comment on that. Nobody will like it, but it's one--we've heard two different ways of doing these things. One is to, you know, take fresh platelets on the day of infusion. But there is a lot of charm in infusing the test and the control on the same day because then you've got the double label and matched pair.

You could, let's say you're studying 7-day

platelets. And you draw your platelets, and you do a fresh aliquot out of those platelets the way I think Dr. AuBuchon described. And then 7 days later, you do your 7-day platelets. But at that time, you also do another fresh platelet. So you've got one single label up front on the product that you're really testing. Then you've got fresh platelets, which can be labeled with a different isotope on day 7, when you're studying your 7-day platelets.

Then if your two controls show a difference because you don't have the same product, you would see it. In other words, that would confirm that you're testing your controls are the same, and therefore, that you would have a better comfort level that the--your tests on two different days were the same or that your fresh platelets drawn that day were the same as the product that you're actually testing because you tested those fresh platelets earlier.

DR. SLICHTER: If the product is an apheresis

product that you've collected on day 1, I can tell you right now that the fresh platelets that you collect 7 days later are not--are going to be a different population of platelets because of the fact that you've now got thrombopoietin stimulation of a new population of platelets. We've already done that study.

DR. ZUCK: Larry?

MR. DUMONT: If I could address the point about why prepare a standard from whole blood to prepared platelet? Because I might have been the one to suggest doing that.

Well, to prepare the standard, the control platelet from a venipuncture and prepare the platelets like Ed has done.

DR. SLICHTER: Yes. Right.

MR. DUMONT: Okay. I think I'm the one that suggested doing that. The reason I suggested it was because I was concerned about technology variation over time. And that if we were always comparing, let's say, a

Trima day 0 platelet to a Trima day 7 platelet, and then we've got, you know, Amicus version 43 compared fresh and Amicus version 43 day 7, that there are changes in that base technology over time. In fact, we've already seen that.

You just showed me some data about an hour ago, where there was another older technology that gave much lower platelet counts or platelet recoveries fresh than we're seeing now with our current technology.

So my concern was that if we have base technology varying, that then we have a continual varying standard and that it would be better to have a method to prepare a uniform standard that could survive all of our careers, if they're still doing this, for people to compare against no matter what the technology does. So that was the basis, I think, of the proposal.

DR. ZUCK: Yes?

DR. NESS: I would agree with Larry, but I think that the problem that we're seeing today is that, you

know, Jim did some and Ed did some. But the numbers, they admitted, were very small. So we don't really know how reproducible that technique is or how best to do a platelet standard from an aliquot of freshly drawn whole blood from a donor.

That seems to be a good goal to work towards, but I don't think we're there yet because we don't know how best to do it and whether it will not have the variability that we saw today based on small numbers.

DR. ZUCK: Gary?

DR. MOROFF: I think the key, the way I see it is--I think I'm seeing it the way you're seeing it, Larry. There should be a uniform standard for all platelet products to be compared to and factor out technology. And the way to do that is by using a whole blood sample for the fresh sample.

DR. ZUCK: Yes?

DR. LEITMAN: What Larry just described, Amicus version 43 and Trima version 10, is an upward creep. So

if you keep comparing day 1 of the new technology to day 5 or day 7 or new storage solution, you would be comparing it to a base that's better than it was 2, 3, 4, 5 years ago. So I don't have a problem with that.

I came into this meeting thinking that the study Jim published two years ago in Transfusion, where the control was the sample drawn--a sample taken from the same unit that was then held longer for the test case, would be the optimal control because it completely controls for the process of collection. And so, the variable, the test case would happen later.

It struck me, as we talked about this, as much more inconvenient for the donor because if you infuse both products on the test date, that's, you know, hour 1 and hour 3, and day 1, they're all the same day. And I asked Jim privately whether it was convenience for the donor that made him change midway through his last study, and he said no.

It was exactly what we're talking about now,

establishing the gold standard method of determining what the--what that standard is for recovery and survival. And that for all time, you know, it's the en-tube method from a small aliquot, and that will resist and stay the same across different labs and different techniques. I have to say that I'm feeling very equivocal. I can see the benefits of both.

What I absolutely feel is you can't set a number. FDA should not be setting a number and not asking investigators to establish their own control per study. Because, number one, it validates the laboratory. This is very complex. The analysis and the performance and the tubes, it's complex processing. And one wants to demonstrate that what one is doing in one's laboratory is the same as what's been published as the method that yields these results. So I think one has to have a control per study. It can't be like we have for red cells, a 75 percent recovery.

But past that, maybe it depends somewhat on the

test question you're asking. Is it a length of storage question, or is it a different instrument question? I'm not sure.

DR. ZUCK: It--I'm sorry. Toby?

DR. SIMON: I guess the question that Sherrill is--or what Sherrill is saying is that this may be good, but the gold standard that's been proposed appears to give us results that are at variance with what we'd anticipate.

DR. SLICHTER: That's exactly right.

DR. SIMON: Yes. So it makes one pause before accepting this particular gold standard, though the concept seems appropriate.

DR. SLICHTER: Well, what my data has shown is basically that over time the recovery stays pretty constant. What does start to decrease is the survival. So if you have a gold standard in which the recovery is less than what you can achieve with fresh apheresis platelets, then it becomes very easy because the

comparison is with a 60 percent recovery for the gold standard and a 75 percent recovery for the stored, or at least fresh for the apheresis.

And then the survival, however, is now 9.5 days or 9 days instead of 7.5, which is what we get for the apheresis. So that, you know, if you're expecting a comparison, then the gold standard has a longer survival, which is going to make a license of a stored product then more problematic with that as the standard. And conversely, the recovery is going to be an easier standard to meet. But at least based on my data, we don't need an easier standard for the recovery. We may need an easier standard for the survival.

DR. ZUCK: So you would say, Sherrill, that you wouldn't agree with Murphy's proposed--that he just published in Transfusion, proposed algorithm?

DR. SLICHTER: No. I haven't said that. I'm just--I'm surprised that--I mean, I think we all felt or at least I thought that the aliquot for the radiolabeled

would not give the data that we've heard today. And the fact that it has given the data that we've heard today makes me then concerned about using that as the gold standard because it is lower recovery and longer survival than what we get with a fresh aliquot from either a COBE or a Haemonetics machine.

DR. SNYDER: But that presupposes that the device you're evaluating works well, and you were looking at devices that have been licensed. What if you got a machine, as I think Jim mentioned early on this morning, that chews the platelets into smithereens? Well, you won't get decreased survival and prolonged recovery.

I get the sense of baby and bath water here. I think it would be a good idea, what we need to get coming out of this meeting is some evidence of where we can go forward with this. We have like six or seven companies, or five or six, that want to move forward with studies, and we're sitting here sort of dabbling about, you know, which is the best way to do this? Am I getting a little

metaphysical?

What I would to see--first of all, Jim's data and my data certainly in aggregate were not necessarily done exactly the same way. I didn't discuss my techniques with Jim. I used a two-inch crystal. Stein Holme just told me I shouldn't be using 1-, 2-, and 3-hour counts. I should be using 3-hour counts.

What I would think to put on the table might be to get a working group set up among the people who've presented today to come up with a common protocol for doing fresh studies all done the same way, have the three or so labs do--or four labs do a small number, so we can get at least a sample from all four different laboratories, hopefully all giving the same results.

