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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

WORKSHOP:

SAFETY AND EFFICACY OF METHODS FOR REDUCING PATHOGENS IN CELLULAR BLOOD PRODUCTS USED IN TRANSFUSION

VOLUME II

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Jack Masur Auditorium
Building 10, Clinical Center
National Institutes of Health
Bethesda, Maryland 20892

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Washington, D.C. 20003-2802
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P A R T I C I P A N T S

Speaker and Moderator List

James AuBuchon, M.D.
Celso Bianco, M.D.
M.A. Blajchman, M.D.
Mark E. Brecher, M.D.
Michael P. Busch, P.D., Ph.D.
Richard D. Diamond, M.D. MPA
Roger Y. Dodd, Ph.D.
Sunny Dzik
Jay S. Epstein, M.D.
Mahmood Farshid, Ph.D.
Hanan Ghantous, Ph.D., DABT
Mindy Goldman, M.D.
Margarethe Heiden M.D.
Sukza Hwangbo
Harvey Klein, M.D.
Matthew Keuhnert, M.D.
David A. Leiby, Ph. D.
Gary Moroff, Ph.D.
Albert E. Munson, M.D.
Scott Murphy, M.D.
Paul M. Ness, M.D.
Anita M. O'Connor, Ph.D.
Betsy Poindexter
Toby L. Simon, M.D.
Sherrill J. Slichter, M.D.
Edward Snyder, M.D.
Suzanne R. Thornton, Ph.D.
Jaroslav Vostal, M.D., Ph.D.
Steven Wagner
Mark Weinstein, Ph.D.
Hannelore Willkommen
Roslyn Ymtovian, M.D.

C O N T E N T S

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C O N T E N T S (Continued)

P R O C E E D I N G S

SESSION III: DECONTAMINATION PROCESS EFFECTS ON CELLULAR TRANSFUSION PRODUCTS

DR. VOSTAL: We Will get started with the first session, that will be evaluation of toxicity to platelets.

The moderator for this session, and the first speaker will be Dr. Scott Murphy. It's fitting that Dr. Murphy's here with us to help us evaluate platelets because he has spent his whole career trying to teach us how platelets work and how to understand them.

So—Dr. Scott Murphy.

EVALUATION OF DAMAGE TO PLATELET PRODUCTS

DR. MURPHY: Thank you very much, Dr. Vostal for inviting us to be here. I think this is an exciting and timely event. And I was not able to be here yesterday. I apologize for that. To the extent some of my comments may reflect that, I apologize.

I think we have a lot of material to go over, and I have a feeling when we finish our discussion period there will be more to say.

So why don't we get started.

Actually, according to the program, the first speaker is Dr. Edward Snyder from New Haven, Connecticut, and Yale University, who will probably then introduce me.

Platelet Viability Evaulation and Testing In Vitro

DR. SNYDER: Thank you very much. It's a pleasure to be here, and I'd like to thank Dr. Vostal and the Agency for holding this workshop.

What I'm going to do in a brief period of time is to discuss the phase I testing. As we'll talk about in a couple of seconds, the agency submitted a draft guidance document in 1999 about how platelets should be evaluated when submitting information for potential licensure. And I asked Jar about this, and he told me that there's another document that is working its way through the system. So what I intend to do is to structure my comments on the response to the document from 1999, and to give some thoughts as to how at least the in vitro assays perhaps should be viewed with relation to pathogen reduction technologies.

First, the conflict of interest statement: I am conflicted up the proverbial wazzoo. The Pall Corporation—Cerus—we did phase II and III clinical trials for S-59. In the process of doing it, S-303 for red cell. We did the radio-label survivals for phase II for Vitex, and I've been involved with clinical trials and advisory panels for Baxter.

I do not own any stock in any of these companies, however, at all.

[Slide]

This is the platelet—the entity that Scott has spent his life teaching us about, and which we know is the center of the universe. I think it's fairly familiar to the group, so I don't need to discuss it very much, except to say that the purpose of a platelet is to let the goodies inside get to the outside so it can do its good works, and also to provide a surface for clotting to occur and generation of fibroblasts and other things, which can be helpful or, at times, harmful, depending on what vessel we're talking about.

A platelet should look something like a chocolate chip cookie on electron microscopy, and generally circulates as a disk. And this is what we want. So, with pathogen reduction and technology analysis, we want to be sure that a platelet that's drawn out of a donor, processed and then given back to a recipient is essentially the same entity that it was, and hasn't been transformed, during that process, into a product that is basically either just antigenic or useless.

[Slide.]

This is a slide from a paper that Sandy Shateel published several years ago, and basically just speaks to the concept of signal transduction; that there are receptors on the outside of a platelet that tell the inside

of a platelet what needs to be done; the mechanisms, the various metabolic processes I'm not going to go into, because I want to focus on the analysis of platelets in vitro.

[Slide.]

Again, this is an old slide from, actually, a Baxter advertising slick, showing what appropriate platelets look like. Again, the disks that have chocolate chip, or these which, for those of us who live with platelets, give you that empty feeling—literally and figuratively. They've undergone the release reaction, the granule contents have been expelled, the alpha granule and the dense granules, which are the chocolate chips, if you will, because of the calcium contained therein. And you have, basically, products that are not very functional, although there's some evidence that they actually might work, and part of this guidance document was evaluation of platelet microparticles as a product, which I'm not going to get into here at all.

[Slide.]

This is an example of what Yale's going to be moving to. We've joined forces with Betty Crocker --

[Laughter.]

—and we've decreased our platelet dose from 12 unit random donor pools to four. This year we've gone to a

virtual platelet transfusion, where we just show a picture of platelets, and next year we're going to the Italian herb form, and we just add platelets for faster clotting. This, I think, is not addressed in the guidance document, but one never knows.

So, all of this relates to the platelet storage lesion, which is the untoward effects on platelet structure and function that occurs after product collection in all of its various aspects.

The mechanism of action we now know is likely multi-factorial. There is no one bullet that everyone talks about. The bad news is that in vitro assays, per se, are not very predictive of in vitro function. The good news is that, when used in conjunction with radiolabeling and in vivo post-transfusion assessments, you do get a good handle on whether the platelets are usable or not.

This concept of Phase I, Phase II and Phase III, which is different from the classic Phase I safety and Phase II and Phase III and IV post-marketing and so forth—it's a different Phase I, II, and III.

So, "Phase I" I'm referring to in vitro, Phase II is radiolabel survivals in normal volunteers, and Phase III is transfusions to the thrombocytopenic recipients—or patients.

I think all discussions of platelets should doff the fedora to Dr. Morad at the Red Cross, in '68, who changed the way we do things. Part of that time they used to add citric acid to platelets to prevent them from clumping. His decision was, in a physiologic way, to leave a tender moment alone and just allow the platelets to rest for 30 minutes before re-suspending, which we now understand is necessary to prevent the aggregates from forming.

So, basically, al of these studies are looking at in vitro testing, which was spelled out in this guidance document which came out in May of '99. And there were four categories: in vitro morphology, biochemistry and function; in vivo—which was Phase I—in vivo circulation—in vivo survival in the circulation, being radiolabeling; clinical hemostatic efficacy, being Phase III; and then the platelet substitutes, which I mention we're not going to discuss here.

There also had been a suggestion that rabid platelets might be useful to evaluate. This was from Dottie Zucker Franklin's picture in the New England Journal, as well.

[Slide.]

So what are the assays? Now, these assays—there's a large list here which were taken from a paper

that Murphy et al. published in Transfusion Medicine Reviews, which really referred to what the Best committee had put forth as a lit of quality platelet assays.

The ones in yellow are the tones that are generally recommended as being reasonable or primary, if you will. The ones in pink are those that would be considered supplemental, because this is a two-slide deal here.

So the first was pH, which I think is still the best—measurement recommended at 22 degrees. And this relates to swirl. And I'm going to get to these as we go forward.

Swirl is the ability of platelets that are in the disk form to refract light. And you can get a feeling of this opalescence here, which imply that platelets are in the disk form. When they've undergone the disk-to-sphere transformation, they lose that ability and they become this sort of dull, sad looking entity here, which shows—one would assume that the pH has fallen, and the question comes up, is this a poor-person's pH meter, the lack of swirl?

Well, this was addressed by Dr. Bertolini in Transfusion in 1996, and those platelets lacking swirl are to the left, and those that have swirl are to the right.

The problem is that here's pH, and what you find here is a large number—percentage of platelets in the 7-pH

range—7.5 and so forth—that don't have swirl which, if you use this as a pH meter would be thrown out. And I don't think there's any evidence these days that people would just like to throw out platelets that are potentially useful, because that does translate into financial recompense for the blood center.

So, I don't think platelet swirl—although several people have recommended it, including some people from New York have recommended that all platelets be evaluated by swirl before they're handed out, because on the basis of some litigious problems that happened. I don't think swirl is a very reasonable test of in vitro platelet function. So I don't particularly feel that that's the case. And this was listed again—but I think it would be considered supplemental; pH is still, I think, the winner and still champion, at 22.

PO2, PCO2, bicarbonate all relate to platelet metabolism. We now know much about platelet metabolism and the importance of oxygen. This is from Baxter's slide, as well, showing the increase in oxygen permeability. The same is true for Med-Sep bags and for bags made by Tarumo and so forth.

And we now know if you have enough oxygen, the platelet undergoes the Krebs cycle and produces CO2 and water; if left to its own devices will undergo anaerobic

metabolism and lactic acid will build up. And as Scott Murphy has again shown, if acetate is present it will use that as its primary source for some of the platelet additive solutions that are being evaluated.

This was not recommended necessarily in the guidance document, but is certainly something that we would recommend be evaluated—blood gasses, basically.

Platelet count is critical. There are a variety of machines to measure platelets and not all of them are created equal. But an electronic platelet counter—in fact, Dr. Moroff as part of the Best Group evaluated this in a multi-center evaluation many years ago.

Lactate and glucose, again relate to the concept of metabolism. The lactate generation would be considered bad. Glucose consumption would reflect that as well.

Morphology was recommended and oil-phase is what is recommended generally as being the best, because this tends to correlate as well as any in vitro assay would do, with in vivo function morphology, although many people feel the best of all of them is the extent-of-shape-change.

LDH is an evaluation of rupture of the platelet. Beta-thromboglobulin is an activation marker, or CD-62P if you want to it by flow cytometry is also suggested.

[Slide.]

This is a slide from Rinder, showing that fresh platelets have about a one percent, the GMP140 was the term Bruce Fury—granular membrane protein of 140 molecular weight—which is now known as either P-selectin or CD-62P, and then with a four-day-old platelet it's about 40 percent.

This was evaluated—some people—everyone of these has its one little devotee who feels that's an appropriate assay. It's fine. We don't really think you need to spend too much time looking at platelet activation because it really doesn't correlate all that well.

Let me go back. Oops, I'm going ahead. There we go.

[Slide.]

The hypotonic shock response, or osmotic recovery, that Dr. Handon developed; the extent of shape change—I think these two are considered among the best assays to evaluate in vivo function.

The mean platelet volume is not particularly very useful, although you get it every time you get a platelet count with the right kind of machine.

Platelet factor 4, again, is not any better, or doesn't give you that much more information than the BTG or the CD-62 would.

Other assays here are listed in pink, because they're supplemental, starting with the PF-4. The ATP content, morphology—electron microscopy, not as—I think I have a picture here—

[Slide.]

—again, it's much more subjective than the phase aspects are. So the light with oil—phase-oil evaluation is to be preferred over the scanning EM.

Platelet aggravation data, as Ted Spate, may he rest in peace, used to talk about—agonist-activated aggregation with dual agonists--ADP and collagen, epinephrine and so forth—is, again, considered supplemental, although in the guidance document the agency spent a fair amount of time discussion that, I think most people feel it's not very helpful. The stored platelet requires two agonists to activate it, and it really doesn't give you that much information.

The other assays: size distribution, GP1B and the 2B3A with the CD-63, pack one—that Sandy Shateel has the antibody to, really are not very useful. Serotonin uptake and releases—difficult assay to do, requires radioactivity generally, and is not very much helpful.

Platelet micro particles are very difficult to quantitate, although that was mentioned, by flow or by

other methods, and is not considered reasonable by the Best Group and myself.

Swirl, we have discussed. Thromboxane B2 is not—again, supplemental, not very helpful platelet adenine nucleotide content, this shape assay not helpful as a primary.

White cell content—again, if you're looking at leukoreduction. And then what about 1 and 2-D gels? Well that's available. This is a map of a 2-D gel that we have looked at. Again, if you're looking at certain aspects it may be helpful, but certainly supplemental would be primarily what you're interested in.

And then what about the new markers—apophtotic markers. We all know about the various things that are released, and as is often the case, Dave Pruder has published on this extensively, showing that platelets do contain cast phases; platelets do contain various cast phase apophtotic, BacTs and BCL and so forth are also present, you know, in platelets as well.

These markers are very—are nice. It's high tech but, again, not as a primary, more as a supplemental type. And this is a scanning EM—I'm sorry, a fluorescent—a picture of JC-1, which was used to stain platelets—platelet mitochondria, as shown in yellow. The red is the actual platelet. And we did this to look at whether evaluation of

JC-1 correlated with platelet function. And Pete Parada—we collaborated with Pete Parada from Stonybrook in this, and we did not find that a platelet that was clearly activated had any change in the—what this measures is the membrane potential. And as the membrane potential changes due to mitochondrial death, you get a change in color. So the question is, will this be a useful assay to show that platelets are not functional, and we found that platelets had undergone activation, with release of BTG, for example, and next N-5 appeared on the surface, but the mitochondrial membrane potential didn't change. So we don't think that there's—it's very useful to measure JC-1 change in membrane potential, and that paper's been submitted to Transfusion.

So, that's sort of the forest.

So let's take a look very, very briefly at what the guidance document recommended.

Looking at just the in vitro aspects, they wanted pre- versus post-testing, platelet counts, morphology was discussed, quantitative scoring they felt should be required by light and by EM. Biochemistry, they had listed ATP assays, glucose, lactate, LDH and pH—didn't mention blood gasses as—down here, and bicarbonate was not recommended.

Importantly, I think that they started saying the pH was critical and 6.0 was no longer acceptable. It

should be a minimum of 6.2. Many people—some on the Best committee, also felt that 6.8 would be a good minimum platelet pH, but I think many people would quibble with that, thinking platelets are hard enough to get these days, and a platelet at pH 6.6 should do just fine. But this is a move in the right direction.

There was also, for the first time, a listing of the upper limit of 7.6—that it should be that or below. Again, Murphy had done some work showing that very alkaline platelets had decreased survival in vivo. Again, I mentioned, blood gasses weren't mentioned.

They also had activation markers. They mentioned CD-62, the CD-63 PAC-1 that I mention; the various other assays.

Physiologic responses: they did measure a shock response, shape change, but they also were fond of platelet aggregation. Serotonin uptake and secretion was mentioned. Stimulated CD-62—they thought that might be useful. The Best committee felt that this was not optimal to do stimulated assays for this.

And they discussed quantitation of micro particles also—very difficult.

The key issues to be considered—and this was from a letter form the members of the Best committee—Dr. Murphy was the chair of that subcommittee, Dr. Rebulla, Moroff,

Dumont and myself—this is what we considered in our response to the agency.

The original guidance was issued in '81, and the current one in '95 at that time reaffirmed in vivo survival as the gold standard, which Dr. Murphy will talk about in a minute.

There was no single best in vitro surrogate assay. If you have to pick one, pH seems to be a good—the one that most people use, and then platelet count.

A battery of in vitro assays should be needed, and they all should look positive—glean that it's okay to move to the second phase, which is to put a normal volunteer at risk by giving them radioactivity. So there is a role for in vitro assays.

The assays, importantly, need to be standardized and reproducible from lab to lab to lab. You have to be able to compare apples and apples, and not apples and applesauce.

And you also—you need an assay that correlates with in vivo performance, which—not many in vitro assays do.

Tests, we all agree, should be—this, again, is from the Best committee letter—should be run as paired comparisons. Protocols should use FDA approved containers. And they have a discussion about volumes, and cell

concentrations, and comparable re-suspending media. And this was a paper that Tracy Mondero and Dr. Vostal published, discussing that aspect of the storage solution and what the platelets were suspended in, which was agreed to by the Best Committee that this was appropriate concern.

And then, again, the maxim that in vivo circulation does not equal in vivo function. That's why you need to do these additional types of assays.

So what was recommended is that we felt there should be one assay from each category. That a paired protocol design is critical. I do not think you can do these studies unless it's a paired design of test and control, unless you have a humungous number of patients in each arm.

Serial assays need to be done, either on day-zero, day-one and day-five or seven—more than just one assay, obviously.

In vitro conditions should mimic in vivo. And in pink, here—PowerPoint is wonderful. I actually learned how to use it, finally—platelet counts should be done for metabolic assays, pH, blood gasses, bicarbonate, glucose and lactate.

Activation markers: CD-62P is fine, and measurement of LDH release.

Function assays: hypotonic shock, extent-of-shape-change, and oil-phase by morphology. That, we felt was the minimum in vitro assays that should be submitted. And then Phase II and Phase III are needed, and then parallel supplemental assays as necessary.

So, specific questions on the last slide is: does pathogen-reduction present new concerns regarding platelet function due to collection, processing, filtration, storage and so forth? If so, these supplemental assays should be kicked in.

Is more extensive testing therefore needed to protect the safety of the volunteer donor, which is the Phase II—the radioactive giving to a normal volunteer.

And does the Phase I, II and III paradigm still apply? And we believe that it does—or I believe it does.

And are supplemental tests needed? And, if so, which ones? These are for the agency to discuss with the corporations that are presenting pathogen-reduction materials for potential licensure.

So we have the core group here, and then supplemental ones as deemed necessary.

Thank you very much.

[Applause.]

DR. SNYDER: Our next speaker is well known to us all—Dr. Scott Murphy, who will be discussing the in vivo radio-label survival aspects of platelet evaluation.

Platelet Viability Evaluation In Vivo - Phase II

DR. MURPHY: My presentation will be a highly personal one, about isotopic evaluation of platelets. Much of the data will be from my own lab, therefore it will be predominantly data using chromium-51, and the data will go back as far as 40 years.

I think there's still—some of the principles under which we operate evolved as long ago as 40 years, and I think it's important to review them.

My title—"Platelet viability evaluation"—most of that activity over this 40-year period has been for platelets that have been stored for transfusion.

May I have the first slide, please?

[Slide.]

And because the results go back so far, we're talking predominantly about platelet concentrates made from platelet-rich plasma. And I'm sure you're all familiar with this process by which we make platelet concentrates.

In the last 10 years, what I consider to be some very challenging data has come forward from pheresis systems, which we'll have to discuss and deal with.

Next slide.

[Slide.]

It was Dick Aster who first successfully labeled with chromium human platelets, anti-coagulated with citrate. He found the recoveries and survivals far superior, compared to the use of EDTA, but he noted some important things.

First of all, he did not recover a hundred percent of radioactivity after infusion, and that excess radio-label seemed to be in the spleen.

Next slide.

[Slide.]

And when he studied patients congestive splenomegaly, he found that the recovery was markedly reduced, and that the bulk of the radioactivity could be localized in the spleen.

Next slide.

[Slide.]

This slide is somehow or other—there you go. Thank you so much.

This is data from our own lab—all in patients, actually, with normal size spleens, large spleens or patients who had had their spleens removed. We found a mean recovery of about 70 percent if the spleen was of normal size—just as Dick did—low recovery with

splenomegaly, and close to 90 percent if the spleen was out.

Next slide.

[Slide.]

This led to a concept diagramed on this slide, indicating that in the normal circulation, only two out of every three of the body's platelets are in the circulation. The other third are in a pool in the spleen, in free communication with the circulation; and, therefore, that when you infuse labeled platelet into this milieu of circulation and spleen, you only find two out of every three of the platelets you infuse.

Now, I assume that if that was going on in 1962, it's still going on in 2002. But, as the French would say, "On vera." We'll see.

Next slide.

[Slide.]

Okay. These are studies that Frank Gardner and I did in—published in 1969. We looked at fresh platelets—the open circles—fresh platelet survival. Again, it's 69 percent recovery predictable from the presence of the splenic pool. These are normal volunteers. And then a shortening of survival at 18 hours of storage at 4 degrees centigrade.

From the very beginning of doing these studies, I wasn't sure how to interpret these curves, because although some of them look reasonably linear, there were many that did not fit a model very well. So we took a very simplistic approach--next slide—

[Slide.]

—of simply getting the mean of the recovery values in the first three hours after transfusion and calculating the percent yield, and simply noting where the survival curve crossed the 50 percent line, and reporting the T-1/2, with four days being pretty good.

And this just shows the relationship between temperature of storage for 24 hours, and yield and T-1/2.

Next slide.

[Slide.]

I'm sorry this—it may not project as well as it should in the back.

I've never been convinced that the modeling that has been done since then has led to a great deal of new understanding. There was a paper in Transfusion last month from Dumont and colleagues, who used a computer program in compiled BASIC for the IBM personal computer to calculate the mean platelet survival time with the multiple hit and weighted means methods.

I've interpreted our survival curves with both of these. They give quite similar results in many circumstances. I'm not sure we're learning more by using this than just noting the T-1/2. The model didn't really have anything to do with physiology, that I know. They had the great advantage that they take advantage of all the data points that you collect going into the calculations.

I believe that we should continue to use them. We should choose one, stick with it, and use it consistently. I don't think it's a fertile area for new investigation.

Next slide.

[Slide.]

When we tried to take platelet storage to 22 degrees, for platelet concentrates we found, as Ed mentioned, that the pH often went down, even after three days of storage—35 percent of the concentrates. But even without pH fall, the mean recovery was only 30 percent at three days which would, of course, be totally unacceptable today.

Next slide.

[Slide.]

And here you see the decline with three days of storage of yield, and also of survival. Probably a reduction of 75 percent of the life span.

Next slide.

[Slide.]

Now, the major clue here was the—bringing on bags with increased gas permeability. Ed has mentioned that, but there were other improvements going on at the same time that I think were crucial, in addition to this concept, to getting us to where we were in the mid-'80s. And I want to give some examples of that, and also try to use the examples to establish a few principles.

Next slide.

[Slide.]

There were other harsh conditions in the early '70s. The small platelet concentrate volume was used. Poor plastics, PL-130 and PL-146, and poor agitation. In our work—initial work—we used 20 cycles per minute on an aliquot mixer.

Next slide.

[Slide.]

When we got to study a second generation container we found—and these are just in vivo recoveries—low results with a 30 ml volume. Got not such good results with seven days storage, and we had poor results with several types of agitation. And Ed Snyder did a lot of very important work in defining which ways worked and which didn't.

But I—when we published this, I drew a line across here—what might be considered a line in the sand—below which I thought we shouldn't go, in terms of an in vivo recovery. It's at about 38 percent. And I'll talk more, as we move on, about potential lines in the sand.

I also want to point out the wide spread in values amongst these normal donors. Come back to that. It reflects on Ed's contention that all of these studies need to be paired.

Next slide.

[Slide.]

Here you see this correlation, in a study of platelet agitators—a consistent correlation between the good-recovery people and the lesser recovery people.

Next slide.

[Slide.]

Let's just skip this one. It just shows the superiority of PL-146 over PL-130—gradual improvement in plastic, in addition to increased gas permeability.

Next slide.

[Slide.]

Keep going, I guess. I don't know what happened to the last slide, but it was another paired study.

[Slide.]

This slide's fine—contrasting PL-146 and CLX. And what you see is, again, a paired study in which the recoveries and survivals for each individual donor for the two plastics were graphed, and you see these quite high r-values, again suggesting the poor—the poor-recovery people and the high-recovery people.

Next slide—again, I think mandating paired studies.

[Slide.]

Now this wide—this is studies in CLX going out to seven days. Mean recovery at about 40 percent at seven days. But I use it more to, again, show that this wide variation among normal donors is present even with fresh platelets.

And I just—not ever let—one thing Ed often says—sometimes Sherrill—drop is—looking for a correlation between an in vitro test and in vivo results. Now, I would expect that you would get extremely good hypotonic shock response, extent-of-shape-change if you measured them in fresh platelets from these donors. And yet the correlation—a rather tight standard deviation. But the r-value is bound to be poor because there's so much variation in the chromium recovery. And although I have no doubt that the in vivo studies are the bottom line, I don't think

these poor correlations necessarily invalidate the in vitro studies.

Next slide.

[Slide.]

This just shows half-lives—again, wide variation, down to seven days.

Now, I think it's not known what these variations in inherent donor characteristics are. Do we really know what their blood volumes are? We estimate them from height and weight, in general, and then that figure goes into the calculation.

Do normal people have variations in the size of their splenic pool? I suspect that's true.

Nonetheless, these kinds of data were used to, I think, enunciate a rather unspoken paradigm—a line in the sand—that 40 percent, after seven—because the platelets were licensed for seven days—that 40 percent was okay. We could live with what amounts to a two-thirds reduction in viability, and a half-life of 2.6 days, which translates into a 5.2-day median cell life—also okay. We'll come back and think about that in a minute.

Then in the '90s there was news from apheresis.

Next slide.

[Slide.]

All these previous studies with PRP platelets. News from Dr. AuBuchon, from New England, indicating that CCI's in thrombocytopenic patients which pheresis platelets were just as good on day five as they were on day one. And a group from Stockholm very shortly thereafter published a paper saying exactly the same thing.

Next slide.

[Slide.]

And then just in the past year, Dumont et al., including Jim AuBuchon and the group from Red Cross in Norfolk showed a 63 percent recovery with platelets prepared with a spectra, and a 6.7 day survival. And I found this absolutely astounding. What happened to the platelet storage lesion? This is what you would expect with fresh platelets.

Gerald Slichter at ASH, last December, reported 79 percent for five day stored platelets in plasma—again, with a survival at six percent. What's happening to the splenic pool?

And one wonders if a PRP concentrate control had been included, whether the pheresis products would have done substantially better. Is the line in the sand drawn from the data in the '80s too low?

And, more important—for me, anyhow—what would the results be with fresh platelets for these preparations? I

hate to think of the fact that the investigators might find that the recoveries were over a hundred percent, or a hundred percent—or even a hundred percent.

Next slide.

[Slide.]

So the current paradigm indicates that you have to have a paired control in the same donor. Typical control has been—in quotes—"ROP"—regular old platelets, the oldest you can find that are licensed. At the end of the license storage interval, perhaps you're looking at the worst case scenario.

I think this places at a disadvantage the—those studying ROPs that have a high in vivo recovery, if you were going to look at an experimental manipulation—pathogen inactivation, what have you.

Next slide.

[Slide.]

Problems with the paradigm—again, is there's still no line in the sand, question 40 percent recovery, five-day mean cell life. No delineation of acceptable inferiority for test versus control—if any. ROP will vary widely from study to study, and you have a concern of creeping inferiority. This is licensed. This has a lower result than this one, but it's not statistically different

so it's licensed. This one—same thing. So this gradual inferiority creep—potential.

Next slide, please.

[Slide.]

So here's a proposal. I think somebody needs to stand up and say, "This is what we should do," and then hide beneath the table over here.

I think that the control should be—the paired control should be fresh platelets. And that the experimental results should be expressed as a percentage of that control. Offered for thought as acceptable after storage would be decrease in recovery to two-thirds of fresh, and in the survival, half of fresh. And I'll come to why I'm so lenient on the survival.

And it's acceptable to have a pre-determined reduction for the experimental, relative to the extent of patient benefit that might accrue. A pathogen-reduction method that would save lives; an extension to seven days to allow implementation of bacterial testing, which would save lives. I would not favor weakening these standards just to get seven-day storage for economic advantages for the blood center, even though the staff at my blood center would love it.

Next slide.

[Slide.]

So here are—perhaps there are more recent recoveries and survivals for fresh platelets, but I got these from blood in 1985, Sherrill Slichter's lab and my lab, and the recoveries then were 57 to 66 percent for fresh platelets, with survivals of 80 to 96 percent. So this is translating—if the controls stay in this range—fresh platelets—then you would have a 41 percent projected recovery in a 4.4 day survival.

Next slide.

[Slide.]

The rationale for being a little lenient on the survival is really based on the thinking of Sherrill Slichter—the data, rather, and her thinking about it, that as patients become thrombocytopenic, their platelet survival time declines, because an increasing fraction of the platelets participate in hemostasis.

Next slide.

[Slide.]

And, therefore, this study from Neroll, in Paris, giving fresh platelets to stable thrombocytopenic patients—time to next transfusion was three or four or five days, depending on the dose of platelets given. And here the patients are being transfused with very high doses, up to 100,000—rarely done. And I'll offer that the idea that since in patients, patients don't survive for six to eight

days, we really don't have to maintain that with storage. There are some caveats which we may want to talk about.

Since I talked about recovery and survival, I'll just say a word about corrected count increments.

Next slide.

[Slide.]

This is a whole bunch of studies done from '86 to 2001; storage intervals, corrected count increments time 10-to-the-third. And you see that the results are generally from 10,000 to 16,000. And think these are difficult studies to do. The results are highly dependent on the patient population you choose, hard to get platelets. All the—sometimes impossible, all of the same age. But I would suggest that a newly licensed product show some CCI data where the mean was greater than 9,000.

So, thank you for listening to this presentation. I hope it, at minimum, provides some food for thought.

And now it's my pleasure to present—well--

[Applause.]

—thank you—a woman who needs no introduction—because I've already talked about her—Dr. Sherrill Slighter, who will talk about platelet viability evaluation in vivo.

Platelet Viability Evaluation In Vivo - Phase III

DR. SLICHTER: Thank you. It's nice to be here. Just—the conflict of interest statements: I have participated in a Phase II study and Phase III study with CERUS for pathogen inactivation. I'm also a consultant to CERUS, and I think maybe on more than half the days they wish I wasn't. So, I think I will present the data, and the data speaks for itself.

[Slide.]

That's, I think, not the first slide. We need to back up.

[Slide.]

There we go. Okay.

My charge today is to talk about in vivo platelet transfusion responses in thrombocytopenic patients. I think a lot of what you've already heard, it's as though, I think, Ed, Scott and I are kind of all on the same page.

I'd like to make one comment about the in vitro measurements. The in vitro measurements, as you've heard discuss, are extensive. They are expensive to perform. I guess I would encourage the FDA to—and I think all of us have been trying to find Ed's Holy Grail, which is the in vitro assay that will correlate with either platelet recovery or survival. I think none of us have seen it. I personally think the value of in vitro assays is to allow you to proceed to the next step, so that they should be

done to prevent you from going to in vivo studies of things that aren't worth evaluating. But to actually require extensive in vitro testing as a licensing requirement, when they don't correlate with in vivo, except as a "yes/no"—either they're acceptable and you can proceed, or they're unacceptable and you're wasting your time. I think other than that, there shouldn't really be any requirements for in vitro assays or even suggesting. I think it's up to the manufacturer to do a variety of in vitro tests—whatever they want to do—and then determine whether they should go ahead.

Now, with that said, we'll talk about in vivo evaluation. What we're looking at here, in thrombocytopenic patients, is assessment of number of platelets circulating following a transfusion; how long they survive, and whether or not they function.

So, for platelet number measurement, these are the things that we look at. We look at the increment, which is the post-transfusion minus the pre-transfusion. And then two other measurements which basically incorporate into a formula some measure of blood volume, and number of platelets transfused. So this is now the corrected count increment, or the percent recovery.

And then, in thrombocytopenic patients, platelet survival is measured as the days to next transfusion.

Can I have the next slide, please?

[Slide.]

In terms of platelet function there are three ways to evaluate platelet function. The first is to look at the bleeding time versus platelet count measurement, and there's a direct inverse correlation between bleeding time and platelet count that can actually be determined by this equation, so that for any platelet count you can say following transfusion, this is the correlative bleeding time that we should see, and therefore this product is either functional, which means it fits this equation, or it's dysfunctional, meaning the bleeding time is longer than predicted for the post-transfusion platelet count.

