DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

WORKSHOP ON FACTOR VIII INHIBITORS

Friday, November 21, 2003

8:00 a.m.

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C O N T E N T S

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C O N T E N T S (Continued)

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P R O C E E D I N G S

DR. LOZIER: I would like to welcome you to the FDA International Association of Biologicals Workshop on Factor VIII inhibitors. My name is Jay Lozier. I am the Chairman of the Organizing Committee for this event.

Before we start the program, I need to go through some housekeeping announcements. First of all, you would all have a registration package of written materials with speaker slides and handouts. Be sure you get the extra handouts that were made available at the last minute which should have been given to you at the front desk.

The registration handouts include an evaluation form that we would like for you to turn in at the end of the day to help us with the design of future workshops. We will have a transcript of the proceeding available about fifteen working days after the meeting and you will find sheet in your handouts with the web page and the address for obtaining that.

There will also be a videotape available from FTC Reports for a price yet to be determined, which I am given to believe is on the order of $500 to $700. So

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that will be available later than the transcript, obviously.

The rest rooms are in the lobby of the Lister Hill Center. When you go in and out of the auditorium, please use the back or side exits. Please turn pagers and cell phones to silent ring or vibrate mode as a courtesy to the rest of us. There is a message center at 301 496-4062 that you can use to relay messages.

Lunch, we presume, will be at noon if we keep on schedule in the cafeteria that is down the stairs in the lobby. There is some renovation there and it may advisable--some people may want to go to the Natcher Building which has a larger cafeteria which is just essentially across the parking lot. I think if you follow the NIH regulars, you will be able to find that.

We have breaks scheduled in the middle of the morning session, in the middle of the afternoon session. Please come back from those promptly to keep our program on time.

Finally, I would like to thank our speakers for their hard work in preparing their talks and getting the material to us. Some of them come quite a long way, as

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you will see, and I think you will appreciate their hard work.

At this time, I would like to introduce Dr. Jay Epstein, the Director of the Office of Blood Research and Review in the FDA Center for Biologics. He will make introductory remarks to start the conference.

Introductory Remarks

DR. EPSTEIN: Thank you very much, Jay. It is my pleasure to welcome everyone to this workshop Factor VIII inhibitors. Before we start, though, I would like to acknowledge the hard work of the Planning Committee and, in particular, Jay Lozier who was the Chair. I think that their effective planning is reflected in the excellent turnout that we have and I am very pleased to see the luminaries of the field here among us.

I just want to acknowledge Mark Weinstein, Andrew Chang, Nisha Jain, Anthony Miraslus who put the program together and Joe Wilczek who has provided administrative support.

Let me also note that this is a co-sponsored workshop with the IABS and that we are very pleased to host this workshop as part of a continuing effort to

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assure the safety, efficacy and quality of the products that we regulate.

Today, with HIV and other viral contaminants under control, inhibitor formation presents itself as the chief adverse event associated with the use of antihemophilic factor. Therefore, the central question at this time, with respect to safety, is how can we ensure that new Factor VIII products or products that have undergone significant manufacturing changes won't induce inhibitor formation in previously treated patients.

Therefore, to reduce the occurrence of inhibitor formation, we need to understand to what extent immunogenicity is a property of the products rather than a phenomenon intrinsic to the patients.

The answer to this scientific question will lie in conducting appropriate preclinical studies, clinical studies, and postmarket surveillance. Today, at this workshop, we will devote most of our time to defining what are appropriate means in regard to preclinical trials inhibitor assays and surveys in patient populations.

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Our specific objectives, then, will be to improve clinical-trial design, to review the available data on the prevalence and the incidence of inhibitor formation, to examine the limitations and the potential of assays for Factor VIII inhibitors, to attempt to foster international harmonization in this area and to explore the future directions that are feasible and collaborations that could emerge.

I would like to just quickly review the structure of the program for you. The scientific program will begin with an overview of Factor VIII inhibitors and the historical context, particularly noting the Dutch and the Belgian experience with inhibitor formation to marketed products.

Next, we will be examining environmental and genetic factors that may influence antibody formation. Can we separate inhibitor formation that is caused by neoantigens in the products from inhibitor formation that is due to the genetic makeup or environmental circumstances of the patient?

Following this presentation, we will hear about the potential of preclinical studies to predict inhibitor

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formation. What role, if any, can animals play in predicting inhibitor formation?

One of the major dilemmas that we face in evaluating clinical trials is deciding what should be counted as a positive inhibitor response. This depends, in part, on the sensitivity, specificity and reproducibility of the assay used to detect the inhibitor. Two of our four speakers will address these topics as well as the results from a surveillance study and the development of new assays at increased sensitivity.

I think most of you are aware that the International Society on Thrombosis and Hemostasis has recommended that previously treated patients be studied first when conducting clinical trials of inhibitor formation. We will be hearing about the rationale for this recommendation as well as ISTH recommendations about what should constitute a positive and what should constitute a high-titer result.

The next two speakers will describe surveys of Factor VIII inhibitor formation in Canada and in the United States. The results of these studies should give

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us insight into the prevalence in inhibitor formation and provide a baseline for expected inhibitor formation when switching patients from one product to another product.

We then get a lunch break. After lunch, we will focus more on the regulatory aspects of inhibitor formation. Colleagues from the U.S. Food and Drug Administration and the European EMEA will discuss their agencies' current thinking about preclinical testing, clinical trials and postmarket surveillance for inhibitor formation.

These discussions will help us to better understand the rationale behind our respective policies and thereby help us to move toward greater international harmonization. We will further examine the FDA rationale for clinical-trial design with a presentation on statistical considerations that influence the number of patients required for a given trial.

Then, after a report on the role of the Data Safety Monitoring Board in clinical trials, we will hear in-depth presentations from industry representatives about specific clinical trials that they have conducted. These reports should allow us to compare the various

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trials with respect to patient numbers, patient exclusion criteria, the assays used and threshold levels considered as positive and the results of postmarket surveillance.

The last part of the meeting will focus on future directions. We will discuss preliminary ideas on prospective international collaborative studies on product-related Factor VIII inhibitor formation. This presentation will be followed by an open panel discussion where FDA staff and audience members will be able to pose questions to the speakers.

I should note that a brief question period will also be available following most of the talks.

So, before we begin our scientific program, it is my great pleasure to introduce Mrs. Laurie Shumway who is the mother of a son with hemophilia and a Factor VIII inhibitor. Mrs. Shumway has graciously volunteered to give us the personal perspective on coping with an inhibitor in addition to hemophilia.

It is my pleasure to invite up Mrs. Shumway. Thank you.

(Applause.)

Consumer Perspective

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MRS. SHUMWAY: Thanks, Jay.

Good morning. My son, Scott, is a 14-year-old high-school freshman with severe Factor VIII deficiency and he has an inversion. When he was two-years old, we discovered that he had a high titer inhibitor. We had no family history of hemophilia so we had very limited knowledge of it and no practical experience at all.

However, through our hematologist, our local chapter and the treatment center here in D.C., we had a huge body of knowledge and experience that we could draw on and we learned a lot in those first months and years. Hemophilia really did seem manageable.