Then if, Sherrill, the results show that we get the same 55 percent recovery and 10-day survival from all four labs using the same technique, then, you know, maybe we could have a problem. But the thing is that's going to take some time, and everyone here is waiting for us to

come up with some answers. Jaro was going to give us a talk. Slides are out there, and there's some missing numbers in one of this slides, which is what we're waiting for.

So I would hope that we would at least agree that we could get a small group of people putting together one common protocol that we could get going, with maybe under the BEST aegis or something to move quickly with it.

DR. ZUCK: Well, would it have to be two protocols? That's the question, isn't it?

DR. SNYDER: Well, I'm assuming that--I would rather use fresh.

DR. ZUCK: Do you sense in this room there's a complete consensus on the best way to do it?

DR. SNYDER: From my perspective, I would rather see fresh done either on the day of collection or the day of infusion. That I'm sort of open about. But I definitely think that the person should be their own

control, and it should be fresh, drawn as gently as possible and prepared however you do that.

I don't think it should be sampled from the main container. For me, my perspective.

DR. ZUCK: Yes?

DR. MOROFF: I agree with what Ed is saying about a standardized protocol. I think there was one that was drafted in 1986, or published in '86, and I think it's 20 years later, and I think that's definitely what's needed to form a work group.

I know there are some immediate questions. But I think a standardized protocol will not only help the companies, but it will help investigators and new investigators. So I think that should definitely be an output of this meeting, a group to put together a standardized protocol.

DR. ZUCK: Toby?

DR. SIMON: Your question about two protocols kind of intrigued me. And from a regulatory point of

view, I guess you're usually wanting comparison to a predicate device that's approved or something that's approved.

So if you had a device that was already approved and you wanted to extend by 2 days, then a fresh sample from that device would be appropriate. But if you had a totally new device, then you would need a new standard. So I don't know if--I guess that's not appealing to the other members.

[Laughter.]

DR. ZUCK: But a 7-day platelet isn't a new device. There was a 7-day platelet in the mid ‘80s. It got derailed not for reasons of efficacy, not for reasons of safety of the platelets, but for reasons of bacterial contamination. The reports kept rolling into the agency and, trust me, they did.

I saw a couple of hands up. Jim?

DR. AUBUCHON: I understand the desire to move forward with some type of guidance, and I understand from

Jaro that the agency is anxious to put out a new platelet guidance, which would include definition of how one would perform radiolabeled studies and analyze those data.

However, we've probably raised more questions than we've answered today. And even with a number of labs looking at these issues, we're probably looking at several years' worth of work before we come up with a final conclusion. I don't know if there is some interim that would be scientifically acceptable and regulatorily approvable until we get to that point. I don't think we're talking about--I would love it, but I don't think we're talking about seeing the licensure of 21-day platelets in the next year.

We're probably talking about primarily going from 5 days to 7 days, and the agency has already seen a couple applications in that regard and has accepted some amount of delta between 5- and 7-day platelets.

Perhaps the agency could quietly or without official notice perform some "back of the envelope"

calculations as to what Murphy's law would expect to yield in terms of an absolute recovery and absolute survival and use that as their criterion for the time being, even if it's expressed as a difference between 5- and 7-day platelets. It's going to take them a while to answer these questions.

DR. ZUCK: I would suspect the agency could handle that. I mean, we don't think about it much, but there are many different anticoagulants for red cells, and by no means does the survival data on all of them look exactly alike. They just aren't. There's a variance. So I think the agency can handle that. I'm sorry.

DR. DAVEY: Jim, I'm just surprised that your suggestion it's going to take so long to answer at least the one question that I see on the table, and that is defining what is a fresh platelet for a standard. What is the one standard that we can use for fresh platelets to measure all other new instruments, extension of

storage, et cetera, et cetera?

Now to Ed's point, if you and Ed and Stein and whoever can pull together, with your combined expertise, in one protocol and do some studies fairly quickly, I would think we could arrive at an answer shorter than years.

DR. AUBUCHON: Maybe we could take the "S" off of that. Make it a year.

DR. NESS: Sounds like an unfunded mandate.

DR. AUBUCHON: Or at least an unfunded study, unfortunately.

DR. ZUCK: Scott?

DR. MURPHY: I was going to ask Jim, well, which study would you do first? I mean, I think that the finding of the gold standard is now kind of hopelessly complicated by the fact that pheresis is giving higher recoveries than bagged pheresis platelets do.

And I think the--if you picked one, the 66 percent and the 50 percent are totally arbitrary. And if

we think that centrifuged platelets in tubes is rather like what we used to get with random donor platelets, we would have to decide whether we needed to up--raise the numbers of 66 percent and 50 percent somewhat higher because the control we're comparing it to now we realize is probably less than we get with pheresis platelets.

But if you use the pheresis platelets themselves, they put the stored product at a disadvantage because it has to do a lot better than supposing you had an improvement for random donor platelets and compare them to the control. They're at a great advantage versus pheresis platelets. So I still think that standardizing the control is very important, but I'm not quite sure I know how to do a study at $2,000 a pop.

DR. ZUCK: Jim?

DR. AUBUCHON: If I had my druthers and time and resources, the first study I would like to do is similar to what the gentleman proposed a few minutes ago--to answer the question about when we should be drawing the

fresh standard. If we could enroll subjects in a study where they would get four different reinfusions to begin with on the day of collection, we would perform an apheresis collection on day 0 and give them an aliquot of that unit back on day 0.

At the same time that we collected a manual fresh collection, 50 mLs, and reinfused that with chromium, indium double labeling, we would then have a comparison of fresh versus apheresis--I'm sorry. Manual fresh versus apheresis fresh.

We would have to let those radioisotopes disappear. Have the individual come back another time, collect an apheresis component from them. Reinfuse that on day 5, day 7, pick a number. And on that same day, draw a manual fresh and compare it. And then we might be able to begin to see just how important the issues are about thrombopoietin pushing more young platelets out of the marrow and whether the manual technique is somehow different than the apheresis technique for fresh

platelets.

Unfortunately, those four reinfusions, at least at our institution, unless we were very careful about how much we reinfused, would push someone over the limit in terms of what the Radiation Safety Committee would allow them to receive in a year. So it's problematic, and that's even before we tally up how much all these radioisotopes are going to cost.

DR. MURPHY: I think that's--if you say you're going to accept a control and you're worried about collecting a pheresis product on day 0 and then a fresh product on day 7, I think there we're working with a definite absence of adequate data. And that study of just the control on day 0, make apheresis collection and see what the results are on day 7 for the control, I think those studies should be done.

I don't know how to solve the problem with the study about the higher recoveries we're getting with pheresis as opposed to tubes. I mean, that's--and I'm

very--I'm a little invested in this idea, but I really am concerned about going back to comparing to day 5. I think that's not looking forward.

DR. ZUCK: Yes?

MR. : I tend to agree with Gary. I think it's perfectly acceptable to make a relative determination based to whole blood drive platelets. And the reason for that is, more than anything else, I would term those regular old platelets, those were not produced by any apheresis technology. And if those are acceptable as a transfusion product, anything better than that should be acceptable.