We've also had a fair amount of experience—and I'll show you the data—looking at fecal blood loss as a measure of platelet hemostasis, so we can actually quantitate the amount of blood lost in the stool as a measure of bleeding risk through an intact vascular system. And then, as all of you know, you can actually do clinical assessment of bleeding, based on some criteria. And most people use the World Health Organization guidelines.

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[Slide.]

Now, loss of platelets occurs actually by two mechanisms. One is senescence—removed in the RE system.

Maximum platelet life span has been calculated to be about 10.3 days. And in addition to this senescent loss, there's a random loss of about 7,000 platelets per microliter per day, which we think is the number of platelets that you really require as endothelial support, so that you don't bleed through your vascular system.

Next slide.

[Slide.]

As Scott has already shown, once you get to a platelet count in the range where you would actually be considering a transfusion, there's a direct relationship between platelet count and platelet survival, so the lower the platelet count the shorter is the platelet survival. And I would concur with Scott that we need to make sure that we have a survival of the transfused platelets that's as long as the thrombocytopenic patient can use, and that's not this.

However, we need to make sure that we keep in mind the fact that if we have pathogen-inactivated platelets, or we have stored platelets, they not only go to thrombocytopenic patients who are being transfused prophylactically—often at trigger levels that are very low, and so we can anticipate a short survival—but they're also going to be used for other patients, specifically, for example, open-heart surgery patients who are bleeding, so

we have to make sure that we keep in mind the broad range of patients who require platelet transfusion.

Next slide, please.

[Slide.]

Now this just is kind of a caricature that looks at the fact that the recovery of platelets following transfusion in normals is somewhere around 60 to 70 percent--Scott's already gone through that—with a survival like nine or 10 days. In thrombocytopenic patients, with platelet counts less than about 50,000, although the post-transfusion response is about the same, as we've already said, the survival is reduced and averages about five days.

Next slide, please.

[Slide.]

Now, this is the relationship between bleeding time and platelet count that I previously discussed with you, and just shows that at platelet counts of less than 100,000, there's an inverse relationship predicted by this equation.

And then, next slide--

[Slide.]

—this now looks at stool blood loss, again at a variety of platelet counts. This was studies done in the late '70s. These patients were not being transfused with platelets at the time the stool blood loss measurements

were made, often because they were allo-immunized to platelets and we didn't have compatible donors. But what's of interest here is that at platelet counts above 10,000, basically you have no increase in stool blood loss, start to get some wiggle in the data here, and at less than 5,000 there's a substantial increase in bleeding. And I think this reflects the fact that you don't have the 7,000 platelets that you need in order to plug the endothelium, and that's reflected in the increased stool blood loss.

Now, what I'm going to do with the rest of the talk here is just, in a sense, show you some examples using data that we've either generated in my laboratory, or from the literature, about how you assess, on a practical level, platelet transfusion therapy in thrombocytopenic patients.

Next slide, please.

[Slide.]

Now, the study population that we usually look at is patients with a hypo-proliferative thrombocytopenia, because that's the majority of platelet transfusions—about 80 percent, at least in our community, go to this particular patient population. They should be patients who are selected to require at least two platelet transfusions, and that's because the experimental design that you want to use in patients—the same as Scott discussed in normal volunteers—is to either do a cross-over design, in which

the test transfusion is compared to reference, so you randomly give the test or reference as the first or second transfusion. You want to make sure that these transfusions, hopefully, are sequential transfusions, so that the clinical condition of the patient doesn't change dramatically because there are a lot of things, as we all know, that can affect transfusion responses. Or, alternatively, you can assign all the patients in a—to receive all their transfusions as test or reference, over a thrombocytopenic interval.

So, either one of these two designs is acceptable.

Next slide, please.

[Slide.]

Now, this is a study in which—I think Scott should maybe pay me for kind of following his lead—but this is a study in which we had two questions. The first one was: how long can you store platelets—either platelet concentrates or apheresis platelets—and get exactly the same answer as fresh?

And so—and then the second question was: are apheresis platelets better than platelet concentrates?

And so these are the number of thrombocytopenic patients who were entered into each of these study

assignments. In the first 24 patients, their storage time for the stored product was only two days.

So this is fresh—meaning these are less than 24-hour-old product. These are reported as CCIs. So this is fresh platelet concentrate, stored platelet concentrate, fresh apheresis, stored apheresis. All four of these transfusions were given in random order to these 24 recipients. And there's no difference among the recipients within a product type for this short storage duration.

However, if you look at platelet concentrates versus apheresis, the platelet—the apheresis platelets consistently give a statistically significantly better CCI at one hour post-transfusion, but there's no difference between fresh and stored. So you pool this, pool this to get—and then pool this, and pool this, to get fresh-versus-stored, no significance; a trend starting to develop, and it only reaches statistical significance here, and that's because within each group there's a statistically significant difference between fresh and stored for platelet concentrates and also for apheresis platelets.

Next slide, please.

[Slide.]

And then this is days to next transfusion. And here what you see is in a very large group of thrombocytopenic patients, you're talking about average

platelet survivals of somewhere around two to at most three days between platelet concentrates and apheresis concentrates no significant differences. Between fresh and stored you're trying to achieve statistical significance. Don't make it—the only statistical significance between the same product, over time, is a five day storage fresh, in fact, is better than five-day stored.

Next slide, please.

[Slide.]

Now, this is a study in which we looked at 16 patients in a paired cross-over design, looking at standard apheresis platelets compared to CERUS, Baxter helinex-treated platelets—they keep changing the name. So that's one of the reasons I'm only good for them on half the days that I consult with them, because I have trouble keeping up with the numbers.

But these are pre-transfusion platelet counts. No difference between the two groups.

In the paired measurement at one to two hours post-transfusion, this is the post-transfusion platelet count for control versus treated—a statistically significant difference.

This is platelet increment—again, a statistically significant difference.

And then calculated as CCI, again a statistically significant difference between treated and control.

Next slide, please.

[Slide.]

This is the 18 to 24 hour post-transfusion data. Again, statistically significant differences in post-transfusion platelet count increment and CCI, but no difference in days to next transfusion in this study.

Next slide.

[Slide.]

And the major reason why this study was done was to actually look at platelet function. And so this is 11 of the 16 patients from the prior slide who had bleeding time measurements done at both one hour post-transfusion and 18 to 24 hour post-transfusion. The pre-transfusion bleeding time was unmeasurable in both groups at greater than 30 minutes, and that's because they had very low platelet counts pre-transfusion.

Post-transfusion, bleeding times improved in both groups, with no statistically significant difference between the groups. And, again, in the subset that had this done, there was a statistically significant difference at one hour post-transfusion.

And I think what this data really says to me is that although there is a clear and statistically

significant difference in post-transfusion responses, in terms of platelet increments, those platelets that circulate following transfusion of the treated platelets are hemostatically actually quite effective.

Next slide, please.

[Slide.]

Now, I'm going to—so I talked to you about bleeding time as a measure of platelet function. I'm now going to show you some data looking at stool blood loss as a measure of platelet function.

Again, this was a study in which we were trying to determine what is the lowest platelet transfusion trigger that might be allowed to be used without compromising the patient. And, remember from the first slide that I showed you on stool blood loss, it looked as though a 5,000 level was where you needed to protect the patient. So we looked at 5, 10 and 20. All patients had an aliquot of their red cells labeled with radio-chromium, and then all of their stools were collected following labeling.

Next slide.

[Slide.]

And this just shows the data. Now, there were between 24 and 31 patients enrolled in the arm. This is the total stool blood loss over their thrombocytopenic

interval. And then this is the stool blood loss corrected for the days o thrombocytopenia, which was considered to be any day in which their platelet count was less than 20,000.

And what you can see is that, in fact, even at a 5,000 level, if they're transfused at that trigger, they are protected from excess bleeding.

Next slide, please.

[Slide.]

And then this just shows data on the effect of the trigger on the number of platelets transfused, and just shows that the lower the trigger, the fewer the platelet transfusions you give. And if you correct for thrombocytopenic day, there's no difference between 5 and 10, but both of these are different than 20,000. And this just shows that the one-hour CCIs, regardless of the platelet count that you transfuse at, all turn out basically to be the same.

Next slide, please.

[Slide.]

And then this just looks at total red cell transfusions given, which I think is a relatively surrogate marker for, again, platelet hemostatic function. And you can see here that the red cell transfusions pe thrombocytopenic day were basically the same in all groups.

Next slide, please.

[Slide.]

And then this just, now, looks at a clinical hemostatic assessment as the primary end-point. This was the recently completed Phase III CERUS Baxter study of the helinex-treated platelets. Hemostatic evaluations were done by trained observers daily. The evaluations were done pre- and post- each transfusion. The observer was blinded as to the product received. There were eight organ systems looked at on a five point scale. And, basically, grade two WHO bleeding is any bleeding that's more than kind of just petichiae and ecchymosis, but does not require a red-cell transfusion, which puts it into the grade three category.

Next slide, please.

[Slide.]

And this just shows—again, as would have been predicted by the bleeding-time data, that within each arm there was about 58 percent of patients who had grade-two bleeding, but no difference between the arm. Again, no difference in grade-three or higher bleeding. Grade four is basically substantial bleeding that may be associated with mortality.

The mean days of bleeding was greater in this arm, but that was because of some outliers. The median days were the same. Duration of platelet support was basically the same between the arms.

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[Slide.]

And, again, as we saw in the Phase II study, where we did a cross-over design, there was a statistically significant difference in all measures of post-transfusion response; post-transfusion platelet count, corrected increment, CCI, between treated and control at both one your and 24 hours post-transfusion. And these were all statistically different, with a p-value of 0.001.

Next slide, please.

[Slide.]

And, again, as opposed to the 16 patients in the II-C study, now we're starting to see, actually, a decrease in the transfusion interval in the treated compared to the control platelets, so that the number of platelet transfusions required in the treated arm is, in fact, greater by about 25 to 30 percent. That's a reflection of both a decreased increment and a shorter survival, meaning that you need to transfuse these people with more platelets.

The average platelet dose is less in this arm, which partially accounts for the differences in increment and interval between transfusion. As Larry Corash, I think, mentioned yesterday, there's about a 10 percent, maybe 15 percent processing loss. That accounts for some

of it, but it doesn't account for all of these. And, at least to my mind, suggests that, in fact, there has been some damage to the platelets by the treatment process which affects both their recovery and their survival, but as we've previously stated, the hemostatic efficacy of those platelets are, in fact, fine.

So, total dose of platelets has to be greater to compensate for the processing and damage received by the platelets. Mean platelet—red cell transfusions as another marker of hemostasis is the same.

Next slide.

[Slide.]

And that's it.

So, Thank you very much.

[Applause.]

Panel Discussion

DR. MURPHY: Can we bring the panelists up, please?

[Pause.]

DR. SLICHTER: I notice I'm the only one with a handwritten placard. I wonder if that's a message.

Laughter.

I think they didn't expect me.

DR. MURPHY: If people will come forward.

I'd like to congratulate Ed Snyder on sticking exactly to time. And to rap both my wrist and that of Sherrill's for going over a bit. But Dr. Vostal thought that this was so interesting that we could probably have a little extra time for discussion.

And we have, behind us, some questions. And I thought—you can all read them. I'll read the first one, and then ask the panel for comments, and then perhaps the audience will want to comment and expand.

How many questions do we have total, Jar? Three? Three total questions here?

"Are current in vitro methods of evaluating platelets sufficient for evaluation of potential damage from pathogen-reduction methods? Should be a list of required in vitro tests for evaluation of platelet damage? For pathogen decontamination, should there be additional in vitro tests required?"

I think I would simply as Eric, who dealt with this extensively, perhaps to summarize and specifically try to address the questions, and then I'll ask the panel to comment.

DR. SNYDER: Well, I think a—I agree completely with Sherrill that we shouldn't make too much out of in vitro testing, other than as a toggle—a go/no-go—for in vivo evaluation for Phase II.

So I would think platelet counts, a test of metabolic assays, with one blood gas you get pH, your CO2, O2 and bicarbonate. If you want a marker for activation, CD-62P is fairly well standardized. And then for function assays, either extent-of-shape-change or hypotonic shock response, or both, and phase microscopy under oil I think would be sufficient—maybe LDH, as well, could be used because it's relatively simple. And then, we get metabolic lactate and/or glucose.

Those are fairly simple assays to do. If they all give you the same good results, you have a good sense of confidence.

As far as additional assays for pathogen-reduction, you could make a case, considering some of the toxicities we've talked about, maybe you'd want to look at some other more high tech assays—apophtotic assays and so forth.

I don't think it's necessary if you have good results on what you've seen. Sherrill's point that if it isn't worthwhile looking at in vitro, it's not worthwhile looking at in vivo is probably appropriate. And I would think starting with those simple in vitro assays would probably be sufficient, with additional ones that could be done by the company as they saw fit.

Possibly to change some aspects—this has been published already in abstract form, but we did in vivo radio-label survivals, along with Dick Astor, many moons ago, for S-59 platelets, and did find that there was a decrease in recovery and survival with the S-59 treated. So I'm not surprised at the comments that were made. I think it fits exactly what we're saying.

I don't think, however—and, again, I have no financial relationship to the company—that—I think there's no free lunch. I think there is a price to be paid, and I think Scott's point is if there's a benefit to the additional therapy that may have a benefit to the public health, consideration should be given to that. And I think Vitex saw the same thing, with some slight decreases in the survival of their red cells, that this is maybe the nature of the beast as we enter this field. So—keep-it-simple-stupid, I think, as has been said, is the way to go.

DR. MURPHY: Any other comments from the panel?

DR. SLICHTER: Yes, I'd—I think we've all given our assessment of the in vitro assays, my only point being that I would encourage the FDA not to mandate some particular in vitro assay.

I mean, I think the message to the people who are interested in bringing new products is that they better start with a variety of in vitro assays to make sure

they're okay, but I'm not sure why the FDA would even have to see those in order to license the product, because that—I think they're not going to license—or, at least, I think they shouldn't license a product just based on in vitro results. I think they should require, at a minimum, the radio-label platelet recovery and survival measurements in normal volunteers.

I think they only need to go to thrombocytopenic patients if the change that's being proposed is substantially out of the box. So I think they're requirement that the pathogen-inactivated platelets be evaluated in thrombocytopenic patients is right on, because that's out of the box.

I think, in addition, I would also concur that we need to look at the quality of the product and what advantage it's going to give to a patient. And so even though the pathogen-inactivated platelets have taken a hit, it may well be that that hit is not enormous, and that the benefit to the patient with getting a pathogen-inactivated platelet may justify some damage to the cell, in terms of quantity. And I think the marketplace is ultimately going to decide, probably, whether they want to pay for having to transfuse more platelets. But I think they should have some—actually great deal of confidence that those platelets, even though they don't have the expected

recovery and survival, they're hemostatically doing the job which we expect them to do.

DR. MURPHY: I'd like to ask two questions, and then I'll recognize Dr. Reed.

Sherrill, in response to that—and it correlates with isotopic studies, in the CERUS study, the control corrected count increments were as high as you'll ever see in the literature—16,000. And the test was 11,000, which is in line with dozens of previous studies in the literature.

How do you deal with a situation like that?

DR. SLICHTER: Well, Scott, I think you—I mean, I received by fax, last night—because my lab was so excited—we had a nine day platelet storage in plasmalyte, with a 62 percent recovery and a seven day survival. And as you pointed out, from the studies of Dumont, and our recent studies, I think unbeknownst to us, the manufacturers are supplying us with a better product.

And so I think, you know, they are better, Scott, than we've seen. But I think it again brings up the point of doing paired observation.

DR. MURPHY: Well, I think the point I wanted to bring out is that when you're using regular old platelets as your control, and the regular old platelets are changing over time—which is, I think, what you're saying--

DR. SLICHTER: Mm-hmm.

DR. MURPHY: —then you may, indeed, see a substantial difference between control and test, and yet test may be a pretty good product.

And just in the interest of time I think we have to keep moving.

Ed?--o f course I'm hogging the microphone—but you used a great term, Ed, that the in vitro test should be looking positive. Do you think we—the FDA would benefit from having a little better definition of what range of results they should expect to see from the in vitro assays?

DR. SNYDER: Well, I disagree a little with Sherrill. I think that Phase I should be limited, but I do think the agency should see the data, and I think there should be some tests listed—minimal though it should be. I don't think they should just do whatever they wish. I think everyone should be held to a similar standard.

I don't know—to answer Scott's question—exactly what those results should be. I think you could get a group of people together, with a cup of coffee that they would buy on their own dime, and come up with the list and exactly what the ranges ought be.

I think it's very difficult to nail things down. It may be almost like, you know—pornography, I know it when I see it—what would be an acceptable—

[Laugher.]

—I had to say something—for those of you waiting, I just said my one thing.

DR. MURPHY: The only point I'd make is, I think I know what's good for extent-of-shape-change, hypotonic shock--

DR. SNYDER: Right.

DR. MURPHY: —but I have no clue what's god for PO2, PCO2--

DR. SNYDER: Right. Some would be a little more open to discussion than others.

Dr. Heaton?

DR. HEATON: Yes—Andy Heaton, San Francisco.

I've got a couple of comments. One is a very good predictor of platelet post-transfusion quality is lactate production rate. And I'm curious to know whether you did either rates of glucose metabolism or lactate production rate on the platelets that have been processed into the helix solution.

And I'd make a second observation--

DR. SLICHTER: Into the what?

DR. HEATON: Lactate production rate, or glucose consumption rate—either has a very, very strong correlation with post-transfusion recovery.

And then the second issue I think that we need to look at carefully with the results that you're presenting is that you're looking at two different effects, one of which is the soralin effect, and the other of which is for the first time in the U.S. we're now seeing platelet-additive solutions containing acetate being used, and that much of the outcome you're seeing is an offsetting effect between the loss of platelets and their activation during processing, and then the addition of acetate in a modified PSM-3, in effect, which contains acetate, which affects platelet recovery.

But I'd be interested to know, have you got any results of glucose consumption rates, Scott? Or lactate production rate?

DR. SLICHTER: Specifically in the helinex platelets?

DR. HEATON: Yes.

DR. SLICHTER: I'm sure the company does.

DR. HEATON: Okay. But you didn't do them.

DR. SLICHTER: I didn't do them.

DR. HEATON: Okay.

DR. MURPHY: I think we'll hear from Dr. Corash, and then one more question, and then we'd better move into the second question.

DR. CORASH: It was done, and not very substantially different between the two groups. I don't remember the exact numbers, but very close together after five days of storage—actually, after seven days of storage lactate and glucose looked pretty similar in the two groups.

MR. RAWLEY: Robert Rawley, Amulet Pharmaceuticals. Given that the in vitro tests are such bad predictors, is there anything that—or appropriate animal model for pre-clinical studies?

DR. MURPHY: I'll just comment that the man with whom I first worked studied freezing platelets in dogs over about five years. And after he'd finally done that and applied the method to humans, it didn't work.

The second thing I'll just say, for about the fourteenth time, that some of the variability in comparing in vivo studies with in vitro studies comes from the inevitable variability in the in vivo studies, which I documented on the slides.

Does the panel want to add anything more before we go on? I think we should. Mark?

DR. WEINSTEIN: First of all, I'm sort of humbled sitting here with all these experts, but I agree with Ed that keep-it-simple-stupid, and I'm sort of reminded of a tricycle—you know, the sort of three wheels, and that we

shouldn't limit what tests—the FDA should not stipulate what tests we should do, but I think that there are three areas that you ought to look at in vitro, which is some sort of functional assay, something that indicates metabolic pathways, and morphology. And that's how I would recommend that the FDA sort of outline it, and then leave the researchers some latitude to do what they think is relevant. Because new tests are going to be coming along.

DR. MURPHY: Jim?

DR. AuBUCHON: You asked about animal models. I don't have any personal experience with this model, but Mo Blackman has done a lot of work with thrombocytopenic rabbits who have received different platelet preparations and infusions of potential platelet substitutes, and has shown some nice data over the years with this ear bleeding model. I don't know if it has been used in all of the photochemically treated platelets that are being considered currently, but it's an interesting approach to consider.

My only comment about in vitro testing is that I think the place for the agency to look for those data would primarily be when the IND is submitted, because that is an appropriate time to apply the go/no-go decision. And after that, Phase II and Phase III, I think in vitro data has very little impact and could probably be safely ignored if everything else was looking good in Phase II and Phase III.

Dr. Moroff wrote—15, 20 years ago, Gary?—something, if I may paraphrase you, something on the order that if a platelet circulates, it's probably functioning properly. And so far, that's pretty much what we have seen.

DR. MURPHY: Yes, I'm sorry, I was a little narrow-minded in my answer about the animal models. I think most models—a very good one for measuring functionality, but I don't think it's predictive of capacity of platelets to survive.

Very short, please.

MR. ????: I'd like to make one comment about the in vitro studies. Despite the lack of correlation with in vivo results, I think you also have to keep in mind that there needs to be a quality check over time for the process methodology, so that a product that's made this year can be assessed in some way, other than doing a clinical trial, so that next year's product is either changed or different. And so that's a reason to have those data available—not just to the FDA, but also to the users.

DR. MURPHY: Thanks.

Let's move--

DR. SLICHTER: I think that's a good point.

DR. MURPHY: I do, too.

Let's move on to the second question. In terms of evaluating the platelet product viability in vivo, are there minimum acceptable criteria for radio-labeled platelets in vivo recovering survival? What is the amount of damage to a platelet from a decontamination treatment that we can accept and still have a clinically useful transfusion product? What is the appropriate control for these studies?

I think I'd like to direct that directly to the panel, if I may, since—I tried to be as direct as I could about that.

DR. AuBUCHON: I liked your suggestions, Scott, particularly because they anchored a test result with a sort of an immutable control; that is, a fresh platelet—although a fresh platelet may be slightly different if it comes off an apheresis instrument as opposed to a PRP or opposed to a buffy-coat method. At least it's a standard that should be able to be reproduced over time, free of any interference with changes in storage conditions, storage bags and the like.

I would have to give a little bit of thought about the practicalities of implementing that, however, before endorsing it unequivocally, because it would cause a change in the way that most of these studies are currently conducting, because platelets can be successfully labeled

and tracked with both chromium and indium. The trend in recent years has been to have a test versus a control, using indium for one of the reinfusions, chromium in the other, with appropriate randomization, and using one collection in both the test and the control methods—for example, splitting them between two bags. And that's a nice way to remove a lot of the variability that can occur in one subject over time. So you really do get a very appropriate paired comparison.

Adding a fresh reinfusion causes a third reinfusion then to be needed, if one still wants to compare what we're currently doing—say, five-day platelets with what we might want to do in the future, say, seven-day platelets, and then have a day-zero or day-one as well. We don't have a third radio-label that works well with platelets, and there's a limit to how much you can make one normal subject glow in the dark. And so we may have to re-think exactly how we would construct these studies.

DR. MURPHY: I guess my proposal would be to skip the standard five-day product, and just come up with numbers reflecting the quality of—and this is when you're proposing for licensure. I mean, there are other questions you might want to ask experimentally, where the control—you want to compare two plastics at five days, of course, you do them at five days. But if you draw a line in the sand

about a decontamination product, compare it to what the patient would get if there was no manipulation whatsoever.

DR. AuBUCHON: I understand, although I believe the agency has generally looked for comparison with something that's already licensed, and it would require a different thinking on their part, I believe. But I don't wish to speak for the agency.

DR. MURPHY: I hope that's why they're here.

DR. SLICHTER: If I could speak to the question, I am much in favor of what Scott has proposed, and the reason for that is because of the one slide that he showed with the creep. You know, you licensed five days, and then is seven days different than five? Well, it's not really different than five, but it may, in fact, be substantially different than fresh.

And so I guess I didn't track exactly what you were saying, Jim, because we do only have two labels, but we can label one product fresh, and then one product stored for the same amount of time, and at least we've pretty much done many of these studies using apheresis platelets where you do an apheresis collection, pool them into one bag, and then re-split them in two bags so you've got exactly the same product. And we've collected our donors in the late afternoon so that our testing is done at night, and by the next day, which is within what I think is fresh, which is

24-hours from collection, we're ready to radio-label and re-infuse.

So—and I think even the thought that Scott had that the recovery needs to be about what we would expect, which is two-thirds of what we've got, and the survival being about five days—I would agree with completely.

DR. WEINSTEIN: I think we should take a lesson from red cells licensure, where there is a distinct cut-off. If you can go 75 percent, 24 hours survival, your recovery, that's good enough. And then you look at—we're going to 42 days, or 49 days. And I think we need to do the same for platelets. Just—there is a minimum recovery and a minimum survival, and if you can accomplish that on however many days, that would be acceptable, if you get around the problem of bacteria contamination.

DR. MURPHY: But, Mark, I would just say that I think—as I—I don't do any red-cell work—but as I understand it, it has the same problem. No lab checks itself to see what it would get for fresh red cells.

Supposing someone's technique, applied to fresh red cells, gave in vivo recoveries of 120 percent. Then surely most of their stored red cells are going to give greater than 75 percent.

I personally believe the same thing about red cells as I just enunciated for platelets.

DR. SNYDER: Actually, that's not exactly true. We've—red cell radio-labels we've done, we do fresh—a normal volunteer's fresh radio-labels, and we've done some platelet studies fresh as well.

They're easily done, and you validate that you're sort of burn-in, that your assay is working well, and your labeling technique is appropriate. You've got a good labeling efficiency. So that's easily done.

I would have to agree with Jim and with Mark that I think a number should be given. I think you want to know—you want to compare the test to the control, and I think if your control is always fresh, and you've got to go two-thirds of something, it's somewhat—not quite as settling as being able to make a direct comparison between platelets stored under exactly the same conditions, only one's treated and one isn't.

Now, that's a little difficult if you've got seven-day versus five-day, but if you want to get seven-day pathogen-reduced, you should compare it to seven-day stored.

I think it's an interesting thought. I would have to consider it. But I think I agree with Jim's point and with Mark, in that regard.

DR. ADAMS: Christopher Adams, PurePulse Technologies in San Diego.

My question is: we've heard a lot about decontamination methods causing damage to platelets, however we haven't really heard anything about the importance of investigating what the nature of the damage is.

Could you just comment on what you think the importance of those types of studies are for regulatory submission?

DR. SNYDER: No.

[Laughter.]

DR. SLICHTER: Well, I think we've done a fair amount of studies with UV irradiation to prevent platelet allo-immunization. And they're in dogs, and there is a study looking at UV irradiation to prevent platelet allo-immunization in thrombocytopenic patients.

There is some damage with UV irradiation, regardless of whether or not there's a photochemically added agent. What the mechanism of that damage is, I'm sorry, I don't know.

DR. AuBUCHON: You also asked the question would it be important to know, or would it be important for a regulator to know.

I mean, clearly, it would be interesting from an academic perspective to know what was happening in order to try to ameliorate any damage in future versions of the

treatment. If the platelets have been damaged in some way but seem to work acceptably clinically, then possibly a black-box approach is acceptable.

But to improve the process, obviously, one would need to know more about it.

DR. MURPHY: I'm afraid my charge is to allow one more question—sorry, Steve, and move on to the third question on the board.

DR. GOODRICH: I'll try to make it a good one. Thanks, Scott.

Ray Goodrich, I'm with Gambro BCT.

I was just—in regards to the second question, about the definition of the "routine-old-platelet" or routine platelet that's being used, we've talked about the control potentially being a product which is at day-zero of storage.

What about the considerations of—is a routine platelet, you have random-donor platelets, you have buffy-coat platelets, you have apheresis platelets. All of those give you acceptable performance characteristics and they're in routine use. Does the control need to be something which is stored and treated under identical conditions to the treated, except for the treatment step? Could that control be taken from anything which is in routine use? For example, if a control is a sample that's stored in media.

which may not be a standard, that's used in some situations, and the treated is in media with the treatment step is that appropriate?

Just looking at a definition of what that "routine" platelet might be?

DR. MURPHY: Well, my proposal was an attempt to get around the problem you're referring to.

Carry on, if anybody else wants to--

DR. AuBUCHON: You raise a very good point, and point out one of the niceties of Scott's proposal.

We have seen this problem pop up more, actually, in red-cell storage studies than in platelets, where often we end up picking as the control arm a treatment that is licensed and that we can reference to the literature, and use as a backstop in case we get a peculiar result in the test arm. And so if we end up with a particularly low recovery--or survival, if we're measuring it in the red-cell study—in the test arm, and we see it also in the control arm, then we will tend—with a lot of waving of the hands, often—but tend to exclude that subject's data from the final analysis, saying "There's something funny about this person's red cells"—or platelets.

If we were to use a fresh control, and then make all of our subsequent analyses in comparison to that control we could get away from that perhaps.

So that's one of the niceties of using the fresh control.

DR. MURPHY: Let's go on to the last question.

If an inactivation process produces cells with a higher rate of isotope elution in control cells, is it appropriate to correct the increased elution in 24 hour recovery calculations?

I would like to get a response from people at the table who are in the trenches doing these studies at this time.

Jim? Or Ed or Sherrill?

DR. SNYDER: Well, I think the key issue is: is the elution due to a damage to the red cell such that it is no longer appropriate for clinical use. If it turns out that the—and that may require that you actually go and do clinical studies while you're still evaluating that.

I would think if you find that it is—the elution is an artifact of some technique, then you should be allowed to do that. If you can—I think it relates to what the mechanism is of the elution.

In one company's work, incubation of the red cells with the product for shorter periods of time was not associated with a more rapid elution. Longer periods were.

There are overriding issues relating to the ability to pathogen-reduce, which—and I think the whole

issue of pathogen-reduction comes down to: its importance will be most important when there's a credible threat to the blood supply. If the credible threat is bacteria, then you're already there. If you're waiting for the Bin Laden virus to be introduced and decimate everyone, then that will be your particular point at which you take it.

So, I think you do have to have allowances for the benefit of the pathogen-reduction technology. And I think if you find that there's some slight damage, there not being any free lunch it's often been said, to find out what the problem is. And if correcting for that elution, for example, is appropriate, I don't have any problem with that.

The key thing is are the red cells functional clinically.

DR. WEINSTEIN: Another option—we saw this with freeze-dried platelets where both chromium and indium eluted off extensively. But we found yet a third radio-label that would stick to the platelets. So you could try different labels.

DR. WAGNER: I have a comment and a question.

The first comment is that for photosensitizing agents that produce reactive oxygen species there's a lot of material in the literature that shows that the action on membranes results in a leakage of ions—an ion leakage. And

so there's a potential for that to be associated with photochemical treatment.

The second comment is a kind of a question, and that is: why not make parallel requirements for survival, and require three-quarter survival rather than two-thirds for platelets? That's a three-quarters recovery.

DR. MURPHY: needless to say, I just decided that in the middle of the night one night when I couldn't sleep.

[Laughter.]

DR. MURPHY: I don't think there's any good answer to that one.

Jaro's on his feet. Larry, is it—15 seconds, do you think?

DR. CORASH. 15 seconds. I think as a corollary to item number three, you really need to measure radio-labeling efficiency. You should actually do it as a control. I mean, something we picked up from Andy Heaton's work—I think it's very important, because it tells you whether or not you're getting a good label. And they are different, between chromium and indium. They're dramatically different.

But both labels work, I think. But I think you should characterize your product for labeling efficiency every time you do it.

DR. MURPHY: I'd like to make one more comment before closing the session.

I think there's more than enough room at this table for all the laboratories in the Western Hemisphere to do these kinds of studies. I'd be delighted to be corrected about that. And some of the people sitting here are eligible for Social Security checks.

[Laughter.]

DR. SLICHTER: I received my first one last month.

[Laughter.]

DR. MURPHY: I think this is a concern for everybody, including the agency.

DR. SLICHTER: Can I make one more comment? And that just is, again, to try and have the FDA help us. And by that I mean that, you know, in the good old days when we used to practice transfusion medicine, we could make clinical decisions and—about the products that we want to transfuse. And I think in this whole discussion today about pathogen-inactivation—today and yesterday—one additional technology that really is available, that will allow us to extend platelet storage is the question about whether you can detect bacteria.

And the reason I bring this up is because, as I mentioned, we've got seven-day platelets that we've got 21 observations now, with 67 percent recovery and five-and-a-

half day survivals. And with a pathogen detection system, we don't have to compromise on platelet quality. We can do that.

