今、多くのスタディがそうであるようにSPRINT studyも答えよりも疑問を多く提起することとなった。そのいくつかは私がここに整理した。たとえば、そのひとつ、なぜARDSの有害事象はPhase IやPhase IIの段階で出てこなかったのだろうか。これに対する答えはわからない。しかし、初期のスタディとSPRINTのPhase III臨床試験とでは異なる点がいくつかある。たとえば、Phase II臨床試験のサイズは小さい。20から24人のボランティアに対して、投与した製剤の量もわずかである。ボランティアは当然健常人であり、ARDSは特定の臨床状態の場合にのみ起こるのかもしれない。最後に動物を使った毒性試験も健康な動物においてのみ行われるのであって、特定の臨床状態を反映しないという同様の問題点を持っている。これらの観察から起きてくる別の質問としては、血液疾患患者に不活化血小板を使用した場合に多くARDSが発症したことに対する説明可能な妥当な機序は何かということである。それはおそらく活性化された血小板と肺に存在する好中球によるARDS発症機序である。この機序についてはDr.Kueblerによって報告されており、その論文で彼は炎症性肺疾患におけるセレクチンと血小板の役割に注目している。この論文の中でどのように血小板が好中球を引き寄せて血管内皮細胞に繋ぎ止めているのか、また特に活性化した血小板はP-セレクチンを放出し、肺の中の好中球をトラップすることで炎症性様の反応を引き起こし、急性呼吸器障害やARDSのような臨床的な病態へと誘導するとされている。不活化処理された血小板が実際そのように働き、活性化した血小板の代わりをして好中球の蓄積と似たような状況へ誘導するのか興味深い。次の質問は不活化血小板が肺の炎症性疾患に関与するかどうかを評価するための動物モデルがあるかということであるが、そのような動物モデルは存在する。これらの動物モデルのひとつでは酸誘導性の急性肺傷害を引き起こし、この傷害は血小板を除くことによって阻止することができる。従ってこのような実験を計画することは可能であろう。つまり血小板を不活化血小板に置き換えた場合、肺において不活化血小板が好中球凝集を助けて蓄積させることができるかどうかを調べれば良い。ではこれらの知見により我々は不活化をどのように前進させることが出来るだろうか？その議論のためにいくつかの可能な選択肢ある。まず、はじめに

臨床試験を繰り返し、有害事象についてよりよい焦点を持てるかどうか、特にオリジナルのスタディで我々が見つけたことについて確認する。スタディは前方視的、無作為比較によるブラインド試験でなくてはならない。それから・・血小板の数を合わせることがひとつの条件である。有害事象、特に肺炎、ARDS、意識消失、低カルシウム血症といったGrade3や4のものをモニターしなくてはならない。そしてスタディのサイズは比較可能なもので、これらの有害事象を見落とさないようにしなくてはならない。

考慮の必要があるかもしれない別の選択肢は既存の臨床データの利用である。欧州からバイオビジランスネットワークまで利用可能なデータがあると言われている。そこでこれらのデータを利用するためには呼吸器系への有害事象を積極的に拾い上げるための適切な感受性を持つ必要があり、受動的なサーベイランスでは不十分であろう。こういった特殊な製剤における有害事象にすぐに気付くためには、スタディ（臨床試験で）は普通の血小板のコントロールを置く必要がある。そして最後に更なる選択肢として安全性のデータを把握するためのヨーロッパの既存の輸血データを使ったサーベイランスを作ることが挙げられる。それは今回我々が臨床試験の中で見た有害事象に関して重要な意味を持つだろう。

そこで血液製剤の不活化に対する現段階の我々の評価における考え方をまとめる。まずはじめに輸血後感染症のリスクを同定することであり、既に述べたように敗血症や感染症発生率を追跡することでこれは可能である。次に臨床試験による製剤の安全性と効果の評価を行うことと、有害事象発生率を定量的に収集して輸血感染症の発生率とを比較することである。その比較が好ましいものであれば不活化血小板を使用することを承認することができる。しかし、その処置と血小板に対する傷害に関する問題があれば、たとえば、予防的介入の代わりに治療的な介入とするなど、製剤の使用に制限を設けなくてはならないかもしれない。
最後にリスク対ベネフィット比が満足の行かないものであるとしたら、輸血後感染症のリスクが増大した場合や伝染病が蔓延した場合といった状況の時のみ不活化製剤の承認を考えるべきである。以上が不活化に関する我々の考えである。
有難うございました。