DR. VOSTAL: I think I would agree with that because I don't think we have a problem with having a standard, that most of the devices are better than the standard. And I kind of liked the fresh platelets approach because it's independent of any type of device, and it also standardizes the donor when he comes in the door, whether, you know, we can accept them as a subject

for the study or not. So, you know, that sounds reasonable to me, using fresh platelets.

Now whether there's a question if there is a thrombopoietin effect. I was wondering whether you could do the fresh platelet study up front, you know, before you collect the apheresis product? Would that get around, you know, having the later effects?

DR. MURPHY: Oh, I would think if you agree with using fresh platelets as the control and you accepted the tube technique that we've seen today, as I've just said, you could test the fresh platelets on day 0 and day 8 and just do a pheresis after you've done the fresh study and to see whether that impacts on day 8 or not.

I mean, I think we're assuming that that might happen, but I don't think we've got a lot of data to show that it really is a significant problem. And I think if you don't infuse the fresh at the same time that you infuse the test, I think you lose a lot of advantages. The donor is absolutely the same. Much easier study to

do technically.

But I guess what the group has to think about is supposing we called it a silver standard?

[Laughter.]

DR. MURPHY: But it is a standard.

DR. ZUCK: Can we go back to Ed Snyder's suggestion, if we're going to try to wring a work product out of this workshop? If, Ed, it were to come by that you put an expert group together to try to struggle with this--we're clearly not going to get a consensus today--who should sponsor that? Should this be a Heart/Lung endeavor? Should this be an FDA endeavor? How would you consider structuring it?

DR. SNYDER: Well, I think to get it from NHLBI is not going to happen. FDA is not going to happen. So it's either a bake sale and a car wash, or we turn to industry. And I hate to say that, but I don't know any other source of dollars, or we don't have the disposal in universities to come up with these kinds of dollars.

Someone can convince the Gates Foundation that this would be worthwhile funding, that would be another source. But realistically, it's the companies that one would turn to. I just put that on the table because that's what we do. And it should--that would be one possibility.

I think the group should be put together. I think Jim should probably be the lead, and I think there should be four labs--

[Laughter.]

DR. SNYDER: No, we'd support it. No, I just meant because he's sort of--he's taller and has a three-piece suit, and he looks--

DR. ZUCK: Anybody in the group want to comment?

DR. SIMON: Well, I was trying to remember 1986, was that industry supported? Although I guess we didn't do as much extensive work.

DR. MOROFF: We didn't do any studies. Basically, we--

DR. SIMON: Right. It was just travel.

DR. MOROFF: It was that document, Toby, as I remember it.

DR. SIMON: So it was a consensus document that came--

DR. MOROFF: It was a document, and there was three of us, Ed, you and I, and then headed up by Ed. And then it was reviewed, and we incorporated some of the comments, the way I remember it. But there were no studies. But I agree with what Ed is saying about there should be some studies performed.

What about this being under the FDA auspices, but with money coming from the industry? Is that something, Jaro, that you would think is viable? Money is going to be important, there is no doubt about that, to do this study. That's what you're referring to, Ed, right, when you say industry?

DR. SNYDER: Yes. Well, even if it comes from BEST, all roads lead to Rome. It still comes from

essentially companies.

DR. MOROFF: Still the companies.

DR. SNYDER: But it's in their--you know, help us help you kind of thing.

DR. MOROFF: Well, what about under the FDA auspices to get this going?

DR. SNYDER: Well, I think it's possible because this is how this workshop was funded. It was funded by the manufacturers, and the money was actually handled by the Hitchcock-Dartmouth Foundation. So it's possible, you know? So I think we can probably do that.

DR. SLICHTER: Can I just clarify what we're talking about here? So we're basically talking about potentially doing a study in which we do an apheresis collection. Before we do the apheresis collection, we collect an aliquot, and we infuse that aliquot on the day of the apheresis collection?

DR. ZUCK: Sherrill, what I heard is that was one potential suggestion how to structure it. But the

group we were talking about would be to look at those options and design a protocol that everybody could execute.

DR. SLICHTER: Well, I was just--

DR. ZUCK: I'm sorry. I didn't mean to interrupt you.

DR. SLICHTER: Yes. Okay. So, and then we store the platelets for whatever period of time we decide we're going to store the apheresis platelets. And then at the time that the apheresis platelets are infused, we then on that day, draw another aliquot to inject fresh.

So we've got two fresh aliquots, one at the time of collection of the platelets, another at the time of infusion of the stored platelets, and then we're going to look at the fresh baseline time 0 and the fresh at injection to try and address the question whether those two fresh have been influenced by the apheresis collection. And then that will help us then decide what should the fresh be?

Should it be so that we wouldn't continue to require two fresh for the storage but, rather, answer the question about the influence of that? And it seems to me that we're going to have to do some stored at different time intervals to see if that makes a difference. Because we know that the max effect of thrombopoietin is like 10 to 14 days.

So maybe if that's what we're talking about doing, I think there are enough manufacturers in the room that we ought to maybe get a sense from them about whether they would be interested in trying to fund studies to see what the standard should, in fact, be for the silver or gold standard, or what the fresh should be and when it should be collected?

I mean, if we're talking about a stored platelet concentrate and extending storage of a PRP platelet concentrate or even doing a buffy coat, then I would think that the fresh collection, in fact, could be done at the time of reinfusion of the stored because we

wouldn't expect that there would have been enough platelets collected with a single red cell collection to make a difference.

So the issue really is with the apheresis platelets and whether the fresh should be done at the time of the initial collection. And because at least in our hands, as opposed to I think what Andy has said, when we've done sequential studies in the same normal volunteer at 2- or 3-month interval, we basically get the same answer.

So that a fresh done on the time of collection, not reinjected at the same time as the stored, at least in our hands, doesn't seem to make as much difference because the donor tends to be, in our experience, really a pretty constant thing.

DR. ZUCK: Does any representative of the manufacturer want to answer Sherrill? Yes?

DR. ELFATH: I don't know if I want to answer, but I wanted to actually share with you our experience on

Baxter. Because we were trying to do, design our platelet studies for the 7-day storage of platelets, and we knew that this was coming. So although we were very excited about the comparing platelet quality to a fresh standard, we struggled a great deal in identifying what is the fresh standard and how to prepare it?

And we quickly realized that there are so many variabilities in preparing a fresh platelet collection. And after discussion with Stein Holme, who knows how tricky preparing fresh platelets is, we quickly realized that we need a very detailed protocol on how to prepare the fresh standard.

We believe that actually adopting the model proposed by Dr. Murphy is very good, and it's a step forward. But dealing with unequivocal or unclear protocols that may generate unequivocal data, or equivocal data and then we have problems with it, will cause complications for manufacturers to have their products cleared by the FDA.

So I think we need to or I urge the FDA, as well as the experts here, to work towards defining how to prepare fresh platelets to be used as a standard before we move on to this model.

I have a second point, actually, that previously or historically, when a manufacturer came up with data or study designs, they were--the first question that came back from the FDA is, "Show us the data." Now I think the situation has reversed. We have a model that we are asked to move to and use, and we are asking, "Where are the data?"

You know, who said that this preparation and this model actually, with the current state of knowledge, would lead to the kind of studies that we hope will clear products from the FDA? So that's my comment.