So I would hope that the FDA would license a variety of things, and let the marketplace and the consumer, and the doc-in-the-box decide how he wants to solve the problem.

So he may want to extend platelet storage by having a pathogen-inactivated system. Or he may want to extend platelet storage by doing bacterial detection. And I would hope that the FDA would allow us the opportunity to make those kinds of decisions, because, as we've said—you know, most of the viral stuff is already in place, in terms of looking at. There are other kinds of things that we need to worry about that the pathogen-inactivated may take care of.

But I would hope that they would allow some—some ability to license a variety of things, and then kind of let people decide how and when and why they want to use it.

DR. MURPHY: It's an appropriate time to turn the meeting over to Dr. Vostal.

DR. VOSTAL: I think it's our intent to try to get a reduce in pathogens in the blood supply, and how we get there is—we don't really care which method will get us there. And we would be happy to approve all of them.

However we need guidance from you in terms of, you know, what is the acceptable platelet product that could be marketed. And this is why we're having that discussion. You know, what will be the least functional thing that you'd be willing to transfuse and still call it a platelet? You know.

Now, we could also discuss whether there's a different product—a transfusion product that's like a platelet but not—similar to a platelet, you know. Whether some of these things that produce platelets damaged in some way could still be useful to make a product that would be for specific clinical indications, but not be a platelet.

So—I think we're running late. We're going to try to take a quick break, maybe 15 minutes. And then we'll get started on the red cells.

Thank you.

[Break.]

DR. VOSTAL: Please take your seats. Next session dealing with red cell testing.

EVALUATION OF DAMAGE TO RED CELL PRODUCTS

DR. MOROFF: We're ready to start the next session, which will address issues pertaining to evaluation of red cells.

And the way it's listed on the agenda is: "Evaluation of Damage to Red Cell Products." I like to

look at it as "Evaluation of the Retention of Red Cell Properties with Treatment."

Our first speaker is Dr. Toby Simon.

Dr. Simon is the chief Medical Officer and Chief Operating Officer of TriCore Reference Laboratories in Albuquerque. And he has a longstanding interest in measurement of red cell properties going back to his days with Blood Services.

His topic is "Red Cell Viability Evaluation and Testing In Vitro."

Toby?

Red Cell Viability Evaluation and Testing In Vitro

DR. SIMON: Thank you. It's a pleasure to be here, and it's particularly timely for me, since I returned to Albuquerque only about a year ago, and had, as Gary had said, been doing these studies for many years, but had been absent for about ten years, and now have resumed my interest, and resumed the laboratory that's doing these studies.

So at the present time, I have no relevant conflict of interest, but I hope when I speak in the future that I'll have many conflicts of interest to talk about.

[Laughter.]

But this is an important topic, and I think it builds very nicely on the sessions yesterday, because we

have the standard ways of evaluating the in vitro aspects of red cells on storage, and with different anticoagulants, and I look at that as sort of a passive effect that we have seen in the past, whereas now we have, with the inactivation methodologies, direct action on the cells that could create additional problems.

So we need to look at, I think, what the classical means of evaluating in vitro effects of storage and anticoagulants, and add to that, perhaps, some additional studies based on the specific actions of the inactivating material—with the viral inactivation and bacterial inactivation methodologies.

Now, also, in a timely fashion, in the third edition of Rocee's Principles of Transfusion in Medicine that's just come out, we again have two excellent chapters, as in the previous two editions, by Ernest Boytler on this subject. And for any of you who want a fairly brief review that gets into most of the major points, I would strongly recommend those chapters.

We can simplify this subject fairly readily, and look at the red cell in vitro viability in three ways. Number one, does the cell circulate. And that is related to the ATP levels that we can measure—and, to some extent, to glucose.

Does the cell function? Does it offload oxygen to tissues? And we could look at the 2-3DPG measurement.

And then do the cells remain intact after they've been transfused? And we could look at hemolysis and secondarily, also, at potassium.

Now, if we focus first on the circulation, we need to look at some relevant—reference some relevant biochemistry, the glycolytic pathway, the Embden-Myerhof, and the energy that the red cell requires is derived almost entirely through the breakdown of glucose to lactate or pyruvate in this pathway. And this particular pathway is phylogenetically very old, and so what the red cell is doing is what is done in many, many earlier forms of life.

Eleven enzymes break glucose down to lactate. So the six-carbon sugar glucose is phosphorylated, isomerized to fructose; phosphorylated again and cleaved into three-carbon sugars that are again phosphorylated. And the phosphate gained is transferred to ADP, producing ATP—and which is used ATP-ase to pump ions against concentration. So it's the production of ATP that is critical to the red cell's ability to circulate.

Now, what the red cell does have that the phylogenetically earlier pathways do not, is the production of 2-3DPG, through the Rappoport Luebering shunt. And 2-3DPG—now, in some articles, and by some scientists is

referred to as 2-3BPG—biphosphoglycerate. But it's a unique feature of glycolysis in the red cell, and it's that interaction of DPG with hemoglobin that has the special role in allowing the red cell to offload oxygen efficiently to tissues. And there's a special side pathway, then of the glycolytic pathway that allows the formation of 2-3DPG.

There's also the hexose-monophosphate pathway, which is the source of NADPH, and this becomes important in individuals who lack the enzyme G-6PD.

But returning to ATP and going through that rather simple paradigm, it was Clement Finch and his colleagues in Seattle in the 1950s who recognized that when red cells lose organic phosphate, especially ATP, one has compromised survival. And this makes the measurement of ATP critical.

What this led to in a practical sense was recognizing that adenine could replenish the adenine nucleotide pool through adenine-phosphorylase transferase reaction, and better maintain the levels of ATP. And so adenine became an additive which improved red cell circulation by—presumably by maintaining the levels of ATP.

Now, with 2-3DPG we shift from circulation to function. The 2-3DPG relates to the offloading of oxygen, and higher levels of 203DPG shift the oxygen offloading curve of hemoglobin rightward to release more oxygen.

The important practical consideration here was that CPD was better than ACD, because of its pH, in maintaining the levels of 203DPG. However, when adenine was added to improve the storage and allow us to keep red cells for longer intervals, there was actually a little bit faster fall in DPG.

Now, the issue of the importance of 2-3DPG is largely unresolved in the literature, despite the fact that this has been a topic of interest since the 1970s, studied related to the massive transfusion in the Viet Nam War by a number of the military services.

2-3DPG we know is important to offload oxygen. W know it's lost in stored red cells. But its importance was thought to be diminished when it was recognized that the red cells rejuvenated the 2-3DPG after transfusion. So within about 24 hours, the individual who's been transfused has red cells that have normal oxygen offloading properties, or functional properties.

However, there are subsets of patients who need immediate improvement in their release of oxygen, in whom this might be important. And there are no good clinical data on this, but, in general, neonatologists are concerned about this; some trauma surgeons; the treatment of hypothermic patients—there are number of situations. And even though 2-3DPG is lost with storage, we know that red

cells of lesser storage interval—five to seven days—would still have good levels of 2-3DPG, so the clinician can select those in these instances.

If there is something in the process of the inactivation of pathogens such as—to lose 2-3DPG, we would have to think about the inability to respond to those clinical situations. And the number of those patients might be larger than the number of patients who would benefit from pathogen-inactivation. So that is a consideration to keep in mind and that requires, I think the measurement of 2-3DPG early in the storage interval after pathogen-inactivation.

Other biochemical changes of the red cells--as they consume glucose, lactic and pyruvic acid are formed, and these can be measured. There's a loss of potassium and a gain of sodium in the red cells that's unrelated to ATP, but relates to the membrane changes with storage. And you can measure the increased potassium in the supernaten.

Measurement of membrane proteins over the years has not correlated with viability. However, if we see that the pathogen-inactivation procedures chemically affect these membrane proteins, one might want to add such a measurement in in vitro studies.

There are also physical changes of the cells with storage. Their shape changes from a discoside to

acsenoside or sphero-asenoside. And lipid are shed to vesicles.

Hemolysis also occurs, but actually the plasticizer that is used most commonly in red cell storage—DHP—stabilizes the membrane and reduces hemolysis. And mannitol, which is present in man of the additive solutions, also reduces hemolysis.

Boytler points out that osmotic fragility is not actually increased, although red cells do lose deformability. Again, this has not been something we have focused on, because it hasn't correlated well with viability function or circulation. But if there's a specific effect from the pathogen-inactivation chemical process, this might be an additional measurement.

Now, what we do when we do these in vitro studies is to measure ATP, 2-3DPG and free hemoglobin, and these measurements are readily done by measuring the NADH to NAD, decreased absorbence at 340 nanometers. And Sigma has kits that do this for both ATP and 2-3DPG, and also for free hemoglobin—all spectrophotometric methods.

And, as we have indicated, ATP is a very valuable measurement, which we believe staringly should be done. It is predictive of viability at low levels. But to continue the theme from the platelet discussions, in vitro studies,

we believe, cannot substitute for in vivo viability studies.

If you have low levels of ATP you can predict poor viability. At better maintained levels, you need to do in vivo studies in order to assure that you have adequate circulation.

And what I've listed on this slide are some sample results from studies that we have done in our laboratory over the years. And these studies have all been published and presented publicly. And I've rounded off some of these results, and this is to create a feel for—and perhaps some definite numbers that should be used in evaluating red cells after any pathogen-inactivation.

And, in general, we anticipate that the hemolysis level should be low, but almost certainly below one percent. ATP will generally decline to no less than half the initial values, so that one should be able to maintain ATP at the end of storage at this level.

2-3DPG, as we'd indicated before, is rapidly lost. And 2-3DPG would have to be measured shortly after storage, at some interval like five to seven days, in order to assure there is adequate 2-3DPG for those limited clinical instances in which a clinician it was important.

Glucose measurements will vary somewhat depending on the glucose that's in the initial storage medium. And

potassium will go up to a measurement of about 50—and we would not want to see it much exceed that.

PH ranges are in the range of 6.6 to 6.9 at the end of 42 days of storage, and is another measurement that should be made to assure this.

So these are what we would expect to be the basics of measurement, and then to this would recommended that be added anything that is specifically indicated by the specific process that's being used.

So that concludes a quick review of the in vitro aspects of red cell viability that we believe are important for an evaluation of any new method of red cell preservation or red cells for transfusion.

[Applause.]

DR. MOROFF: Toby, thanks for your presentation.

Our next speaker is Dr. John Hess, who is Associate Director of the Blood Bank at the University of Maryland Medical Center in Baltimore. And previously, John has been Chief of Blood Research for the U.S. Army, and has had also a longstanding interest in red cell property issues.

The title of John's presentation is "Red Cell Viability Evaluation and Testing In Vivo."

John.

DR. HESS: As Gary said, I used to be with the U.S. Army. That has a lot to do with conflict, and not much with interest.

[Laughter.]

DR. HESS: I was asked to talk about red cell viability. And in the hemoglobin-baggie view of red cells, we assume that the body removes dead red cells, so the ones that circulate are alive. And the problem with this is that red cells, after you reinfuse stored red cells are cleared rather quickly over the first 15 minutes, somewhat more slowly over the rest of the first day, and then almost uniformly at about one percent per day thereafter.

And so the common measures that are used are the recovery—that fraction of the infused cells that are still circulating at 24 hours, and the survival—that fraction of the recovered cells that continue to persist beyond 24 hours.

Now the problem with this is that there is a phenomenon called "red cell rejuvenation." You can soak red cells in solutions of materials that will drive ATP synthesis; materials like phosphate, and inosine and pyruvate and adenine. And under appropriate pH for a couple of hours, and you can markedly improve the recovery. And so what this says is that some cells are marked to die, and in some way this marking can be removed.

What we don't know is, is whether all solutions or time points are equal in this process; whether better rejuvenation would safe more cells, and what this really means in terms of the definition of viability.

Now, the first red cell recovery measurements were actually made with the first red cell storage study. And Rouse just simply measured the increment in hematocrit and corrected for the reticulocyte count. The first survival measurements were made by differential agglutination, by Ashby. And what she did was she added O cells to someone who was B, and then agglutinated their B cells, and followed the clearance of the background unagglutinated O cells until they were gone, and plotted this. And so she was able to show that normal red cells live for a hundred days.

To improve these measurements, we have gone to labels. And there are a handful of classic ways of labeling red cells. One can use the existing genetic labels. One can radioisotope labels. One can stable-isotope label. One can use affinity labels, like biotin. And the problems are that biotin appears to cause immune response in at least some people. The genetic labels require allogeneic, which really became impossible in the age of AIDS. The stable isotopes require mass-atroscopy,

which was not routinely available in most red cell labs. And so radio-labels became the de fact standard.

Davey has told us what an ideal label should be. And certainly, Steve Wagner told us yesterday that the world is far from ideal.

Of the common red cell labels, only chrome has both the long half-life and the low elution that basically make it stable and easy to use. And so it has become the standard. Eighteen years ago Dr. Moroff chaired a committee in which 15 major experts reviewed all of this and agreed on two methods which they said would certainly make the world a far better place if everybody would use one of the two.

If you read the report carefully, 14 of them were actually advocating one method, and that was the chrome-51 method.

There are two excellent reviews in the literature of how to perform labels—apart form Dr. Moroff's report—one by Dr. Davey and one by Andy Heaton. And between them they contain about—most of the collective wisdom on this subject that exists.

Now, as I mentioned there is an early phase of fairly rapid removal of effete or marked red cells, early on after they're infused. And so the way chrome-51 labeling is probably best done is to measure the

radioactive counts at 5, 7-1/2, 10, 12 and 15 minutes, and then back regress to time-zero to come up with a time-zero absolute dilution measurement; that is, your zero time.

And the problem with that is that actually in the early phase, some very effete cells, or very heavily marked cells, or just unlucky cells, are removed at an even faster rate in the first five minutes. This can be shown by using a second label, and now the standard one is technetium-99, to come up with a true time-zero. This value will be 1 to 3 percent higher than the regression value if the—the time-zero point—if the recovery is above about 80 percent. And it's usually not much larger even if it's below.

The problem with doing the double label is that there are significant inaccuracies in both these measurements. These inaccuracies result from all the various measurements that go into doing a red cell recovery measurement. And if one adds all for the various sources of error, one discovers that these measurements can't be more accurate than about 5 percent. But these tend to be random errors. And so large numbers of patients will allow you to correct for them.

Now, the current FDA thinking—as I was given it to be for this talk—was, is that we would like to—it looks like my machine put some strange—

[Laughter.]

—that the sample recoveries should be—these are "greater than" and--

[Laughter.]

—you know, that they want the mean recovery to be greater than 75 percent with a strong statistical case that it's better than 70 percent.

Now, when we look at the way these kinds of tests have been used in the past, in the classic studies that licensed CPDA-1 and AS-3—performed by—what Dr. Zuck did, back in 1977, was collected 37 patients from four different sites, and measured the single label chrome-51 recovery, and found that it had a mean value of about 81 percent. Now, you will notice that it ranges from 95 to 66 in all of the 37 individuals, and that what that means is that the fraction that's clears varies from 5 to 36 percent, or overall, almost a seven-fold range.

Now the problem with CPDA-1 was, by the time—when it was invented in 1968, the world used whole blood. By the time it got licensed 11 years later, we had gone to packed cells, and the tighter you packed CPDA-1 blood, the worse the recovery became. And so when you used CPDA-1 packed cells, the mean recovery was down in the low 70s.

It was for this reason that we went to adding constant volumes back to the stored red cells, in the form of additive solutions. And here in a study of 20

volunteers at two locations, performed by Dr. Simon, you can see that there is quite good mean recovery—about 85 percent—at six weeks, but much poorer recovery at seven weeks.

And so one can use these as historic, you know, controls for other studies. And this is she original work that Tybor Groenwald an I did on looking at systems that drive ATP higher during storage. And so here are two small groups of 10 people each that suggest that these systems can work out to seven and eight weeks.

But the problem of course is, is that there are a few people, even in a system that appears to look bad, that have values that are very similar to those. And so it is possible to bias these studies, either accidentally, or on purpose, by knowing who people who are good recovers are. And so there are some tricks that you need to play to watch these studies.

And certainly one of the easiest is simply to increase the numbers. We repeated that study a year later with 10 more volunteers, and then 10 more at nine weeks. But here, when we actually got 20 people, we saw a reasonable approximation of the normal range of recovery. And so by the time you get out to 20 people, you hope to see a fairly broad range of recoveries.

About one person in—about 3 percent of people are missing inosine triphosphate pyruphosphatase, and are known to have very poor ATPs, and correlates with poor recovery. Not everybody who stores poorly has that enzyme defect, but, you know, we're beginning to break down some of the understanding of what causes these wide population differences.

Another way of using 20 studies to get information is to use 10 people twice in cross-over studies. And here is a study that we published two years ago, comparing probably the most studied solution in recent times—Adsol—with our 10-week storage solution.

Now, there are excellent studies of six-week storage of Adsol by Dr. Heaton, Dr. Moroff, and others—Jim AuBuchon—that all have values that are right there in the 80 to 85 range. And so when you have a study that both has a reasonable spread and gives you the answer that you've come to historically expect, that too can be convincing.

The problem with doing large cross-over studies that look at lots of things—and this was a cross-over study we did to look at warming red cells up to 25 degrees for a day—is that essentially all of the variability in these studies has to be with inter-donor variability. And when you extend a study that has radiation washout periods, and multiple six to eight-week storage times, and then do it

for 16 donors in a center where you can do, maybe, two a day, two days a week, one of these studies will drag out over the course of a year. And so you suddenly have volunteers dropping out—soldiers who get reassigned, people who come in on a day in the middle of a hurricane where, you know, you lose the electrically so many times that you can't calibrate your radiation sources. It's a problem. Or, you know, snowstorms occur and people can't get in.

And so these become quite difficult studies to perform, and time consuming, and labor intensive and expensive.

[Pause.]

Having said that about recovery measurements, let me just mention that survival measurements have, at least in my experience, always been normal in all the systems, both liquid and frozen that we have tested; but that in these coming tests, where we're actually putting in things that are potentially metabolic poisons—now, they're directed at DNA, but we really don't know that they don't find appropriate activation sites in some of the enzymes that exist, and they may subtly poison things that will affect survival. And so I think looking at some survival studies would be useful.

Just in conclusion, I think the conventional chrome-51 study still remains the standard. We could look

for changes in early loss that might be associated with the process, with the double-label systems and the numbers that we're going to be using to test. They really won't detract from the precision of the numbers that come out. And so I think several unequal ten studies is a perfectly reasonable study.

Rejuvenation studies will probably just muddy the water, in the sense that the more badly you store cells, probably the more you can rejuvenate them. So that measuring rejuvenation and calling the ability to rejuvenate a positive value may, in fact, not be telling us much.

I think red cell survivals for at least a couple of weeks, you know, during the washout phase of the chrome, to watch to make sure that the survivals approach the normal hundred days would also be useful—and certainly will be critical if we're going to use these products to treat people like thalassemics, where shortened survival would increase their exposure to iron.

Unsaid in all of this is that apart from just passive measurements of recovery and survival—you know, we are beginning to realize that red cells have real functions; that they secrete ATP to lead to microvascular vasodilation that may well affect the fact that they don't flow very well early on, that caused our trauma surgeons to

complain about "old blood." And we know that stored blood may contain breakdown products, such as phospholipids and fatty acids that are actually pro-inflammatory. And so, you know, there are really no standard tests for looking at those yet. But because they are related to issues like trolley, and like the resuscitation of trauma—which probably affect far more people than are presently affected by infectious disease transmission—they are important and we need to keep thinking about them.

Thank you.

[Applause.]

DR. MOROFF: John, thank you.

Panel Discussion

We'll now turn to the panel discussion, and the panel will consist of our two speakers, Toby Simon and John Hess, and Jim AuBuchon, who is Chair of the Department of Pathology at Dartmouth-Hitchcock Medical Center, will join the panel also.

Let me take 10 seconds before we put the questions up to just review this slide, which is current FDA thinking. And John Hess showed that for a few minutes—a few minutes ago. And I just wanted to go over two points.

I wanted to go over two points. First of all, for hemolysis levels at the end of this storage period: the

current thinking is that all products in the study—in the study arms—should have less than 1 percent hemolysis. And with regard to 24-hour in vivo recovery autologous at the end of the storage period, a study at two separate sites should be conducted with at least 10 volunteer subjects per site.

In terms of the 75 percent level which we've talked about a few times this morning, the sample mean level should be equal to or greater than 75 percent, with one side at 95 percent, lower confidence limit greater than 70 percent.

This is current FDA thinking, and I just wanted to start the discussion with that.

We have three questions, and we're going to change the order, in terms of how the platelet discussion was approached. We're going to start with the in vivo question, and then go to the in vitro question - in vivo questions related to in vivo studies.

The first question—and this really is a follow-up to John Hess's presentation: for the evaluation of in vivo red cell viability with normal subjects, what methods should be utilized to measure the 24-hour recovery parameter, and should red cell survival be also measured?

John, we heard your viewpoints. If you want to maybe just summarize your points, and then we can just go right down the panel.

DR. HESS: yes, I would hope that everybody uses at least—uses chrome-51, with or without technitium, and reports the study both ways, if they use the second label.

Second, I hope that they will measure survival out for at least two weeks. And, certainly, if they're thinking about using these products in thalassemic children, out somewhat longer.

And I think that's it.

DR. MOROFF: Let me also say, if anyone in the audience has some comments or questions, please come to the microphone and we'll recognize you during the discussion.

Toby, you want to address this question?

DR. SIMON: No—I would agree. I don't have anything to add to that.

DR. MOROFF: Jim? Would you like to comment?

DR. AuBUCHON: I think the proposal that John put forward is entirely appropriate. One question as to what to do with the results when sometimes they're anomalous. For example, if the survival is close to the 75 percent benchmark, it's indeed possible that the single-label mean survival might be greater than 75 percent and the double-

label slightly less than 75 percent. And as I understand it, the FDA does not usually round up, so 74.9 is not 75.0.

What do you do in a case like that?

If one has recovery data at the same time—I'm sorry, survival data at the same time indicating survival is good, that might be an additional nudge to say go ahead and accept this. But there are times when the single-label and the double-label are not exactly concordant.

And we have come to accept using a double label, particularly after some vigorous discussions 20 years ago, surrounding the licensure of Adsol. And I don't know if Dr. Heaton would like to engage in any of those discussions again, but there certainly is a theoretical advantage to using the double label. However, in most all subjects that I've reinfused, I really haven't seen that much advantage; have not really seen that the double-label technique gives a more accurate—quote-unquote—determination of red cell volume in order to determine the t-zero point.

There are certainly some times when, for technical reasons, the single-label study can't be interpreted, and then the technitium determination of red cell volume becomes very important in terms of being able to salvage that subject.

But I'm not really sure that the double label helps all that much.

DR. SIMON: I think historically, if I remember correctly, the double label that Dr. Valery was using at that time was iodine—125, right?—

DR. MOROFF: I think he still is.

DR. SIMON:—and he still uses that.

DR. MOROFF: He's still using that, which has been giving him lower results than the other double-label procedures.

DR. SIMON: And then, you know—then Dr. Heaton developed, I think, the technitium-chromium combination, which then became favorite.

It seems to me that the drift of the discussion here is that we might be able to go back to the single label, and it might be—that it should be satisfactory.

DR. MOROFF: Toby, I feel the same way, and I want to put some data up on the screen.

This is some data where the double label is the technitium procedure, and the single is the chromium. And look at the bottom. There are 10 studies with stored red cells, and there's really very little difference with this data—double-label, single-label. And the means are 2 percentage points different, which could tie in with the errors that you were talking about, John.

So I think this is a question.

And also, if we do stay with the double label, I think it's time that we standardize between the iodine procedure and the technitium procedure, historically and it continues to this date, to give lower results than the technitium procedure, when you're looking at double-label procedures.

There are some questions—or some comments and questions pertaining to this from the audience. John? Dr. Chapman?

DR. CHAPMAN: Hello. My name is John Chapman. I'm from Vitex.

I think we have about the collective wisdom of experience in radio-labeled red cells and recovery, except for Dr. Valery not being here.

And my question is: talking about stressing the cells and how that may change things. Like we're using in Inactine. But previously, we had been stressing cells with gamma radiation.

And I was curious to know if there is data not only of the red cell recovery after gamma radiation, but what is the effect of gamma radiation on red cell survival?

DR. MOROFF: Jim, do you want to answer that? You did a lot of studies with us on that?

DR. AuBUCHON: Yes—with irradiation, the study that Gary masterminded through the Rd Cross, looking at

irradiation and either at day-28, or of storage for 28 days after irradiation, indicated good recovery, but we did not assess survival in that study.

And, as John said, everyone's experience up to the time of using pathogen-reduction technology was that if the red cells circulated at 24 hours, they had a normal survival. So you didn't need to go beyond that 24-hour point.

But now we're seeing some data that indicate there might be something different in the long term survival of the chemically treated cells.

So you raise a good point, John, that the long term survival may be different in irradiated units. And we don't know.

DR. MOROFF: I would say that there should be a common protocol for measuring the survival. There's a lot of different ways of expressing the data from a survival protocol, and there's different protocols for when you do sampling.

So I think there needs to be a little standardization, in terms of how the long term survival of red cells are measured.

Dr. Heaton? Has a comment or question?

DR. HEATON: Yes, indeed I do. Andrew Heaton, San Francisco.

Bear in mind that, you know, as you consider the difference between single and double label, most of these studies have been performed on relatively modest differences in the red cells; AS-1 versus CPDA-1, AS-2 versus AS-3. So you've been looking at relatively small differences, and it's true under those circumstances the gap between single and double label is small.

But as you look at pathogen-reduction, you're looking at major treatment of red cells, and chemical activity which might compromise—cause a very dramatic reduction in early loss and, secondly, which might also reduce survival.

So I would suggest that for simple red cell licensing studies there really is no difference between single and double label. But if you're going to think to pathogen-inactivation, you do want that absolute standards of having your red cell volume. And so I would strongly suggest that for pathogen-reduction, you should maintain the double-label standard.

And then on the survival—you know, John comments 14 days. I would suggest that you need to go 28 days or 35 days, depending on the amount of chrome, or IRB will allow you to inject. Because long term survival is important. You're transfusing red cells—to keep the patient's red cells in their circulation for a hundred days post-

transfusion. And, again, in the context of pathogen-reduction, we are adding highly active chemical ingredients to the red cells. I think survival over a 28-day period would probably be important.

DR. MOROFF: Andy, in studies we've done with you we've always measured for at least 28 days.

DR. HEATON: We have. Yes.

DR. MOROFF: I would say that should be part of a standardized protocol.

DR. AuBUCHON: But—I agree with you, Andrew. Our measurement of survival is fraught with some not necessarily obvious difficulties. Blood volumes change over time, which could cause either dips or apparent increases in the survival of the red cells. Any inter-current blood loss would obviously change the survival. And since the Federal government, appropriately, would like us to include subjects of all genders and all races and all ages, that means that we will have some menstruating females whose red cell loss over time will show up in a long term survival study.

Then there's the rate of elution, which Dr. Mowson told us was 1 percent, but even he knew that it was anywhere—1 percent per day—but he knew that it was potentially double that in some subjects, and was not absolutely the same over time.

So, what you see is not necessarily what you get.

DR. HEATON: You certainly will have to use computer modeling to adjust for elution. And Gary's suggestion that there be a standardized protocol and a standardized method of data analysis, I think is entirely appropriate.

DR. MOROFF: One last comment and then we'll go to question two.

Larry Corash?

DR. CORASH: Yes, I'd just like to ask the panel: there's some excellent work that was done years ago by Knadler and Block that's created a cubed height-weight formula for determining blood volume, that's based upon both red cell mass and radio-iodine plasma volume studies.

Is that acceptable? That seems to me to be a very solid body of work, and I wonder what the panel thinks of that for using a blood volume—to make blood volume calculation?

DR. MOROFF: Any comments?

DR. SIMON: We've used it, but it's an estimate. And I think it's not precise, because it assumes the average person, and you have variability.

DR. MOROFF: Let's go on to the second question. And the second question says: for Phase III clinical trials, what parameters should be measured to evaluate any

influence of the treatment procedures on red cell viability properties? What study design parameters, such as the type of appropriate control study, should be utilized?

Who wants to handle this first? John? Do you want to handle this? We'll just go right down the line.

DR. HESS: I guess I like the cross-over study designs. And I think, apart from that, you know, doing them well in a couple of centers that are good at doing them is important.

DR. MOROFF: What parameters would you measure? Frequency of transfusion? Number of units transfused? This is for Phase III, where you're not going to be using isotopes.

[Pause.]

DR. HESS: I'm not sure I have an answer.

DR. MOROFF: Toby?

DR. SIMON: Well, you know, I think they are difficult. Ido think that, you know, there are some things that we can measure—and I think you mentioned them: the frequency of transfusion, the extent to which the hematocrit and hemoglobin are increase, and the various oxygenation parameters that have been suggested as indicators of the success of the—transfusion oxygen content.

DR. MOROFF: Could you elaborate on what would you measure in terms of oxygen? Parameters—could you elaborate on that a little bit, Toby?

DR. SIMON: Well, the ones I think that they used in the fluosol studies which—I'm not in detail—but the oxygenation of the tissues, oxygen extraction ratios—although you have to have catheters in place and samples, so they get fairly complicated, but those are the—I think the fluosol studies are probably the best model that we have at the present time.

And you would—if you could cross over and do controls with your standard red cell methodology, and then the pathogen-inactivation red cells.

DR. MOROFF: I with the cross-over design. I think that's a good way of approaching that.

Jim?

DR. AuBUCHON: I have two different comments.

First, several years ago I had the opportunity to participate in a clinical trial of enzymatic conversion of group B cells to group O, and then infusion of these converted cells into group O patients to control—it was a paired study. The control was infusion of native group O cells to the recipients.

This was a radio-labeled survival study—recovery and survival study.

The results with the enzymatically converted cells were very interesting, and it seemed that they worked. But what I found almost more interesting was the outcome of transfusing compatible group O cells to these group O recipients, and seeing the wide variability in response, in terms of recovery, survival and time to next transfusion. It was, to put it mildly, all over the map.

Therefore, any clinical study that uses patients is going to have to be extremely large to take into account this inherent variability, because patients who need transfusion are, by definition, sick. And we had a number of patients in the study die during the study—not because we did anything to them that was terrible—thankfully—but because of their underlying illnesses.

So all of this needs to be taken into account, and that is going to really require monumental size studies on the order of the sized studies that we've seen recently with the S-59 platelets, in order to be able to assess statistically what is really happen.

Another comment pertains to some discussion we had during the platelet panel about the creep of—creep downward, slide downward of our standards. And should we take the opportunity of these various manipulations that we are putting red cells to now to improve the quality of red cells? You know, we have come to accept, and I think

clinicians have come to accept, that the red cells we give them have no DPG. There are some data—admittedly debatable—that that may not be the best thing for patients. And just from the logical point of view, and looking at how red cell transfusions are handled nowadays, it makes sense that we may not be doing the best thing for our patients.

Anesthesiologists wait until the very last minute to transfuse red cells in the hope of being able to avoid the transfusion. And when they give the red cell transfusion to a patient, that patient indeed needs oxygen delivery to their tissues. But what we're transfusing them are little red cell sponges—oxygen sponges that pick up oxygen in the lungs very well and don't offload it in the tissues for the next 12 to 24 hours, instead just increasing the cardiac workload.

So it's not surprising that there are data in the literature suggesting that older red cells aren't as good for patients as fresher red cells. I'm not convinced that that really has any clinical standing, but it's something to think about.

So, as we begin to undertake significant chemical modifications of red cells, should we also take the opportunity to see if we can improve them? And should we make sure that we aren't doing anything worse for patients, not just in terms of having 75 percent of the red cells

recovered the next day, but are we not precipitating more of a storage defect—if you want to call it that for--red cells that causes some clinical harm to patients.

DR. MOROFF: Jim, for the Phase III trials that we're talking about now, what parameters would you measure? Would you measure frequency of transfusion? With the caveats that you have mentioned?