Dr. J. Vostal (FDA-CBER. Chief of Laboratory and Cellular Hematology in the division of hematology, the Office of Blood Research and Review)

So, this has to balance out against the risks that could come as a result of application of these processes to transfusion products, and these risks could include damage to the transfusion products, adverse events to the recipients of such products, also toxicity to processing personnel, because those people actually could come into contact with very high concentrations of the chemicals, and also the toxicity to the environment because if those chemicals are mutagenic or potentially carcinogenic there may be an issue about their disposal.

And, here you can see that the benefits are, the target for pathogen reduction is, the reduction of viruses, bacteria, and parasites, and especially the potential reduction of

emerging and unknown pathogens.

So, to think about what the benefits are, I have to review the data that was presented earlier by Dr. Dodd, and this is for the current risk from bacteria in transfusion products and this is very nicely documented in this paper published by the American Red Cross and Dr. Eder, and this is a very exciting study because it has such a large number of products tested, and it pretty much single handedly defines the contamination rate of untested products to be about 1 in 5,000 and also defines the septic transfusion rate at 1 in 75,000. So, this is for products that were actually tested and determined to be negative. And for fatalities the risk is 1 in 500,000.

Now, after a collection of this data, the American Red Cross reviewed their collection and testing procedures and found places to optimize it even more, and they think that by applying their diversion strategies and increasing the sampling volume for bacterial testing, they can reduce their septic rate by 70 percent, 75 percent, which could bring it down to 16 one in the 1 to 300,000 range.

So, the current level of transfusion product safety is achieved by testing and prevention. And testing has a very good risk-to-benefit ratio. It's performed on a sample of the product, testing does not damage the transfusion products, it does not present a toxicity risk to the patient because nothing is added to the transfusion product, and overall testing has made the blood supply very safe. So, the risk-benefit analysis is very favorable, and if you look at our little teeter-totter, the benefits significantly outweighs any type of risk that may be associated with testing.

Now, if you try to apply this type of an analysis to chemical or photochemical pathogen reduction, we put on this side benefits, and we have the target, and the target would be a reduction of the current viral risk, which is 1 to 150,000 and a reduction of bacterial septic risk, which is at 1 to 75,000. So, in order not to shift the risk from transfusion transmitted disease to some other adverse event, this side of the teeter-totter should be somewhere around also 1 to 75,000.

And, this is a relatively tall order because this next slide shows you the size of a study that will be required to assure that you're eliminating a risk of 1 to 75,000. And the size of that study to achieve 95 percent upper confidence limit would be over 200,000 patients. So, it's not likely that any sponsor or company will be able to achieve a

study of this size up front. So, more likely you're going to be able to see studies in the hundreds patient range. And, so, the strategy has been to conduct studies that will look at efficacy in some adverse events and hope that if the study does not demonstrate any adverse events, then it could be approved and sizes of this type of a population could be achieved by doing a postmarket study. So, what are our concerns about novel pathogen reduction methods? The pathogen reduction process creates a novel mixture of chemicals and biologic products that is infused intravenously to a wide range of patients of different ages and condition states of health. So, the concerns are that the pathogen reduction chemicals interact with nucleic acids, they are frequently mutagenic and frequently carcinogenic, and may require a long-term postmarket study to determine if there is a risk associated with carcinogenesis. An additional concern is the application of light energy which can damage cells and can certainly damage the products themselves, and then the chemicals are nonspecific in that they can also bind, once activated, to proteins, lipid and cell organelles. So, the damage or the potential damage caused by these chemicals can be widespread and may be difficult to detect with the current testing strategies that we have.

So, the strategies that we have for approval of products such as these is to go through the classical FDA pathway, and as we go through phase one study, starting with phase one in vitro study, and these study identify gross lesions to cell biochemistry, to cell morphology. In addition to that phase one you would have animal studies to evaluate toxicity, and earlier today and yesterday we heard about the pathogen reduction chemicals that have been tested, that have gone through this in vitro study process, and actually they are found to be relatively safe based on the outcomes of these studies. Because they had a

relatively safe profile, they progressed through to phase two clinical trials, which included radiolabeling studies in human volunteers to define the transfusion product kinetics. And, some of these studies actually indicated that there is a loss of the ability to circulate and decreased recovery in healthy human volunteers. That by itself does not actually indicate whether there's any additional loss of functional efficacy.