As far as the financial support of such studies, I think the contribution to put this workshop together was much less than what's being discussed now, that we will have to support studies. And I'm not sure that with

the current situation what that actually--at least I know that Baxter would struggle a great deal with this issue of supporting studies of such magnitude to adopt a new model to study platelets.

DR. ZUCK: Any other manufacturer want to comment?

MR. DUMONT: I think at Gambro, we would consider it. But you have to consider also that we're nearly half way into our fiscal year. You know, the budgets are long set. We don't have a lot of cash laying around for things like that. So it would probably be considered in the budget for next year. I couldn't, of course, promise how much money.

DR. ZUCK: Ed?

DR. SNYDER: Yes, when I suggested that we look at fresh, I wasn't thinking of doing with an apheresis, looking at thrombopoietin effects, and yada yada. I don't think it's appropriate for us to ask companies, personally, to go looking at pheresis, you know, effects

on thrombopoietin because that's really not--you know, we're asking them to fund things way beyond what I think we're trying to get at.

In addition, you'd have to pick a machine, or you'd have to validate it for every machine, and it just gets way out of hand. I was just looking at validating indium and chromium fresh platelet survival and recovery. And from what Dr. Vostal said, that if it turns out that it's a little less than an apheresis product and the apheresis product therefore looks better by comparison, so much the better.

And I think that would be minimizing whoever is going to be providing funding, which is going to be painful regardless, it would also be more chewable, and it could be done in a shorter period of time. And I think we could get consensus on that one simple thing, since Jim and I have contributed some, you know, the footings for which we can build a small, little house instead of a huge mansion.

You can get from point A to point B. You don't have to go in a gold Cadillac. You can drive in a little mini Cooper, and you still get there.

DR. ZUCK: Anybody on the panel want to comment?

DR. LEITMAN: I was just listening to these comments. Sometimes the excellent is the enemy of the good. And I was really sort of straddling the fence. I didn't really--couldn't quite figure out whether we should strive for maybe what is the excellent or the absolute standard and define why we think that's it. Maybe it's good enough to use a whole blood aliquot, a tube method that's standardized across every lab that will use it, accepting what we know about it.

And as has been mentioned many times, some of the problems may be small number, so it's not clear that there's that much of a difference. But you would generate more data and get more knowledge using that method. I don't have a problem with that.

DR. DAVEY: I would just support both what Ed

and Susan had said. I think we ought to keep it simple. There are some simple answers. We can get with a fairly targeted study a standard fresh platelet from a tube. I think maybe we can all move toward that. And the other studies can come later.

DR. MOROFF: I also agree, as I've said before, with that, and I agree with what Ed is saying. It should be a simple study dealing with fresh whole blood platelets.

DR. ZUCK: Yes?

DR. SNYDER: And I think it's possible to avoid the thrombopoietin effect by just simply waiting in the same donors for some period of time several months later and doing another study. In other words, do the study in two parts rather than at one time. Because in the same donor, they're going to react similarly. And most people believe that.

DR. SLICHTER: So you're suggesting doing the fresh study to validate the donor. And then at some

later point just doing--so that you're not talking about concurrent transfusions. But if you do what you've just said, then you could even use the same isotope?

DR. ZUCK: Jim?

DR. AUBUCHON: I like the concept of simple. But let's say we do a study where we determine amongst multiple labs that have a reasonable size N that the average recovery of a fresh manually prepared platelet preparation is 60 percent. It's not 75 percent. It's 60 percent.

Then how do we decide what multiplication factor to apply to that to create the standard that Scott has proposed? And I think Scott was thinking--I don't want to put words in your mouth here, Scott. But I think you were thinking that 70 percent was as good as you could get, and then two-thirds of 70 percent recovery was what we would be shooting for.

But if as good as we can get is 60 percent, then do we apply two-thirds to that, or how do we decide what

fraction to apply to that number? What if we come up with 50 percent for the manual technique?

DR. SLICHTER: Well, that was the point that I was struggling with is then how do you determine what the quality of the stored platelet should be if your fresh standard comes out to be less than your stored, which we hadn't anticipated?

DR. AUBUCHON: I was told when I was growing up here at NIH that the standard for red cell recovery, which at that point was 70 percent, and later it morphed to 75 percent, had been derived on the back of an envelope in an English pub one night because it was thought that a soldier could withstand immediate clearance of 30 percent of red cells units, hemoglobin, without causing hemoglobin error which, of course, would be very concerning and would stop things from moving forward.

So I can clearly see that that number is steeped in a lot of scientific tradition. I would hope that we

could come up with something that's a little bit better founded on data as we move into platelets.

DR. ZUCK: Yes?

DR. ELFATH: I have a suggestion. Actually, I think the first step, I think, is for us to agree on a protocol for preparing fresh--how to prepare the fresh standard. And I think we--at least I can see from Baxter's side, they could actually with each platelet study that gets done, this standard will be used to generate data that the FDA can accumulate and decide on, you know, compare it to different things, generate the data required to actually move onto this model.

So I think the first step is if we agree on a standard protocol for preparation of fresh platelets that everybody can use. I think that will be the first step to begin to generate the data that we are looking for.

DR. ZUCK: What I'm trying to get to is how do we get to agree on a common protocol? It doesn't seem like we're very close to that at this point, and that's

what I've been trying to probe a little bit.

DR. SLICHTER: Oh, I don't think that's true. I think that basically Ed and Jim have a method of radiolabeling in tubes. They've basically shown that whether they radiolabel with chromium or indium, they get the same answer, and that basically the absolute answer that they got is relatively the same.

So I don't think that we have a distance to go in terms of establishing the standard for the fresh platelets. I think that that's--I don't know if the rest of the people agree, but I think we're pretty much there. So I don't think that's an issue.

For me, the issue is what Jim has just addressed is once we know what the fresh is, then what are the criteria going to be for the comparison of the fresh to the stored to get licensing? And I think, from my standpoint, that's what the issue is.

DR. AEBERSOLD: Paul Aebersold, FDA.

DR. ZUCK: Yes?

sj

DR. AEBERSOLD: Again, an unpopular suggestion. There may not be one control. There are times, as been mentioned, when it may be very important that the control be the same platelets that are being tested. So if you want to say are 7-day apheresis platelets okay, you would maybe want the control to be a sample from the apheresis platelets on day 0, and so that you wouldn't be infusing them simultaneously.

In other situations, it may be critical that the infusions be on the same day because that's very important in testing. So there may be different circumstances where in one case the control should be infused on the same day. In other cases, it may be very important to have the control be of the same collection that's being tested.

And you know, you don't want to have a control, as Dr. Slichter said, that comes out below your stored platelets.

DR. SLICHTER: It just got more complicated.

DR. AEBERSOLD: You made it complicated.

DR. VOSTAL: One thing is--I mean, I think what we could do is take a cut at what Scott suggested and say it should be 66 percent of fresh, and start collecting data. And if the scenario comes up, as Jim suggested, that there will be 60 percent recovery instead of 70 percent recovery, we can always adjust the standard, just like it was--just like happened for red cells.

You know, they started out with 70 percent, and then it got pushed up to 75 percent. So you know, we could reevaluate after a year or two of data collection and see if we're doing the appropriate thing.