DR. AuBUCHON: I think for a cell that is being manipulated in a way that there is a reasonable probability that it will not survive as long, I think at least survival needs to be measured. And if one could have a group of patients who are relatively stable clinically, and who are being chronically transfused—sicklers and thalassemics, one might think of—to measure the inter-transfusion interval. I think that is going to
be very difficult, however, to get any meaningful data out of, because you would require hundreds of these patients, probably. And the studies are just too daunting.

DR. MOROFF: Too difficult to do.

Any other comments on this question? Toby?

DR. SIMON: Well, we've—you know, over the years studying anticoagulants and blood bags and filters and leukocyte reduction, I think we've felt fairly confident from what, I guess, we're now calling our Phase I and Phase II studies, and generally have not required these. And I'm

presuming that the additional requirement relates to kind of a new phase of activity in the unit, in terms of the chemical inactivation, and suggests that we need these studies.

But I think the problem is—the issues that Dr. Hess brought up in his presentation, about the things that happen as a result of—like the trolley and the fatty acids that accumulate and those kinds of problems—it would take a very huge number to detect, I think, any kind of difference—meaningful difference.

So, if one could do a smaller number and at least confirm that oxygen extraction and consumption went the way it should go with transfusion, or was comparable to the control—and transfusion interval and other things—then hopefully you could get enough data to be confident and at least look for any toxicities that would be unusual.

DR. MOROFF: Larry Corash? Do you have question? Then we'll go on to question three.

DR. CORASH: yes, this is an issue that we've actually had to deal with, because we are currently enrolling patients in two Phase III studies for our product. One is an acute study, in which—basically, it's surgical patients, and it's largely a safety study, because you really can't measure hemoglobin increments or

transfusion frequencies in surgical patients, because the stuff goes in and out, and there are too many variables.

But in the chronic transfusion setting of sicklers and thals, to develop this study we went to chronic transfusion clinics and got databases for patients that characterize their chronic red cell requirement--for some of these people, going back 10 and 12 years.

And, in fact, there's a lot of information to model off of, where you can look at grams of hemoglobin consumed per kilo of body weight, per day of support—and, I think, get at some very valuable information. And these studies, I think, lend themselves to be of a manageable size, in fact, to get good statistical precision.

DR. MOROFF: Last—a point on this question? Sunny Dzik?

DR. DZIK: Yes, Sunny Dzik. I just wanted to put some word of caution into cross-over trials, which the panel seemed to support. Just to echo what Larry just said, that in surgical patients and in—you know, unless you're in the thalassemic, sickler chronic model, even there there's a lot of individual patient variability over time. But once you're in the hospital model—surgical patients—you just can't do a cross-over study.

So I don't think we should walk away from our talk today with the idea that all of these should be done

using cross-over only, because that puts you in a little box just with out-patient sicklers and thalassemics.

And the other point I wanted to make was just to put in a pitch, perhaps for tissue oxygen levels. There are—there is technology and probes for demonstrating that tissue PO2—tissue PO2—is elevated following transfusion. And that seems to be what the point of the exercise is about.

So, you know, we might consider that.

DR. MOROFF: Thanks, Sunny.

Rich—last question.

RICH? DR. DAVEY: Again, with respect to red cell function, has there been any thought to looking at the equilibrium curve to see if there are any bulk changes in cooperatively or electropheresis to see if there are any hemoglobin species—other ones

—generated by these agents that are put into the red cells.

DR. MOROFF: Any comments?

[Pause.]

The panel does not have any comments, Rich - on your question.

Let's turn to the last question, which pertains to in vitro testing.

Should there be a list of required in vitro tests to be used in initial studies prior to Phase I

in vivo studies. If yes, what parameters should be measured? Should storage cells be subject to rejuvenation to assess the functioning of the red cell metabolic processes?

Toby, do you want to start this one?

DR. SIMON: Yes, I think—as I indicated, I think there should be a set of in vitro tests that would be required, and most logically they would be Phase I and you would do them before you did the in vivo, and that would be the standards of ATP and potassium and glucose and pH at the end of the storage interval, and then I would check 2-3DPG at an earlier point to see that at least—something like five days of storage.

And then, I think, you would look at each of these processes and determine whether there was anything unique that they did to the cell, or anything—any chemical process, membrane activity or whatever that you were concerned about, and might require additional tests based on that.

DR. MOROFF: What about the use of rejuvenation with storage cells, just to show that the ATP and 2-3DPG machinery is still intact?

DR. SIMON: Well, I mean, I think it's interesting. I don't think that I would—to the point of wanting to require that. It could muddy the waters, I

think, as John indicated. And we haven't traditionally done that, so it would be difficult to compare—although I guess one could compare—to do a trial and compare it.

I think it would be of interest, but I don't know that I—at this point I don't think I'd recommend that it be required, because I think if the cells are intact and functioning and circulating in the way we expect them to, then I think that should be the requirement.

DR. MOROFF: John?

DR. HESS: About 70 percent of all the information that you can get out of an in vitro trial is in the ATP concentrations--depending on how you look at it and what you believe about the errors in those measurements to begin with. They're not particularly accurate. And we're talking about differences over very small ranges.

As I say, my reservations about the rejuvenation--

DR. MOROFF: I think you've already stated those.

Jim?

DR. AuBUCHON: I have some reservations about rejuvenation, as well. What you put a red cell through in rejuvenating in the laboratory is not exactly what the body is going to put the red cell through. And I would like to see some correlation experiments performed in order to know

that it really—that this test is actually showing something that has some clinical relevance.

The other in vitro test—an in vitro test I would like to mention also is just red cell morphology. Harry Merriman, for many years, has advocated red cell morphology to be performed at the end of storage, saying that the red cell recovery that is seen with radio-labeling is usually approximately the same, but is never better than the red cell morphology score, which is also given in a percentage fashion.

And that appears to hold until you get to the hypo-osmotic storage solutions that both Harry and John and Tibbi have worked with. But in the standard solutions that we're used to using, that would appear to be potentially a useful in vitro measurement.

DR. MOROFF: Steve Wagner yesterday mentioned about the depletion of glutathione by some of the treatments that ware being used to pathogen-inactivate components.

Are there any comments about measuring glutathione? We don't do that routinely. I don't think we ever measured glutathione, per new bags, per leukocyte-red cells. There's a lot of glutathione in red cells and you can get rid of a lot glutathione and probably still have good redox potential.

But is there any thought about measuring glutathione?

DR. HESS: Actually, we've done a little bit of that. And as long as there's ATP, there will be glutathione.

DR. MOROFF: Harvey Klein?

DR. KLEIN: Well, I would argue that point, John, because in the past the studies that most of us have done were in cells that have been stored, and they've simply been running down. But here we're actually putting in components that may poison systems in the cell.

And I'm—I'd like to actually extend that question to ask you—since we've heard that some of these may result in low glutathione in cells, shouldn't we be looking for an in vitro test of oxidative stress, especially since some of the cells we're going to be storing may be low in G-6PD, because there are people who have low G-6PD and other protective enzyme systems.

Maybe these cells will pass all of the various in vitro studies, survive normally, deliver oxygen normally, and be particularly susceptible to oxidative stress in vivo.

DR. MOROFF: Harvey, what would you suggest as a test of oxidative stress?

Are there any comments? Probably some drug—there's all sorts of drugs that--

DR. HESS: Phenylhydrazine would certainly be an easy one.

DR. MOROFF: Jerry—other comments in response to Harvey's comments? Jerry Holmberg?

DR. HOLMBERG: I was heading up to the mike at the time that Jim was answering the question on the morphology, and I'm just concern—I'd like to hear some other comments there from the rest of the panel on the cell morphology scores, especially with a lot of the systems that are using additive solutions at the end for re-suspending.

DR. HESS: Well, certainly we have shown that just by making very simple salt gradients you can make the relationship between morphology and recovery go away.

Klaus Hugman has done a lot of work with the morphology score, and he's had some scoring systems. And I think there's some value—I agree with Jim, I think there's some value to the morphology score.

DR. MOROFF: Bernie Horowitz? Jerry, did you have another point or--

DR. HOLMBERG: I just wanted to hear what Toby said.

DR. SIMON: I don't have any experience with it. And at least from my reading, it didn't appear to be a significant factor, but obviously--

DR. AuBUCHON: I wouldn't want to see the agency discard a proposed system with good in vivo recovery because its morphology score was bad. It should be used as an early screening tool to indicate whether or not you've got a problem.

DR. MOROFF: I agree with that.

Bernie Horowitz?

DR. HOROWITZ: I'm not getting the sense of the committee as to what you as individuals think is important in the measurements that are being made. Obviously, if there's no difference between the control and the treated, everybody's happy.

But what do you do when the measurements show a difference? And there are real-life examples of that. We used to use a particular system which is not being pursued for a variety of reasons now, but as an example, potassium levels went up, there were changes in osmotic fragility. On the other hand, circulatory survival, at least in animals, appeared unchanged—or, you know, unmodified.

So, for these in vitro measurements—although I agree with you as to what should be measured, I'm uncertain as to how to handle differences when they're observed.

DR. MOROFF: That's a good point, Bernie. Are there any comments? You're right, there are no standards, other than for hemolysis—and that's an absolute standard.

Toby?

DR. SIMON: Is the question you're asking the same one that the prior panel discussed? That is, if you feel there is an advantage to pathogen-inactivation, how much diminution in the quality of the red cell do you accept?

Is that--

DR. MOROFF: In vitro.

DR. SIMON: Not in vivo, just in--

DR. MOROFF: He's talking about the initial testing, I think.

DR. SIMON: In vitro.

DR. MOROFF: What would discourage you—if your in vitro results were way down, so to speak, for certain tests, how far down do they have to be to discourage you from going to in vivos? That's a tough--

DR. SIMON: Yes, I think that's difficult. I mean, I would be reluctant to proceed if the cells didn't appear to—you know, if the ATP did not appear to be maintained and we didn't have at least some, you know—level DPG, and I think it's suggested what this should be. If it's lower than that, then I would have some concerns about proceeding, because, you know, we don't have an ideal

product now for patients. And I would at least be concerned about proceeding if there's a significant difference.

DR. MOROFF: I think one point I want to make here: controls are very important—to do matched controls. There's a lot of inter-donor variability, as you talked about. And I think controls are very important. We didn't, maybe, stress that enough in the last half hour.

Last question, from Larry Corash.

DR. CORASH: Just a point of clarification, Gary. In the beginning of this panel discussion you put up a slide that you said was current FDA thinking about the 75 percent recovery level, and the confidence interval around that, which I think is fine.

Is that actually published and reference-able someplace? Because I've been asked that question, and I can't find it in the literature.

DR. MOROFF: It's not published, and that was the reason why I wanted to show what current thinking is. I called Jaro about that. And, to the best of my knowledge, that is not published.

DR. SIMON: And we've looked—I've looked for it also, and couldn't find it.

As far as I know, back in the—I think with the CPBA-I, around that time—Tom might know better. He's

shaking his head, so I think that's correct—the agency went from 70 to 75, as thinks were changing and they wanted to make sure that they didn't get a less good product. And I don't know.

DR. MOROFF: There was a workshop in 1988, and the minutes from that workshop—the transcript from that workshop lists the 75 percent standard without any explanation, and also lists the 1 percent standard without any explanation—whether it's a mean, or whether it's for all the units. That's the only place where I think there is anything written down, the way I understand it.

Jaro, do you want to comment on this?

Tom?

TOM: I know they seem like a long time ago, but CVA-I studies—they did, when we had a pre-IND meeting to qualify the first aniqua of viskine with adenine, we were told the mean had to be 75, the standard deviation was left open—if my memory serves me right—which it probably doesn't because that was 30-odd years ago.

DR. MOROFF: Jaro Vostal?

DR. VOSTAL: Well, my colleagues and I actually inherited the 75 percent cutoff, but it was just that. It was a number. And we've been approached by a number of manufacturers with studies, and they want to know, you know, is that a plus-or-minus some standard deviation? And

so we had to, by necessity, add these statistical parameters to make it so it would be useful for these studies—or interpretation of the studies.

So that is actually what we've come up with, you know, dealing with manufacturers. And that's what we're going by now. But it is not published anywhere.

DR. MOROFF: Thanks, Jaro.

One last question. Are there any comments about the appropriateness of 75 percent for the 24 hour recovery? Is that the appropriate minimum level—the way it is now being interpreted?

DR. AuBUCHON: So, Jaro, you're telling us that it's not published but it is carved in stone.

[Laughter.]

DR. MOROFF: Jim, do you have any comments about the appropriateness?

DR. AuBUCHON: Yeah—80 is better than 75 and 85 is better than 80, because it is clear that that's still a large proportion of cells that are going to be removed from circulation, and that the patient is not going to get any benefit out of.

But we have come to recognize, through a half-century of blood banking, that a unit that can yield 70 to 75 percent recovery seems to do pretty well clinically. That doesn't mean we shouldn't try to make it better.

DR. MOROFF: John, do you have a comment on this? John Hess?

DR. HESS: I think most of the current liquid systems, except CPDA-1 certainly have values that are probably above 80. And whether that represents a kind of operational standard—we certainly accept lower values, down to the 70s—high 70s—for the frozen systems.

DR. MOROFF: Question? Mike Busch?

DR. BUSCH: Just on that point—what's the denominator for calculating the percentage? What we're seeing is additive loss of red cells through the procedures—leukoreduction—Some of these procedures involve further manipulations, these adsorbents, etcetera. So, you know, is the denominator the number of cells that actually are transfused, or is it the starting content of the red cell unit before treatment?

DR. MOROFF: John, you want to answer that?

DR. HESS: No—at each point, at least the way the recoveries are measured, they're an independent measurement—you know, whether one—as in the frozen systems, uses the freeze for a wash. In vitro—you know, the whole system recovery, we usually report that separately from the actual measured in vivo recovery. But you report both values.

DR. AuBUCHON: But you raise a good point, Mike, and I'm going to get into this this afternoon when I speak.

Because calculating the recovery based on what is infused will allow us to predict the physiologic response of the patient to the transfusion. However, if one takes into account the initial number of cells that entered the process before the treatment or filtration or whatever, and look at more of a therapeutic efficacy standard, one may find that one needs more transfusions in order to get the patient to where the patient needs to go. And that has ramifications for the system—and possibly also for the patient.

DR. MOROFF: Jim, you have the last word—we're beyond our time. We could have probably talked about this issue for another hour, in different ways and forms.

But I now turn the microphone over to Sukza Hwangbo, from the FDA.

DR. VOSTAL: Thank you, Gary. That was a very helpful discussion—this one and the one for platelets.

And we'll be studying those responses as the time goes on.

SESSION IV: EVALUATION OF TOXICITY TO RECIPIENTS AND TO HANDLERS OF TREATED TRANSFUSION PRODUCTS

DR. VOSTAL: Now, we're going to switch a little bit. We've been talking about toxicity to the products

themselves, but now this upcoming session will look at toxicity to the recipients of the products.

And to help us lead us off, we have Ms. Sukza Hwangbo, who's a toxicologist in our group, and she has been helping us evaluate some of these treatments.

DR. HWANGBO: In terms of toxicity evaluation of a product, this is a very unusual situation. Needless to say, this is not a drug in a traditional sense, but a chemical residue that should be removed as much as possible.

Yesterday our three manufacturers described their procedure very nicely. The most likely procedure may be—you know, if I can oversimplify—a chemical is mixed with the blood, and irradiated with UVA light, and incubated for a certain period of time, and then washed.

So, the level may be very low, however we are talking about nucleic acid targeting agents and, you know, they may modify DNA and RNA. So we are talking about genotoxing material.

When we design pre-clinical study protocol, we simulate the actual use condition in clinical setting. So the route of administration in this case will be IV. We have five speakers today to discuss toxicity.

First, I would like to introduce Dr. Anita O'Connor. She will give us an overview of how review in CBER. She is a toxicologist in CBER, FDA.

Overview of Toxicity Studies for Biologic Therapeutics

DR. O'CONNOR: Thank you, Sukza.

As Sukza mentioned, this morning I"m going to give an overview on the types of toxicology studies that we generally look for in biologic applications.

[Slide presentation.]

So, how do we define the problem? Biologic products can be very complicated. And they can have many different components. There's obviously the biologic product itself—and we're frequently wondering whether this product has been altered by the manufacturing process. Does it have some increased immunogenicity? Has it been altered by chemicals and processing? Are there carry along, ride along or carryover residual cellular components in the product? Have these been altered somehow by the manufacturing so that they are mutated or immunogenic.

And, lastly, are there chemical impurities which are the result of in-process impurities, and have they had a direct or indirect effect on residual cellular components or the host tissues?

So there are many things that—many different components of the product that we look at in the toxicology evacuation.

Well, why are we even looking at this problem? Blood is perceived by many to be at a very low risk from pathogen infection, and there are methods of preventing this risk through donor screening and nucleic acid testing, for example. So why do we even want to do toxicity studies with transfusion products?

Well, one thing, obviously, the agency is concern about is emerging pathogens; bacteria, viruses, other things that could emerge as pathogens. We are concerned about this.

So the role of the toxicity evaluation has a very important role in risk assessment, in safety package, and we balance that will efficacy and risk communication—which is traditionally done with labeling.

Well, biologic products have unique challenges; we have a unique guidance document, ICH-S-6, which concerns itself exclusively toxicological evaluation of biologics.

biologics aren't traditionally thought of as being mutagens or carcinogens. So there haven't been a lot of carcinogenicity studies with traditional biologic products.

But one that is very challenging with biologics is the immunogenicity concerns. Has the product acquired some altered immunogenicity, and will this affect—will it become an immuno-toxicant when given to the host—or the patient.

Now, chemicals, which has been the focus of this workshop to a large extent—chemicals or in-process impurities, resemble more traditional concerns. And the type of toxicological testing that we do with these chemicals is more along the lines of the types of tox testing that is asked for by the Center for Drugs. And some of our speakers this afternoon are from the Center for Drugs and will talk more specifically about those types of tests.

So when we do a toxicology evaluation, we look at both general and specific toxicities. And for the general toxicities, we often have animal models, and we look at basic effects on organ systems, such as blood pressure, liver enzymes. But sometimes—some biologics, we don't have good animal models, and the risk is really verified by a clinical creep approach. In the Phase I study we start very slowly, and the risk is verified by an increasing amount of clinical experience with the product.

There are special toxicities. We look very closely at things like carcinogenicity and reproductive

toxicity for chemicals—more so than chemical impurities—more so than a traditional biologic product. And these are—toxicities are rarely verified from clinical trials. We will go to animal models and then project the toxicity from the pre-clinical study to the clinical situation.

Now, the results of the toxicity evaluation is really—what's really important on the effects on the population of interest. The population that will receive the product. And we look very closely—what is an acceptable risk for the population that will receive the product? Is this a product that will be used by—that there will be chronic infusion? Or is it just a product that—the toxicity could result from an occupational exposure?

So there are many different situations. And we relate the toxicities to acceptable risk relative to the population that will receive it.

Some populations of concern are special populations. Some products will just be used in the general population, or healthy adults. Other special populations that we're frequently concerned about are children, who have an immature immune system; pregnant women, who have an altered immune system as well as cancer patients and trauma patients, which can have any—all sorts of different physiological impairments.

So, these are—this comes into the equation when we think about the risk of various infusion products.

When I speak of risk communications, we're primarily talking about labeling. And we have some knew efforts in labeling products for pregnancy. As many of you probably know, we are transitioning away from an alphabetical categorical system for labeling products for pregnant women, into a process where the results of the reproductive toxicology studies are actually described, if there are meaningful results, in the labeling. And there is a new guidance document out on this. It's primarily a CDER document, but we will be using it to some extent in CBER also.

Just a couple of final comments.

When we evaluate the toxicity of a therapeutic—of a biologic—we're going to look at all the components. Sometimes we—we frequently look at the whole product in a model, and/or we look at the individual components and chemicals.

And there are many ways to look at this. We will frequently look at the worst-case scenario. If there is a chemical product, we will calculate what is—if this product is going to be used in a chronic infusion situation, what is the worst case? What is the maximum amount of that

chemical that a person could potentially receive over their lifetime?

And, lastly, it is a risk versus benefit process. And we'll hear more about that this afternoon, I believe.

And, with that, I'll conclude.

Thank you.

[Applause.]

MS. HWANGBO: Questions for Dr. O'Connor?

[Pause.]

Then we are going to adjourn our meeting. So we will meet at 12:30? Okay. We will meet at 12:30.

Thank you.

[Luncheon recess.]

MS. HWANGBO: Please have a seat.

We will continue our toxicity session.

Our next speaker is Dr. Hanan Ghantous. She will discuss mutagenicity and carcinogenicity studies.

She is with the office of New Drug Applications, CDER, FDA.

Mutagenicity/Carcinogenicity Studies for Evaluation Compounds To Be Added to Transfusion Products.

DR. GHANTOUS: Thank you.

Good afternoon, everybody. My talk today is going to be on genotoxicity and carcinogenicity studies for pharmaceuticals in CDER—that's the Center for Drug

Evaluation, which I don't think is different from what you do at CBER.

I will be talking about the influence of genotoxicity data and the need for carcinogenicity studies; the influence of genotoxicity data on the dose selection, the design and the interpretation of carcinogenicity studies.

I will also talk about the timing of these studies—when do we do them—and some alternative models for carcinogenicity testing.

This is a list of all the guidances—or most of the guidances concerning carcinogenicity and genotoxicity studies. I also have the safety study guidance—the M3. All these guidances are on our website. Their all ICH, so they're on the ICH website, too.

There's a new carcinogenicity guidance that's not listed here. It just came out. It's about protocol submission to the Carcinogenic Executive Committee.

The standard battery for genotoxicity testing—we usually like to see three tests; two in vitro and one in vivo. We like to have the tests that will show us gene mutation and chromosome aberration.

Examples of these tests are the Ames test, as an in vitro test; a mouse lymphoma as an in vitro test, and the micronucleus test as an in vivo test. Any other tests

are okay—as long as they show us the gene mutation and the chromosomal aberration.

Why do we like these tests? They are well-established tests. There are internationally accepted protocols out there: the OECD protocols. They're validated against rodent carcinogenicity tests—well, some people believe in this and some don't agree.

This battery—or these tests are complementary to each other. Like I said, they show of gene mutation and chromosomal alterations. And we don't have one test that shows us all the DNA damage, so we have to do more than one.

Interpretation of data—of genotoxic data. When we have a positive result in any one test, identifies genotoxic potential. Well, what does that mean? A positive result in one strain in the Ames test cannot be ignored. At the same time, a negative result in an in vivo test cannot overrule a positive result in an in vitro assay—although some people think that the in vivo tests are more accurate prediction of rodent carcinogenicity.

Our carcinogenicity guidance says, "The assessment of the genotoxic potential of a compound should take into account the totality of the findings and acknowledge the intrinsic value and limitation of both in

vitro and in vivo tests." We have to look at the whole battery, and we have to take the weight of evidence.

The carcinogenicity guidance also states that "a single positive result in any assay for genotoxicity does not mean that the test compound is going to be genotoxic to humans." We have to S2A genotoxicity guidance, which contains a checklist for evaluating relevance of positive results.

Timing of genotoxicity studies—the M3 guidance says "prior to first human exposure" we should have the in vitro tests done. If we have a positive result there, maybe more testing should be done. But the whole battery, including the in vivo and the in vitro should be done prior to the initiation of Phase II studies.

If we have any concerns over the genotoxicity of the compound—we have positive results, and depending on the indication and the dose, this might result in a hold—until further testing is done.

Usually we start with the battery—with the two in vitro and one in vivo tests. If we get positive results, we might do additional studies with the same endpoints. Then we might do a cell transformation assay, the CHO assay, and then we go into carcinogenicity testing. A two year rodent bio-assay or a short alternative method.

The sponsor really doesn't have to go through this sequence. They can go from the three-test battery into the carcinogenicity testing if they want.

Now, the need for rodent carcinogenicity studies—why do we do carcinogenicity studies? To identify a tumorogenic potential in animals and, if possible, to assess relevant risk to humans.

The drugs that are going to be given for more than three months in the U.S., and six months in Europe, should be tested for carcinogenicity.

Drugs that are going to be used for less than six months, but are going to be used repeatedly over a long period of time—like drugs taken for anxiety or depression. Drugs that are going to be used in a delivery system and the patient is going to be exposed to them for a long period of time.

We have some pharmaceuticals that are not used for a long time, they're used for a short period of time, but due to their nature, they might need to be tested.

The considerations we need to take before we do carcinogenicity testing is class alerts--if the drug we're looking at comes from a class of drugs that show carcinogenicity effects. Structure-activity relationship should be the first thing to look at; evidence from 90 day

toxicity study, any evidence of pre-neoplastic lesion should be looked at.

Long term tissue retention of the parent compound or any of the metabolites, and any changes happening to the tissue where the retention is occurring.

Genotoxicity—if we have a compound or a drug that is known to be genotoxic in a number of species, and implies hazard to humans, or we know it might be genotoxic to humans, then we really don't need to do carcinogenicity testing. If the drug is going to be used for a long period of time, just a chronic study would be enough.

Experimental approach—we usually ask for one long-term rodent carcinogenicity study. The species selection depends on the pharmacology, the repeated-dose toxicology, metabolism, kinetic, route of administration. This is usually a two-year rat study or could be a different species.

The second study we ask for is a short or a medium-term study in transgenic or neonatal mice, or a long-term study in second rodent species. What we used to ask for is the two-year rat study, and a two-year mouse study. Now we have the alternative models that are replacing the two-year mouse study. So, the sponsor can use these models with the two-year rat study.

Other studies are mechanistic studies, which are always useful, to know what's going on. If they can detect cellular changes, biochemical measurements, more genotoxic testing to tell us more of what's going on.

The dose selection for carcinogenicity—the high dose selection could be done from toxicity-based endpoints. We always have to get an MTD—a maximum tolerated dose—from pharmacokinetic endpoints; saturation of absorption; pharmacodynamic endpoints; maximum feasible dose, and limit dose.

I'm not going to talk more about this. There's the guidance—the S-1C—explains this very well.

The selection of the middle and low dose—we usually don't like to see those calculated mathematically. We like to see these factors taken into consideration: the linearity of pharmacokinetics, the human exposure, the mechanistic information and the rest of the points here.

Genetically modified mice—these are the models that are being used now instead of the two-year mouse studies. The p53 model, which is a tumor suppressor gene knock-out model, which identifies the genotoxic carcinogens, and it used mostly in the U.S.; the Tg.AC model, which is a tissue-specific oncogen model—example, the skin-paint model. And it identifies the non-genotoxic

carcinogens and promoters. The TgRAS H2 model—and this identifies genotoxic carcinogens. It's used in Japan.

Induction and promotion model—also identified nongenotoxic carcinogens; and the neonatal mouse model, which is sensitive to genotoxic carcinogen, and it's being used as an alternative to the P53S, say, for genotoxic drugs.

Most of these models are still new and they're still being validated by NTP—the National Toxicology Program, and ILSI.

What is the best choice for an alternative model? The model that will add the most valuable information to the safety evaluation when it comes out negative. It's good to have—to test the drug in a model and have a negative result, but what does that negative result mean? We still have to take it case by case, and we still have to take all these points into consideration. We still have to look at the results of the genotoxicity batter, the route of administration, the metabolism. So we have to look at the whole picture and take the weight of evidence.

The protocol design—usually, a four week dose ranging study is needed. It might be needed to be done in transgenic animals, too. Standard positive controls is always needed because, like I said, these are all new models. They haven't been validated that well, so we have

to make sure that the model is working. So we need a positive control.

The number of animals for the p53, between 20 to 25 for the Tg.AC-15. Number of dose group, 3 plus vehicle plus a positive control. And we always need to achieve an MTD.

And the duration is six months, but now FDA is talking about going out to nine months to make sure that we're seeing something there. And like I said, they're still being validated by ILSI and NTP.

This is from the European Agency for the Evaluation of Medicinal Products. It's their Safety Working Group. This actually does not have anything to do with FDA. But I put this here because it summarizes nicely my talk.

This working group looked at the ILSI validation of the models and came up with this conclusion. And their conclusion is: "In compliance with the ICH document, the TgRAS H-2 and the p53 model can be used as alternatives to the mouse long-term study in conjunction with a long-term rat study and genotoxicity studies." And that's exactly what we ask for at FDA—the genotoxicity, the rat two-year study, and then the mouse two-year or it can be replaced by one of those models.

The outcome of an experiment with transgenic animals should not be considered as the decisive factor in assessment of genotoxicity. I would say here "carcinogenicity"—but rather as par of the weight of evidence. The results of a transgenic assay may be used to prove or disprove a hypothesis derived from genotoxicity data, rather than to decide whether or not a compound is genotoxic or, I would say, carcinogenic.

Thank you very much. If you have any questions--

[Applause.]

MS. HWANGBO: Do you have any questions?

DR. WAGNER: Yes, I have a question. Do you make distinctions between whether a compound by itself is genotoxic, or whether a compound after it has been treated in blood, and is ready to be transfused is genotoxic?

DR. GHANTOUS: A compound is treated by blood and becomes—?

DR. WAGNER: Treated in—

DR. GHANTOUS: You mean added to blood?

DR. WAGNER: Right. The compound may change when it's added to blood, so you're always talking about a compound, but do you think it's important to look at the genotoxicity of a starting compound, or at the end of a process?

DR. GHANTOUS: Both—yes. I think both. The end of the process is important, too. Yes.

MS. HWANGBO: We will have another question-and-answer session at the end of the session. So--

Now we will move to the next speaker.

Dr. Suzanne Thornton will discuss reproductive toxicity studies. And she is a member of Reproductive Toxicity Subcommittee within CDER.

Reproductive Toxicity Studies

DR. THORNTON: Thanks. I hope everyone's still awake after lunch.

As she said, I'm Suzanne Thornton, and I'm currently in the Division of Reproductive and Urological Drug Products at CDER.

My talk today will basically be a brief history of the reproductive toxicology guidance. We'll go through the actual study designs of the studies.

We'll also—as Dr. Ghantous spoke—discuss the timing. When should these studies be conducted during the clinical development?

We'll also talk about some recent pediatric legislation which impacts new drugs which are trying to be marketed. And then we'll switch to special considerations for these special pathogen-reduction products.

So, prior to like mid-1960s, there were really no reproductive toxicology standardized study designs. However--I'm not old enough to remember—but there was the Thalidomide incident, which raised a lot of questions about—"We really need to get a standardized guidance to assess, in non-clinical animal studies, these new drugs and chemicals before we actually give them to humans." So we're trying to assess the human reproductive risk assessment.

So in 1966, actually at the FDA which was referred to—or he was in the Department of the Health, Education and Welfare—Dr. Edwin I. Goldenthal developed the Guidelines for Reproductive Study for Safety Evaluation of Drugs for Human Use.

And what Dr. Goldenthal identified were three important portions or segments which he felt were important to assess the reproductive human risk assessment—or potential. And he called these segments I, II and III. The Segment I was designed to identify the study of fertility and general reproductive performance. Segment II dealt primarily with teratological study—or birth defects. And Segment III dealt with perinatal and postnatal study in animals.

Now, the Goldenthal guidance, as this was referred to—or "the Goldenthal rule"—actually was the Gold Rule of study designs from 1966 until the mid-1990s.

So, what happened in the mid-1990s was the establishment of the ICH. They were actually established in 1990—and ICH, just in case you don't know, is the International Conference on Harmonization.

The composition of the ICH are basically regulatory authorities in Europe, Japan, the United States, as well as experts in the pharmaceutical industry. And their objectives are mainly fourfold, but it's basically to help with the registration of new chemicals and drugs to alleviate any roadblocks that might be there, and by doing this—the way to do this is to harmonize your study designs so that everybody is on the same page.

And in 1994, the ICH released their guidance, which is the ICH-S5A and—as I'm scrolling down—you can see that they basically kept the same three segments that Goldenthal identified in 1966. They're divided into Section 4.1.1—which is referred to as "the old Seg I," which, again, deals with fertility and early embryonic development. The Section 4.1.2, which is the old Seg III, deals with pre- and postnatal development. And then the 4.1.3 deals with the embryo-fetal development, which is the old Seg II.