But once we discovered the inhibitor, it suddenly didn't seem so manageable anymore. We didn't know whether we would be able to treat Scott effectively. Scott was diagnosed with a bleeding disorder on a Saturday in January, 1989, when he was eight-days old. You know, it is always on a weekend. The hospital we were at was able to diagnose that he had bleeding disorder but they couldn't, until Monday came, tell us what type of bleeding disorder he had.

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So they treated him with fresh-frozen plasma and that did stop the bleeding. In the next few weeks, we discussed possible treatment options and their pluses and minuses. Since recombinant factor was sort of on the horizon at that time, we decided that we were going to treat our son with donor-directed cryoprecipitate.

Cryo was an effective treatment for Scott in the first two years of his life. During that time, we really didn't perceive any changes in how he responded to treatment. So the discovery of an inhibitor in 1991 was a real surprise. His inhibitor was 280 Bethesda units. So the first serious issue we faced was how were we going to treat Scott in the presence of such a high-titer inhibitor.

It is really startling when you realize that your son may not be able to be treated effectively. Porcine Factor VIII might work but what happens if the bleeding doesn't stop or he needs treatment again? For the day-to-day bleeds that he might have experienced, our treatment choices were basically Factor IX products that really, at that time, were not considered as safe as the monoclonal Factor VIII products that we had rejected two

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years earlier. So that was a really difficult thing for us to sort of come to terms with.

From 1991 until 1995, we were forced to treat Scott with Factor IX products. They didn't work particularly effectively. When Scott would have a bleed, we would often have to treat him several days in a row, immobilize the joint, and we used a lot of ice. During that period, our refrigerator quit and we bought a brand-new refrigerator with an ice crusher. I still say it is the best investment we ever made.

We also became pretty adept at managing pain. Scott spent many nights sleeping in a bean-bag chair because it was really the only way we could support a bleeding knee so that he would be comfortable enough to sleep. It was really frustrating not to be able to stop a bleed.

We knew that immune tolerance was out there and that it offered a way to deal with the inhibitor. But, back in the early '90's, there really wasn't a lot of information on it or experience with the various protocols. Because Scott had a high-titer inhibitor, we were advised, at that time, that we should wait until it

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was lower before we started immune tolerance. We might not get that same advice today.

Also, we knew that we would need to place a central line for venous access before we started such a program. There was a precipitating event that really pushed us into immune tolerance and to try to improve venous access. I shut his arm in the van door. So we treated him but his forearm continued to swell. You know, he had tingly fingers or couldn't quite feel them. And then we were--after two or three days, we really could not get access to his veins.

So off we went to the hospital where he was treated more aggressively with higher doses and more frequently than we had at home. But we were really worried about getting clotting where we didn't want it and knew that there was a danger of that. But we also knew there was a danger of really damaging his arm without treating.

So this event made us realize that we couldn't wait any longer. If Scott had a more serious bleed, we could have lost him, is the bottom line. In fact, during

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the time when Scott was young, there was a child in our chapter who had inhibitors who did die.

But the decision to begin immune-tolerance program was program was primarily left up to us. There were no definite answers as to when to start or which protocols had higher probabilities of success. In fact, as I said earlier, we were advised to wait until the inhibitor titer had come down.

Waiting had significant impacts for us. We couldn't treat Scott effectively for a four- or five-year period. Once we started immune tolerance, there was a higher cost associated with it. Scott was larger. He weighed more and required more factor.

We started the immune tolerance in 1993. His inhibitor had come down to 28 Bethesda units. We had a central line placed using porcine factor. It was a Broviac catheter which Scott had for nine years.

Scott hit his peak inhibitor titer two to three months after starting immune tolerance. His peak titer was about 3500 Bethesda units and he had an antiporcine inhibitor titer of about 1,000. So we had lost our

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ability to use porcine factor to treat any serious bleeds for a while.

What we did in immune tolerance was we treated Scott twice a day with fairly large doses of Factor VIII for about two years and then we treated him daily for another two-and-a-half years. During that time, we were getting ready to give up because we didn't seem to be making any progress.

While Scott's inhibitor titers had been unmeasurable or low for several years, his progress towards normal Factor VIII levels had sort of stagnated. Like, his one-hour post-infusion levels were, I think, around 30 percent. So we were thinking this is it, maybe; this is as good as we are going to do.

But a lucky conversation with some Scandinavian hematologists after a session on inhibitors at an annual NHF meeting caused us to continue. They had had a patient and they had kept going for six to seven years and had achieved near normal Factor VIII levels. So we decided, okay, let's stay the course. So we continued treating for another year and a half to treat him daily

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with factor. We did gradually see improvements in his Factor VIII recovery.

Since 1999, Scott has received factor every other day. We haven't been able to able to go to a three-day prophylaxis regimen. We tried it once, but, in the first months, on the second day without factor, every single time he had a bleed. So we went back to the every other day. That prevents virtually all bleeding. Maybe two or three times a year, we have something we need to treat but it is usually because he has had an injury or something.

You can imagine the cost that is associated with an immune-tolerance program. Scott's factor costs were over $1 million in several years. Both my husband and I work for the federal government so we have access to insurance that does not have a lifetime cap. We consider ourselves very lucky.

However, every year, the first time we get factor in January, we reach the catastrophic limit in our insurance and we owe the maximum out-of-pocket expense for the year, which is $4,000 or $5,000, depending on

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where they set that in a particular year. Then, after that, our insurance pays 100 percent of factors costs.

However, we still face some challenges from time to time in dealing with insurance. For example, we periodically take a look at the price we are paying for factor to try and keep the cost of Scott's treatment to a minimum. We are obtaining factor from a preferred provider with our insurance company but found another provider that had the same factor, the same product, for a significantly lower cost.

We switched providers and that meant we would save our insurance company tens of thousands of dollars. However, the new provider was not a preferred provider so, under our insurance company, there was a higher out-of-pocket expense that we needed to pay. So the insurance company wanted us to pay another $1,000 towards factor in that year even though we had talked to them about changing providers before we did it. So, after several phone calls and letters and stuff, they did, ultimately, waive that $1,000.

But, in closing, let me leave you with a few questions that we and Scott face. For instance, this

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past June, when we went in for his annual clinic visit, Scott's inhibitor titer was 0.5 Bethesda units but his recoveries were not quite as good as they had been.

When things change, we are always concerned about what does this mean. In this case, we haven't seen any change in his bleeding patterns or in his response to factor but, in the past year, he has grown five or six inches and put on twenty or thirty pounds and probably what happened is we just didn't increase his dosage sufficiently during the year.

While Scott is aware that he has hemophilia, its effects on him on minimal. He plays all kinds of sports and participates in other activities. How are we going to help him understand the importance of continuing regular infusions to keep that inhibitor at bay? What role will insurance issues and possible discrimination in employment play in determining what he does and where he lives?

How could changing products affect his inhibitor levels and recoveries? How will his inhibitors affect his ability to take advantage of a possible cure? Inhibitors really added more uncertainty to our lives.

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But, for us, after immune tolerance, hemophilia, once again, seems manageable.

That's it. Thanks.

(Applause.)

DR. LOZIER: Thank you very much for bringing us that perspective. I think it is an important one.