DR. AEBERSOLD: But Dr. Vostal, what are the companies supposed to do on May 4th? Are they supposed to wait a year? Many of them are waiting for guidance, and I only bring that up because they did fund this with the hope that they would be led into the promised land somewhere. So if I could just bring that to the table on their behalf.

DR. VOSTAL: Well, I think--

DR. SLICHTER: Can I just say one thing before you do, and that is that, I mean, I think it's not only the manufacturers, but the blood centers. Because we are now being required to do bacterial testing on platelet concentrates, and that has now reduced the effective storage time that we have from 5 days to 4 days, and we are really in a major state of disrepair.

So I think all of us feel an incredible sense of urgency to, in fact, do studies to extend storage and really, you know, look to the FDA to help us help patients because we're really--we're really suffering right now, I would say. And so, all of us want to have extended platelet storage, and so I think the onus is on those of us in this room to really come up with a way to proceed out of this consensus conference.

DR. VOSTAL: Well, I think that's why we are here. And so, how about if we propose that we set up the studies using fresh platelets as a standard, and the

collection will be done at the time that the donor comes in? So you get a value up front. And then you can do the apheresis after that and do a study on the apheresis product, you know, 5 days, 7 days later and then make the comparison.

I know it's not the ideal way of doing it, but it gets around the thrombopoietin effect. And you probably--it's a compromise, but you'll probably get some kind of a validation of the product.

DR. ZUCK: Is this to provide a model, if you will, for industry to proceed? Could you--currently, the numbers that you've asked for has been very high, and try to get a study group together and get the funding for it is not going to be trivial.

DR. VOSTAL: I'm--

DR. SLICHTER: But I think is what--I think what Dean Elfath has said is that if we can agree on some kind of approach here today, which I think what Dr. Vostal has just said is a reasonable way to proceed, then industry

can presume that that's what's going to be required by the FDA in order to get licensing. And they can then start their licensing studies, and we can start to accumulate the data and see what it looks like. So that nobody really has to, if you will, provide money to just do fresh studies.

We can--I mean, I think there still needs to be, you know, maybe a group that Ed was discussing that would really sit down, "This is the protocol. This is how you draw the sample. This is how you label the sample. This is how you infuse the sample," and da-da, da-da. And agree on a common protocol, which I think we can do.

And then industry then is said, "Okay, this is the fresh. This is the stored." And then is the FDA, Jaro, prepared to--I mean, then if they get 66 percent of the fresh for recovery and 50 percent for survival, is that the criteria that's going to be used for licensing now with the expectation that maybe in the future those numbers will change?

DR. VOSTAL: I think that is a reasonable approach. The only question I have in that is whether survival should be 66 percent as well? And I was hoping that the panel would help me sort that out a little bit.

DR. ZUCK: The red light on, that same answer for Jim?

DR. HEATON: Yes, I'd like to make a couple of comments relative to all the discussion about the apheresis. In effect, the observation that apheresis has slightly higher recovery is being used to undermine the standard that's proposed. The reality is that the standard that we should compare platelet products to is those platelets which you collect fresh after the donor at the time.

It's true that if you pherese a donor, you may be able to select a younger population of platelets that has a higher recovery. That's like selecting neocytes out of red cells, for example. But I don't believe that that should encourage the panel or the FDA to think that

that's now the new standard. The standard should be what is currently in the donor at the time the donor donates.

And so, if you can do a double label control study and show what fresh platelet recovery is from a unit of blood that's collected and the platelets separated, and that your test material is equal to 66 percent or better than that, then you've met that standard. I certainly think it's very attractive that with pheresis, you could get 120 percent of that. I think that's wonderful.

But I don't think that you should allow that to undermine the standard that is proposed today, and I certainly don't think that manufacturers want to spend millions of dollars supporting a trial to find out whether 120 percent standard is what's desirable or a good idea until the FDA has established what its basic standard is. And the basic standard should be that which is in the donor now.

DR. ZUCK; Yes?

DR. DAVEY: Just a couple comments. Number one, I appreciate, Jaro, the FDA's willingness to really move ahead on this. It's really very, very encouraging.

And from what Sherrill and others have said, I think that it makes a lot of sense for Ed and Jim and others to get together and develop a protocol, and let's skip the testing if it's going to take a year or two and a lot of money. We can maybe provide that for you.

In terms of the 50 percent, I'm not able to comment on that. I'd like to hear from Scott again why he suggested 50 percent, so that we can be a little clearer on that for--

DR. MURPHY: You mean 50 percent for the mean cell life? The reasoning there was that in practice in clinical medicine with prophylactic transfusion, platelets never have much more than a 2- or 3-day lifespan. You have to transfuse them again. So I thought that--but the first of all, for surgical patients, the increment you get is very important, and we

know also that the mean cell life--the time to next transfusion is extended by a high yield of the transfusion.

So I thought that we could use a less rigorous standard for the mean cell life than for recovery because retaining the capacity to live 8 days is not going to be relevant in the patient. Now, you know, I can see Jaro's concern, though, because it is true that the two lesions--the lesion of being sick and thrombocytopenic and the lesion of storage may somehow or other be additive. I mean, that seems to me to be unlikely, but it's something you could think about.

I think the whole thing about picking these two fractions, two-thirds and one-half, is really getting us into difficulty today because we've suddenly realized that what's probably going to be the gold standard is a silver standard, and perhaps we should ask for higher percentages of that silver standard. In other words, maybe even two-thirds is too low. But I think it's going

to be impossible to know that until we get some experience.

MR. : I'd like to bring maybe some more simplicity into the discussion with the suggestion that perhaps instead of having two measurements, we have one. And that be the area under the curve and perhaps two-thirds of the area under the curve might serve as a single standard of fresh platelets?

DR. HOLME: Could I also comment on that? Scott, let's say if you have a case where the percent recovery is 95 percent while the survival is 49 percent of the standard, would that be a good product? That's what I'm saying, I think the area below the survival curve would be a best--better measurement because it takes in consideration both the recovery and the survival at the same time.

Because like I said, you can have cases where you have--you have very good recovery, let's say 95 percent of the fresh, and then you have 49 or 48 percent

survival. Do you then make a decision this is not an acceptable product because it doesn't meet the survival criteria?

On the other hand, it more surpasses the criteria for the recovery. However, by taking the area below the survival curve, you consider both of them at the same time.

DR. MURPHY: Well, I'll just answer that the same way I've answered it in the past, and that is that that gives equal weight to the survival as opposed to the recovery. And as I said, I think the survival is not as important as the recovery.

DR. HOLME: Yes, but this takes into consideration both, the area below the survival curve.

DR. SLICHTER: Yes, but I think what he's saying, Stein, and I actually agree with, is that the recovery is the most important characteristic for both thrombocytopenic patients and for the surgery patient. So we give probably 50 to 80 percent of the transfusions

as prophylaxis--

DR. HOLME: But that is what I'm talking about. You have a product that has 95 percent of the recovery of the fresh, but it doesn't meet survival criteria.

DR. SLICHTER: Well, yes. But what if you have the converse, Stein, where you're at 30 percent recovery, but at 105 percent survival? We don't need 105 percent survival.

DR. HOLME: There could be cases where there must be some judgment call. It's going to be very difficult to look at those two separately because we could have cases where either barely meet none of the criteria. You can have cases where you have, let's say, 67 percent recovery and 51 percent survival.