Now, these studies are primarily designed to be conducted in rodent species, with the exception of the 4.1.3, or the old Seg II, where is required a rodent and a non-rodent species.

Now, in the U.S. we generally—the rodent species is the rat. But in Europe and Japan, it tends to be the mouse, and the non-rodent species is generally the rabbit.

Now, since 1994, there really have been no changes to this guidance, but there have been two addendums which have been released, and these are the ICH-S5B and S5B(m). And these primarily affect changes in the dosing for the fertility reproduction studies, or the old Seg I.

So let's look at the design of these studies.

So this is the Seg I, and this is just schematically represented, but I wanted to go through the three study designs, talk about the dosing period, and then talk about what are the endpoints or outcomes that you're trying to get out of these studies?

So, for the Seg I, or the fertility reproductive studies, there are three different study paradigms. One is where you treat males during pre-mating, and then you mate them with untreated females and you see what happens. Another paradigm is where you treat the females, pre-mating, you mate them with untreated and you see what happens.

But the most common design is where you treat both the male and female—this is where the addendums have come in. The pre-mating can either be two or four weeks. It depends on what you want to do, but you have to justify in your protocol why you're doing two or four weeks. And for females, you dose for two weeks, then you have a mating period, then you have a post-mating period. And for the females, you actually conduct a caesarian section on gestation day six.

Now, as you can see, there's a problem here. Because happens if, when you do a C-section in your females, you find something wrong and you've treated both your males and females? How do you know who is the contributing factor here?

So one way to do a slight modification of this protocol is to actually keep your males after you do your C-section on your females—keep treating them, and then, if you see something on your C-section, you can go back and now treat your mated females [sic] to untreated females. And that's a way to try and tease apart—is this due to a male fertility problem or female fertility problem.

So the reproductive parameters that you're trying to achieve, or what are the answers you want to get from this study for both males and females—you're looking at

gamete maturation, as well as mating behavior. You're looking at mating indices and fertility indices.

Now, for each of the genders—for males and female rats, of course, there are additional outcomes. For male rats, you're interest in spermatogenesis. You do this by conducting sperm analysis, where you're looking at sperm in the testes, epididymis; you're looking at sperm viability, motility and morphology.

Now, according to the guidance this is optional, but we are seeing more and more of the studies having this component. And, to me, I really like to see the sperm analysis.

And also you're looking at reproductive organ weight and histopath, mainly testes and epididymis.

In the female you're also, during the study design, you do estrous cycling. You want to make sure that they are cycling properly. And then for Caesarian-section data, you're looking at the number of implants, the number of resorptions or corpora lutea; the number of viable fetuses, and you're also looking at reproductive organ weight and histopath.

When you go to the old Seg II, or the embryo-fetal development, again, like I said, this is the only study where you require a rodent and a non-rodent species. And generally the dosing occurs during organogenesis, which

is defined as the time from implantation to closure of the hard palate.

Now, implantation in rodents, as well as non-rodents, especially mouse, rats and rabbits, happens about gestation day six or seven. And the closure of the hard palate is anywhere from gestation day 15 to 18.

So this is just an example of dosing. This is actually dosing from gestation day six to 17 in a rat. But remember that you can dose anywhere from gestation day six to 15 to 18, depending upon how you would care to design your study.

The study endpoints—oh, and then again, you do a Caesarian-section on gestation day 20, which the day right before they would normally deliver.

Study endpoints—again, optional is the gravid uterine weight, which is a good idea to measure. You don't necessarily see that quite frequently but, again, it's a good way to see are all the fetuses delayed, is the weight lower.

Caesarian-section data is the same thing. You're looking at your number of implantation sites, resorptions, corpora lutea, your number of viable fetuses, as well as your gender ratio. And you're also looking for body weight in your fetus, as well as malformations and variations, and you're looking not only at external malformations and

variations, as well as soft tissue or visceral and skeleton.

Now, according to ICH guidance, for skeletal identification you only have to do one study—I mean one stain, which will stain the bone. But now we're seeing where you're getting dual staining, where you can see cartilage and bone, and that helps to determine if, say, your fetal body weight is low, you can look at the skeleton and say, well, they're developmentally delayed. And OECD guidance actually requires double staining.

This is just the rat. It's the same paradigm

Now, for the prenatal and postnatal study, this is probably the longest—or it is the longest of all three studies. And the dosing period is from gestation day 6 to lactation day 21. And then on lactation day 21 you stop dosing, but then you select the pups which have been exposed in utero, as well as during lactation, say, potentially through milk during lactation, and you're looking for indices as they grow, as they mate and then as they reproduce.

So the endpoints that you're looking at for both generations are parturition difficulties. Do they have difficulty in delivering the pups? Once they deliver them, are the mothers sick and don't take care of them, so you have total litter loss?

You're also looking at litter data—mainly pup viability. How many pups survive after they're born? For this F-1 generation who were just chosen to grow up and mate, you're looking for a lot of landmarks—pre-weaning landmarks such as incisor eruption, eye-opening and pinnae detachment. And if you have fetuses or pups that were born and have low birth weight, you do see delays in these developmental landmarks.

You also are looking for post-weaning landmarks like vaginal opening and prepuceal separation.

You also want to look at behavioral—are there anything behaviorally that's happened during in utero exposure. The common paradigms are learning and memory study. You're looking at locomotor activity, as well as auditory startle.

And you're also looking at their mating behavior. Again, mating indices, fertility, as well as estrous cycling in the females.

There are also two additional special reproductive tox assessments that are not necessarily in ICH guidance, but depending upon the application and the compound or drug, you may want to do.

They are placental transfer and milking. Of course, with placental transfer studies, they are technically feasible, but it really does require a lot of

technician time. But it's good in that you get both maternal fetal exposure. You can actually collect the blood, spin down the plasma, and actually get toxico-kinetic analysis from both mother and the pup—which could be very important, because that way you know how much you gave the mom, and you know how much was actually transferred to the fetus.

And then there's also the milking studies, if you're concerned that the drug is being transferred to the pup or the offspring via the milk. There are actually very feasible ways, where you separate the mom from the pups for about four hours, and then you give her oxytocin, and you actually milk her like a cow. They actually have little sucker devices that you can do that.

In my previous life, I had a great little—what we called "Ecuadorean milk maid." He had the right sized hands, and he could like manually do it. So he had a really good niche there.

So, when should these reproductive toxicology studies be conducted? As Dr. Ghantous implied, the timing of all of these studies are outlined in the ICH M3 guidance. And for the fertility and repro studies, and the embryo-fetal development studies—i.e., Segment I and II—the M3 guidance says that they must be conducted prior to Phase III human clinical trials.

For the pre and postnatal developmental studies—i.e., Segment III—they need to be conducted prior to submitting the NDA. However, the caveat that is outlined in that guidance is that all of the reproductive tox studies should be completed prior to inclusion women of childbearing potential with the caveats that if they're not using highly effective birth control or pregnancy status is unknown.

So, while it states that you don't really need the Segment I and Segment II before Phase III, we generally see it before Phase II, because you're starting to enroll additional people who are of childbearing potential.

Now, there are some recent pediatric drug legislation, which I realize are for drugs. But since these special pathogen-reduction products are considered compounds, then they will probably—although I'm not sure of that—fall under these potentially.

So in 1009, there was a pediatric rule which required manufacturers of new and marketed drugs or biologics to evaluate safety and efficacy in children, if the product was going to be used in children. And then recently, this year, there is the 2002 Best Pharmaceutics Act for children, which actually reauthorized the pediatric exclusivity reauthorization, meaning you could receive a written request if the agency—the FDA—feels that the

product will be used in pediatric patients. You could receive a written request that you must do evaluation in this population.

So out of this legislation has also come a new directive and a new need for guidances, because of course before you go into a pediatric human population, you want to do something in a non-clinical animal model. So it's becoming very important to design studies with guidances using juvenile animal models to try and get an understanding, is there a higher risk or a different risk in pediatric patients before you actually go there.

So, to step back a minute, reproductive and toxicology in these juvenile animal study designs—why are they even important? Well, of course development is a continuous process. Of course there are structural and functional maturational differences which affect the drug safety.

Postnatal toxicity is more likely in tissues undergoing this postnatal development. These are just some of the organ systems, of course, which are developing. Of course they're all developing during in utero.

And the studies really need to be designed so that the critical window of susceptibility is assessed. As you saw in the study designs, we have mandated when we think the optimal window of dosing—such as in the embryo-

fetal development gestation day 6 through 18, because that's organogenesis. But there are certain other windows of susceptibility, such as if you know that your skeletal systems is a target organ, then you will want to dose your animals during that most susceptible time when the skeleton is developing—just as an example.

So what are some special considerations for these special pathogen-reduction products? The first thing that came to my mind, of course, is dosing. And I know right now that when you dose, just because the way these products are given, you know, the products are in the blood themselves. But the question is: do you really want to simulate the human exposure, or maybe, the other alternative, that you want to maximize your exposure. And we know that you can't necessarily maximize the exposure with these reduction products, because you're limited by volume that you can actually give.

So another consideration is the vehicle. Maybe the most appropriate vehicle—which, like you're using now are your platelets or your blood products. Maybe to get that maximum dose you may need to change the vehicle—sterile water, saline, etc.

Another consideration with dosing is your appropriate dose levels. In CDER, like Dr. Ghantous said, we're looking for the worst-case scenario; the maximum

tolerated or MTD. And when we design or look at dose selections for reproductive tox studies, our high dose we want to just kiss that maternally toxic level. Your high dose should be where you get some maternally toxic dose, which could or could not impact the fetus. Your low dose, on the other hand—we don't want anything. And your mid dose, of course, is in between.

And another consideration: are you really concerned about the parent chemical, which really is not active until it's irradiated? Or, maybe the thing is you're looking at those irradiated photo products.

And, again, coming from a different point of view, maybe a single dose, even during these study designs is not going to be enough. What happens—okay, this is just my non-clinical side coming out—if you have a person come into the emergency room, which will then have to go to the OR, which could go to the ICR, they're going to potentially be getting transfusions during this entire period. They're going to get accumulation of these products.

Again, it comes back to the worst-case scenario. What's the highest number of units that potentially someone could be infused, and what's that going to do if you have these products in there?

Some additional considerations are, of course pharmacokinetics and toxicokinetics. It would be very

helpful to us to have both maternal PK or TK for the parent and photo product.

And since these are DNA intercalating agents, especially in the fertility and reproductive studies--like I said, the sperm analysis is optional—I think it would probably be prudent for these drugs, because of they're actual pharmacological activity.

And one thing which we don't see, which I really—it helps us a lot, is to make sure that if you run a study that you really have a maternal and fetal "no adverse affect" level, because lot of times we get studies, and there's no—we can't really assess it, because there's no level where there's no adverse event.

So, in conclusion, there's a new day that seems to be dawning for studying the reproductive tox risks of this class of drug, and that there are some considerations—these are just a few and, of course, they're open for discussion—which you need to consider, such as do you really need to achieve or get the worst-case scenario, such as an MDT?

PK/TK data is very useful, and you may also need to determine the critical window of exposure and toxicity for the developing embryo.

And, in closing, I just wanted to let you know that on the CDER website there is a new draft reviewer

guidance which was released in October of last year. And it's the integration of study results to assess concerns about human reproductive and developmental toxicities. And this is a reviewer guidance, in that it provides us with a tool to take in account all of your non-clinical toxicity studies, your reproductive toxicity studies, your reproductive toxicity studies, and actually assign or determine what human risk is. And that is a draft, so it is open for comment.

Thank you.

[Applause.]

MS. HWANGBO: Do you have any questions for Dr. Thornton?

Pause.]

Our next speaker is Dr. Walter Dzik. He will discuss neoantigenicity. He is the co-director of Transcipitant Blood Services, Massachusetts General Hospital. He is also an Associate Professor of Pathology in Harvard Medical School.

Studies to Evaluate Neoantigenicity of Blood Products

DR. DZIK: Thank you. I have a new appreciation for the work at CDER—milking those rats every morning—

Laughter.]

—something.

It's a pleasure to be back at NIH again. And I wanted to actually, maybe, point out first—in your handouts, my address is wrong. This is the correct address. And e-mail address there is correct. So if you want to take exception to what I'm about to say and wish to e-mail me something, just go ahead and use the e-mail in your handout.

I want to address my conflicts, also, up front. I'm chairman of the Scientific Advisory Board for Vitex Corporation, and a paid consultant to Vitex. Though, to be fair, I must say that I have not discussed what I'm about to say, really, with anyone—so—including any of the people from Vitex.

So, the opinions I'm about to offer are completely my own.

I'm going to talk abut neoantigenicity and neoantibodies. And I'll also use the term "immunotoxicity," kind of interchangeably with that. So if I switch back and forth between those two, you'll understand me.

And I also, at least for my presentation, would like you to consider the word "drug" and the word "chemical," to be essentially the same thing. I mean, drugs are given with a therapeutic intent, but the topic now that we're discussing is the immune system and its response to foreign molecules. And so in this context of

immune response to molecules, it doesn't really matter whether they're chemicals or drugs, I believe.

I ant to start with a case example. There was a 53-year-old surgical patient, has a prior history of orthopedic surgery, who comes in now—is in the surgical ICU with pancreatitis. He's got an arterial line in and various other lines in his body. And on the sixth day he's noted to have a low platelet count, and someone sharply decides to test him for heparin-induced thrombocytopenic—HIT—and that test comes back positive.

A day later he's acutely short of breath and has a inferior vena caval filter placed. A couple days after that, he has positive blood cultures, and on day 12 he has died, with multi organ failure and sepsis.

And if you work in a hospital, you'll understand that this is not an unusual case. This is a real case, and one abstracted from many episodes of heparin-induced thrombocytopenia that are seen in my institution.

HIT with thrombosis is a very serious problem. It causes substantial morbidity and mortality in the United States every year, and results in unexpected thrombotic complications and death. It's an unexpected syndrom, and obviously an unintended syndrome, and it's due to an antibody to a neoantigen. It\s also an example of a

neoantibody problem which really would not be detected if heparin were to begin its pre-market licensure today.

The neoantigen is rather well understood. What occurs is that an otherwise completely uninvolved protein, platelet factor 4, which has some lacing residues, just turns out that biologically, these positively charged molecules will join up with the negatively residues on heparin if there's at least 10 of those heparin residues, and form this unique new structure—this neoantigen to which some people will form an antibody. If the patient does make that antibody to the neoantigen, it results in platelet activation and thrombocytopenia as these platelets begin to clump, and for certain unlucky patients, there's probably also cross-reacting antibodies that bind to heparin-P4 on the surface of blood vessels—on the endothelial surface. And so you basically begin to get an endothelial attack and endothelial damage, and increase your opportunity for thrombotic complications.

What is interesting, and quite relevant as an example for today's discussion, is that this is not a dose dependent feature. It certainly follows full dose heparin, but has also been described even with patients who are just—who are not actually receiving heparin as a drug, but who have indwelling catheters which are heparin-bonded, and

as the little bits of heparin leach off of those catheters, that's enough to trigger the antigen-antibody response.

It has all the typical characteristics of an immune response, and naive individuals take about a week to form their antibodies, whereas individuals who've been previously exposed will typically make an antibody in just a couple days and form a higher titre IDG type response—just typical of the standard immune response.

The antibody's not a rare event. Some people have fine that as many as 35 percent of patients who are getting heparin repeatedly will make the antibody. But, again, not everyone has the clinical syndrome. In fact, only a minority of patients will have these devastating thrombotic complications. And why some people have the clinical expression and others do not, is really somewhat of a mystery, but there's certainly been a suggestion that there are co-morbidities. And so now this further complicates concerns of clinical risk in neoantibody problems because you must consider not only the antibody, but the context of the patient in which the antibody is formed.

So some people believe that during period of sepsis and inflammation that thrombosis is more likely to occur in HIT; others believe that patients have underlying

thrombophilias—perhaps genetic thrombophilias—that will predispose them to thrombotic complications of HIT.

So that's just as an example context where we come to pathogen-reduction. And the cartoon—I'm going to walk you through a series of cartoons that kind of conceptualize what might be the candidate neoantigens which could potentially form neoantibodies, and then give you examples from other chemical and drug literature of some of these concepts.

So, in its simplest form the idea would be that you would take some treatment and treat a cell. And I cleverly made something that looks neither—I hope—like a red cell or a platelet to you, but treat some blood cell and it might alter that cell and the patient might make an antibody to it. And that's kind of your first-pass thinking about neoantigen formation and neoantibody.

And in this overly simplified view, the assay that you would want to do is to react serum from recipients of pathogen-inactivated blood components—so, let some human beings get this stuff, and then let some time pass, and they might make an antibody —and react their serum against target cells that are treated with the chemical. And you can obviously include untreated target cells as a control.

And the kind of assays you might do, or the readouts for this very straightforward approach—for red

cells, obviously hemoagglutination would be probably the best system. It's tried and true. And for platelets you might use either flow-cytometry or amepa assay—again, well-described assays for showing antibody to platelets.

Unfortunately, I think that is an oversimplification. What the patients are actually being administered in these processes is a bit more complicated. It obviously includes some level of residual chemical which may serve as an antigen for the recipient. But I think we also need to consider that there are both degraded cellular material of the intended product, or degraded red cell pieces, or degraded platelet pieces, as well as damaged donor DNA—which may be part of the infusion. And then there's also a degraded residual chemical—the pathogen-inactivation chemical—either photo-aytics of it, or breakdown products of it which occur in a biologic system.

So the patient gets all this stuff, and a number of potential things might arise. So this is the—in the center here, the blue cloud is meant to be the intended therapeutic product—the red cell or the platelet—but as the residual—some residual chemical goes in, it may bind to a completely independent third-party protein. So, if you're following me, this is the concept, like, of how heparin bound to platelet factor-4. And this could be anything—

third-party protein. It's not easy to predict what it might be.

There's the residual drug itself, of course, which may serve, itself, as an axis for an antibody formation. But there's also the fate of that drug. It binds to an enzyme to metabolize it, and then it gets broken down into metabolites. And both the chemical-enzyme complex are an interesting opportunity for neoantigen formation, as are the metabolites, either themselves or as they bind to enzymes.

If you then extend that to consider these little—these are the residual cellular elements or breakdown products of the treatment, then you get this kind of very complex matrix of potential opportunities for different classes of neoantibodies.

So the object here was just to not think of this exclusively in this one context, but recognize that the reality is that other possibilities may arise. And I want to give you some examples now, to show you that this is not just completely mindless imagination.

So what are some well-described examples of neoantigen formation,, in addition to the heparin PF-4 one we mentioned?

Well, to start with—an example at the very top—where the person makes an antibody to the intended

therapeutic product. We don't actually have to stray too far from hematology circles because of the recent problems which occurred with the Eprex brand of erythropoietin, and patients' making antibodies for that EPO, obviously well also recognized is the antibodies people have made to thrombopoietin or to interferon.

Drugs or chemicals can also bind to blood cells and form a complex resulting in the immunologic drug reactions. And drug purpuras are—there's hundreds of them. I mean they fill up pages in textbooks. So there are many kinds of drugs which can cause drug purpuras in some patients.

Quinidine is probably the granddaddy of this, and has been heavily investigated drug for which people make an antibody and then develop thrombocytopenia.

Drugs also, because they are chiefly metabolized in the liver—this, again, chemical-enzyme complex often becomes the locus of the neoantigen. And a good example there is halothane. Halothane hepatitis a well-recognized problem, results—the immunogen, the target of the patient's immunotoxic response is the joining of halothane to its enzyme in the liver that metabolizes it.

Other organs outside the liver also metabolize drugs, and the skin and lung are primary sites. The skin is especially a frequent site for problems where chemicals

bind to their enzyme in the skin, because you add into that soup the additional aspect of light. And because light can change the way chemicals are structured, as a result of UV energy, you have an additional opportunity for a neoantigen there, and so there are photo-allergic reactions which manifest in the skin.

Also—metabolites of drugs. Sometimes it's not the chemical itself, but it's what the chemical gets turned into that is actually the source of the new antigen to which patients will make antibodies. And a good example of that are some of the hemolytic anemias which occur—and also thrombocytopenias which occur in response to ingested chemicals and drugs.

Finally, there's this—just an example, even a little closer to home—the issue of perhaps—not perhaps—but that the intended article itself undergoes a certain degree of degradation as a result of the pathogen-inactivation process, and that then alters the intended article so that the patient makes an immune response to it. And we saw that in one of the European experiences in their production of plasma-derived Factor VIII, in which there was then an outbreak of Factor VIII inhibitors—antibodies to Factor VIII—and the problem was attributed to degradation aspects.

So the clinical manifestations of these—I've already touched on them, but just to give you a little

flavor—the liver is a common site, as we already mentioned. It's because drugs bind—drugs and chemicals form adducts with hepatic enzymes, and then that combination is the site of the neoantigen. It's not just halothane—and this slide just lists as many as would visually look nice to a large audience. Just understand that there are many examples of this; there are many different hepatic enzymes and transformation enzymes which become the antigenic site for an immune attack. And this has been nicely discussed in an article in Immunology Today, if you want to do a little more reading on this.

The skin, as I mentioned, is a common site because of this activation aspect which occurs. Now, these are called photo-allergic reactions. This is different from photo-dermatoses. This is due truly to an immune response to an altered moiety of the ingested drug, and it's expressed in the skin of the patient, and some of them are exfoliative.

If you've ever seen a patient with Sevens-Johnson syndrome, it's an unforgettable horror show of the worst possible thing that can happen as a drug reaction.

And then in the blood, for example, blood cytopenias. These often are either hemolytic anemias o thrombocytopenias, where the drug is binding to a blood

cell and the combination of drug and blood cell form the target.

What's interesting about these is that they're often—they are metabolites of the chemical, not the original chemical itself. And, just as an example of that, is nomifensine. This was an oral antidepressant that was used in Europe but never made it into the United States, and it really never made it long in Europe. It was withdrawn due to multiple episodes of this sever drug-induced intravascular hemolysis.

And when it was studied and reported by Dr. Salama in the New England Journal, it was shown that the recipient antibodies would really fail to react with drug-coated cells. But if you took cells and coated them in metabolites of the drug, and you could get those metabolites by letter some normal person swallow the drug, and then using either that individual's plasma, which would contain the metabolites, or even his or her urine, which would contain the metabolites—so you would coat innocent red cells with urine from a normal person who took the drug, and then the patient's antibodies would attach those cells, because they were coated in the metabolite of the drug. This is an old experience from Salama that's been repeated many, many times. A recent report in Transfusion from Dick Astor's lab showed this in the case of a non-

steroidal a lady took and developed a severe hemolytic—drug-induced hemolytic anemia, with an LDH over 1,000, and she went into renal failure and required dialysis, and it was an obviously severe case of hemolysis. And when they used flow-cytometry—and this is now degree of fluorescence on the x-axis, and number of cells on the y-axis—the positive response they've shown here is when you took the patient's serum which, of course, had the antibody, and then reacted that against innocent red blood cells which had been coated with urine from a volunteer who took the drug. And it was only in that circumstance that you could get the immunologic—that you could detect the neoantibody-neoantigen combination—because it was against a metabolite.

So, to kind of summarize the assays—I think when we do a direct analysis of the recipient against altered red cells, that's pretty straightforward, and you can use classical hemagglutination for red cells, or mepa tests or flow for platelets.

The problem, I think, for all of us is that for all these other kinds of antibody-antigen situations—which are known to arise—we really don't normally have any way to test for them during pre-approval studies. Especially in this kind—like the heparin-PF-4, because you have no idea what the chemical is binding to, so you can't even—until a problem finally becomes—until it surfaces, you cannot guess

in advance which of the million proteins in the body it's going to pick out to bind to in order to form the antigen.

So, with that problem in mind, you might wonder are there higher risk groups? Could we get a better handle on this by looking more carefully at certain kinds of people. And the literature here is very unsupportive. There isn't a good way to identify people who are clearly at higher risk for making neoantibodies to chemicals. Obviously, individuals with positive anti-nuclear antibodies might be of interest to technologies that involve alteration of DNA, and clearly people who are atopic, and people with asthma—high allergic people—are more likely to make antibodies, we think, in general.

Patients with repeated chemical exposure are obviously higher—or should be at higher risk. Other medications may cause drug-drug interactions, which are well known in clinical medicine.

Interestingly, patients with defects in DNA repair mechanisms might be an interesting group to at least think about, and it's certainly clear that there are genetic risk groups—in fact, there's a whole field that time doesn't allow me to talk about of pharmacogenetics, which is seeking to identify certain risk groups for toxicities to drugs. And we do know that there are certain HLA types, for example, who are more likely to make

antibodies to penicillin, and we know that there are slow acetylators and people with inborn errors of beta oxidation who are more likely to encounter a drug immunotoxicity to a variety of different hepatically-driven drugs.

And then there's the unexpected. If you have allergies, like my wife, and you live on Seldane in the spring, you've recently learned not to swallow your Seldane with grapefruit juice because of its interaction.

So I'm going to then just close up by talking on two issues, because having giving you a flavor for the—really, the unpredictability of the problem of immunotoxicity, it's something that has to be considered in the overall risk-benefit considerations we'll be hearing more about this afternoon.

To do that, it's necessary to set the stage, I think. And it's important at this meeting to drop back and understand that this is really the profession that we're all, in one way or another, a part of. And it starts with the donors and goes through to the recipients.

And, of course, in the last 20 years—or, at least, in my lifetime—in this field, there has been from the Food and Drug Administration, a tremendous emphasis on the safety, purity and potency of the product—get all those "p's" in line. And while we've made tremendous progress, I think, in improving the quality of the fluid, I would want

to remind everyone, from my perspective, that we have a tremendous problem over here on this side of the equation in many of the processes that are required for safe clinical transfusion practice; and that unless we address these problems as well, patients will not actually receive benefits from improvements that occur in the product.

The analogy which—for those of you who have heard it too many times from me, I apologize—but the analogy with air travel is very clear. If you just have safer and safer airplanes, but no one knows how to fly them, and there's no air traffic control, and that sort of thing, then you will not have safer air travel.

So it's very important that Federal agencies — which actually once they speak people listen and practice is dictated—that they be very sensitive to not making a problem worse rather than making it better.

Now, looking at those risks, this is the Paling scale, which—Dr. Blockman is in the audience, really taught us to recognize—this is a nice way to show risk: vertical lines. This is powers of 10. This is certainty, one in 10, one in 100, one in 1,000. Here is our viral risks. That sort of thing.

In the neoantibody-neoantigen considerations, we do have to recognize that if—"if"—supposition now—if an immunotoxicity resulted in morbid complications, like the

way HIT does with heparin, even in one in 10,000 patients, that would be a bad trade-off, obviously. And this very fact teaches us about our inability to really detect low frequency events in pre-approval studies.

An example—and I could have chosen from hundreds of examples—but an example you may recently be aware of, is the problem of ticlopidine-induced thrombotic thrombocytopenic purpura—TTP—a more dreadful ailment, a bad thing to have happen to you, you wouldn't want—does occur at a low frequency in patients treated with Ticlid, and the frequency is in this order of magnitude—1 in 1,000 to 1 in 5,000. Well, that's high, compared to some of these other viral concerns.

And so, this is just a challenge for all of us, that low frequency complications may be relevant when you're trying to fix a low frequency problem.

So what is regulatory's responsibility? Well, in my view, one needs, of course, to consider the fact that there's—and I apologize on the previous one not including bacteria. This gives you a sense of its higher frequency. We already talked about that yesterday. But there are a variety of other transfusion mishaps which desperately need attention. And, of course, my concern is that if we—that we be cautious about our zeal to work on the product and to promote things which will cause billions of dollars to be

invested in improving that product, but in the fixed-reimbursement health care world where I work, that money will go over to here at the expense of money that's over here.

And if you don't believe that, maybe then we can come back to that in a question-and-answer period. But you really should at least be thoughtful of that concern.

So, in conclusion, there are drug-related immune reactions, occur at a low frequency, among many commonly-used drugs. In fact, almost all commonly used drugs some people are going to make antibodies to them, whether its thiazide diuretics or just simple topicals.

Immune responses are not dose related, which is a problem for us. The clinical manifestations are not predictable. Antibodies may react with the original test article—you know, the chemically treated therapeutic blood cell—but, in fact, are more likely to react with metabolites, with third-party structures—like that heparin PF-4 complex, with drug-enzyme complexes, or with antigens created ex-vivo as part of the products of a process.

The current assays that we have have low sensitivity and are not standardized. And antibody formation and its clinical side effects are really not likely to be measured or even observed during pre-market studies.

Thank you very much.

Applause.]

MS. HWANGBO: Do you have any questions for Dr. Dzik?

Pause.]

Maybe we will discuss further at the end of the session.

Our next speaker is Dr. Albert Munson. He will discuss environmental issues, and occupational safety issues for handlers.

He is the Director of Health Effects Laboratory Division, National Institute for Occupational Safety and Health.

Environmental Toxicity and Occupational Safety for Workers Dealing with Concentrates of Decontamination Chemicals

DR. MUNSON: Thank you.

NIOSH does not require the statement on the bottom—the disclaimer kind of thing, like some of the agencies do. In fact, we've had some pressures at some time to do that.

But I put this down there because this is really the first time that a process like this has been looked at or actually talked about around NIOSH. And nobody—absolutely nobody—and we have around 3,000 people in the

National Institute of Occupational Safety and Health, spread around the country—nobody had heard of this particular process.

Laughter.]

And when I was asked to find someone to do this—as you might expect—I got a lot of "no's" and so that's why I'm here.

Laughter.]

But, you know, I've had an absolutely enjoyable couple days, and I'll tell you way. It was that—I guess—I'm a pharmacologist and toxicologist by training, but when I got out of the service a long time ago—and I guess there's not many people here that are older than I am. And you can look at my hair or lack of hair and know that—that, indeed, when I got out of the service I was a lab tech. And I found my way to Roswell Park Memorial Institute as a lab tech back in the late—mid to late '50s.

Now, I ended up in the hematology lab, and at that point in time this institution was still relatively young, and there was a lot of bright, energetic scientists and clinicians there. And they were all going to cure cancer—in a relatively short period of time.

So being a hematology tech, I was put in a lot of interesting positions. There was a young physician, his name was Don Pinkel—pediatrician. I don't know how many

have heard of him. But he was there. He ended up leaving there not too long after this incident occurred, and he went down, called by Danny Thomas, and he was the first director of St. Judes.

And one day—this was when he was at Roswell Park—he called up and he says, "I have this eight-year-old child, and she has aplastic anemia." Now, I don't remember—many of you remember—but clorampentocol was widely used at that point in time, and I believe—although I don't know for sure—I think she was one of the individuals that succumbed to that disease of aplastic anemia. And he was treating her with blood transfusions. And one day he says, you know, "I can't give her more blood transfusions. She's bleeding, and we really need to be giving her platelets."

Now, in 1957 we had glass bottles that we drew blood in, and we had to siliconize everything, and—so you can imagine the issues that were associated with it.

To make a very long story short—which is interesting to me, it may not be to you—in these years—30 years has gone by since I thought about this—my career went in a different way. And what occurred is that just about that time we worked really hard to isolate some platelets, and were semi-successful at it, and actually used some gradients and other kind of things, and we did infuse her with platelets. And I think it was once or twice a week

she got blood, and then we gave her these platelets—or at least what we thought were platelets, or they appeared to be platelets. And they seem to help to some extent.

The whole idea was, could we get this little girl through a very critical period of time, and try to stimulate the bone marrow at that point in time? This was actually before we know that there was a T and B cell—just to give you an idea.

Level of success—and we were one of the first to get some of the plastic bags, and then we could do a little differential centrifugation, and eventually learned how to get platelets a little bit better and infuse.

And this went on for, I think, six months—something like that. She eventually had tremendous reactions—febrile reactions—and it turns out that we weren't isolating the white cells away very well, and she ended up with antibodies again white cells. And we purified the white cells, and that really helped.