Overview of Factor VIII Inhibitors

DR. LOZIER: At this time, I would like to provide a succinct, I hope, overview of something that took two-and-a-half days, I guess, to present in its entirety in Chapel Hill, recently, and that is an overview of Factor VIII inhibitors.

First, I will talk about the definition of inhibitors and their characteristics and briefly mention their frequency of occurrence and discuss the problem of inhibitors from the regulatory standpoint, then discuss the issue of how we assess the inhibitor risk in clinical trials and then just briefly explain the workshop agenda.

During the course of prophylaxis or treatment of hemophilia with Factor VIII concentrates, antibodies to Factor VIII can develop in patients with hemophilia A. These inhibitor antibodies will manifest themselves by

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neutralizing Factor VIII activity and/or accelerating the clearance of Factor VIII from the circulation.

The inhibitor-neutralizing ability is measured in vitro by assessing the Factor VIII activity after incubation of patient plasma with normal source of Factor VIII, usually plasma, the so-called Bethesda assay and all its variants. The in vivo assessment of Factor VIII inhibitors is the fall-off study where the elimination of infused Factor VIII from the circulation of a patient is measured over time.

Factor VIII inhibitors are interesting for many reasons but one of the interesting points is complement fixation and immune-complex disease and anaphylaxis are relatively rare in contrast to the Factor IX inhibitors. The Factor VIII inhibitors are typically IgG4 antibodies with specificities for Factor VIII epitopes that may, in fact, interfere with binding of von Willebrand factor, phospholipids, Factor IX or Factor X.

Recent evidence also shows that some of these inhibitor antibodies may catalyze proteolytic cleavage of Factor VIII.

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The inhibitor epitopes are typically clustered at the A2, A3, and C2 domains as well as the acidic animo acids that fall between the A1, A2, domains and the B domain and the A3 domain.

The antibody response to Factor VIII is characterized by the titer at any point in time of antibody as measured in vitro and also as measured dynamically in the patient by the nature of the anamnestic response. There are inhibitors that are high titer versus low titer and the anamnestic response, meaning the increase in the titer upon reexposure can be high or low.

Factor VIII inhibitor incidence depends on a number of patient factors, environmental factors and sometimes the Factor VIII product, itself. We have all read and written, in many cases, about the overall rate of Factor VIII inhibitor development being on the order of 20 percent. There is, though, quite a bit of variability in this data depending on the severity of the patient population that you are assessing, the frequency of the assessment for inhibitors will change data and the

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threshold for a positive inhibitor will certainly factor into the calculation of the incidence.

The greatest inhibitor incidence, as far as new inhibitor development, is in those patients who have no prior exposure to Factor VIII, the previously untreated patients or so-called PUPs. The lowest inhibitor incidence is in those patients who have previously been treated and not yet shown an inhibitor, the so-called PTPs.

As you can see from this summary slide from Earnest Briet's metaanalysis of seven prospective inhibitor studies in patients with severe hemophilia A and high-titer Factor VIII inhibitors, there is, when you normalize to a common starting point--and the follow-up here is in years as opposed to exposure days. But the point is, over the course of time, we will asymptotically approach the 20 percent inhibitor frequency in these patients.

Of course, the greatest rate of new development of inhibitors is in the early time frame, typically in the first year or two which would correspond typically, on a weekly-treatment basis, to about 75 exposure days.

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After five years, the new inhibitor development rate is much decreased, but there is still, even out late in the course of hemophilia, a small but finite incidence of new onset of inhibitors.

The patient factors that play into this are the severity of the hemophilia, the nature of the mutation--namely the inversions, deletions and nonsense mutations--have a greater incidence of inhibitors than missense mutations and small deletions. Presumably, this difference is a reflection of the issue of how much cross-reactive material that may tolerize the patient over time or the so-called CRM-positive status versus those who have no cross-reacting material.

Other genetic factors that may play into this are possibly HLA haplotypes, race. African-Americans may have a higher incidence of Factor VIII inhibitors than Caucasians. There may be cytokine and immune-response modifier genes that factor into this and I will be presenting some animal data suggesting that may be the case, at least in a mouse model, that I have developed. I will talking about that at the ASH meeting in San Diego in December.

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Comorbid disease states including infection, perhaps autoimmune conditions, pregnancy or malignancy, which are really more relevant more acquired inhibitors, not specifically our topic today, but these are factors that can influence the incidence of inhibitors in hemophilia.

Concomitant surgery or trauma, perhaps acting as an adjuvant, so to speak, for the immune system. There has been discussion of whether the infusion method or treatment intensity is a factor for influencing the rate inhibitor development.

The Factor VIII concentrates that we have available are those derived from plasma which we have had from the 1960s beginning with the low specific activity, cryoprecipitate, preparations which have usually more protein than units of Factor VIII. In the '60s, chromatographic and precipitation maneuvers were developed that gave intermediate purity, Factor VIII products that had on the order of 10 to 20 units per milligram of protein. Then, finally, the monoclonal-antibody purification process yielded the high-purity

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concentrates that typically have 2,000 or 3,000 units of Factor VIII per milligram of protein.

In the 1980s, recombinant product came along which were derived from fermentation of Factor VIII transduced cells and purified by monoclonal antibody or other affinity chromatography preparations or methods and also have the very high specific activity of 2,000 or 3,000 units per milligram protein.

The manufacturing process, which we are interested in, of course, can influence immunogenicity of Factor VIII and seemingly minor changes in virus inactivation procedures are associated with an outbreak of inhibitors in heavily treated patients using one particular product.

This is the Dutch-Belgian inhibitor epidemic alluded to earlier which 8 of 140 previously treated patients with extensive Factor VIII exposure with severe hemophilia A developed inhibitors in short order after use of a new plasma-derived Factor VIII concentrate that was solvent-detergent treated and heated at 63 degrees for ten hours.

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The patients demonstrated both low- and high-titer Factor VIII inhibitors that had complex inhibition kinetics and appeared to have specificity for the Factor VIII light chain. When this product was discontinued in these patients, the inhibitors gradually declined.

The problem we have at FDA and the other regulatory agencies that we communicate with is to evaluate the new Factor VIII products for safety, efficacy and potency and inhibitor antibodies now are the chief adverse event that we are concerned with since the virtual elimination of HIV and hepatitis risk.

The inhibitor risk assessment depends on the definition of what an inhibitor is, what is positive and what is negative, where is your cutoff. What is the significance of a transient inhibitor and how do we define and decide what the cutoff is between a high- and low-titer inhibitor?

We are interested in who should participate in trials and we are going to hear from Dr. White on the ISTH recommendations on studying PTPs preferentially in initial trials of new products. We want to know how clinical trials should be designed.

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With regard to clinical trials, we are interested in what size trial, how many arms we should be asking sponsors to bring to a licensure proceeding. In addition to how many arms, what are the appropriate comparators? Should we use historic controls? Should we compare with current products, with plasma products, or comparable recombinants? We will also hear from Dr. Aledort about the role of the Data Safety Monitoring Board this afternoon. We also need to know how, once we have this data, do we evaluate the clinical trials and to assess the inhibitor risk for new Factor VIII products and can this regulatory approach be harmonized between the FDA and the other worldwide authorities. We will certainly be hearing from Dr. Rainer Seitz on that topic this afternoon, to some extent.