Is this a better product than when you have 95 percent recovery, but 49 percent survival? Which the latter one didn't meet the criteria, even though it had far better recovery than the first product.

DR. MURPHY: Well, you know, that's the problem

with drawing a line in the sand is that every once in a while your result is going to be a little bit below the line.

DR. HOLME: Exactly. That's what--

DR. MURPHY: To get away from that problem, you don't really draw any line in the sand at all. And we're back to where we are now.

DR. HOLME: That's why I'm saying I think the area below the survival curve is a better way because it takes into consideration both survival as well as recovery.

DR. MOROFF: I think we need data to show that, Stein, from multiple laboratories. We've been using recovery and lifespan as the criteria for many, many years. And I think at this point, we should continue to do that but, at the same time, start calculating the area under the curve and see how the data looks over the next year.

DR. SIMON: One other question, Scott. My

impression is that most of the platelet products that have been approved have been well more than 50 percent. Is that correct?

DR. MURPHY: Fifty percent mean cell life?

DR. SIMON: Survival.

DR. MURPHY: Yes. Yes.

DR. SIMON: Lifespan. So one could probably go a little bit higher without--it's arbitrary, but somewhere in the 50 to 60 percent range?

DR. MURPHY: I'm not sure that everything we have licensed today would have two-thirds of the mean cell life of fresh platelets. That's my impression from reading the literature.

DR. SIMON: So it should be somewhat lower on the survival?

DR. MURPHY: That's my opinion, and I haven't heard an argument yet that makes me want to change.

DR. LEITMAN: This 50 percent survival is not of--it's of a fresh standard. So it's even lower because

the standard is already--well, it's a number of hours or days, and this is 50 percent of that. So it's not 50 percent of what one infused. It's 50 percent of a number that exists from a fresh aliquot.

I saw no--this is a very soft number. It's a moving target. It's arbitrary. So I guess I second Gary's motion that we--that more data be collected.

As I was listening to the presentations, I found the numerical survival to be another soft number. So it--the area under the curve was conceptually easier for me to grasp as having clinical significance than this number that's a tangent drawn to the first couple of points, when the first couple of points sometimes were difficult to draw a tangent to.

DR. MURPHY: But I think Stein presented some data today that, at the end of the day, it doesn't make much difference whether you do a T 1/2 or mean cell life or--

DR. SLICHTER: Yes. The programs, even though

he's got his survival curve going like this and his tangent to the line like this, even if he uses the area under the curve, the program calculates the area under the curve as being the area under the tangent. It does not?

DR. HEATON: It calculates it as the total area.

DR. SLICHTER: Okay. All right.

DR. LEITMAN: The only problem with the area under the curve is it weights the recovery again. So the recovery is weighted up front as the recovery, and it goes--

DR. HOLME: Absolutely.

DR. LEITMAN: But that's a very clinically relevant number.

DR. HEATON: Yes, it is. I mean, if you have 10 percent increase in recovery, you get a much bigger increase in the area under the curve than the same commensurate survival.

DR. SLICHTER: But I would agree with Gary's

approach, since we've never used that as a standard, the area under the curve, I think to continue to use recovery and survival measurements and then generate the data to see how that looks is the most reasonable way to proceed at this time.

DR. HOLME: There's a lot of data out there already. In Norfolk, they probably collected more than 400, their radiolabeling studies, with platelets stored under a variety of conditions. They can easily count the area below the survival curve and compare that to the numerical expected lifespan--

DR. MURPHY: Well, I would be--

DR. HOLME: --or the recovery.

DR. MURPHY: I would be inclined to try to solve one problem at a time, and I think we have a big problem in the relationship of the control which seems to be lower than we would like, and I think to do more than try to cope with that is more than is practically possible.

MR. : I just want to make one more

comment. From a regulatory point of view, the area under the curve is very analogous to me to viability of a drug.

DR. AUBUCHON: A possible compromise for which I have no data whatsoever--

[Laughter.]

DR. AUBUCHON: --would be to look at the area under the curve, but for only the first few days. If we think that a platelet transfusion is--whether administered to a thrombocytopenic patient or to a surgical patient really is only going to provide therapeutic benefit for 48 or 72 hours, perform the integral under the curve only to, say, 72 hours.

At that point, then prolonging the survival to some unrealistic number, 15 days, is not going to give a platelet unit any particular--a platelet collection method any particular advantage. The question is what's the bang that can be delivered in the first few days?

DR. ZUCK: There's a red light in the nosebleed section.

MS. WHITLEY: Yes. Pam Whitley from Red Cross.

From the lab standpoint, I'd like to go back to the study design, where we're talking about a fresh sample on day 0 of collection. It might be the better sample. But however, logistically, from the lab standpoint, it's really better to have it on the day of infusion because your donor is going to have--I'm thinking about the donor is going to have a lot of blood sticks if he's collected on day 0, then a fresh sample. And then an infusion on day 0, and then sampling up until the infusion, and then more sampling.

Your isotopes, you don't have a tight control on your double label. In fact, it isn't really a double label. So your infusions are separate days. Your blood collections go on for longer than 10 days for sure. So I think that I would like to propose a fresh blood sample on the day of infusion, from the lab standpoint and the donor standpoint.

DR. MURPHY: The other aspect to that is what is

the effect of undergoing a pheresis just after you've received labeled platelets? All right? You give them after the pheresis, but then the patient, the donor is recovering and changing during the first few days of the survival.

I mean, I agree with Pam. I guess that's what I'm saying. But I think there are enormous advantages. And as I've said, if we're ever going to do a study, that's something we could actually study to know how much of a difference it makes, whether it's day 0 or day of infusion.

DR. ZUCK: Jim?

DR. AUBUCHON: The only disadvantage I can see from collecting the fresh sample on the day of reinfusion of the test platelets is the potential effect of thrombopoietin. And since it looks like--I can't say for certain--but since it looks like our manual collection method is giving recoveries that are a little bit lower, perhaps if there is a thrombopoietin effect, that

will--it will counteract that to some degree, and it won't be quite so prominent.

I'm not expecting this is going to make 10, 15, 20 percent difference. You know, a few percentage points will probably get lost in the shuffle of all the data.

DR. MURPHY: I think that's true is that's what will happen.

DR. MOROFF: I also agree that the sample, the fresh sample should be injected on the same day as the stored sample. I think that's a very important point that Pam is reiterating from a practical point of view.

DR. ZUCK: Jaro, has the agency gotten any help from this?

DR. VOSTAL: I think so. I think this last proposal is what we've really wanted to see in the first place. And that was, you know, a dual injection on the same day of the test of the product itself because we do care about those donors and volunteers, and sticking them twice with the same thing is really--I mean, I don't

think I'd want to go through that. So I think that's a concern.

I think we're actually making some progress, and a lot of these issues are getting discussed, and we're getting a lot of feedback. It seems to me that we're not going to have the perfect answer. It's going to be some kind of a compromise, and it's got to be more or less arbitrary number.

But I think what we should do is, you know, push forward and try to come up with some kind of a compromise so, as Dr. Snyder says, you know, the companies can get started, and more data will start coming in.