So, with blood transfusions, platelets and a lack of white cells—she got the white cells when she got the whole blood and that seemed to be okay. She actually was one of the first aplastic anemias that survived. And I think he published this in the Journal of the American Medical Association many years back.

I tell you this story because I stayed in the lab for awhile as I got my graduate degrees, doing that kind of thing. And now a whole period of time came by and I see the issues related to platelets, and I now see that we're going to clean the platelets up and purify them even more. And this is truly exciting to see. The engineering that's going to go into this is going to be an interesting kind of thing.

My job today is a very simple one. I came trying to figure out what I could talk about—the toxicology of these compounds, not having seen the first piece of information or being able to get very much.

But my job is a simple one—the reminder—and that is to consider the worker.

I don't know how many of you—you don't have to think about this generally on a day-to-day, when you're thinking about individual patients, and individuals that are sick. But every single day there's 9,000 people—9,000 workers that walk to work in the morning and they are disabled that day.

Now, albeit this is often very acute or traumatic injuries —it's often in the construction and farming and things such as that—but this does happen.

Every day that people go to work there's 17 of them that don't even come home alive. And maybe things

that we deal with an awful lot is 137 of them die because they went to work, but they die of diseases at some point in time later. And what are they? And that's what NIOSH is sort of about.

Linda Rosenstock came to NIOSH about eight years ago. She's now left to take the deanship of the School of Public Health at UCLA. But what she did is when she came on board she said, "What should we be doing in NIOSH? It's a research organization. We're supposed to help as much as we can by providing research help, in terms of occupational safety and health." And through a fair amount of activity for a year, 21 priority areas were identified, and there was a national framework—I have a reason for talking about this, so if you'll bear with me.

She went out and talked—the institution—this is before I came there five years ago, there was a strong consensus that there was about 21 priority areas, and when you see them—I'm going to show you them—you'll see that many of them look like "motherhood" statements, but they do represent an awful lot of areas in which the worker is—has problems: allergic and irritive dermatitis—billions of dollars are lost, and a tremendous amount of problems associated, related to this. And you can go through this list and see these areas that NIOSH does deal with, in terms of trying to do research in each of these areas.

I put infectious disease here in red. We are a part of the CDC, but when it comes to worker protection, NIOSH has a responsibility. And, as you probably have seen, many of the alerts associated with worker health and nurses—whether it be ergonomics problems, needle-sticks, such as that—these are things that are dealt with, or research is being dealt with, whether it be field research or surveillance in this area. And infectious disease really would be one of the things that would—you have to think about, in terms of the problem that we've been talking about for two days.

Now, if you look at the rest of these, most of these issues—most of these areas—could be a result of this new technology. And so any one of them might possibly be important.

There is one here called "emerging technologies." And this—what happened is that when we had these 21 priority areas, committees were set up, both internal and external committees—stakeholders, everything else—and they said, "What should we be doing in this area?" And they said, when the 21 priority areas was established, somebody said, "We're going to have emerging technologies come on, and NIOSH should be there at the lead, trying to help out as these emerging technologies come into play, so that

maybe we can have some impact and protect, essentially, the workers."

Well, I was on the lead team, and we've been working on emerging technologies for, I think now, four or five years, and had these committees working, and nobody knows what to do with emerging technologies. You start going out and trying to find what the emerging technologies are, and you can find them, but nobody wants to deal with worker health and safety, you know, when they're trying to get something off the ground.

And so this could be the opportunity—and probably the main reason why I wanted to come and talk to you today—is that this is emerging technology. It's going to be new industry. Keep in mind the worker as you're involved.

What things do we have available to us that we know from history?

Well, the health care workers is a large area. And NIOSH has many activities in looking at health care workers. They're one of the highest at-risk groups in the worker population. We have, of course, miners and construction workers and other people, but the health care workers as a whole group are one of the highest at-risk groups.

Related to the issue we're talking about here—we're talking about infectious disease, HIV, hepatitis,

tuberculosis—these are things that the health care worker is at risk.

The short personal story is that when I was working with blood, back then in the late '50s and the early '60s, and did many other kind of things, I ended up with hepatitis—serum hepatitis—fulminating serum hepatitis, spent two months in the hospital with every particular fluid in my body came out yellow. And I got it form working with human blood. Back then it was called serum hepatitis. I still have decent titres of hepatitis B antibodies.

The health care worker is at risk, due to infectious disease. They also are allergic to—not allergic—that's my area of research—they also are at risk from a number of drugs that they have to deal with. In this new industry—and I was going to talk about this, the new industry that we've been hearing about—there's going to be thousands of new workers, everybody from the lab personnel to the manufacturing, the blood banking, the clinical staff—that are all going to have certain at-risk because of the new activities that are on-going.

If we look at history—and this was one of the biggest surprises to me—is that, indeed, health care workers have increase in cancers because of handling of the cancer chemotherapy agents, and the handling of drugs that

are used in the treatment of AIDS. There is a whole literature on the risks and the diseases that health care workers have because of being associated with these particular agents.

This is a surprise—knowing full well that the way we prepare drugs, and the way they are handled, both in the pharmacy and on the floors, it is—it never made much sense to me that these individuals have an increased incidence of cancer, fertility problems—I think we have a list of them—yeah—these—there's literature for all of this. And it was alluded to, I think, yesterday. I think Dr. Wagner had indicated that this—these agents are—these are fairly hazardous kind of drugs. They are all associated with the inhibition of cellular replication and growth.

NIOSH has a major activity, trying to figure out where this exposure is really coming from in the—pretty much now in the clinical settings. I thought it would go away. It doesn't seem to be going away. These individuals are still at risk, the ones that are associated with these particular hazardous drugs.

And so I come back to just talking a bit about the individuals, and raising the issue, for those that are involved in this developing industry—I like the lab techs, because I was one, and I was injured by not knowing. I trained 17 graduate students to get their Ph.D., and they

are the most careless individuals that one wants to see. And unless, you know, someone is monitoring, and someone is educating, they put themself at a fair amount of risk, as do post-docs.

Now, I suspect—although I don't know—that out there in the community, developing these new agents, I think what's it? 303 was the—maybe the last one—the 320, there's a chemist someplace, and technician someplace, and they're handling these compounds, and they're using UV light, and they're activating, and they're measuring binding, and—ladies and gentlemen, they're at risk. And we may save a lot of people by purifying the blood supply, and we do not have to kill other people in that particular process.

We heard yesterday—and it was alluded to a couple of times in the questions—that in preparing a new—or developing a new industry, we are going to end up with a lot of engineering. We're going to end up with plastic bags, and we're going to be heating things, and breaking off tubes, and doing all kinds of great things to protect, essentially, the worker to some extent, but also toe get the product so that it's given to the patient.

And blood banking and whatever—however this industry goes, needs to have the attention not only to protect them from maybe the material as it's being

irradiated or whatever, that the little drip off of the tube may end up on the counter, but also as we do this we have the opportunity to engineer the blood banking facility in such a way as maybe we can help a little bit with the ergonomics aspect because we have tremendous amount of shoulder and arm pain and many other musculoskeletal problems. And, indeed, if you've got to engineer something to do it, it may not cost very much, indeed, if you have these things in mind.

These are just some other—I don't know—I don't think the home nurses give blood transfusions or blood products, do they? Do they? So, again, we have even another place where this can be a problem. Probably the physicians are the least at risk.

Laughter.]

Most of these things have been alluded to. I did this slide before I heard the comments yesterday, and I haven't had the opportunity to look, really, at the toxicology that's come out. Some of these things—just looking at the compounds, boy, I said, these are inherently reactive molecules, and indeed, the handling of these molecules one needs to be careful of. You actually might think about this, particularly as they—in the production part, that some of the same concerns that we had for cancer

chemotherapy agents, you may want to think about in terms of these.

And the other part is that I saw UVA being used an awful lot. One comment was made is that this is the thing—caused suntans. If you ask a dermatologist, he says "That's going to burn your skin. It's bad. It's injurious. It causes skin cancer." And if we're going to have UVA light and use it in the best protected kind of way—this can certainly be done, but we have to be sensitive to it.

We heard a lot about hypersensitivity responses of various sorts, particularly, in this case, type II and type III hypersensitivity responses due to neoantigens. But we also have the activation of the psoralens, in this case, which are known to be—cause contact dermatitis, and indeed, this has to be something to think about.

I was tremendously impressed that, indeed, the lymphocytes from the UV activation of—the two compounds, I think, that were shown, the one-way MLR response to mitogens were essentially flattened out totally. There was no response—they really wipe out the mitogenic activity, or the proliferative activity of the lymphocytes in this blood. It's remarkable. I've done a lot of these, even with irradiation—gamma irradiation, or use some mitamyacin

C or many of the things—it's really hard to totally wipe out the one-way MLR. You'll see a major decrease.

This is obviously a tremendous reaction that's occurring, that intercollates with the DNA and wipes that out. And the question one might ask is that even though the lymphocytes are there and they can't divide, will there be one or two of them that escape, and they have had a genotoxic kind of thing. And in the years down the road, will there be a lymphoma? But that's not worker health and safety, that's a different issue.

I thought I was going to talk about these things, but they've all been talked about so much that I don't need to say much more.

Again, this is a new industry. These are some of the risks—at risk because of the specific product action; at risk because of work organization.

I would suspect—and, again, I don't know what it's like today, but I remember being in the blood bank, and it was a stressor situation—getting up in the middle of the night doing type-and-cross-matching, and trying to make sure that you had the right kind of thing was very, very stressful. Now, again, I think everything's pretty much automated now, and some of these are—but look at the stressors that you're going to create with this new technology—maybe not with this new technology, just maybe

in blood banking in general, that one can make a real help in this particular case. The physical stressors, which I have alluded to; potentials for accidents—anything that can happen will happen. Will you have drippings on the counters, and will bags break, etcetera.

"At risk because of—"—and you fill in the blank, because I don't know what this industry is going to look like. All I can do is stand here and say it may be something that you can see along the way, where it won't cost that much additional money, that may save lives or save morbidity.

Some basic comments—set up good safety programs in the lab, blood banking industry to the clinic; reduce the potential exposure; look for the places for exposure. And yesterday it was mentioned that, at least with one of the products, that the occupational safety and health people are intimately involved from the beginning. This is absolutely super. The industrial hygiene people should be able to develop, or use some of the assays that you have to detect exposures in various places.

Personal protective equipment is a big item in NIOSH—but nobody uses it. And I'm not sure—and I understand why. For the most part, most of it's uncomfortable, even to the extent that—for the most part, we've even moved away from using some of the UV hoods

because of the way we keep the laboratories these days, we don't really need to use them for maintaining an aseptic area.

So, finally, just educate, educate, educate the worker. Let's let this industry—it needs to be developed. I'm really excited about it being developed. And let's make the place a safer place for the worker. Remember the worker. They're at risk.

It appears that there's going to be thousands, maybe tens of thousands, of these individuals coming in contact with it.

Thank you.

Applause.]

MS. HWANGBO: Thank you.

I'll stay here just for a few minutes, okay?

You said you had some difficulty finding the right person to talk for this session. I'm glad you gave us the talk. And I also had my own difficulty finding you.

Laughter.]

DR. MUNSON: I tried to stay hidden in Morgantown.

MS. HWANGBO: Yes, I found your name, and the name of your division from website—NIOSH website, and I explained to you our need here for today. And I asked you—"Am I calling the right person, or right place," and you said "yes." I'm glad you gave us talk. Okay.

Applause.]

Panel Discussion

MS. HWANGBO: Please come all the toxicity speakers to the podium. We are going to have a little discussion session.

Pause.]

Invite questions from the audience.

DR. SNYDER: Ed Snyder from New Haven.

Dr. Munson, now that we've all met each other, and we know we each exist, what happens next? Now that we know about you, you know about us.

In the blood bank field we're interested in—this is the first time that we will be taking blood products and sending it out to be processed, and then having it returned. My understanding is that it is not intended for many of these pathogen-reduction technologies to occur in the hospital. It will be centralized.

What obligations do we have? Are there mechanisms —what are the mechanisms to ensure—I mean, we're concerned about labeling to make sure that we send out, you know, 55 units of O, we get back 55 units of O and it's properly labeled. Those are the things I'm sure that people involved with developing these technologies involved in the field, they're intelligent, there's due diligence being done.

But what about protection of the worker? What involvement would CBER or CDER have with this at all? If anything?

DR. MUNSON: I don't know if I can answer that right now. Let me just—but I'll try.

This is a research organization—NIOSH is. That's—OSHA is in the Department of Labor, and they do the ruling kind of things.

The resources that are available to you from NIOSH, to some extent, would be scientists and individuals that might be able to help, particularly when it comes to, maybe, quality assurance. But I would think that quality assurance is something that you would have built in pretty strongly—and also the engineering part.

We have physicians, but they would really have to come up to speed on what you're talking about here. We don't have anybody that's dealt with blood banking, per se.

But you have a really good point, and I'm going to take this back, because it may be the first place where we might be able to have an impact and at least assist in some fashion.

DR. LePARC: Yes, German LeParc from Florida Blood Services.

A couple of questions: one relates to toxicity. One of the hottest issues now is therapies that include

gene silencing—gene silencing by binding to RNA as opposed to DNA. And that affects, of course, the expression of certain genes.

There have been a lot of studies about this compounds interacting with DNA, but I wonder if there are any—since these things are going to interact with nucleotides, are three any toxicity studies that will be required with RNA and the possibility of gene silencing side effects by these compounds?

DR. MUNSON: I don't—I'll take a shot at this, because what you're talking about—as I understood what I heard yesterday—and we probably want to hear from the individuals—if you look at the molecules—the couple molecules, the reactivity, when they're activated—yes, they have a tendency to collate with DNA. And that's essentially one of the places that's being targeted.

But it's my understanding that they also will—you have something that's going to bind pretty quickly, so I'm sure they'll bind to RNA, and they'll bind to protein also.

So, I don't think that—I don't think that there's any problem—

DR. LePARC: Somebody will have the answer, then.

DR. MUNSON: Maybe somebody else wants to.

DR. DZIK: German, just to also—I mean, I would expect in the treated donor product there's a lot of

silencing going on in the bag, right? I think it would be the expectation that in the recipient, the quantity of these chemicals that the recipient finally sees—assuming the products have been handled as intended—would be so low that you wouldn't expect to see that.

I mean, if you were going to get RNA shut-down, then you're—from residual chemical, well then you're going to be—that's probably going to be an unacceptable level of even DNA binding, let alone the RNA binding.

So I would expect—I'm not an expert here, but I would expect that we wouldn't anticipate a lot of RNA damage.

DR. LePARC: And the second question has to do with environmental issues. At least one of the compounds will have to be washed away, and presumably will generate millions of gallons, you know, when you—we collect 14 million units a year. If we wash them all, or at least some of them, we end up with millions of gallons of wash material that—you know, I don't—if it has some carcinogenic—I know the State of Florida is not going to allow me to put it down the drain.

So, I don't know how we're going to deal with that.

DR. DZIK: Well, you know, I'm from Massachusetts, and you know, you saw Erin Brockovich, where, you know,

they had the glass of water and said, "you drink the water."

Laughter.]

DR. CORASH: A point of clarification about the chemistry. And I can only speak for our chemistry and our compounds.

Psoralens—first of all, you eat psoralens, so you're getting your own little occupational exposure every time you have a sprite or a 7-Up, but not S-59. But the process is an ex-vivo process that takes place in a device which has controls to prevent the worker from being exposed to UVA light. And it's done in a closed system, under the types of worker protections that are used for handling blood products for protection against blood-borne pathogens which, unfortunately, you obviously came into contact with many years ago—as many of us probably did.

But S-59 photodegrades and then, of course, we use a compound absorption device to pull the residual down to very low levels. And then it also has a very short metabolic life, and doesn't appear to have any covalent chemistry, at least when—you know, in the absence of long-wave-length ultraviolet light.

S-303 completely degrades to the levels that we are able to measure it. It was designed to be an unstable compound that in an aqueous environment, with a half-life

of about 25 minutes, would undergo multiple half-lives down to undetectable levels. So they're —by design, some safety aspects have been built into this.

Now, I think that, you know, a number of speakers have raised, obviously, very valid points. But in addition, extensive pre-clinical studies have been done. But I think Dr. Dzik has touched on an important aspect also that we face all the time in the development of new pharmaceutical compounds.

DR. MUNSON: The question previously, I interpreted that he was asking, "in the bag" did it bind to RNA, not—

DR. CORASH: Oh, absolutely. Because you have RNA viruses, and—

DR. MUNSON: Right.

DR. CORASH: —and so it's very effective.

DR. MUNSON: That's what—

DR. CORASH: Yes—absolutely right.

DR. MUNSON: And I think it's my understanding that you may have done your carcinogenicity study by binding it to plasma proteins?

DR. CORASH: No. There's very little reactivity with plasma proteins. And in the carcinogenicity studies that were done by transfusing animals with plasma—because it's very—it's not really possible to expose these animals

to chronic transfusions with platelets because the animals are too small and you can't harvest all of these platelets. But exposing them to the compound itself, to plasma containing the compound that has not been photo-eliminated, plasma containing the compound that has been photo-eliminated but without the compound-reduction process, and then with the complete product with the compound-reduction process as well—so that the animals are seeing exposure of all different types of—you try to simulate—at least we tried to simulate, the product that's being transfused as close as possible to be the test vehicle in these model systems.

MS. HWANGBO: Do we have any more questions?

Oh, yes. Good.

DR. CHAN: Penny Chan, National Blood Safety Council in Canada.

I was fascinated, I think, by Dr. Dzik's presentation where he raises the issue of neoantigenicity. And I guess there's two main issues that that raised in my mind. One was how do we assess for neoantigenicity. There aren't particularly good animal models as far as I know for looking for neoantigenicity. But then linking it to some of the other effects—the non-infectious risks of—quote—"normal" blood, platelets, etcetera, and things that we really don't have a very god handle of the risk on.

And surely we're talking about pre-markets, looking at all the drugs and things that are put on the market. Aren't there any plans for post-market surveillance, and really close follow-up of these? How are we going to look for long-term effects? How are we going to link them to transfusions? And that goes for the normal transfusions as well as the new products—and the things that we really don't know what we're introducing, particularly in the carcinogenicity, the terato—well, you know—the reproductive and the effects on embryos and that sort of thing.

So, is there any FDA efforts that are going to go on, looking at post-market surveillance in a really close way?

DR. DZIK: I mean, I can't—I think your question is perfect. I mean, I can't speak for the agency, I don't work for the FDA.

But I—the point of my talk is reflected exactly in your question, that we really—I would agree with you, we really need—we will a need post-market surveillance system to even observe and detect the—any low-frequency adverse effects with may arise. Because the assays for immunotoxicity are—I think we—everyone does the best they can in the right spirit, but we would be foolish to take those negative results and walk away confident that there

will be no problems. That would really be stupid—and not in line with what we know to be the facts in any number of other circumstances.

So we need a post-market thing, and I'm sure the people from the FDA—they may not be answering today, but I'm sure they're listening.

MS. HWANGBO: Yes, we do have post-marketing surveillance, either for new drug application or pre-market approval. We have such a system.

DR. DZIK: I think your question also speaks to the second question that's above us there, which is this issue of being able to track events after approval—yes.

MS. HWANGBO: If we don't have any more questions, we would like to discuss the prepared questions.

Can you hear me?

For this kind of chemical we look at very careful of carcinogenicity study data. In carcinogenicity studies, the test animals—for example, mice—have a small blood volume. To deliver the chemical repeatedly, mimicking clinical circumstances, sufficient volume of the vehicle—for example plasma for platelet study—is needed.

Our question: Can homologous plasma—here, when I say—when we say "homologous plasma," this means another mouse plasma, there is no ABO typing. For example, mouse

plasma be used as a vehicle without causing immunogenic complication in the animals during a long study period.

DR. GHANTOUS: To me, I mean, it's both mouse to mouse, so I don't think there will be any problem. But I will leave it to the immunotox expert to answer that.

For me, doing a carcinogenicity study, the important thing is to reach an MTD, and to go—and high doses, same thing as Suzanne said here. So I don't know if we can do that with platelets or with plasma, and I'm not sure if with these products you can use different vehicles, or do you have to use the platelets or the plasma.

DR. DZIK: I'm not—I wouldn't be concerned about the fact that the plasma from the donor is not in any way matched to the plasma of the recipient in an inbred mouse model. That wouldn't concern me too much.

I would have a little concern—and this is not based on knowledge, I think—I would have a little concern about saying, "Well, we're going to use plasma and not platelets." Because the—you get comforted when the final product that you've prepared doesn't cause any toxicity in the recipient. But if—using plasma as a surrogate for platelets assumes that the distribution of the chemicals will be in plasma as they would be in platelets. So if, for example, you had a very lipophilic compound, it

probably wouldn't be the same distribution in plasma as it would be in cellular membrane.

So I would—I'm less—so there's kind of two questions here. The question is brought up in the sense of these animals' not being necessarily well-matched for each other immunologically. That doesn't both me so much. But I'm a little bit troubled by the stand-in of using plasma as a test stand-in for platelet transfusion, or for red cell transfusion, for that matter.

From a neoantigen standpoint, it's no good at all, because—you know, if you were doing quinidine experiments, the quinidine antibody is to a glycoprotein-quinidine complex. So if you don't have the glycoprotein there you'll never see it.

So—but from a—so it can't work at all from a neoantigen-antibody concern. I think it can work—grossly—for carcinogenicity endpoint.

MS. HWANGBO: Thank you.

Do we have any more—okay. Yes.

DR. McCULLOUGH: Tim McCullough from Cerus. And, as you know, we've completed a six-month—

MS. HWANGBO: Can you talk a little closer to the—louder, yes.

DR. McCULLOUGH: As you know, we've completed a six-month mouse study in transgenic mice, where the vehicle

was 35 percent plasma. And as blood groups for, say, canines are very, very well known and very well defined, and for all of those studies we've done in that species we have had to blood-match each individual dog to do the study, which each unit to be transfused.

For rodents, it's less well-known. For rats, there are, in the literature, about four blood groups cited. For mice, there is essentially nothing.

And so when we began studies using this as a vehicle that was a big question. There are multiple questions using plasma as a vehicle: would the constant volume expansion of daily dosing, or very frequent dosing over a long time cause its own problems? Would there be immunogenic complications and consequences? And it was unknown when we began short-term testing out through—up through six-month testing.

And throughout that period we found no complications from long-term treatment with homologous plasma in the mice.

With respect to the other point you raised about its appropriateness—one thing for platelet concentrates, our clinical vehicle is 35 percent plasma, and the platelets actually only comprise about 1 percent of the volume of the platelet concentrate. If you do—equivalent

to hematocrit, if you do a platelet crit, spin them down and measure the volume, they're only 1 percent.

So most of what people are getting exposed to is the additive solution and the plasma as the vehicle.

Further, we've done analytical characterization to show that all the photoproducts, and all the products all the same in the vehicle, with or without platelets present. So —to justify that as an appropriate vehicle.

And so, that supported this use as a vehicle, and we were successful without any complications in long-term IV dosing of rodents.

Thank you.

DR. DZIK: That's helpful information—yes.

MS. HWANGBO: Yes, thank you.

Okay. We can go to the second question.

Pause.]

Were pathogen-reduction components to be approved for clinical use, is there an advantage to requiring the standard, which is non-pathogen-reduction product, be retained in nearly equal amounts nationally, thereby permitting not only a ready alternative in case of toxicity discovered post-market, but also permitting post-market analysis of the frequency of adverse events in pathogen-reduced versus non-pathogen-reduced products?

DR. DZIK: Is the question to the audience or the panel?

Laughter.]

DR. PEHTA: I'm Joan Pehta from Connecticut.

Well, I have some questions regarding this. I'm thinking of different patient categories that might have a problem that we wouldn't know about. And I would think, those, in particular, patients with autoimmune hemolytic anemia. And I know Ed Snyder said yesterday he had some concerns regarding using these products in neonates if the clinical studies didn't include them.

But, in particular, patients who already have antibodies on board, how they might react to this product which may affect blood group antigens in particular, little "e."

DR. BIANCO: WE are discussing question number two, right?

DR. DZIK: Yes.

DR. BIANCO: So I'd like to ask the panel, that if you were in the hospital and you were offered the choice to receive a unit of blood that was pathogen-reduced or one that was not touched—that is, a standard one—what choice you would make?

DR. DZIK: It just depends. I think—if the person said, "Would you like a piece of blood that has a carcinogen versus one that doesn't?" So, you know—it's—

DR. BIANCO: We have confronted this type of question. And, certainly, for those of you that have not been so close to transfusion, over a number of years, since the AIDS tragedy, and this happened—we moved to introduce the test for HIV too slowly. It took a few weeks. And many centers were—and many transfusion services were sued because it coincided that units that were not tested within a short period of time, to the point that we started introducing new tests overnight.

And I think similar questions will come regarding that. I don't think that the question has an answer. I think the answer will come from you, in terms of assessing the risk associated with the components that are there. Because from the perspective of the population and the recipients, they would like to have the safest possible product.

DR. THORNTON: But if you ask that question to a normal person in a hospital, are they going to know what you're talking about?

DR. BIANCO: Oh, they don't.

DR. THORNTON: You know, that's my question. Me, as a toxicologist, I'm going to say, "Well, how exactly did

you reduce that pathogen—"—if I was cognitive, of course, and not intubated—you know, and then now we get into a discussion with you.

But is a normal person off the street—they're not going to know, so they're going to trust you to make the most important decision for them. Do you give one that's reduced, or do you not? And you will have to make that decision, based on us regulators, in conjunction—have you done the right testing on that? And I think that is our concern, as a regulatory agency—what is the right testing? Because it's so new, it's—you're breaking ground.

DR. BIANCO: But if you did the right testing, like you did with a drug, and you've approved that product for transfusion—

DR. THORNTON: Mm-hmm.

DR. BIANCO: —then, an issue—all of us will have to confront and make that decision. I have two products on the shelf—

DR. THORNTON: All right.

DR. BIANCO: —and which patient will receive which product?

DR. THORNTON: But do you think that the decision eventually won't be there? That it will all be reduced products.

DR. BIANCO: With honorable exceptions—that has been the tradition, particularly when it involves HIV. In other products that involve just medical issues—for instance, we have two products, some are leukoreduced, some are not—

DR. THORNTON: Mm-hmm.

DR. BIANCO: —solvent detergent plasma—there were concerns because it was a pooled product, so there could be a balance and it coexisted with regular plasma. But in all the tested products—HIV, for instance, tested, or tested by nucleic acid amplification, as we do now—those products did not coexist on the same shelf.

DR. THORNTON: Right. But you know, to the general public, they're trusting us to make those products save. And so, really, the decision as to whether they're safe it's going to rely with the physicians and the regulators, not necessarily, in my opinion, with the patient.

DR. BIANCO: Oh, I was just giving you the responsibility—

DR. THORNTON: Yes, I know. I'm just saying, you know, normal—

DR. BIANCO: —and saying that we will rely on you for that decision.

DR. THORNTON: Well—and we'll rely on you, as well.

Laughter.]

DR. DZIK: Dr. Bianco raises, I think—the relevant question is that, traditionally—you just said it, I think—traditionally our response has been: do it for everything. SD plasma was a bit of an exception there.

And the question is—the question is, is there an advantage? Do you see an advantage in having the opportunity to compare two systems, or do you see an advantage in an all-or-nothing approach? That's a good question.

DR. BIANCO: I see an advantage in comparing, as a physician, as a scientist. Unfortunately I don't think that ethically or legally we could do that. It's very hard to carry out a double-blinded trial today for certain things, particularly when there are issues—where there are dread issues, with a perception of safety.

And those are the concerns I have.

I'd like to see it happen, and I'd like us to have, actually, even a base-line of incidents that are reported to date. That is, yesterday we were talking about potential anaphylactic reactions. They happen every day. And my fear is, if we don't have this base-line, is that this product may be approved, two or three patients in the

country die, and we remove a technology that could be very, very useful to protect for many things because of fear—like happens with some drugs and other things—that this is a horrible.

And I think that this will depend on informed consent. It could only be done as an experiment, not as a practice.

DR. DZIK: Correct.

DR. BIANCO: And how do you do the informed consent?

????AUDIENCE: Just to reiterate something Ed Snyder said yesterday about pregnant patients—one of the problems, when we had SD plasma, was the package insert said something to the effect "must be used with caution in pregnant females." And if that kind of wording, if a boxed warning, that it has not been studied well in pregnant females, goes on these products you're going to stuck—you're going to have to have two inventories.

And I don't think we really want to get into that situation where you have two inventories. Either we do it or we don't. So the wording that goes into any approval, we have to really think about that.

DR. HOROWITZ: Bernard Horowitz.

I also wanted to address the issue of two inventories, and whether we should strive, or set up as an

objective, of having adequate data available in order to eliminate the currently licensed product simultaneous with the licensure of the new virally-inactivated product.

And I personally believe that's a mistake. It's a mistake because the amount of data that are required to fully replace the existing product is so large that it would delay the introduction of the new product.

Moreover, you do lose the capability of having the additional data come available, in at least a parallel manner, and to the extent that it can be organized, a simultaneous manner, comparing the two products post-licensure.

So, for those two reasons, I think it's an awfully important discussion, because with SD plasma, in particular, it caused a two-year delay, by having a conference—meeting—such as this, come away with the conclusion that Vitex, the manufacturer had to be able to satisfy the full nation's need for the product. And we were not talking, at that time, about not having data in neonates. We were simply talking about manufacturing capacity and distribution capacity.

And at this meeting we've raised a whole host of additional questions, including those in your own talk on immunogenicity, many of which were not relevant—or less

relevant—for SD plasma than they are for these cellular products.

So I think, as a community, we should be ready to have dual inventories, and all of the difficulties that that implies a the blood banking level, but the alternative is to cause undue delays.

DR. GOLDMAN: Hi, I'm Mindy Goldman, from Canada. I think I'd like to—

MS. HWANGBO: A little louder, please.

DR. GOLDMAN: Hi. I'd like to agree with some of what Dr. Horowitz has said.

First of all, I think that adding a new test will never be a risk to a patient, unless there's no blood because you're eliminating all your donors, while changing your product may have a risk. Not all patients are in the same risk group. And probably about 30 percent of prescription drugs—you know, all those "new and improved" things—end up with a "Dear Doctor" letter going out within three years of being on the market, because some side effect, some rare thing has been picked up in a given patient population or in a given circumstance.

And within one month of this thing being approved, we'll have transfused more patients than in all the studies put together. And so we'll have much bigger data pool to say, "Yes, we could actually safely use this

in neonates, and pregnant ladies and exchange transfusions, and—"—you know, a whole host of things that you will never be able to really address in any way in the studies.

And we're comparing with a product that's very safe at the moment—remember. And so even if there is an anaphylactic risk, or an alloimmunization risk of 1 in 10,000, that's more than the risk of the product at the moment—perhaps with a little bacterial culture as done there for the platelets.

MS. HWANGBO: Dr. Slichter?

DR. SLICHTER: Yes, I would just like to also, I think, support what Dr. Horowitz has just said, in the sense that—you know, when Dr. Bianco is talking about getting sued, I think there's a big difference between conceivably transmitting Shiver's disease, or malaria and getting HIV. I mean, HIV you're gonzola, or at least you were when we were trying to institute the HIV tests.

So I think there are risks which this technology should help us avoid, but I don't think there the kinds of risk that we're talking about.

And I think as long as the FDA—and this supports a little bit what I said earlier today—doesn't require, or mandate this, that it still becomes somewhat of a physician's choice, and there are certain patients that I might very much like to have this for. There are others

that I'd like to wait a little bit and have more information on what the long-term consequences are going to be. And, you know, at blood centers we have multiple inventories of everything. I'm not advocating that we have even more, but we can deal with those things. We deal with ABO types, we deal with leukoreduced, non-leukoreduced.

And it's kind of—you know, to my sense that, you know, maybe the FDA will still let us practice a little medicine. I mentioned that earlier, that when I started, we got to do some of those things. We don't get to do nearly as much of them anymore.