We will be interested to know opinions of those in the audience about the role and the importance of postmarketing surveillance in the regulatory decision-making process.

The morning sessions will address definitions and laboratory issues and measurements of inhibitors and will discuss some of the clinical epidemiology from the

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United States and Canada. Our afternoon sessions will address the design of clinical trials including FDA and industry perspectives. Donna DiMichele will be a moderator of a panel discussion at the end of this conference and will be presenting, immediately before that, her thoughts on the possible role for postmarket surveillance.

So I would like to thank you and try to keep on time here. Immediately, I would like to introduce Dr. Joan Cox Gill of the Blood Center of Southeast Wisconsin. She has been a hemophilia care provider for many years and has done a lot of work on inhibitor and hemophilia research. She will be discussing the environmental and genetic factors that may influence inhibitor antibody formation.

(Applause.)

Joan?

Environmental and Genetic Factors

That May Influence Antibody Formation

DR. GILL: Thank you very much. I want to thank the sponsors for the privilege of presenting at this meeting this morning.

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I am going to talk to you about environmental and genetic factors that may influence inhibitor antibody formation. We all know that inhibitors impact the outcome of replacement therapy and also impact the assessment of efficacy and safety of new therapeutic replacement products and is likely to impact the outcome of gene therapy. Therefore, it would be highly desirable to predict risk for inhibitor development and to identify factors that predispose to inhibitor development.

What is the evidence for genetic factors playing a role in inhibitor development? First of all, just by natural history, we know that inhibitors develop early within a median of nine to 11 exposure days to replacement therapy. This suggests that there is a predisposition to inhibitors on the part of the patient's genetic makeup.

There has also been shown to be an increased inhibitor risk in African Americans in several studies that have been published. Finally, there are animal studies that show that the introduction of an out-bred female into a hemophilic dog colony in Canada resulted in progeny with inhibitors whereas other lines in that

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colony, the dogs did not develop inhibitors. Also, there is a differential development of inhibitors in hemophilic mouse strains.

We decided to try to address this issue by doing a survey of sib pairs across the United States and Canada. This slide just summarizes the number of families that were surveyed and the inhibitor incidence. So, as one would expect, 30 percent of the severe hemophilia-A patients, families, there was at least one family member with an inhibitor whereas in moderate hemophilia-A patients, it was lower. Again, as has been shown in many studies, the incidence, or prevalence, of inhibitors in hemophilia-B is much lower.

In this slide, I would like you to concentrate on these purple numbers. If one calculates the expected number of families in which there would be two patients affected with an inhibitor and the incidence of inhibitors is 15 percent, one would expect, by chance alone, five families.

If the inhibitor incidence is 20 percent, one would expect nine families. We observed a significantly higher number of families in which there was concordance

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of inhibitor development in 28 of the families and this was highly statistically significant.

This study was a confirmed study by Astermark in an international survey of sibling pairs of 460 families. Again, African Americans or blacks had a higher incidence of inhibitors than Caucasians. Concordant inhibitor families were higher than expected by chance alone. There was a much higher risk of an inhibitor if you have a positive family history of inhibitors, so about 48 percent would be expected to develop an inhibitor if their family members had an inhibitor.

What are some of the factors that might be influencing this increased risk of inhibitors in families? Well, first of all, the factor-mutation type has been shown to be in influence so the more severe mutations result in higher prevalence and incidence of inhibitors than the less severe mutations.

However, if one looks at intron-22 inversion mutation which accounts for about 40 percent of severe hemophilia only 20 percent of the patients affected by that mutation do develop an inhibitor. So this suggests that there must be other factors involved in inhibitor

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formation. Indeed, if one looks at all of the other mutations causing severe hemophilia A, one has a similar incidence of inhibitor development.

We then asked the question that, since each hemophilic member of a single family has the same Factor VIII mutation, if additional important genetic factors play a role, the risk of inhibitor development should be greater in the hemophilic siblings of an inhibitor patient than in the extended hemophilic relatives; that is, grandfathers, cousins, nephews, et cetera.

We looked at data from two studies. One was the inhibitor survey undertaken by the Hemophilia Research Society where we identified 113 inhibitor patients with severe hemophilia A and found that 41 percent of those families had one or more family members affected. There was a 52 percent risk of inhibitor development in your sibling had an inhibitor whereas only 11 percent inhibitor risk, if only your extended family members had an inhibitor and not your brother.

This was born out also in the sibling study I just mentioned where, again, we saw only 9 percent in extended family members. So, again, this suggests that

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there are other genetic factors important in inhibitor development other than the specific Factor VIII mutation in the family.

So what could some of these other genetic risk factors be? First of all, we know that T-cells are important in inhibitor formation. There is concomitant disappearance of inhibitor with loss of CD4 helper T-cells in patients who are infected with HIV.

We know that the IgG isotype is predominantly IgG4 and this is evidence for the TH2-like nature of the response; that is, the response requires T-cell help for B-cell differentiation and immunoglobulin isotype switching. In addition, tolerance to Factor VIII can be induced in inhibitor patients, again suggesting a role of T-cells and in animal studies tolerance induction has been shown by blocking accessory molecule interaction in some of the mouse models.

We know that the initiation of the immune response occurs when antigen-presenting cells present peptides in the context of MHC that are recognized by the T-cell receptor and, in order to have a proliferative response, one needs to have interaction of accessory

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cells that provide a second stimulus for proliferation and expansion of the immune response.

Then, depending upon the cytokine environment in which that response occurs, one can have antibody synthesis or we now know we can have suppression of antibody production and, if there is lack of secondary responses, then tolerance occurs.

One of the genetic variability factors that has been studied quite extensively is that of HLA. This is a fairly logical step to look at. We found that, in several studies, there was no significant difference in HLA type in patients who developed inhibitors or who didn't develop inhibitors and only weak associations were found when only patients with intron-22 inversions were studied.

So, in looking at the overall immune system, these are some candidate genes that may be important in inhibitor development. MHC, of course, as I have mentioned already, immunoglobulin genes, T-cell receptor genes, cytokine and cytokine receptor genes that have defined cell subsets and then accessory molecules.

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I think that there is a lot of suggestive evidence but, to date, we don't have any firm polymorphism or mutations that have been found that would substantiate this hypothesis. So we have a lot of work to do in this area.

So what are some of the factors that may predispose to inhibitor formation that are non-genetic or environmental? There has been a lot of work done to examine the type and purity of Factor VIII concentrate effect on inhibitor development. In a nice, systematic overview of studies over time by White published in Hemophilia, a number of these studies were examined.

This slide shows the weighted mean percent cumulative risk of all inhibitors and the weighted mean percent cumulative risk of high responder inhibitors in studies of patients who receive multiple low and intermediate purity plasma-derived concentrates, single plasma-derived concentrates and recombinant concentrates.

What we can see from an examination of these studies is that the overall cumulative risk is about the same, if one looks at plasma-derived concentrates, multiple plasma-derived concentrates versus recombinant

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concentrates. But, interestingly, a few studies, there was a much lower risk in people who had received single plasma-derived concentrates.