DR. SNYDER: If I can summarize what I think I've heard? We want to compare fresh--we want to use fresh platelets as the standard, but we're not going to look at indium versus chromium fresh in vacuo. We're going to collect in a randomized fashion fresh platelets and inject them on the day of the--at the end of storage, whether it's an apheresis product or it's a whole blood

collection that's made--a whole blood platelet that's made into a concentrate.

And we're going to label the fresh platelet with one isotope and the pheresis or the stored product with the other isotope on the same day. Infuse and collect data as we go along, looking at some area under the curves.

The companies would sponsor the studies, and therefore, we'd be able to get the data on the fresh so that we'd be able to get the study completed. And the data would be generated for fresh indium and fresh chromium by virtue of basically doing the studies.

So if I've heard this right, what Jim and I have presented in its de minimis fashion is considered enough to move forward, that there's enough consistency that people don't think we're going to be, you know, doing a "Thelma and Louise" off a cliff here, wasting a lot of money, and that the area under the curve will be looked at.

And then as things move along, we'll have--we'll use recovery and survival for now. We could fine-tune it. I mean, if you have the data, you can always look at it and massage it after the data is collected.

The key thing is the protocols would be different among the different companies, but the technique used for labeling will be standardized by this group that's going to get together to standardize it for fresh platelets, for an apheresis platelet, and a whole blood platelet.

So there would be three protocols that everybody would use in different fashions, depending on the product the manufacturer wants to use. Does that seem what everyone else is hearing?

DR. ZUCK: A fair summary on my part.

MS. ROSE: Leslie Rose, Haemonetics.

Also I just wanted to see if I could just add to that that it would be great if we could get Jim and Ed together, and whoever else is going to work on this, to

not only talk about exactly how those labelings are going to be done, but also exactly when the sampling should be done. The sampling time points.

I mean, whether it's 1 hour or, you know, 10 minutes, 30 minutes. One hour, 3 hour, or is it just a 3-hour, day 1, 2, up to day 7, or is it up to day 10? And what kind of corrections are going to be used?

DR. MOROFF: That should definitely be part of a standardized protocol, Leslie, to have the sampling times spelled out in detail.

DR. ZUCK: Jim, I think we answered most of the questions one way or another that were woven in your tome here.

DR. SIMON: Tom, there's one more that--I noticed the one that didn't get is this one. "Platelet stored in the test system be labeled and reinfused on the last day of the intended storage period or on the day after the last day." Question 4.

And I think in some of our hallway conversations

there's--I'll start off by saying I thought I heard a consensus that we would want to keep it the way it is and do it on the last day. So if it's 5-day storage, we do it on day 5. Seven-day storage on day 7, 8, or 6. I don't know if there's objections.

DR. MOROFF: I think there was a consensus, Toby. I agree with you.

DR. SIMON: The other questions I think we did hit.

DR. LEITMAN: I have a comment that's not on these questions here. I understand there may be some data already submitted to the FDA on length of storage in studies that were paired and controlled. And if the FDA is considering those, I certainly would hope they would not hold them up, hold up that consideration while these studies are organized because there may be a pressing need to consider that data.

DR. NESS: I just wanted to make one point. In Scott's formulation where he says survival may not be as

important as recovery, which in general I agree with.

Since I don't see any pediatricians around here who would ordinarily make this comment, I guess I will make the comment that they would ordinarily make. Which is, in some cases, when you're thinking, you know, you don't need an interval of only a couple of days for an adult patient, particularly for pediatric outpatients with oncologic diseases, if the survival stays up, one infusion a week can often be enough to keep them out of the hospital for a long period of time.

So I just think in our thinking we need to consider that not everybody is the sort of every 2-day infusion patient in the acute care setting and that, particularly for kids, the survival may be more important, which is one of the reasons why I like the area under the curve, which I thought might be important for that group.

DR. ZUCK: Yes?

DR. SNYDER: We just sort of all decided that we

would stop at day 5 as a consensus. But I don't know if Dr. Vostal wants the FDA to be consensed in that regard. Are you willing to consider this? Or we think it's a great idea but, you know, you're the dude.

DR. VOSTAL: You mean stop--you mean calculate the area under the curve for the first 3 days or 4 days or--

DR. SNYDER: No, I mean not having to do survivals on day 6 to validate a day 5, or do it on day 8 to validate a day 7 storage. We just sort of whizzed that past you when you were--

DR. VOSTAL: Oh, okay. Right.

DR. SNYDER: You were noticeably silent.

DR. VOSTAL: Well, to us, I think the way it's done currently, you do it on the day of expiration. But you do it, as Jim pointed out, you do it during the day, and there is, you know, 12 hours of additional time until midnight of that day that you have to sort of extrapolate the data that you get from that experiment to confirm

that those platelets will work at midnight.

So we think that it's actually better or a more conservative approach is to do it on the following day so you would get--make sure that the time--the full storage time is covered by the experiment.

DR. MOROFF: One problem with that, Jaro, is that all the data that has been achieved in the last 20, 30 years has been collected on the day of storage. Day of expiration, I'm sorry.

DR. SLICHTER: And platelets don't--I mean, it's kind of like talking to the house staff, you know, and you tell them platelets don't go from something to nothing. I mean, you know, there is a continuum.

So I don't have any concern that that 12-hour period, I'm now going to have just garbage because I think there's no data to suggest that things just, you know, that there's a drop off the cliff kind of thing. That if you go that 12 hours--plus which I think Jim has already pointed out that, you know, the manufacturer is

not stupid. And they will just collect the product in the afternoon of one day and infuse it in the morning, you know?

So I would hope, Jaro, that the FDA would continue to allow us to have it that if we document 5 days, that even though we may not have documented that additional 12 hours, that I don't think we're putting anybody at risk. And I think we should not expect to have a 6-day answer for a 5-day licensed product. I think it should be 5 days.

DR. VOSTAL: But I think just the fact that we're talking about this means that there is concern that those products will not make it to day 6. You know, that they will fail somewhere in between?

DR. SLICHTER: Yes. But, Jaro, they're not going to go off the cliff. I mean, I think they may not be--I just don't think--that's not going to happen, and I don't think we should make that requirement.

DR. SNYDER: We were talking about it because

you raised the question, basically. And I think we were a little surprised because if the pH was 6.2 on all these products at midnight on day 5 or something, you could say, "Well, yes, maybe it's going to do something." But the pHs are good, and I'm using pH as my sort of in vitro evaluation.

I have no problem extending it, and I understand you can really, you know, get down to nitty-gritties, but I think if it's for the--I just think it's much more difficult to go especially out to day 8, if you want to get day 7.

I agree with Sherrill. I don't think it's necessary. But somehow I think my comments are too weak to convince you, but other than just I think it's a really good idea not to do it.

DR. AUBUCHON: If I could offer some logistic reason not to do what has been suggested and also a little bit of data? The logistic reason I would offer for not testing on the day after the last day of

licensure would be the fact that for the individuals for whom it is probably most important to have a good recovery and good survival would be the thrombocytopenic patient. And most of these patients--not all, but most of them--get transfused when the morning platelet count comes back and it's too low.

And we've looked at when we release platelets from our transfusion service, and it's between 10:00 and 1:00, and that's because that's when the house staff sees the morning platelet count. So what happens to the platelets at midnight is really immaterial.