And I think allowing things to kind of come on the marketplace, see what the marketplace does with them—I think we had, you know—what Dr. Horowitz said about Vitex and their plasma I think is very relevant, because, you know, here was a fully pathogen-inactivated product, and when we went out and talked to our hospitals we didn't get a single order, you know, because they were concerned about the cost, and also the issue about being a pooled product.

So I think, as a community, we should try and get these products out there. I mean, I think one of the things that's happened at this conference today is that somebody got up and said, you know, we had the conference three years ago and we're still in the same place. Let's let things come if they can meet the standards that have

been discussed here, but let's not mandate that everybody get them, because we just—they are a new product. They're brand new. We don't know what the potential long-term consequences may or may not be, and, boy, we would sure be in deep trouble if it turned out that, you know, we converted the whole marketplace and then we found out there was some God-awful whatever—toenails turned pink, or something—and that was what our blood supply was.

And so I think there's some value in letting things come along as they become available, and incorporating them and making sure that they're okay and safe to use, but not requiring that they just blanket—everybody does it.

DR. SNYDER: Ed Snyder.

One of the concerns I have, also—some people have mentioned Thalidomide. There's also the problem that occurred with the DES daughters, and the fact that it skipped a generation and clear cell carcinoma occurred in the children of the mothers—the women who received this when they were pregnant.

I think the post-marketing surveillance needs to be done to a degree that perhaps might be more extensive than has been done in the past. And I think that's something that's—I know the transfusion community would

certainly be willing to work with both the companies and the agency to ensure that this was done.

And I agree completely with Sherrill and Bernie that this should be brought it slowly, so that we can not have converted everything and find out, "Oh my God—"—although pink toenails might not be so bad. It might be—you know, it would be useful to have a little safety valve that we're not really as omniscient as you may think we are.

So I agree with those two previous comments.

MS. HWANGBO: We hear you.

DR. THORNTON: And there are many pregnancy registries that are being established now, and that will help us just with the post-marketing surveillance for women who received—and their children.

DR. MARTINEZ: Bill Martinez, Gainesville, Florida.

I think that the way things are going to roll out, it's going to be that platelets are going to come out in the market first. And when they come out, pathogen-inactivated, there will probably only be one inventory, and that will be pathogen-inactivated platelets—before red cells come out.

And maybe some data that comes out of the use of platelets that would be pertinent to the use of red cells later on.

And I just wanted to bring that up as an item for discussion by the experts.

DR. DZIK: I'd just comment—you know, if the processes were exactly he same we really would learn form the first one. Because there are so many differences—technical differences in the processes for platelets and red cells, even within a single company, we may get great knowledge from whoever's out there first, but I wouldn't be too hopeful, because I think the other techniques involve different chemicals, different manipulations, and what works in one setting might not work in another.

I would almost argue the opposite: that each of these—it's almost like a different drug, like a different medication, and may have its own toxicity profile and its own advantages and disadvantages. So we may have to learn about these one by one.

And I think the fact that, you know, very companies have more than one object in the pipeline is a very wise thing for the industry as a whole, because the one we start with may not be the one that we end with, you know.

MS. HWANGBO: Dr. Bianco?

DR. BIANCO: Yes, I don't want to leave you with the wrong impression. I also support what Dr. Horowitz said, Dr. Slichter, and Dr. Snyder.

I think that it should come. It should come as the manufacturers can do it—except to raise the issue—two essential issues.

One is that we will have to make decisions. And those decisions are not easy decisions to make. But we do have to make—and we discussed it a lot at the time of the solvent detergent plasma, which patients should get it if not all the patients, with leukoreduction.

And so that's our responsibility, not necessarily yours as regulators.

And the second issue is that you can help us, too, with the issues of perception about HIV. HIV is different. And there is one case of transmission of HIV, or two cases of transmission of HIV after three years of introduction of the NAT—nucleic acid amplification testing—and this makes the newspapers all over the country.

So, those are the two levels that we have to consider.

Thank you.

DR. DZIK: I'd like to just follow on Celso's comment, because I think you're right about the—I mean, if we were to reach a consensus opinion that bringing these

out slowly rather than mandating them was the wiser choice, that kind of takes it to the SD plasma concept. But we might want to think through whether what we did with SD plasma is the same thing we'd want to do with these new products. For example, in the case of SD plasma, I suspect that most places did what we probably all did, which was this was discussed at either your pharmacy and therapeutics committee, or within the setting of a transfusion committee, or some other hospital quality-based committee.

We didn't take it to our IRB. We didn't include patient discussion. You know—we were the wise men in the hospital committees who said, "Okay, this is what we're going to do or not going to do." And maybe that's what we should do for this kind of technology, but maybe we should think of other approaches, as well.

So, I think we have a lot of thinking still to do. If we were to bring this in in a more non-mandated way, like in a more dual inventory way, just how should we come to our decision-making about who gets what. There's a couple ways to do it.

MS. HWANGBO: Okay.

If we don't have any comments, we will close our session.

We are breaking here, and when we'll restart again? Thirty minutes later, or three o'clock? I mean, we can ask audience. Three o'clock? Okay.

We will start our risk and benefit analysis at three o'clock.

Break.]

DR. VOSTAL: Okay.

So in the last two days we covered, initially, the efficacy of these products, and today we covered the risks that are associated with these products—or these methods.

SESSION V: RISK AND BENEFIT ANALYSIS

And so now we come to the most important part of this meeting, is to find out the correct balance for risks and benefits. And in order to do this we wanted someone who could give us the big picture, to look at the landscape and tell us what he sees.

And so we recruited Dr. Harvey Klein, who has seen just about everything in blood safety and transfusion medicine.

So here's Dr. Harvey Klein.

Benefits of Pathogen Reduction vs. Toxicity Risks

DR. KLEIN: Thank you very much. I'd like to thank the organizers for inviting me to speak. It's a pleasure to be here. And thank you all who are still

remaining in the audience. I can probably call each of you by name, I think, at this point.

Laughter.]

What I was asked to talk about was a risk-benefit analysis for these technologies. And when Dr. Vostal asked me to speak about that I pointed out that I'm not really a risk-benefit person, except insofar as, as a physician one makes this kind of judgment every day.

So I thought what I would do is to try to give some guidelines and to look at some of the data that have come up in the last almost 48 hours now, and put together some principles, perhaps, that might be helpful.

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If someone is waiting for a number that they can use to decide whether the benefit is sufficient so that the risk is of little concern. I'm not going to give you such a number.

Benefit to risk ratio is often used as a term, but numeric predictions of benefit and risk don't really exist. And the mathematical division—the so-called "ratio"—is never performed.

So what I'm going to try to give you is some kind of a risk analysis.

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In some instances this is relatively easy to do. Certainly in the vaccine world—and this is probably associate as children start to go back to school, and as there has been a shortage of some vaccines—to look at a benefit-risk analysis for measles, mumps and rubella—the MMR vaccine.

As you know, most everyone who's exposed to measles is infect—mumps and rubella. Of those who contract the disease, about 1 in 20 develop pneumonia, 1 in 2,000 develop encephalitis, and about 1 in 3,000 die from measles.

If you look at mumps, encephalitis occurs about 1 in every 300. I didn't find a figure for orchitis, but that does concern me.

Laughter.]

And, finally, for rubella, if a woman is pregnant and is infected by rubella at the appropriate time, the risk of the rubella syndrome is about one in four.

So those are the things that would be eliminated by a vaccine. And even though the vaccine is not a hundred percent effective—between 95 and 100 percent effective—and the risk of the vaccination is encephalitis or severe allergic reactions occurring about one in a million, I don' think you need a calculation to decide that we need to

vaccinate children. And, in fact, you can make a calculation. There are formulae that have been derived to give a number. But just by looking at that I think everyone can see that the benefits far outweigh the risks.

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Pathogen-reduction technology is a little bit different. First of all, we're not talking about a single technology. Each technology is different. The agents we've heard about are different chemicals. They have different chemical and biologic characteristics. The spectrum of pathogen-reduction is different for each of the agents. The activity for specific pathogens—log inactivation—if I can use that term, having berated those who used it earlier, and when we define it for each of these agents is different.

There's different activity in specific components. For platelets we've been talking primarily about apheresis platelets, but we know about buffy-coat platelets, and single-donor-derived platelets.

We know that there are a variety of adducts. They're different for each of these—and metabolites. And we're not even familiar with all of those that exist, or what they bind to, or what their toxicities might be, or how we might even measure them.

And, finally, the profile of adverse reactions is different for each of these, and so is the toxicity, whether it's a long-term toxicity or a short-term toxicity.

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There are other considerations. One of the benefits might be the recognized component-specific benefits. And clearly they are different for red cells than they are for platelets.

There are the potential component-specific benefits. And, again, we've heard a lot about how this will prevent the next agent, but we've also heard from Dr. Busch that it probably wouldn't prevent the next agent, unless the infectivity of the next agent is much lower than that of the agents we know about.

Having these technologies available would not have prevented transfusion-transmitted HIV, HCV, or HBV, because the titres of infectivity at several stages in these diseases—as we saw from Dr. Busch—would have been much too high.

So, yes, it would be important, but it's not going to prevent the next agent.

We worry about component quality —the biologic activity and the recovery of the cells or the plasma protein—although I'm less concerned about that than I am

about the other issues. Certainly there are toxicity issues.

And then we've heard time and time again about the vulnerable patient groups: the neonate, the premie, the pregnant woman. And I like to think of virtually woman between—in the child-bearing years as potentially a pregnant woman being transfused, because they don't know, how do we know who the pregnant women are.

And then there are clearly issues that involve geography in the blood system risk calculus. Certainly, if you were in a country where there was a big problem with malaria or with Shagus disease, the risk-benefit analysis would be quite different than it is here in the United States.

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We've heard about these risks, and I'll just go over them quickly for you again so that we have them in one place.

We've heard about the risk of transmitting HIV being about one in two million to one in three million, depending upon whether you use NAT testing for single units of for mini-pools. HCV—again, in the range of one infection in every two million units. HBV, probably about one infection in every 200,000 units, or maybe every

400,000 units, if we used a single-unit NAT testing. No one talked much about HTLV, but it's about one in every three million units—estimated.

Then there's cytomegalovirus, Epstein-Barr virus, the other herpes viruses, and some of the other agents we heard about.

Bacteria—severe reactions about one in eery 50,000 units. So if we look at transfusions, this fits in with about what Roslyn Yomtovian said for deaths. And if you believe the literature that says it's not quite that common, then I'll say that these are severe reactions. But that's the ballpark.

And then, of course, there are a variety of parasites—the emerging agents. And at least several people mentioned transfusion-associated graft versus host disease and other white-cell associated events which might be eliminated by using some of the technologies we've heard about today and yesterday.

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So those are the potential benefits.

I would remind you, though, in calculating the benefit-risk analysis, that you also have to think about the survival of the patients. And the survival of the patients, both in terms of whether or not they get an

infectious disease, and whether that disease causes harm—especially the chronic diseases—but also in terms of whether there is, in fact, time for a chronic side effect—a chronic toxicity.

And if we look at studies done back in 1984 in New Zealand, at the end of one year only about 80 percent of individuals who'd been transfused were still alive. And at the end of two years, about three-quarters. And the 25 percent that died, died obviously of their underlying disorder.

Vamvakis and his associates at the Mayo Clinic looked at survival after a year as 76 percent—again, very similar—and at the end of five years, fewer than half a cohort of individuals living in Minnesota were still alive after blood transfusion.

And you can see that this particular figure has been repeated in other studies. In a study performed in 1993—perhaps a little bit more relevant to today's care—at the end of 40 months, 51 percent of the patients were alive. In another study looking at patients in New York in the 1990s—again, done by Vamvakis—showed at the end of five years that only 41 percent of them were alive.

So, again, this feeds into our analysis. Not all of those who are going to be transfused are going to live

longer than five years—and not because of HIV transmission, or hepatitis C.

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And, in fact, we can go even further in determining who's likely to survive and who's not likely to survive. We can use age, we can use gender. We can use the disease and surgical procedure as predictors—those who get transfusion and, in fact, the transfusion dose. So those that gets lots of transfusions are much more likely to be dead at the end of five years because they're more ill to begin with than those who receive fewer transfusions. And, again, I think that could be factored into the decision on whether to use a particular kind of product.

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Now, we also heard that infection is not equivalent to disease. And I don't want you to take these remarks to mean that I don't care whether you get infected with one of these agents, as long as you don't die from it. That's not what I mean at all.

But in looking at benefit and looking at risk, I do think we have to remember that HIV has a long incubation, and those people who are dead at the end of

five years are unlikely to have AIDS. On the other hand, there is secondary spread from this agent, so it is an important one, even though it's unusual today.

Now, our speaker from NIOSH, who is very unfortunate because he actually got severe disease from hepatitis B—and, in fact, you can get acute and chronic disease from hepatitis B. But the vast majority of people, of course, do not. They're infected and many of them have absolutely no illness at all and are then immune.

Hepatitis C is, again, a disease that causes a substantial amount of chronic illness. About 20 percent of people infected will develop chronic illness—after 20 years. So this may not be terribly relevant for the cancer patient who's 70 years old and receiving blood transfusions. And the mortality is very low.

For most of the herpes viruses—certainly CMV and Epstein-Barr virus—most recipients were immune. I think Dr. Busch gave us the figure of 48 percent for cytomegalovirus, so it's only for selected populations that this is, in fact, an issue. Again—for the premie, for the transplant patient, the severely immunosuppressed patient, and for pregnant women.

And parvovirus B-19 is very much the same. Most of the people in this room are, in fact, immune. There's little documented disease, except in the childhood period,

except for specific populations and, again, we can identify those.

We have heard that especially in the case of platelets, there are not only many exposures but there is a substantial amount of disease —probably the most common infectious disease today —in red cells, much less so. But those are the fulminant infections that do kill, and so maybe that's an issue that we should be addressing.

I do think it's unfair to place the regulatory agency on the spot that nothing has been done in the last decade. Many things have been done. On the other hand, there are a lot of other strategies that one could use—arm prep has been mentioned, taking the first 30 cc's or so of blood and deferring it has been mentioned. And there are other strategies as well.

And finally, parasites are an issue, but not so much in the United States as elsewhere.

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What are the risks of the various technologies we've heard about? Well, certainly the loss of cells. We're always going to lose cells whenever we manipulate a product like this. I'm less concerned about that. I think we can deal with that. It's not fair, perhaps, to say that at a time when the country is seeing the largest shortage

of blood, perhaps since World War II, but still I think that's an issue that can be dealt with.

Impaired cell function is a different issue, especially if it has anything to do with the result of transfusion. And I heard one of our prior speakers, in talking about days of bleeding, mention that the days of bleeding were increased in a particular study, but that was because of a couple of outliers.

I worry about "a couple of outliers," especially when studies are small, and I'd like to know that those two outliers didn't need a lot more transfusion for a reason related to the treatment of the cells that they received. Its possible.

Decreased cell survival is related to loss of cells, I think. And while that's an issue, it's more of an issue, perhaps, in patients who are receiving chronic red cell transfusion and accumulated iron than in other patients.

Dr. Dzik talked about alloimmunization to a large extent, and I think that is an issue we need to be concerned about.

Then, of course, there is the long-term toxicity of any of the compounds that we're adding to blood. And then I am concerned about errors—especially errors if a system is particularly complex. We are tainting blood,

after all. We're adding toxic substances to blood. And they have to be toxins because they're killing pathogens. And then we're removing them.

But I am concerned that one error in the system, with 13 million or so collections per year, could negate all the benefit that comes from reducing pathogens.

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now, I'd like to give you a couple of paradigms, if I might. And the first has been discussed already at some length. It's the solvent detergent plasma paradigm.

Now, solvent detergent plasma was not adopted in the United States. It has been used widely in Europe. And perhaps that was because of some marketing mishaps, as many people say. But I can tell you that we at the National Institutes of Health did not adopt it for what we thought were good medical reasons. Five fairly experienced physicians, including one who's in the National Academy of Sciences, decided not to adopt it even though cost was not an issue with us at all.

SD plasma had several large advantages. It inactivated the major agents that cause disease—viral agents—in transfusion: HIV, hepatitis B and HCV. Of course, it had limited inactivation, in that it touched only those agents that were lipid encapsulated.

It was a standardized component —and I thought this was a great advantage —for protein content. And, in fact, it reduced transfusion-related acute lung injury—a major benefit, I thought—which wasn't aggressively marketed, and that's a shame.

It was one of several available options in the United States. There were several other kinds of fresh frozen plasma available. And the disadvantages of SD plasma was that it was a pooled product. And, of course, the next agent, in a pooled product, on a public health basis, would be much riskier than the next agent in a single-donor product.

Then there were the recognized loss of a number of plasma proteins as a result of the SD treatment. And initially, we recognized these. They were very well publicized, and didn't know whether or not this would make any difference. Now, as most of you know, recently there's been a report of possible increase in thrombosis in patients undergoing liver transplants. I believe that was a physician's letter. It is now a black box on the package insert.

I don't know whether that was related to the SD treatment. Possibly not. Whether it was related to the change in balance of plasma proteins? Possibly not. But it was clearly something that was not recognized during the

relatively small number of patients treated in the clinical trials. And, of course, when you have clinical trials, you are going to be treating a relatively small number of patients compared to what you will be doing when you actually license the product.

And as has been stated earlier, if a side effect occurs only once in eery ten thousand infusions, you're not going to see it in the clinical trials, and yet it's going to be far more common than any risk of transfusion-transmitted viruses.

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Then there's another paradigm that I think is probably relevant, and that's the paradigm of red cell substitutes. The red cell substitutes—this is a drug, if one of them were licensed, would be dispensed either by the pharmacy or by the transfusion service, and there would be a medical decision to use this. It would be a prescribed drug. You could use it for a selected patient group, whether that was the patient group that was on the label or not, you could make that medical decision.

It would probably be limited indications. So that if you could decide that if a trauma victim who had no access to blood might benefit, you could prescribe a red

cell substitute. So even if it wasn't quite as safe, you're using it as a physician uses a drug.

There is also the possibility of patient assent—or what's known as informed consent. In most instances, you could ask the patient and tell that patient what the potential risks and benefits might be. Now this would, of course, be more like a pooled biologic, and with all the risks inherent in that.

Pathogen-reduction I look at more as a public health decision. I don't think the agency is going to mandate the first licensed pathogen-reduction technology and de-license the components that aren't reduced. I certainly hope that there is not that thought. I think the marketplace will decide whether or not a certain technology or another technology is adopted universally.

But it is my suspicion that if we have a good pathogen-reduction technology it may well become a single component in use throughout the country. In that case, if there is only one inventory, then it's no longer really a medical decision, it is a public health decision and you have obligatory use. All patient groups are going to receive it. Again, the premie, the pregnant woman, the neonate, the patient that receives the exchange transfusion, the patient who is chronically transfused. It

will be used for not only any indication but all indications.

It will be, in fact, an imposed risk. And, once again, it's thought of right now as being treatment for single units. Perhaps there would be multiple units. Maybe those would become pooled units, but that remains to be seen.

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So, at the end, I suppose I should be giving you conclusions, but you need data for conclusions and I don't really have data. So, instead, I'm going to give you opinions.

From a benefit-risk perspective, blood in the U.S., and in other developed countries, is extraordinarily safe—at least from an infectious disease perspective. And I agree with Sunny Dzik that this may be the smallest area of risk of blood transfusion, but I disagree with Dr. Dzik in thinking that we can't have both guns and butter. If I had my way, I would like to have a totally inactivated, Group O unit of red cells, with a standardized hemoglobin concentration—and the equivalent in platelets and plasma.

Certainly, pathogen-reduction could provide an additional layer of safety.

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For a tolerable benefit-risk profile—and I prefer the word "tolerable" to "acceptable." I don't think we really accept most of the infectious risks but we do tolerate them until something better comes along. A pathogen-reduction technology should offer us broad inactivation, minimal damage to cells, little toxicity potential in the most vulnerable patients and, finally, a fair-safe manufacturing system.

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Now I think that risk perception counts. And while bacteria in platelets may be the most important infectious risk today, HIV clearly is what's on the public's mind. And so, from that standpoint, it wouldn't surprise me to see a single inventory. But that will be a market decision.

And, finally, in looking at risk and benefit analysis, geography is important. If this were South America, Central America, the Far East or Africa, we'd be so much more concerned about malaria that an inactivation technology might make a lot more sense.

Blood donor characteristics are important. The robustness of the health care delivery system is important.

And certainly that may alter dramatically the benefit-risk calculus.

Thank you very much.

Applause.]

DR. VOSTAL: Are there any questions for Dr. Klein?

Pause.]

Thank you very much.

So the next speaker is Dr. Jim AuBuchon. And we've asked him to address the effects on blood bank resources.

Effects on Blood Bank Resources and Blood Supply

DR. AuBUCHON: Thank you very much, Jaro, for inviting me to speak at this very interesting conference.

To begin with, my conflict of interest statements: I have consulted with or done research with most all the companies involved in these fields, probably none of whom are going to be happy with what I have to say this afternoon. But they shouldn't fear too much, because one of the side effects—the negative effects of being the last speaker on the program is that someone else may have said something that you were going to say and, in my case, I think everyone has collectively said just about everything that I was going to say.

What I would like to do is to look at bringing pathogen-reduction technology into practical implementation, with an eye toward what will drive acceptance—particularly by my colleagues. I'll talk a little bit about what patients may want, but what will make this technology acceptable or tolerable to them, and what effect implementation will have on blood bank resources as we try to deliver them to the patients who need our help.

I will look at this from a risk perspective, with the intent of increasing the safety of transfusion, and also from the perspective of—yes, I will talk about it—cost.

So, to begin with risk—of course patients are worried about the safety of a transfusion even. They want to make sure that it's going to be safe. And I think we must forgive them when they want this question answered in a very simplistic manner. And they want the right button pushed here to have a safe outcome.

Of course, we know that we can't make transfusion entirely safe. We can try. And as a collective group of individuals trying to improve transfusion safety we have had incredible success in reducing viral risks over the last two decades. This is a logarithmic scale, of course. The good news is that we have dropped the risk for HCV and HIV by multiple logs, but the other side effect of a log

scale is that you never reach zero. And even with pathogen-reduction technology we probably won't reach zero. But we're making great headway.

But we now have risks that are so low for viruses that the risks that we have been talking about, from toxicity and the like, represent significant competing risks—maybe not numerically, but in comparison to the risks that we're trying to avoid.

So, as we try to squeeze this balloon of risk down, we run the risk of the balloon popping a bulge somewhere else, and potentially creating more of a problem than we solve.

I think it's important for these competing risks that we recognize them—as Harvey was trying to lay out in his talk—define them carefully, and then with that recognition and definition try to minimize them.

So, if one were to construct a decision tree, trying to decide whether or not to bring a new intervention into practice—in this case, pathogen-reduction technology—we would need to look at all the different outcomes that might come from using it or not using it, some of which might be better than others, and then looking at the costs involved, and the health benefit outcomes. And one could look at the cost of delivering these outcomes versus the cost of delivering these, or just what health benefit would

be associated by bringing in this intervention, versus the health benefit of not using that intervention.

Now, in this kind of analysis, all effects are to be considered, not just the desirable ones. And all consequent costs need to be considered, not just the direct costs. So you can probably see where I'm going on this—that the competing risks will tend to reduce the overall health benefit from introducing an intervention and drive up the cost of that intervention, even beyond the direct costs.

We have seen this before in blood banking. In my first exposure to cost-effectiveness analysis, where we looked at pre-operative autologous donation for coronary artery bypass grafting, we had to deal with the possibility that the patient, in donating a unit or two of blood for themselves, might have a peri-donation reaction, and this reaction might cause them morbidity or even mortality. Was this a big problem? Was this just a consequence that we needed to acknowledge, or was it potentially a deal-breaker?

Well, using data in terms of infectious disease risks that were going to be avoided by autologous donation—that are now a decade old—we found that a peri-donation fatality risk of just 1 in 100,000 units collected on these CABG patients negated all the health benefits. We haven't

gone back to repeat these analyses, but this number is probably up now in the million range. In other words, a patient is more likely to be harmed from a peri-donation reaction before coronary artery bypass surgery than being harmed by having an allogeneic unit transfused. And, indeed, one looks at the risks that one would have to include in the analysis—it's little bit scary. Look at a large group of patients who were donating for themselves the risk of a serious reaction was 1 in 400—those patients who had cardiac disease. The risk of hospitalization is one out of 17,000 autologous donations. And, of course, if one was going to donate blood before surgery, one may have to delay surgery for a week or two, and you have to compare the risk of avoiding HIV or HCV with the risk of dying because you have delayed your cardiac surgery. And that's 1 in 200 per month.

So, these kind of numbers, I think, have caused change in the practice of pre-operative autologous donation—at least at out institution. We rarely, if ever, now see a patient being referred before coronary artery surgery. And that may actually be to their benefit.

Harvey talked about the down-sides of solvent detergent plasma. And, indeed, this was another competing risk that we had to deal with. The potential for the spread of a non-enveloped virus through the pooled product

was something that we knew was out there—it was a potential risk—but the primary non-envelope viruses that we are aware of as human pathogens are not bad actors, for the most part. But that's not to say that the next AIDS-like virus couldn't be non-enveloped. And, again, using data that are now eight years old, that kind of virus only had to be present in one out of 71 million donors before all the benefits of avoiding the enveloped viruses were negated. To recalculate that number today, it would probably be close to a billion. So, clearly there are risks which may appear very small, but because the risk that you are trying to avoid is so small, they may ultimately overwhelm the benefit.

We have seen this also—having to be recognized—in terms of the thrombotic risk that may be associated with solvent detergent plasma.

But we also have to deal with this on a daily basis in making clinical decisions. All of the physicians who write orders for red cells always think, "Do I need to really do this? Is this an appropriate transfusion?" At least I hope they think about it. And we've had some of our beliefs challenged recently. For example, a study published last year suggested that those patients who were elderly and anemic, after myocardial infarction—particularly if they had a hematocrit less than 30 percent—

might do better if they were transfused. So the decision not to transfuse because of fear about HIV or HCV might actually have been the inappropriate decision. And the competing risk of anemic morbidity actually overwhelmed the risk of HIV and HCV in many of these cases.

So, for all these competing risks I think we need to balance the risks and the benefits with a certain dose of circumspection, if we can achieve that.

I was very pleased yesterday to see the amount of attention that was focused on bacteria in platelets. Because clearly, that's where the problem is. It's not HIV, it's not HCV—and arguably, it's not even HBV, because of the relatively few long-term consequences of that transmission. The primary risk from pathogens today is clear bacteria, particularly in platelets. And this represents one of the major risks that a transfused patient faces. The other one, of course, is getting the wrong unit of red cells. And between the two of those risks, they dwarf, by at least an order of magnitude if not more than an order of magnitude, the risks of HIV and HCV that really have brought us to pathogen-reduction technology.

Though how much protection do we want to establish against HIV? And instead, should we pay more attention to these competing risks and, indeed, the potential for reducing risk with respect to the agents that

we have already focused so much attention on, is very limited. In other words, if you have reduced the risks to a small amount, you can't reduce it much more.

Looking, for example, at the difference between a mini-pool NAT and solvent detergent NAT, yesterday Mike Busch very clearly showed that there is just not much potential for reducing exposure further by adding an improvement in the technology that we're using. And, indeed, if we totally eliminated the HIV risk—whether you're starting from here or here, that's not many cases. Those recipients who would have otherwise gotten HIV or HCV will be exceedingly happy that we have done it, however, if we put our resources in this basket, we may not have them to use elsewhere.

I've been involved with two different groups that have been looking at the cost effectiveness of nucleic acid testing, and the numbers are sobering. When you look at going to mini-pool or solvent detergent testing, the numbers are in the millions of dollars per quality-adjusted life year. And if you talk about going from this technology to this technology, the numbers get even larger. Please don't take these numbers as final, because they're undergoing some revision, particularly with some more recent information about the cost of the testing. But anytime you have to list cost-effectiveness numbers in

scientific notation, or something that could be expressed that way, it's probably not the best use of money.

But, of course, what we're all trying to do is we're trying to increase the health of the patients that we're serving. And if we can do that, and if we do have the resources to do it, well, that's great. My job isn't to build bridges, my job isn't to put a man on the moon. My job is to try to make transfusion possible for the patients at my institution. Ad if I can do that more safely, and if I have the resources to do it, I'll be happy to do it.

And, of course, not all of the arrows go up at quite that angle. Some have only a slight rise, and that will immediately raise the question of is it worth the effort to bring it in. But none of us want to have this happen. None of us want to see the safety of transfusion decline—with our well-intentioned efforts to make it better. And we face that, of course, with chemical inactivation of pathogens, because this is a perfect example of a competing risk, as we've been talking about.

Are the toxicity concerns going to outweigh the benefits? And if the risk of HIV, or HCV is one in several million units, what new risks should be accepted? And how can we prove that they're this small?

You know, we've heard talk about confidence intervals, in terms of viral inactivation potential. And

we've heard talk about confidence intervals in terms of red cell recovery. We haven't heard any discussion of the confidence intervals around the toxicity evaluations. In fact, the toxicity data that have been released for these chemical inactivation steps have been relatively limited and relatively condensed. Can we be sure that the risk of causing cancer after administering, admittedly, a very small amount of a psoralen is less than one in two million? And can we be sure with a 95 percent confidence that it's less than one in two million. In other words, are we really going to be improving the safety of our transfusion recipients? I think that's a very tough question for the toxicologists to try to address.

As I said, I was very happy to see that we are focusing more on bacterial contamination of platelets as a potential application of pathogen-reduction technology, because this is really the primary concern that many of us have now, in terms of bugs in blood.

And the numbers you saw yesterday are, indeed, sobering. When we're talking about HIV in terms of fractions of cases per million, and yet we're talking fatalities—patients who are just as dead—in the tens per million. This is not just an American problem. Other countries that have looked at it have seen the same thing as well. And for those who prefer numbers in a different

format, you look at the French data, calculate it out, that's 1 in 140,000. And yet we're talking one in several million for the viral diseases.

So, to return then to looking at different potentials for intervention, we probably should restructure this decision model a little bit, if we're actually going to look at pathogen-reduction technology, because particularly if we're going to focus on bacteria in platelets, the bacterial detection methods need to be considered in here as well.

Now, obviously, the bacterial detection methods aren't going to do anything to reduce viral infection. But the probability of no intervention leading to a viral infection is going to be very low. In fact, what's likely to drive an analysis like this is going to be the level of bacterial infection leading to morbidity or mortality, and the toxicity of any pathogen-reduction technology.

Now, my next slide is not a cost-effectiveness analysis based on this model, because it would probably take a small army of decision analysts several years to consider all of the different ramifications that would have to go into a model like this. This is a complex analysis, to say the least. So you won't be seeing it in print anytime soon. But I think it's an important one to be addressed.

Instead, I certainly am a strong advocate for addressing the problem of bacteria in platelets with a method that doesn't inject a new risk—and that is using some type of detection technology. We have been culturing all of our platelets for the last three years. It wasn't an original idea with me. There are blood centers in Europe that have been doing this for more than half of a decade, and yet most of us have been content to just let this happen, and not step up to the plate and address it. That's another issue.

But I think the potential here for applying a technique like this—whether it's culturing or some other newer technique to come down the road, is providing assurance of sterility and removing, or at least greatly reducing, the major risks that platelet recipients are now facing.

Of course, there are other benefits, potentially, to come out of making sure that the platelets don't have a lot of bacteria in them—such as longer storage, and storage after pooling the platelets, as is done in Europe, and reducing the cost for leukocyte reduction.

So let me change gears, then, with the recognition that some of these benefits have dollar-signs attached to them. They have benefits which deal with how we use our resources, because I think that's going to be

another issue in approaching the implementation of pathogen-reduction technology—cost.

We can certainly understand, after hearing all the work that has been done on these techniques, that a lot of R&D money has been spent in bringing them close to licensure. It's only reasonable that the companies are not going to be giving them away. It's going to cost us something to use this technology. It will cost us for the reagents and the equipment that may be needed. It will certainly cost us for the staff time to handle this additional step.