So, the next step after phase two study is to progress through phase three clinical studies, which specifically assess efficacy, will define a transfusion frequency of these transfusion products and identify any adverse events on toxicity associated with application of these products to a specific patient population. Then if the phase three clinical trial works out and the product gets approved and gets on the market, then to identify and follow any type of very low frequency adverse events in toxicity, phase four studies would need to be put in place so we could monitor the performance of these products.

Now, I wanted to talk about the Cerus S-59 treated apheresis platelets because this is the product gone the furthest along this development pathway and I think we can learn something from what we've seen out of the outcome of their phase three clinical study. So that as we heard earlier this study done by Cerus was called the SPRINT trial, and we heard a description of it earlier today, and it was a phase three randomized, controlled, double blind, noninferiority study. The objective of the study was to compare safety in hemostatic efficacy of photochemically treated platelets to conventional platelets. And the primarily endpoint of this study was the proportion of patients with grade two bleeding assessed by a standardized WHO scale. What I'm going to present to you are tables taken directly from this report. And, you

can see this table five here talks about proportion of platelets with grade two or higher bleeding, which was the specific primary endpoint. This was quite a large study, had 318 patients in the treated arm and 327 patients in the control arm. If you look at any grade two bleeding, both of these studies are equivalent to the proportion of patients that had a grade two bleeding. So, from that viewpoint the study was successful.

Now, the sponsors also broke out the bleeding by different bleeding sites. The only thing I would like to point out here is that in the mucocutaneous bleeding -- that's bleeding that's known to be dependent on the level of platelets or function of platelets -- it's not a statistical difference but 18 there's a trend toward being increased mucocutaneous bleeding in the treatment arm. Now, if you look at, the other thing I would like to point out to you, there's also a difference between bleeding in the respiratory organs, slightly higher, not statistically significant, but I think it's something that we should keep in mind because it may come up a little bit later. So, here's table six from the same paper, and this table looks at the platelet and red cell transfusion used during the study. If you look at the platelet transfusion, the total number of transfusions, platelet transfusion in the treatment arm was 2,678 as compared to 2,041, so, about a 30 percent increased use of platelets to support these patients; this is four patients with hematologic malignancies. Now, if you look at, you know, where did that number come from? You look at the mean number of transfusions per patients, that's higher, 8.4 versus 6.2. If you look at the mean interval between transfusions, as the shorter interval, it's 1.9 versus 2.4 days. You can also look at the dose that these patients received, and this may be part of the problem that the processing of the

platelets during the pathogen reduction treatment uses up some of the platelets and so the dose that's actually going into the patients is lower than in the control arm. You can see also here that the percentage of doses that were less than three times ten to the eleventh, which is the standard platelet dose, the percentage in the treatment arm is 20 percent of the patients received less than a standard dose versus 12 percent of the patients in the control arm. The additional thing that should be pointed out is the use of red cells in this trial, and although it's not statistically different, there's a trend toward a higher use of red cells in the arm that's fully supported by the pathogen-reduced platelets, about a half the a unit difference between a treatment arm and control arm. So, on table seven in this paper, the authors summarized the platelet responses following platelet transfusions. And here we're looking at the platelet count and you can see the starting platelet count in those patients was equivalent between a control and a test arm. And if you look at the one-hour posttransfusion, the platelet count in the treatment arm is about 37,000 versus about 50,000¥in the control arm, so already a significant decrease. If you look at specifically the platelet increment, you're going from 34 in the control arm to about 21,000 in the treatment arm, and if you look at the count increment, you also see a decrease. And the same results or same trend is observed in the 24-hour CCI or that 24-hour evaluation, and you can see there's significant differences in the platelet count, in the count increment and also in the CCI. So, based on these results it appeared that the patients are receiving the treatment, a treated product could have been underdosed with a platelet product. Table eight from this paper talks about refractoriness to platelet transfusions, and refractoriness in this study was defined as two

episodes, two consecutive platelet transfusions with a one-hour CCI count of less than 5,000. And, the treatment arm, you can compare the treatment arm to any refractory episode that was examined. It was 21 percent in the treatment arm versus 7 percent in the control arm. The following line would be that any transfusion with CCI less than 5,000, we have a 27 percent versus 12 percent in the control arm. So, it appears that there's significantly more refractory patients that are transfused by the treated platelet.