About the only time we transfuse at midnight is when we have a ruptured AAA come in in the middle of the night, and then we're usually putting enough of everything into them that one or two percentage point different in recovery or survival isn't going to make any real difference.

In terms of data to offer in opposition to this proposal would be our experience in transfusing on day 6

and day 7 in cases of medical emergency, when we have no other platelets, mind you, and also looking at pH on day 8. These are for apheresis platelets. We published the CCIs on day 6, and day 7 are just fine. And even waiting until the morning of day 8 to check pHs, in 98 percent of cases, they still remain greater than 6.2. So, and they continue to swirl.

I'm not concerned. I agree with Sherrill. They don't drop off a cliff at the stroke of midnight. They don't turn into pumpkins.

DR. SIMON: One other practical point. A lot of the apheresis procedures and blood donations will be done in the afternoon and the evening hours also. So you will have fewer hours of storage.

DR. VOSTAL: Is the panel in agreement that this is not the right way of doing it?

DR. LEITMAN: We could go through this. I think that day 5 is fine, for all the reasons mentioned.

DR. VOSTAL: It's a miracle.

[Laughter.]

DR. ZUCK: Any additional comments or questions from participants?

[No response.]

DR. ZUCK: Panel, on behalf of everyone, we thank you. And for a closing comment, Dr. Jaro Vostal from the FDA.

DR. VOSTAL: All right. Thank you very much. We had a very exciting day, and my job here is to finish up with the acknowledgments and maybe some FDA perspectives.

So in case I state an FDA perspective and I have to make a quick exit, I decided to start with the acknowledgments.

[Laughter.]

DR. VOSTAL: So one really wonderful thing about this workshop is that it's been put together by a collaborative effort, and that's because the industry was able to provide the funding. Academia was able to

provide the data, and the government is benefiting from all of these efforts. So we really appreciate this.

And to show our appreciation, we'd like to point out the companies that actually did sponsor the workshop. The money that was donated went through the Hitchcock Foundation at Dartmouth-Hitchcock Medical Center, and it was then handled to pay out the travel expenses and additional expenses such as cookies.

So we appreciate that the companies such as Baxter, Cerus, Gambro, Pall/Medsep, and Terumo were able to help us put this on.

Also I'd like to thank the steering committee that was able to organize this workshop, and this included Jim AuBuchon, Scott Murphy, Edward Snyder, Salim Haddad, and myself. And also we benefited from the input of the BEST committee. That's the Biomedical Excellence for Safer Transfusion Working Party of the ISBT.

And the BEST actually spent a lot of time discussing this and debating this, and I think a lot of

the data that came out--that was presented today came out of those discussions.

Okay. So now moving on to where we stand. The FDA is committed to a gold standard, which may now be the silver standard or the tarnished gold standard.

[Laughter.]

DR. VOSTAL: We think that the regulatory review process will become more uniform and less subjective if we have a standard to compare--uniform standard that we can compare these products to. Specifically, common research protocols will minimize the differences in methodology and improve the interlaboratory compatibility.

And I think this is important because we--there are about four or five labs that do these experiments, and it would be great if they were done in a uniform, standard manner so we could have a uniform comparison between product to product.

And has been mentioned many times already, a

fixed standard can maintain the same level of platelet product quality over time. And this is important to us because we did approve a 7-day platelet that was a little bit worse than the 5-day platelet. And you know, it's just the writing on the wall. It could be additional products coming that will take advantage of that lower quality, and they could slip in under, you know, bring in additional lower quality products.

Especially if you start to think about combining processes that could have additive damage to the platelet, such as 7-day platelets or a 9-day platelet that's been pathogen reduced. You know, you could probably have a significant damage, so we would end up with a transfusion product that wouldn't be really helpful clinically.

And finally, the uniform protocols and accepted standards will facilitate product development in a competitive, but a fair environment. And I think this is important for the companies who are thinking about

planning their studies and planning their product releases. And you know, it's important that they have a definite goal to meet and a standardized way to get there.

So what's our plan for implementation of the standard? Well, based on this discussion that we heard today, we will recommend that future studies are done using fresh platelets as the control.

Now the acceptance criteria that we discussed here today, basically, it will constitute the current FDA thinking. And we still have to go and get the concurrence of the Blood Products Advisory Committee, and that will happen this year in July, this coming up summer in July.

So we've already talked to the BPAC previously in December, where we announced where the workshop was going to take place. Now we're going to come back and say, "The workshop took place. This is the results of the workshop." And we'll ask them if they agree with our

conclusion.

And finally, we plan to incorporate this discussion of these protocols and the criteria into the revised 1999 platelet testing guidance, which we hope to release in the near future.

This is the most difficult page. So our current thinking here is to follow the protocol designs that were discussed here today. That may be easier said than done, and I think the idea of having a working group try to straighten these out is very good.

Until that happens, I would suggest that the work performed by Jim and Ed should be followed, and I think that it would be appropriate to just, you know, collect more data in that area. And based on the additional data, you know, that's going to be done as a part of the validation studies for the products, I think we'll be able to, you know, a year from now or six months from now, be able to see whether we are on the right track.

Now what is the right study size for these studies? Well, we heard today that it really depends on the statistical approach. In the past, we've requested that these studies utilize about 20 donors, 20 volunteers, and that they be done at two separate studies. We like to see that number come out to be about same. I don't know if we'd be much comfortable with a much smaller number, like if it was 10 or a dozen.

I mean, I think if the statistical basis is there, we would learn to live with it. But right now, our comfort level would be to see about 20 different donors participating in these studies.

Now what's the acceptance criteria? Well, I think, based on the discussion we heard, that you can go with 66 percent of the fresh recovery. And since I didn't hear a lot of dissension about the 50 percent survival time that Scott proposed, I think we would agree to go along with that, and with the reservation that as the data comes in, we may be able to reassess that

sometime in the future.

So I think we would recommend that the criteria would be, you know, 66 percent of recovery and 50 percent of survival of fresh platelets. And I think the approach when to use the fresh platelets would be at the end of storage of the collected product. You know, do a simultaneous infusion so you have a double labeled, and you only have one time where you have to collect from the donor. So it would be concurrent infusion of the radiolabeled products.

Now is failure an option? Well, what happens when a platelet product fails to meet the criteria? And fortunately, this may not be the end of the road. There could alternative merits that the platelet products have, such as pathogen reduction or extended shelf life, and these could be licensed if their benefits outweigh their shortcomings.

Products that do not meet criteria can still be licensed but will need to have labeling that indicates

how they differ from platelets. And we actually may need to call these something other than "platelets classic," to borrow a phrase from our soft drink industry.

Now each of these products will have to be considered on a case-by-case basis, as we usually do when we deal with novel products.

And finally, what are the future prospects, or what is our wish list? Well, I think we would encourage that there be a continued search for the Holy Grail in the platelet field. And this has been, for the last 20, 30 years, tests--either in vitro tests or animal test that could replace human in vivo or radiolabeling studies.

In addition, I think we should continue a search for alternative cell labeling methods that could be used to replace radioactivity. And finally, to find a synthetic substitute to natural platelets that will have a long shelf life, be pathogen free, and be non-alloimmunogenic.

sj

So thank you very much.

[Applause.]

[Whereupon, at 4:44 p.m., the meeting was concluded.]

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