Now, these studies were very small and there were some older patients entered into these studies. So I think this is an intriguing observation that will probably not be studied further because we now are using primarily recombinant concentrates, or at least desire to use primarily recombinant concentrates because of their improved safety.

Because some of the older studies did not measure inhibitor titers as frequently as the newer studies and, thus, patients who have transient inhibitors can be missed by those studies. The incidence of high responders was also looked at. What was very interesting here is that cumulative risk for a high-responder inhibitor is actually lower in patients who receive recombinant clotting-factor concentrates versus those that were treated with multiple, low and intermediate purity plasma-derived concentrates.

I think this answers, or at least partially answers, an important question about use of recombinant

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clotting factors which we were concerned for a long time about an increased risk of inhibitor development using recombinant factor but, in fact, the high-responder inhibitors, which are the most clinically significant, actually had a lower risk.

What about differences in recombinant clotting-factor concentrates? Kogenate and Recombinate, as you know, are both full-length recombinant Factor VIII concentrates where as ReFacto is B-domain deleted. You can see from this summary slide that the cumulative risk is almost virtually identical. The median age of the patients treated in those previously untreated studies or PUP studies was virtually identical and the median exposure days to the time of inhibitor development was also almost identical, so suggesting that, at least in these three studies, again small studies, that deletion of the B domain does not have an effect on cumulative risk for inhibitor development.

What are some of the other factors that might predispose to inhibitor formation? The age at initial therapeutic exposure has, in two very small studies by the Swedes and I think it was Spanish groups, suggested

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that if the patient was exposed earlier, the patient would have an increased risk for inhibitor development.

This is counterintuitive where most of us, I think, were brought up to believe that if one received a foreign antigen early, it would be more likely that tolerance would develop. Well, those two small studies suggest that that is not true although I must say that they are both very small and especially the older patient groups in whom the inhibitor risk was lower, there were only four or five patients in that group. So I think we need to do more studies to further define that possibility.

In all of the PUP studies that have been done, the dose and frequency of initial therapeutic exposures has not seemed to play a role. There are some intriguing possibilities that, if a patient were exposed in utero via a maternal-fetal transfusion to maternal Factor VIII, perhaps that may be one of the factors that could explain the difference in inhibitor development among patients who have the same Factor VIII mutation. However, there is, to date, no evidence to suggest that that is true.

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There has been one study by, I think this was the Swedish group also, who examined the possibility that breast feeding might be protective in inhibitor development. There are homologous proteins in breast milk that have a significant amount of homology to Factor V and Factor VIII and therefore might be expected to provide some measure of protectiveness against inhibitor development if oral tolerance is a true phenomenon that might prevent inhibitor development. However, in that study, there was no evidence that breast-fed infants had a lower incidence of inhibitor development.

We also know that there are many concomitant illness--for example, HIV infection--that might predispose to a patient developing an inhibitor perhaps even later on in life. There have been also several anecdotal reports of patients who had serious infections who were treated with high-doses of a new product at the time of surgery who then developed an inhibitor even though they had been previously exposed to many, many doses of Factor VIII concentrate.

I think those kinds of studies to have a better definition of the risk of inhibitor development later on

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in life after many exposures to Factor VIII, those studies really need to be done and we need to have a better definition of that aspect of inhibitor development because, if we are going to attempt to determine whether or not there are neoantigens being formed with new products that are being developed, we need to know what the baseline natural history of inhibitor development is in these patients in whom we are now adding a new product.

So, if one looks at some of the PUP studies--I was asked to address the question as to what we can learn from PUP studies to apply to our design of studies for previously treated patients. As Dr. Lozier has already shown you, if one looks at the cumulative risk of inhibitor development in PUPs, the inhibitors develop early and then the inhibitor incidence levels off. However, there are some patients, as we mentioned, who do develop inhibitors later on in life.

So I think that, in addressing this question, first of all, we need more information about the long-term natural history of inhibitor incidence, either or just-appearing inhibitors, in patients who are treated

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with single products versus multiple products. We need careful follow up of patients during a switch to a new product and we need to evaluate the effect of illnesses and medications on inhibitor development during product changes so that we need to understand the pattern of inhibitor development in order to then evaluate whether or not a new product actually has neoantigens that provoke a new inhibitor response.

So, important variables that we need to look at to evaluate both PUP and PTP studies are hemophilia and mutational analyses, the ethnic background of patients, family history of inhibitors, a previous history of inhibitors, any concomitant immunologic disorders and medications and whether or not an anti-inflammatory disorder might occur at the time of exposure.

I think that there are several organizations in this country and in Europe that should be able to develop new studies that will help us better define the long-term natural history of inhibitor development in heavily treated patients as well as in previously untreated patients. I hope that this workshop will stimulate the development of some of those studies.

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Thank you very much.

(Applause.)

DR. LOZIER: Joe, why don't you go ahead and load Dr. Saint-Remy's talk.

We have time for a few questions. I forgot to mention if you wish to ask a question from the bench here in the front, you can actually activate your microphone by pushing a button at the base of the microphone. Those in the back who may have a question can go to either of the freestanding microphones in the back. We have time here for a question or two for Dr. Gill.

Dr. Golding?

DR. GOLDING: I don't have a microphone so I am going to shout. One of the issues with reducing an antibody response to an antigen, as you pointed out, requires T-cell help. Now, there have been several papers that have shown that there is such a thing as bystander help. In other words, if someone has an intercurrent infection or some other immune stimulus, you can get help to the B-cells without the actual, in this case, Factor VIII providing the help.

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When you look at the patients and the history, you see that, in very young people, they are getting inhibitors. So what I am wondering is how carefully people have looked at intercurrent infections, for example, in young children and the association that that may have development of inhibitors and whether very aggressive treatment of infections or avoidance, if possible, of treatment during times when there is acute infection could be helpful in this situation.

DR. GILL: I think that is a very important point. Unfortunately, we have not, to date, looked carefully at that question. I think, in all of the previous studies that have been published, that possibility has not been looked at and I think that that is something that we need to do.

We could possibly get some preliminary information from some of the PUP studies in which all adverse events were recorded during the study. But, as far as I know, no one has done that to date. But it is something that we certainly should do in the future.

DR. LOZIER: Dr. Chang of FDA has a question.

DR. CHANG: Joan, I enjoyed your talk very much.

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DR. GILL: Thank you.

DR. CHANG: One of your slides, you had a very good comparison on the product type and also the accumulated inhibitor formation versus high-titer patients. I assume that is on the PUPs patients. I was just wondering whether or not there is a systematic analysis on the PTP with a similar layout of the analysis.

DR. GILL: In that same paper, published by White in Hemophilia, there was a discussion of PTP patients as well but the conclusion was that there really wasn't enough definitive data to answer the question, which I think is why we need to do additional cooperative studies.

We are going to be hearing about some cooperative studies, I think, later on this morning with the CDC studies and I know that the Hemophilia and Thrombosis Research Society is interested in initiating some natural-history studies, too, so that we can get enough patients to begin to answer those questions.

DR. LOZIER: Thanks very much, Joan.