Now, the interesting thing in this observation are these, if you look at immunologic refractoriness, there is actually no difference between the treatment arm and the control arm so the refractoriness that we see, the overall refractoriness is probably due to cell damage and not necessarily due to an immunological alteration.

So, this slide summarizes the results of the hemostatic effectiveness from the SPRINT clinical trial. The trial itself met the primarily endpoint of proportion of patients with grade two bleeding. However, it failed a number of other indicators of platelet efficacy, for example, it increased platelet utilization by 30 percent, it decreased the time between transfusions, decreased posttransfusion platelet count response, increased the number of platelet refractory patients and also increased a trend towards a higher red blood cell usage.

So, if you take all these together, they could reflect some potential adverse effects. For example, if you have increased usage of transfusion products, you could be mediating an increased frequency of transfusion-transmitted diseases, particularly if you are looking at red blood cells that have not been treated by this product. And also the 30 percent increase in platelet use and the increase in red blood cell use may eventually have a negative impact on the blood supply.

Now, this study was published in several papers. The one I just went over looked at the efficacy of the platelets.

The second paper that came out looked at the safety of these products in the same trial, so this is looking at the adverse events in the SPRINT trial published by Dr. Snyder and colleagues and was published in Transfusion in 2005.

Now, once again I'm just going to highlight some of the tables that are published in this paper. And, I think the most telling one is table five, which summarizes the adverse events that are different between the treatment groups and these are statistically significant differences between the treatment group and the control arm of the study. And you can see there's actually 11 cases or 11 types of adverse events that were statistically different between the treatment and the control arm.

In each case the difference went against the treatment arm. And, so, we have increased number of petechiae, increased fecal occult blood positive, increased dermatitis, increased rash, pleuritic pain, muscle cramps, pneumonitis, mucosal hemorrhage and acute respiratory distress syndrome.

So, out of these adverse events there were also events that were graded as grade three or four so that means clinically significant, clinically serious, and these four adverse events were hypocalcemia, syncope, pneumonitis and again acute respiratory distress syndrome. It's interesting to point out that in the control arm these significant adverse events actually don't show up. For example, for ARDS there's 5 cases out of 318 patients of ARDS and none in the control arm. Also, if you look at syncope, you have 6 cases in the treatment arm and no cases in the control arm. In hypocalcemia, over 20 cases in the treatment arm and only 6 in the control arm.

So, in this paper the sponsor actually claimed that there may have been an issue in identifying ARDS in some of the patients that were coded as having ARDS and so they went back and reanalyzed the data with a blinded group of experts to see if they could come up with different results. And those experts looked at a number of different respiratory events but in the end, after the reanalysis, the ARDS was still present with 12 cases in the treatment arm and 5 cases in the control arm, a loss of statistical significance that we saw initially but the issue of ARDS or some kind of acute lung problem did not go away.

So, here's a summary of the SPRINT adverse events data. This is actually a typo. It should be nine types of adverse events significantly different between the treatment and the control platelets, and they all went against the treatment platelets. Four types of these adverse events are clinical grade three and four and the organ systems involved here are the respiratory, cardiovascular system, dermatologic system and the parathyroid-renal system possibly based on the hypocalcemia.

So, if you look at the risks that could be associated with the use of these platelets, it appears that 1 in about 60 patients supported by treated platelets could have grade three or grade four adverse events. So, if you put this on the teeter-totter, you have on this side the risks, documented risks from a prospective blinded clinical trial of 1 per 60 adverse events and you're stacked up against trying to reduce a risk of 1 in 150,000 or 1 in 75,000. So, based on this type of analysis, it's difficult to see how this type of risk would be able to justify general use of these products to offset a bacterial and viral risk Now, one of the important concepts in pathogen reduction is the ability or the potential to prevent unknown and emerging pathogen transfusion-transmitted diseases. And pathogen reduction may have a favorable risk-

to-benefit ratio if the pathogen is widespread and has a high mortality rate. There may be populations that more susceptible to the new or actually current pathogen, and pathogen reduction chemical risk may be offset in this type of a group. However, the use of pathogen reduction products in the general population in anticipation of having an unknown pathogen occur years from now is not justified by the current risk-benefit profile.