There are many indirect costs to be considered, as well. They've been mentioned—about product loss, have to consider turnaround time, and potentially product potency concerns.

Now, some of these data have been mentioned before. Some of them went by relatively quickly, but let me put my spin on them. For example, the Phase II studies that Drs. Mintz and Snyder presented at ASH last year, showed that there was no statistically significant difference in the 24 hour recovery, between red cells that had been treated with Inactine and those that had not. But that doesn't mean that these two forms of red cells are entirely equivalent. That hasn't yet been proven. And, indeed, there may be a difference between the recovery of

red cells treated in this way. And there does appear to be a statistically significant difference in the survival of these cells—potentially, about a one-third difference.

Would that make a difference in the use of these cells? Will a surgeon or an anesthesiologist notice the difference in the OR? Probably not. Will the hematologist taking care of a thalassemic over a long period of time notice the difference? We'll see. And I'm glad some long-term studies with S-303 are being established in this manner.

Because the same question arises there, in the study that has been presented in abstract form. There was a difference between the 24 hour recovery, between treated and control cells. And this is only a 3 percent reduction in clinical efficacy. Is that likely to have a clinical impact? Well, we have far more difference in the hemoglobin content of our cells than—of our units than just 3 percent. But, potentially, one could project that this would have some impact on our usage of red cells and the overall cost of implementing this technology.

We see this also with the use of psoralens for pathogen-reduction in platelets. The studies that have been reported from Europe showed that the treated group required more transfusions. They had a lowered count

increment, in terms of looking at how much the platelet count actually rose.

Now, by using the statistical method that the authors of the group decided to use, they were able to note that the count increment and corrected count increment clearly depended on the dose of platelets. But the dose of platelets was about 10 percent lower in the group receiving the treated platelets. So does that mean we are going to have to pay for the technology, and pay for 10 percent more platelets being transfused.

In the U.S. trial, as was noted, the platelet content was, again, slightly less. The post-transfusion count increments were a little bit lower. The group receiving the treated platelets needed more transfusion and had shorter inter-transfusion intervals.

Will this cause our hematologists, and other transfusers of platelets, to change the way that they decide to practice? Because most hematologists, in my experience, don't calculate corrected count increments, or they don't say, "Well, we gave a 10 percent smaller dose, therefore I'm not surprised that the patient's platelet count is a little bit lower." They'll say, "Did the patient's platelet count get to X?" And if the answer is no, they're going to order another transfusion.

So, the count increment is indeed something that's important. And that may impact, ultimately, the cost to the institution--and put more strains on our availability of platelets. In many parts of the country, the collection of platelets really drives the blood collection system—not that red cells aren't used and aren't important. But platelets are what's in short supply more often than not.

And will we have enough platelets in our system to suffer the decrement of these treatments, and continue providing the supports that patients need and that hematologists expect? That is, indeed, a rhetorical question. I don't have the answer.

So, we have been successful with reducing these viral risks, and we are now the victims of our own success. Because we can't really push these down any further without causing these competing risks to rear their ugly heads.

We will have a bit more room to maneuver, with respect to potential toxicities if we compare the benefits to be gained by the pathogen-reduction technology to septic fatality or bacterial contamination concerns—in platelets. Because here there is more risk still inherent in the current system. Therefore we can tolerate more risk in the implementation to remove it or reduce it.

So, my recipe for making transfusions safer is that we should focus on the largest risks. As you have heard today from others, the biggest risks are not viruses. They're bacteria in platelets and giving the wrong unit of blood to a patient—as well as others. And we should focus on those largest risks; put our resources there, put our time and effort there, put our national conferences there—if we expect to really make some inroads and improve transfusion safety.

Also, when we try to implement a solution, let's look for a solution that doesn't bring along with it new risk—or at least try to pick a solution that has the smallest risk attached, so that the net benefit, after you've subtracted the competing risk—the net benefit will be as large as possible.

Thank you very much.

Applause.]

DR. VOSTAL: Thank you, Jim.

Are there any questions?

Pause.]

All right. Thank you.

Public Comments

DR. VOSTAL: Now, the next session is a public comment period. And the host for that will be Dr. Ed Snyder. And I'd like to take this opportunity right now to

thank Dr. Snyder for helping us plan and organize this workshop. His tireless efforts were invaluable in getting this workshop put on.

Thank you.

Applause.]

DR. SNYDER: Thank you very much for those kind words. Steve Wagner was part of the group as well, as I'm sure Jar will be mentioning at the very end, plus the other members of the team that are listed on the back.

We all tried to come up with a conference that would touch on the issues, to let not only the industry members but also the industry recipients, if you will, know what the state of the field was. So I think we've certainly achieved a lot of that.

The purpose of this session is public comment. There were three people who had signed up to present. Dr. Dzik was one. He has since decided to dis-sign-up.

Laughter.]

There were two others: one from Biomerieux. I don't know if that individual is here. Yes? You are?

Off mic remark from audience.]

Laughter.]

DR. SNYDER: Hoisted on his own petard. Yes. There you go.

And the third was—I don't have my sheet with me. Who was the—oh, yes, Steve Binion from Baxter, who I think has also told me he would not be speaking.

Laughter.]

DR. SNYDER: So—I'll tell you about my trip to Maine --

Laughter.]

DR. SNYDER: No. I think what we should probably do is open this up to just statements from the floor. I always like to refer to a comment that Jack Hoke made, who was with NHLBI many years, and talked about people suffering from the pain of undelivered speech. And I think now is the time to relieve yourself --

Laughter.]

DR. SNYDER: —of your speech.

And the only ground rules are to limit yourself to no more than five minutes. And the session is supposed to run until 4:30. We obviously may not take that much time. So—just please identify yourself.

DR. LePARC: German LeParc from Florida Blood Services.

I just thought that to give people here a perspective, I'll tell you a short anecdote that happened just recently.

As you may have heard in the newspapers, TV or everywhere, our institution had the sad experience—a wrenching experience—of having had one of the—the second case of HIV transmitted through transfusion during a window case—while doing that.

The week after this broke out in the media, I could go back to see patients again. You know—that whole week was just gone. And we had a young patient who was diagnosed with TTP, and we are in a multi-center study that is ongoing with one of these companies, where we recruit our patients to treat with pathogen-reduced plasma. Actually, it's a double-blind, so we give something that we think has it or not. You never know.

But, part of it is you have to present to the patient an informed consent, which is a five-page document, that tells him about the known risks of plasma transfusion, and the unknown risk of pathogen-inactivation, or pathogen-reduction. And the patient—a very intelligent person—was still with it, and knew what the situation was, and started to ask me questions, "Well, what about this risk," and you have to say, "Well, we don't know. That's why we're doing the study." And "Will this work the same as plasma?" "Well, we don't know. That's why we're doing the study." I mean, my answer was that kind of thing.

And I really wanted to bring this person into the study, because that's the only way you make progress. And at the end, the patient said, "You know what, Doctor—I know that the risk, from what you say, of getting HIV and what the risk of getting HCV and HBV—and I'd rather take known risks rather than the unknown risk of this new treatment"—and denied us recruitment into the study.

And this is—you know, in the middle of a frenzy where, you know, there were people that wouldn't get transfused for anything in the world, or were banging on doors—"I want autologous blood only," even though they were with three nitro-patches and an oxygen tank.

Laughter.]

DR. LePARC: So, you know, I think you need to know that the public has different expectations, depending on what their outlook on things are. And, you know, we may think that—some people may think that pathogen-reduction is the way to go. And other people say, "You know, there are so many unknowns, I don't want to take that risk, and I'd rather go with what we have now."

DR. SNYDER: Celso Bianco?

DR. BIANCO: America's Blood Center.

This was a wonderful workshop. And you made only one mistake. I think that Harvey's and Jim's presentations should have opened the workshop. They were very sobering.

They were superb—and set the tone, in retrospect, for all the things that we heard.

???AUDIENCE: Harvey made one statement that I'd like either him to explore, or perhaps some of the people who are from the companies—and that is the issue of the pathogen-reduction methodologies' not being able to help with an emerging infection?

DR. KLEIN: No, I'd like to clarify what I meant by that, just so that it won't be misunderstood.

I think that if you have a pathogen-reduction technology that reduces something by four or five or six logs, and your emerging agent has 10 to the 8^{th} , then you're still going to transmit infection.

Now, if we go back and we look at Mike Busch's data, looking at hepatitis C, there's a chronic phase with a very high infectivity. If we look at hepatitis B, there are several times in the course of the infection where there's very high infectivity. If you look at HIV, in the early phases of the ramp-up, there's a lot of virus circulating.

If we have a pathogen-reduction technology that's capable of addressing 10 to the 14^{th} or so, then I think we can feel fairly confident that the next infection is not going to be an issue—and only fairly confident at that.

Now, I don't mean to say do nothing because you can't do everything. But I think we need to be very sober in realizing that it's very easy to say "We're going to add something to kill viruses and prevent the next HIV." I think that based on the data we have, that's very unlikely to be the case.

DR. AuBUCHON: But—could request a clarification from manufacturers about the capabilities of their techniques?

It was my understanding—and I'm not a virologist. I may have this wrong—that when the viral reduction capacities were shown as greater than 5.7 logs, the reason that that wasn't listed as greater than 8 logs or greater than 10 logs was not necessarily because the technology could not go to greater lengths at inactivation, but because the test systems did not allow them to test at higher concentrations than that. They had reached zero. They couldn't start any higher than what they started.

I would request some clarification as to just how much of a concern Mike's projections and Harvey's comments really are.

DR. CORASH: yes, I think—you know, Mike's data, which are --

DR. SNYDER: Please identify yourself for the --

DR. CORASH: Larry Corash, from Cerus Corporation in California.

I think Mike's data, obviously are very informative, but you have to make a distinction between genome equivalents and infectivity. And what Mike is measuring are genome equivalents that are in the—you know, the plasma of those donors.

What's being measured in the pathogen-reduction assays are infectivity. And so you can only, you know, demonstration elimination in the sample that you're testing for infectivity that the highest titre that you can grow it to.

And the wild-type viruses frequently are not amenable to—they're not—they will not plaque in these assay systems. So you can't demonstrate it.

I think the situation is really pertinent in, you know, the hepatitis field, where Harvey Alter has calibrated, you know, viruses that have been proven infectious multiple times over. And the titres of those run between four and five logs, you know, per mil, because that's what's infectious in those plasmas.

That's not to say we don't know that in those plasmas—those—you know, those genome equivalents, although we know that in general there's a mismatch between

infectivity and genome equivalent. So we can neither prove or disprove that—unfortunately.

But I think, you know, in Mike's example of the very early phase of infection in these still vulnerable window periods, where he's finding, you know, genome equivalents, which we would have to assume exceed infectivity, they seem to be in the range of somewhere between three and four log per ml—if I'm quoting you correctly. I mean, you can state your own data.

And so, in that range, at least we know for infectivity we have something which seems to be—have some capacity.

DR. BUSCH: Mike Busch.

I mean, obviously this issue of the relationship between infectious units and genome equivalents is critical to not only pathogen-reduction, but toward the need to implement enhanced sensitivity NAT.

So we've been focused on this, both in animal model studies, and in trying to acquire data in real human transmission settings to answer it.

And I—in the animal studies that—we've done some in collaboration with Harvey—and certainly, even worse, in vitro infectivity assays—I really think are just simply insensitive. The in vitro assays are fundamentally insensitive to—particularly primary isolates, and then the

animal models—for example, in the HIV studies we did several years ago with Chris Murphy and Harvey—it was very clear that the kinetics of primary infection in the chimp is so dramatically different. And then we went back and attempted to transmit primary isolates to chimp PBMC and could never even find and acute plasma panel that would infect chimps.

So those animal models, to me, are really difficult to extrapolate, which is why our focus, recently, has been on acquiring data from human transmission settings—either these look-back cases that have been observed, where look-back has found recipients who got pre-sero conversion, even mini-pool net negative units, and either did or didn't get infected. And trying to generate the data to ask "What was the viral load—" in this case, in the window period, "—that was associated with transmission?" And were surprised to see, you know, transmission seems to be happening with levels of primary viremia that are extraordinarily low, at the limits of detection of NAT—essentially, single-copy, or certainly, below detection in mini-pool.

So, to me, I infer from that that during the ramp-up window phase, that all of that virus that we're detecting with genetic methods is almost certainly

infectious. You've got an exponential growth, it's just like bacteria. You're in a massive growth phase.

What's really surprised me, though, is that the work that we've been doing recently, looking at—correlating infectious units with transmission in people with chronic infections--HIV, HCV--where once again we're finding transmissions with concentrations by genome analyses as low as 10 or 1 or even sub-detectible by genetic methods.

And, to me, again, the inference I draw is that the theory that a lot of this virus was non-infectious, neutralized-effective is wrong. And that even in chronic phase, I think to be conservative—and I think, you know, accurate—we have to assume that genome equivalents are a pretty good reflection of infectivity of these human viruses in the context of transfusion.

DR. CHAPMAN: I'm John Chapman from Vitex.

I appreciate the points that are being made.

I think one thing I would say is that our virologists spend a lot of their effort trying to get to very high titres that they can produce. And then when we have those at the highest level that we can make, then we do our experimental designs to look at the kinetics of inactivation, so as to understand what is the rate of inactivation.

And I think that tells you a lot about what the power of the technology is against that particular virus.

And also, in some cases, like human pavo-B-19, we have data which is showing—because our mechanism of action is to disrupt nucleic acids, we can show by PCR—long-range PCR—whether we're damaging the pavo-B-19. And there we can have titres of 10 to the 11^{th} being rendered to have no detectible PCR implication after treatment.

So, I think it's a case of virus by virus you have to look at. And you have to use all the technology that's available to assess what is the power of that technology.

DR. SNYDER: Dr. Goodrich?

DR. GOODRICH: Ray Goodrich, Gambro BCT.

I think Harvey Klein raises a very valid and interesting point. And I think that there is data that is available to be able to address some of these questions.

For a long time, we've approached this from the standpoint—I know Mike does these calculations—has done them, has published them—looking at detection limits as the issue. And there you're working at high titres and going down, and asking where does your sensitivity, to be able to detect these viruses, become negligible, and no longer effective.

And that defines a window period. And it defines, as a result, the number of transmissions that might occur—and have occurred in some cases—and you could calculate and determine what those are.

With pathogen-inactivation, you're taking a slightly different approach. And I don't know if we've looked at this from the standpoint of saying, "We kill different viruses to different levels." And we're starting at the low end—the low amounts that are present at certain periods we may kill very effectively. At what point on the upper end do we lose effectiveness? And what are the titres during various stages of viremia in donors, who might be infected and might be donating blood.

I think that information is available. And maybe, if we look at it, if we analyze that data, we could come up with some estimates of how many cases, based on performance levels with a variety of different types of viruses, whether it's three logs, four logs, five logs, six log per ml, inactivation that's achieved, how many cases you might be able to interdict.

I don't think that that's been done, and I think it would be a very worthwhile exercise.

DR. SNYDER: Roger?

DR. DODD: Roger Dodd.

Yes, I really got up, I think, to support Harvey's point. I think it's a very important one. And the level of the discussion clearly indicates what the outcome of this position is: and that is that we really do not know and we cannot predict what will happen with the next virus.

I remember—and I know that things have changed—I remember working for some time in the area of pathogen-inactivation and looked, along with Steve and others, at a number of different agents—not the refined agents that we've heard about over the last couple of days. But it was very clear to us at that time that when you were dealing with five or six logs of a relatively sensitive virus, you were also at the limit of survivability or functionality of the cells in the system, whether they be red cells or platelets.

And certainly in the systems with which I was familiar, you were in a very narrow window, where on the one hand you had less inactivation, and on the other you had less platelets, basically. So I think that that's something to be borne in mind.

I think it's very creditable that such high levels of inactivation can be shown, but we don't really know what they mean.

And the other thing that I—two other things I wanted to comment on. And, again, I agree with Ray and with John, that you can tell a lot by looking at inactivation kinetics, but there have been a number of cases that many people in this agency—or used to remember quite well—of predicting infectivity or inactivation resulting in an infectious vaccine, because it just did not continue along the predicted straight line. That's a matter of concern.

And the other thing, as a historical note, I find myself sitting beside Lew Barker. And Lew, many years ago, published really quite a seminal study, which couldn't be done anymore—and I don't think he was responsible for the study, but rather for the analysis. And in that study it was shown that a milliliter of serum from a hepatitis B carrier was infectious out to 10 to the 7^{th} dilution; in other words, at least 10 to the 7 infectious doses per milliliter of HBV in that plasma. And again, I think that emphasizes the point that Harvey made when he stood up that even the materials with which we're familiar will probably only be effectively inactivated in the presence of current levels of highly effective testing.

DR. SNYDER: Bernie?

DR. HOROWITZ: Bernard Horowitz.

Well, I think to answer the question in part, we should go back to the 1980s. Because factor-8 concentrates—every vial of factor-8 concentrate transmitted hepatitis C. And that could be shown in chimpanzees, and, unfortunately, could also be shown in man.

Every vial—not every vial, but many vials contained hepatitis B. And as we sadly learned, many contained HIV.

Viral inactivation came. We know the data associated with those early methods. All of the methods stopped HIV, even though many of them—several of them—were not that potent. Does that mean that they will stop the next, or the current methodologies will stop the next virus? Of course we don't know what the virus is.

But, nonetheless, we do know what the potency of those early methods were, and they inactivated about four logs of virus, for a variety of viruses that it would spike with—not greater than four—greater than and equal to—they inactivated four logs.

The better methods inactivated greater than six logs of inactivation, and they also stopped hepatitis C.

So what can we infer from—now, those are data that we can look at. What can we infer as to the current methodologies?

I think the current methods—the ones that inactivate greater than six, have a good shot at stopping the next virus. No guarantees. But they really do have a good shot at it. And yesterday, when I was asked the question—or, in fact, the entire assembly was asked the question: "What should we aim at—target at—for inactivation?" And I said, "Well, at least six logs, and preferably eight." And I didn't go into all of the reasons for it, but those are some of the reasons that stood behind the numbers.

Some of them just come from the numerology, and definition of a TCID-50, which I don't think I'll bore the audience with at this point—but—and probability analysis that's associated with it.

But from what I can see, at least of the Cerus data and the Vitex data, they at least have a good shot at stopping the next virus.

DR. SIVAN: My name is Yariv Sivan, from Maco Pharma.

I have a question that could have some influence on the cost analysis of these systems.

Post-market surveillance was mentioned, but what kind of size of post-market surveillance would be acceptable for these kind of things? Are we talking about

one in ten thousand, one in a million, depending on viruses?

DR. SNYDER: I'm sorry - what kind of what?

DR. SIVAN: What size of post-market surveillance would be required?

DR. SNYDER: Would our PMA specialists like to respond to that?

No response.]

That will have to be worked out.

Pause.]

Dr. Busch approaches.

DR. BUSCH: I just wanted to follow up, both on Roger's and Bernie's comment. I mean, I think the unfortunate reality for this conference, to me, is that, you know, testing is going to have to stay in place; that these methods will achieve, hopefully, four, six logs, but will not allow us to disband current testing nor, with every new agent, assess whether testing is needed in addition.

Now, I don't think that you can extrapolate from the pool derivative impact of these methods to single units, because you have the dilution factor, where you're actually pooling, you know, one infectious unit into hundreds or thousands. And so your ending titre that inactivation needed to kill, was substantially lower than

might be present in a single donor who's in the window or chronic carrier stage.

If you look at the distributions of viremia in the window or chronic carriers, you know, I think four to six logs killed will eliminate infectivity—per ml kill—will eliminate infectivity in probably 80, 90 percent of chronic carriers. And, you know, during most of the primary window, eliminate infectivity there as well.

But it's not going to safeguard from your high titre carriers in either context.

DR. HOROWITZ: Mike, as far as—it's Bernard Horowitz again, for the record.

As far as I know, there's absolutely no data to support the dilution effect. I've heard it quoted at so many meetings. But, in fact, the total amount of virus that you put into any of these systems is what's the controlling the outcome of whether or not you're killing all of it, some of it or none of it.

And I have never seen any data to say that if you have 10 to the 3rd virus in 10 mls, is any different from 10 to the 4th virus in 1 ml.

DR. SNYDER: Mo, is your comment directed—Celso or Mo, are your comments directed at this? Okay.

DR. BIANCO: Celso Bianco.

I think that this discussion brings up what I think is a marketing lesson to all of us. That is what we heard yesterday and today, that if there is one thing that is important, and where pathogen-reduction could help, is with bacterial contamination.

What the companies have marketed until today, is "the next virus," is the fear of the population. And I think that that's the big change that we attained with this meeting.

And I wish that's the way that the real things were the object of the claims and marketing of those products.

DR. BLAJCHMAN: Mo Blajchman.

I was looking around the room because Tom Zuck was here earlier. I think he's gone now.

But Tom wrote an article that many of you remembered, that was entitled "Can we have a zero-risk blood supply?"—or some such thing.

And his conclusion, as best I can remember, was that this was not possible.

It's still probably not possible. The only problem is, we have technologies that have been talked about in the last 24 of 48 hours, that bring us much closer to a zero-risk blood supply than has ever been possible.

Now, Harvey Klein criticized me during his talk—and possibly appropriately so—because I took to task the regulatory agencies for not regulating some of these technologies. And I think he was right to criticize me.

But I think you have to understand the motive of where I was coming from. The motive—what is inhibiting the institution of these new technologies is the economic side of things—the potential costs of these things, which are not inconsiderable. These costs are significant.

But it's that that's stopping us from reaching closer to that zero-risk blood supply. To leave it to the marketplace to decide to spend the money perpetuates this delay in reaching the zero-risk blood supply possibility. And as far as I can see—and this happens in our country—in Canada—and is likely going to happen in the United States—unless the FDA or similar regulatory agencies mandates some of these things—like doing something with bacterial testing, like introducing other innovations, we are not—we in the United States and Canada and elsewhere in the world—are not going to be able to achieve the zero-risk blood supply because the problem is that there aren't good mechanisms in place, particularly in the United States, for recovering that money.

As I understand the reimbursement system, it's a system that allows—that doesn't readily allow for recovery of those funds. And that is the problem.

So, I took to task Jay Epstein for not doing anything, because I felt that that is the only way that we in transfusion can advocate, and do our job in delivering the safest possible blood to the patients. In order to do that we need to encourage the regulatory agencies to do that.

At a May ABV meeting a few years back, I spoke for the institution of leukoreduction—universal leukoreduction. I certainly acknowledge that the data aren't all there, but I think this is going to be a major advance for our patients—and that is universal leukoreduction.

I was criticized by somebody who I have had a lot of respect, and still have, because they tried to paint me as being on the payroll of one of the corporations.

And that's fine. But what I wanted to do, but have too much request for this person to respond appropriately, or the way I really wanted to—that he too has a conflict of interest, and most of us in this room, including myself, have conflicts of interests, because we represent the institutions that we work for—the hospitals that we work for.

We are protecting those budgets.

I think we are behaving somewhat unethical when we don't do the best job we can for the patients that we look after. It is not up to us to decide where the money is spent. It is up to us, in my view, to argue, to make the case, for attaining a zero-risk blood supply. And until we take that position as a group, we will not have a zero-risk blood supply, and not even get close to it.

DR. SNYDER: Okay. I'm biting my tongue here.

Go ahead.

Pause.]

You were in motion. You get to go.

DR. FARSHID: Mahmood Farshid from FDA.

I would like to comment on Dr. Horowitz's point that—I think the comparison of the single unit reduction with the manufactured plasma derivative may not be completely accurate, because there—even if you have five or six log reduction, the manufacturing fractionation process also contributes to the viral removal. And in a plasma derivative, we require, as indicated yesterday, at least two steps for clearance—removal and inactivation, which should provide somewhere about 10 log reduction in case of envelope viruses.

So the level with indicated for the single unit—five to six log—does not appear to be sufficient to provide assurance that no virus will be in the unit.

And for the limit of clearance, it should be sufficient to basically inactivate or clear the level of the virus at the peak, basically taking worst-case scenario, which would be the viral load at the window period.

DR. SNYDER: I'll let Bernie comment.

DR. HOROWITZ: I apologize, too. This is such a technical question for, I think, the audience. And yet the impact is significant enough, I think, to warrant a response. Because if we believe that the inactivation systems are not going to inactivate the next virus, then—aside from the comment of bacteria—you know, we shouldn't really pursue them, in my mind.

Just as a reminder—I was referring to the 1980s, in part—in large part, not only because we had the viral data, but factor-8 concentrates were little more than cryoprecipitate in the 1980s. And there's evidence that in the cryoprecipitate, the virus was concentrated rather than diluted.

So, as a challenge for the system—it was a very adequate challenge, despite the fact that it was a process derivative. The only step that really had any impact at

all was the cryoprecipitate. And some viruses were removed, and others were concentrated. And, in any case, there were relatively high titres.

So, I think that the evidence from the 1980s still holds for today, and we still have a good shot of inactivating those viruses with the methods that I've seen in the rom.

DR. SNYDER: Dr. Willkommen.

DR. WILLKOMMEN: Willkommen, the Ehrlich Institute.

I want to say, the safety of products comes from the combination of measurements—a combination of actions. In the case of plasma derivatives, we have the testings — testing of plasma pools. We have the different fractionation methods. We have two inactive—or we have two methods which are effective for inactivation or removal of the viruses and this has to be demonstrated.

In the case of the single donation product, I think we have a little bit different situation, because we have not so much opportunity to combine different measurements. We have—but we have two. Also, we have the testing. The testing is enhanced by the established—or the introduction of the obligatory testing of the genomes, and AT testing.

And then we have, if we are speaking here about the inactivation methods--pathogen-inactivation in components—we have then the inactivation method.

On the other side—and I think we have a safe product then, and the inactivation—although enhance the safety.

On the other side, I'm always vary cautious with any conclusions on unknown viruses. We know from all inactivation methods—and we have seen it here also, that the different methods have some limitations, and we have to consider that, and we have to test that, and we should know that, so that we can make real predictions.

I agree complete, if the inactivation—if a donation contains 10 to the 11 maybe povuviruses, it is possible that the inactivation method cannot completely inactivate it. But we will see it from the kinetic, from the real good investigation of this method, which what we can predict from this, and what we can conclude or assume from that.

And so I think this—this, altogether, I think give us a feeling or give us information, and the knowledge about the safety of the products.

DR. SNYDER: Dr. Brecher.

DR. BRECHER: Thank you, Dr. Snyder.

One the one hand, I think we've done a very good job at this meeting, talking about viral inactivation, and arguing the various merits. And we've mentioned pathogen-reduction in regard to bacteria, but we sort of blew past detection systems. And I'm a little disappointed that that didn't get a little more play in this meeting.

But, in any case, whatever we do, we have to do something. And to quote a wise man from the 1999 meeting, "The imperative is to act so we don't have to explain ourselves on Nightline." Ed Snyder said that.

DR. SNYDER: Let me respond to Mo.

In the United States we have Casey Kasim. I don't know if you know Casey Kasim. Casey Kasim has this top 40—or at least, last—when I stopped listening to him. And he used to end every show with a comment, which I think is germane. It says, "Keep your feet on the ground and keep reaching for the stars."

So I can go back to my institution and tell them zero-risk, zero-risk. And they're looking at cutting our cookies, our paper goods, travel budgets—there's really nothing left to cut, except the $7 million blood budget and 26 people that work in it.

So, it's difficult. We can't, I think, lose credibility. And I'm sure you understand. I understand

the "Mo-isms" when you get going. You're very passionate about your points of view.

But I think this is something that we have to temper reality with reaching for the stars here.

I would like to raise a question. And I maybe peeking into Pandora's box. I wasn't sure whether I should do this or not. If it's not appropriate I'll close the box and just turn the podium over to Dr. Vostal.

In talking to some of the pathogen-reduction company folk, I've asked them how close they think they are to licensure of various materials. And they tell me that—as far as toxicity is concerned—that's an issue for me.

And the response I get is that "We've done everything the agency has asked us to do."

When I talk to the agency, I kind of get the feeling they may want them to do more.

And I can't quite put this all together. I just feel—I'd like to know, to some degree—and this is where it may be inappropriate to discuss this in an open forum, which is why I'm continuing --

Laughter.]

— what expectation should we have? Are the companies correct that they've submitted everything they need to, and you're going to think, and it may take you several years to finish thinking --

Laughter.]

— or is there an expectation, based on this conference, you know, there are whole areas that have yet to be looked at. Because part of this has to do what Mark's comment was about do we want to put in pathogen detection systems before inactivation systems, and so forth.

And I think as, you know, people in the United States looking at this, what might we be reasonably willing to expect in the coming months or years.

DR. CHAPMAN: I would correct your statement, I think, Dr. Snyder—respectfully—in that I don't believe we've ever said that we think we're finished. This is an ongoing toxicology program. In fact, when I presented my results, I indicated that our carcinogenicity study is ongoing. And that's—we're at a point now that we're ready to start sharing our toxicology data with the scientific community. We'll be presenting reproductive talks at the ISBT and at the ABB meeting.

So we want to start sharing our data. I think we're at the very beginning of that, not at the end.

DR. SNYDER: I actually wasn't referring to anything you had said, but I appreciate the comment.

Dr. Vostal, are you able to respond to this, or should I just go away.

Laughter.]

DR. VOSTAL: You should have kept that box closed.

Well, I think—these are really complicated biologic products that we're looking at toxicity of. And what we wanted to do at this meeting was to present the way that we're looking at toxicity currently. And we wanted to open up the forum to see, you know, whether we were doing it appropriately? Where there's opinions about how it should be done otherwise.

And I think one—one thing that I'm hearing is that—you know, this is an ongoing process, and it—post-market surveillance may be a bit factor in evaluation of these products.

For the other stuff—you know, there was a lot of things presented, and a lot of opinions presented, so we'll have to go back and go over the transcripts and see if we find something else.

DR. SNYDER: Okay. I think I will thank you all. Unless anyone has any other burning issues, I'll turn this back to Dr. Vostal to close us and take us home.

FDA PERSPECTIVE ON DAY TWO

DR. VOSTAL: Okay. Well, that was two days of discussion of pathogen-reduction. I think we covered all we set out to do, and we finished on time. And that's how the FDA does work.

Laughter.]

Applause.]

So, the objective for us was to have an open forum for discussion of how to evaluate these methods—you know, toxicity and efficacy. And then also to get input from the transfusion committee on how they would look at the risks and benefits. And I think we've achieved all that, and it's exceeded our expectations—certainly my expectations.

I'd just like to also address Mark's point that he made about bacterial detection.

You know, our objective is to somehow reduce bacterial contamination in these products. You know, however we get there is not really that important to us as long as we get reduction of those.

This workshop—the point of it was to help us evaluate decontamination because it's one of the most difficult things we've dealt with before, in terms of, you know, having a device and a biologic product and novel issues—though judging efficacy and toxicity.

So, we needed—this is the one that we needed most help on, so that's why we focused on this one first.

Let me just—so we went through the objectives.

I just wanted to bring up one more point that didn't get brought up in the last two days.

This is terminology—the current terminology that's being debated at the FDA. And we have the—you know, what's "in" and what's "out."

Of course, what's "out"—now, because I don't think we're going to be able to ever claim sterility in these products after, you know, pathogen-reduction. I think we should not call them pathogen-decontamination, pathogen-inactivation, pathogen-free, or some of these other terms is better than "clean," "ultrapure," "clean as a whistle," "pure as the driven snow."

I think the impression should not be made, just by calling it something, that there is zero risk.

So we would prefer if we stuck to pathogen-reduction.

With that, I'd like to also than the planning committee that helped to plan and organize this meeting. From the FDA, there was Nat Wolins, Betsy Poindexter, Sukze Hwangbo, Mahmood Farshid, Joe Wilczek and Trevor Pendley.

And I think the best thing this group did was to call Steve Wagner and Ed Snyder to help us plan and organize this. Those two guys really were invaluable to us.

So, with that, I'd like to close. And thank you all very much for attending and participating.

[Applause.]

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[Whereupon, at 4:35 p.m., the proceedings were adjourned.]

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