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We will go to our next speaker, Dr. Jean-Marie Saint-Remy of the Center for Molecular and Vascular Biology at the University of Leuven, Belgium. He has been a leading researcher in the field of Factor VIII inhibitors and was one of those who showed that certain Factor VIII inhibitors have proteolytic activity against the Factor VIII molecule.

He will provide his talk entitled What Can Preclinical Testing of Factor VIII concentrates tell us; a Cautionary Tale.

What Can Preclinical Testing of Factor VIII Concentrates Tell us; a Cautionary Tale

DR. SAINT-REMY: Good morning. Just to comply with the European habit of starting a talk by making apologies, I have two apologies to offer you. The first one that you should have the handout with old slides and I apologize. I just probably overlooked the mail asking me to send the slides. But you will get them later on. The second apology is that, as you probably already realize, I have a kind of flu-like illness. This is the true influenza coming from Australia. The funny thing

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about this is that I got it in Brussels from one of my patients living in Scotland. So the world is small.

I like the title I have given because this is really open and probably very much in the spirit of what we do in our lab. I would like to start by making just a couple of statements. The first one is that we might not speak the same language but I am trying to combine the scientific approach and the clinical approach because I have been educated as a M.D.

On the clinical perspective, preclinical testing of Factor VIII means that you wish to know in advance which of those Factor VIII concentrates are going to produce inhibitors. But, of course, inhibitors, in terms of immunological setup, comprehension, understanding, does not mean anything, just a case in point. The point is to evaluate whether a factor VIII concentrate increased risk of immunogenicity in general not only the 25 percent of those antibodies which might interfere with Factor VIII activity.

When I am saying evaluate the risk of increased immunogenicity, that is really what I mean. Factor VIII is a foreign protein for every hemophilia-A patient. It

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is going to be fully foreign if you no Factor VIII at all. It is going to be partially foreign if you have parts of your Factor VIII functional and not functional.

If you are in good health, well, you should consider Factor VIII as a foreign body and then make an immune response against Factor VIII. I would be very anxious not to see any kind of immune response against Factor VIII in healthy individuals with or without Factor VIII, as a matter of fact.

Everything has to be considered not in terms of whether or not you see antibodies, you see an immune response against Factor VIII, but whether or not the equilibrium in between immunity and tolerance is established or reestablished as soon as you come with Factor VIII.

You see that this is a very complicated slide. But basically the message is simple. You have a constant exposure to an antigen, Factor VIII in this case. This is going to trigger an immunity which is compensated by tolerance in the periphery. Nowadays, it is almost impossible to open a journal on immunology not to see a paper on a new mechanism of tolerance induction in the

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periphery. The new one is certainly to a version of the B-cell receptor which is a very interesting finding which I think has whole new consequences on the way we should look at anti-Factor-VIII antibodies.

We all, including hemophilia-A patients, have specific B and T-cells in the periphery with the capacity to make a full-blown immune response against Factor VIII with about 20 percent of those antibodies having inhibitory capacity.

The B-cells are produced, as you, I guess, know, continuously during your lifetime and you make each day 100 millions of new B-cells which have the capacity to respond to Factor VIII. On the other hand, the T-cell repertoire, at least in men, is almost entirely fixed at birth so we have a huge capacity there.

As soon as you have a good pair of B and T specific for the same antigen and the right conditions, which might be triggered by inflammation, for instance, you will stop making a full functional immune response against Factor VIII.

The other part I would like to stress which was already alluded to by one of the questions after the

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previous speaker is the fact that once you have been exposed to an antigen, again, if you are a healthy individual including, of course, hemophilia-A patients, you have got to memorize your immune response. It means that you have memory T-cells, memory B-cells. It is a kind of surveillance. They are there just to react whenever necessary.

If you expose, and this is certainly the case, Factor VIII on a regular basis to the same antigen, what the antigen is going to do is first, of course, to trigger the memory response and, in this case, the B-cell memory response against Factor VIII and trigger a new set of somatic mutation from the memory B-cell there from this new set of somatic permutations, you will get a new population of memory B-cells and then your population of plasmacytes which are going to be clone to the bone marrow and sit there probably for a few weeks to produce the high-affinity antibodies. You have to understand that each time you lose the Factor VIII in such a setup, you will, again, embark on this circle and create new memory B-cells and new plasmacytes.

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The point is to decide what happens exactly in Factor VIII. Basically, we don't know. It would be dishonest just to say that we know everything about the immune response against Factor VIII for many reasons. Perhaps, as someone said at a previous meeting, there are not many immunologists interested in this Factor VIII immune response. It might not be very reason.

The second reason by be that Factor VIII is a huge molecule. Whenever you work with mice, you like to use what we call circus antigen, antigen which are as small as possible you can define a single epitope. That is much, much easier.

Now, the knowledge we have acquired over the last let's say ten years about the way those B-cells, when they are activated by interaction with T-cells, what their fate is, is really tremendous. You have basically two pathways. The first one is not a classical one but which is terminal-center-independent which is going to generate B-cells with a short life but producing antibodies with the IgG4 isotype if it is programmed with no somatic mutation but with the capacity of cloning to expand. On the other hand, you have a size 8, a new run

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of memory B-cells and long-lived plasma cells which are going to sit in the bone marrow.

That is about it for the homeostasis for the Factor VIII immune response except that, on the other side, you have regulatory T-cells and anti-idiotypic B-cells. I am not going to say a word about anti-idiotypic B-cells but I would like just to show one slide about regulatory T-cells. This is certainly an emerging field and especially in the field of Factor VIII.

If I have to make kind of a guess for the forthcoming five years, we will learn a lot about the role of those regulatory T-cells in the immune response, the control of the immune of response against Factor VIII.

Basically, we distinguish now a not suppressive but regulatory T-cell which is characterized by the presence of CD25 receptor. This is the IL2 receptor. These cells were trained to the CD4 lineage and, more recently, the transcription-repressor factor, FoxP3, is now considered as the main characteristics of those T-cells.

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We know for a while, for almost ten years now, that the thymus is actively selecting some of those T-cells. They are hosted in the periphery and act there as a natural counterpart for immune response. But, on the other hand, and this is somewhat new and there is as paper last month in the Journal of Clinical Investigation showing interesting data in man that, indeed, even those CD4 T-cells without the two characteristic markers, whenever they are in the periphery exposed under specific conditions, they can just change themselves and acquire to those markers and become part of what we call the adaptive regulatory pathways which, of course, means, the message is there, that this is probably amenable to manipulation.

I would say that, perhaps, we speak much about the role of immunogenicity of Factor VIII as a molecule, per se. We should program another symposium on regulatory T-cells and the way the immune system could program down the immune response against Factor VIII.

Now, what about the animal model? I said and I promised to make a kind of cautionary tale, so I will try to keep on my promises. Optimal criteria for an animal

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model; well, you have to act with an animal where the genetic background is identical. Otherwise, you end up with consideration about extra MHG factors or whatever which could have, indeed, an influence on the capacity to mount an immune response against Factor VIII.

Ideally, the model should be deficient in the antigen, and I mean fully deficient, which is probably not the case right now with hemophilia in mice. You should, and this is maybe the main point, be in a possibility for evaluating the immune response at the clonal level, be it, at least in vitro but, if possible, also in vivo.