Now, as many studies do, the SPRINT study actually generated more questions than it answered. Some of these questions I'm going to sort of try to go through right here. For example, one question can be, why did the ARDS adverse events not show up in the phase one or phase two testing? Well, the answer to this is not really clear. But, there are differences between the earlier studies and the phase three SPRINT clinical trial. For example, the phase two clinical studies were small. They only used 20 to 24 volunteers and only used a small volume of treated cells that were infused into these volunteers. The volunteers were healthy and ARDS may develop only in a specific clinical situation. Finally, the animal toxicity studies were also done only in healthy animals so the specific clinical situation may not have been reproduced in those types of animals. Another question that could come up from these observations is, is there a plausible mechanism that can explain why ARDS developed with the treated platelets transfused into highly complex hematology patients? And the answer here is possibly yes. There is a plausible mechanism that involves activated platelets and a recruitment of neutrophils to lungs. And this plausible mechanism, that was published by Dr. Kuebler, in a summary that looked at selectins and the emerging role of platelets in inflammatory lung disease. And this body of literature talked about how platelets can actually recruit and

tether neutrophils to endothelial cells and in particular in activated platelets they're expressing P-selectin and with trapping of these neutrophils in the lungs may set up an inflammatory-type response and lead to clinical situations such as acute lung injury and ARDS. So, it would be interesting to see if pathogen treated platelets could actually play a role or replace these activated platelets and also lead to the similar type of neutraphil accumulation.

So, the next question could be, are there animal models to evaluate whether treated platelets can participate in lung inflammatory disease? And the answer is yes, there are animal models that can be used. One of these animal models talks about acid-induced acute lung injury, and this injury can be blocked by removing the platelets, so it would be possible to set up an experiment like this. This is done where you could replace protein platelets with treated platelets to see if those treated plates could support neutraphil aggregation and accumulation in the lungs.

So, with these observations how can we move forward with pathogen reduction? Well, there are several options available for discussion. First of all, we would repeat the clinical trial and see if we can have a better focus on adverse events, particularly the ones that we saw in the original study. The study should be prospective, randomized, blinded, with an active control. It should have a -- well, this is up to discussion but one aspect would be to adjust the dose of treated platelets to be equivalent to the conventional platelets. The trial should actively monitor adverse events, particularly the ones that were grade three and grade four, such as pneumonitis, ARDS and syncope and hypocalcemia. And the size of the study should be comparable to the original study so we don't lose out any sensitivity to detect those adverse events.

Another option that could be discussed is to utilize existing clinical data. There is data that we heard about that's available from Europe through the

biovigilance networks. Now, to be able to use this data we'll need to have adequate sensitivity to detect respiratory adverse events and passive surveillance may not be sufficient to be able to do this. And, in order to be able to discern the adverse events that are specific for these types of products, those studies should have a control arm of conventional platelets.

And finally there's an additional option, that is to design an active surveillance using existing transfusion data from Europe to capture appropriate safety data. That will be relevant to the observed adverse events that we saw in the clinical trial.

So, to summarize our current thinking on evaluation of pathogen reduction for transfusion products, the initial step would be to identify the transfusion-transmitted disease risk, and this can be done, as we talked about, by following septic rates or transmission rates. Then the next step would be to evaluate transfusion product safety and efficacy with preclinical and clinical trials and to get a

quantitation on the adverse event rate and then do a comparison between the adverse event rate and the transfusion-transmitted risk. If the comparison is favorable, we would be able to approve the PR-treated platelets for use; however, if there are problems with the treatment and some injury to the platelets, there may be a limitation to the use of those products, for example, they may be used only for therapeutic interventions instead of prophylactic interventions.

And, finally, if the risk-benefit is not favorable you can consider approval of these products only for situations where the transfusion-

transmitted disease risk goes up, and this could be in situations with an emerging pathogen epidemic. So those are our thoughts about pathogen reduction and I thank you for your attention.