Of course, no or limited adjuvants because, as soon as you use adjuvants, like CFA or IFA, or even milder adjuvant, you distort the immune response one way or another.

What are the models available? Well, why not start with the kind of them which is wild type mouse. You have the hemophilia-A mouse, immunodeficient mice, the combination of the Factor VIII deficient, immunodeficient, and more recently the transgenic mouse strains.

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Now, why should I spend two slides on normal mice? Of course, in normal mice, the mice have Factor VIII so whatever you do with your human Factor VIII, you will skew the immune response against the determinants which make the difference between human and mouse Factor VIII.

In this case, the repertoire of those mice should be purged of all the T-cells recognizing not all the T-cell epitopes present on Factor VIII but all the immunodominant T-cell epitope on Factor VIII. This analysis is of very much importance.

The usefulness, I think, and has been over the years is to establish just a library of monoclonal antibodies. What for, you would say. Well, we learn a lot about the mechanism by which Factor VIII is inactivated. We learn a lot about how the structure and the function of Factor VIII are related and I think it is still a good tool to compare the antigenicity of factors.

I will give you an example of this. This is an old study, at least five years old, I think, just to illustrate the point. This is the heat-denatured Factor VIII. It is a plasma-derived Factor VIII which was

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heated as we normally do for factorization, 63 degrees centigrade. We use a mouse monoclonal antibody recognized in acidic A3 domain.

This is the type of activity in optical density on the vertical axis and, of course, increased amount of Factor VIII on the horizontal axis. You see that, as soon as you heat for one minute, at this temperature, you will lose quite a lot of the reactivity.

The message is that an antibody against the acidic A3 domain, which is, of course, a crucial part of Factor VIII not only for the binding of von Willebrand factor, you will lose a lot of reactivity with some of the monoclonal antibodies. This one is a conformation-dependent antibody against the acidic A3 domain.

So what about the knockouts? Everyone is picking the knockout mice. The knockout mice should be fully immunocompetent in terms of the immune response against Factor VIII. So the repertoire should not be skewed towards the non-specific for human Factor VIII.

I think it is interesting but with limitation first of all because it is not quite sure that the Factor VIII hemophilia-A mice which is now available, and there

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are basically two strains, are totally devoid of Factor VIII. To the contrary, the XLM17 knockout mice have been shown to have a part of the heavy chain of Factor VIII floating around which might have an influence on the way tolerance is induced against Factor VIII. We don't know for sure, but it might be the case.

So ideally speaking, in an ideal world, we still are expecting to have a fully Factor VIII mouse strain available. But, with this type of model, it is possible, I think, to directly compare the immunogenicity of different batches of Factor VIII and possibly also to evaluate the impact of added factors, like, of course, von Willebrand factor.

You probably remember this slide coming from a paper we published with a student from Frankfort working at that time in biotests.

AUDIENCE: What are the yellow and white on the graph.

DR. SAINT-REMY: Yes. I am going to explain it. This is Bethesda units. This is the influence of von Willebrand factor on the immunogenicity of Factor VIII concentrate. What we did is to take a population of

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hemophilia-A mice. They were injected with plasma-derived Factor VIII or recombinant Factor VIII or von Willebrand alone.

In some cases, plasma-derived Factor VIII No. 2 and the two recombinant Factor VIIIs--these were, of course, then deprived of any von Willebrand factor in the final formulation. This one is containing a kind of degraded form of von Willebrand factor.

So, what you see in the yellow bar is just the Bethesda units obtained in groups of those mice injected with product as it is commercially available. So, what we did is to add, in some cases, some von Willebrand factor just to see that, in this plasma-derived Factor VIII, additional fully functional von Willebrand factor would decrease by 50 percent at least the level of inhibitors against Factor VIII. The same for this recombinant Factor VIII here.

So the point is not to make kind of a lot of fuss about this but we think that we that we should look much more closely on the way von Willebrand factor is probably modulating the immunogenicity of Factor VIII.

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What I am saying is von Willebrand factor. It may be that other factors do play a role, too.

Immunodeficient strains; we have been playing a lot with SCID mice. SCID stands for severe combined immunodeficiency. It means that those mice have no functional B and T-cells. They are unable to mount an immune response against any protein, any cells and, of course, not against Factor VIII.

The downside of this is that, because of having no function B and T-cells, they have no lymphoid organs so it is impossible to mount a primary immune response in those mice which is basically a limitation. So, what you have to do is to reconstitute those mice with cells and they will accept cells because they are unable to reject them, and you can reconstitute groups of those mice with cells pertaining to different patients that are, of course, already primed cells, B and T-cells.

I think this is useful because you can still compare different Factor VIII preparations or different individuals together injected with the same Factor VIII preparation in a setup which is a mouse environment.

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I will just show you the way we do it. This is the donor. We have to take quite a lot of those cells, but this is not unlimited because, if you go too far, of course, the graft is going to reject the host. So the SCID mice are injected with IP, with a number of those PBMC. Then you inject either nothing or either Factor VIII.

Just to illustrate the point--this is not published--again, you have the inhibition of Factor VIII activity here. You see that when you take the plasma IgG from the patient from which the cells were taken to reconstitute the SCID mice, you have a good capacity to inhibit Factor VIII as a function of total IgG concentration.

This is the result of finding a number of six of those SCID mice reconstituted with the cells of those patients just to make the point that it is possible, as you see, to reconstitute an inhibitory response, secondary response, in those mice. You see that the variation from one mouse to another is certainly very high and this is not the ideal model.

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So we switch to another type of model which is available which is combining no Factor VIII and no immune system. For the time being, this has been extremely useful to evaluate the immunogenicity of different vectors used for gene therapy and especially in collaboration with Ivan Dandridge in our lab.

Now, the caveats, because I promised to be cautious, the number of FH genes in the mouse is about 100-fold higher than what you see in man. So the capacity to start with the diversification of the B-cell compartment in the mouse is about 100-fold higher in man so it is difficult to extrapolate at the molecular level what you see in the mouse to man.

Of course, the MHG-S2 determinants are distinct in the mouse as they are in humans. We know that if you take human Factor VIII, inject human recombinant Factor VIII, in hemophilia-A mice, you will get an immune response. If you do the same with mouse recombinant Factor VIII, you will get not only another type qualitatively different immune response but also the titer of inhibitor is going to be much lower.

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So there is some difference between mouse and human Factor VIII although the two molecules are highly homologous. The effect of inflammation is, to me, something we should absolutely look at. There are many, many reasons why the induction of an acute inflammation or joint bleeding or so could, indeed, trigger the production of inhibitor antibodies and I think we should set up protocols to understand this more.

Of course, the caveat which is probably the first one is that hemophilia, being heterogenous, I don't know if many of the human population where the inbreeding is complete, where everyone has, of course, the same deletion in the Factor VIII gene, which have treated at the same time and the same place and the same conditions.

Now, the way we have chosen to try to circumvent at least some of those difficulties is to say, well, it is almost impossible to take a mouse model and extrapolate everything up to the human situation. So why don't we try to go back to the mouse and only to the mouse? Why don't we try to use an hemophilia-A mouse, inject it the normal way with mouse urine, with murine recombinant Factor VIII, just to avoid differences

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between the two molecules which we do not understand, to be honest.

As I said, the best thing about animal models is the capacity to follow the immune response not only in vitro but in vivo too at the clonal level. So we have opted to work on the transgenic mouse model. But, of course, if you now bombard the genome of a mouse with a few of those receptors coding for the BCR or TCR against Factor VIII, and insert them at random into the genome, you will end up with something which might not be very interesting.

So, what we do is a target replacement, what we call a knock-in system, where you integrate the receptor you are interested in at the right place in the genome. The idea is that, by doing so, it should be amenable to all the physiological modulations later on.

So the usefulness of such a model, which is ongoing now, is to allow a clonal analysis of the anti-Factor VIII immune response not only in vitro and in vivo but also to take the cells, separate the cells in vitro, and reconstitute all the mice with this, just to put

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those transgenic cells into a more normal physiological environment.

It should be useful for evaluating treatment strategies. It should be useful, too, to evaluate more so-called physiological conditions like inflammation, sub-QT injections and so on.

Now, I have to give some kind of conclusion about what I think we should do. I think, with the tools we have now, and this is probably provocative, we should take the pain of comparing the immunogenicity of Factor VIII preparation in the Factor VIII knockout mice we have now. We should evaluate the level of specific antibodies, level in inhibitors, but also run a kind of epitope mapping.

You know, I am pretty convinced that, if we look carefully, it might be possible to have a kind of a footprint, each product having a specific pattern of antibodies recognizing different regions.

The B-cell epitope mapping is now something which is routinely used. We use, on a routine basis, about 55 different fragments of Factor VIII and we can,

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using this technique, identify almost any possible B-cell repertoire on the Factor VIII molecule.

The technique is simple. From the DNA of Factor VIII, we made a vector. This is transcripted in the system containing ribosomes from rabbit reticulocyte lysate. So everything is there in terms of amino acid to make small bits of peptides except one marker which is methionine which is radiolabeled.

Now, if you have your antibody, you can mix it with a solid phase in which you have protein A or protein G. the mixture is then incubated with the polypeptide of interest, one out of the 50 we are now currently using. You have a kind of complex bond which can be precipitated and you just can do radioactivity on it or you can disassociate the peptide and look for the real signs of the peptide you have been precipitating.

That is very handy. It takes less than one day to run the assay and, with the well-skilled technician, you can run about 50 assays a week.

I should skip on this because the previous speaker already said that. Just if we wish to make a prospective trial in PTPs, as I was asked to speak about

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this, I would certainly not restrict myself to assess the level of inhibitor antibody. I would also look for not only the titer, all the antibodies against Factor VIII. I would also try to make the picture more clear about the capacity of those antibodies to interfere with physiological partners like von Willebrand factor and phospholipids and I would run an epitope mapping in a systematic way.

I can tell you that we have now data on the intron-22 inversion where it seems that, indeed, the mapping--this is coming from human beings--the mapping of epitopes made by--antibodies made by those patients, seem to emerge as a clear picture.

Last, but certainly not least, what we should do at the T-lymphocyte level. I have no time to speak about this but this is going to be crucial in the coming five years.

Thank you so much.

(Applause.)

DR. LOZIER: Again, we have time for questions, perhaps one or two. Go ahead and activate your microphone.

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AUDIENCE: I was a bit intrigued about the mice experiments that you did where you add von Willebrand's factor and you saw a reduced, I guess, immunogenicity of the Factor VIII products. I was just wondering how that was done. Was the von Willebrand-containing product mixed with the Factor VIII prior to being administered to these mice, or was it a sequential thing where--

DR. SAINT-REMY: We did both.

AUDIENCE: You did both. And the results were the same. I guess one of the questions is, as a result, can we get any information from that in terms of how much endogenous von Willebrand's factor in a factor and is there a correlation between that and inhibitor risk, and what about giving DDAVP to these mice who presumably do have von Willebrand's factor in them and you can certainly almost duplicate what you did just by increasing their endogenous von Willebrand's factor.

DR. SAINT-REMY: To answer the first part of your question, the answer is no. We have absolutely no clue through such experiments to give you an answer to this.

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About the DDAVP, this is another story. We are working quite hard on DDAVP and we have reached surprising findings; basically, that is to say that the prediction of von Willebrand's factor and Factor VIII might be completely dissociated under certain circumstances and maybe Factor VIII is not only produced from the hepatocyte as many people do think.

We have a system--I cannot speak about it too long--is about the fact that we just perfuse human lungs with a system containing or not DDAVP and it is possible to produce Factor VIII in this system.

DR. LOZIER: Could you identify yourself and give your affiliation for the transcript.

AUDIENCE: Oh; I'm sorry. Manuel Carcao from the Hospital for Sick Children in Toronto.

DR. LOZIER: I think we can take Dr. Aledort's question.

DR. ALEDORT: On the same vein, do you think it may have something--the von Willebrand's factor presence may have something to do with your assay of inhibitor function because of the inhibition of inhibition of catalytic activity that you have also shown in the

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presence of more von Willebrand's factor, that it is less immunogenic but that the ability to measure it may be altered?

DR. SAINT-REMY: That is a pretty good point. I think we already discussed this. In this system, we check for the presence of catalytic antibodies but it was not possible to detect it, of course, at the polyclonal level.

DR. LOZIER: There is just one question here, please.

DR. BERGEDRIVER: Bergedriver, Baxter. I think we have discussed the limitations of these results with von Willebrand's factor in mice for quite some detail in the past. My question is what do you think you see effects of von Willebrand's factor with one recombinant Factor VIII product but not with the other recombinant Factor VIII product? Have you got any explanation for that. If that would be a more sort of general feature of von Willebrand's factor, you should see a reduction in both recombinant Factor VIII products that you used for your studies.

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DR. SAINT-REMY: You are perfectly right. But, I mean, these are experimental data. That is all I can say. We were really surprised to see those data and we thought it might be interesting enough just to try to publish them. But I have no explanation. Of course, you and me, we have about fifteen different hypotheses to explain this, but--

DR. LOZIER: I think we need to move on to our next speaker, in the interest of staying on schedule.

Dr. Sanj Raut of the Division of Hematology at the National Institute for Biological Standards and Controls at Hertfordshire in the U.K. will give us a talk on the regulatory aspects of the Factor VIII inhibitor assay.

Thank you.

Regulatory Aspects of Factor VIII Inhibitor Assay

DR. RAUT: I would just like to thank Dr. Weinstein, Dr. Lozier and colleagues for inviting me to give my talk today. In my talk, I will be concentrating on issues related to the standardization of Factor VIII inhibitor assays and, in particular, I will be showing you some a number of collaborative studies that have

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addressed the issues of the difficulties in measurement of Factor VIII inhibitor.

One of the early studies that actually specifically addressed these difficulties was a study carried about by Austen and colleague who published their data in Thrombosis Hemostasis 1982. In this study, they essentially compared two inhibitor assays, the standard Bethesda and the New Oxford methods.

In the study, they had eight plasma from inhibited patients. Seven of it were from hemophiliacs and one was from a patient who developed spontaneous antibody to Factor VIII. These samples were distributed to eleven laboratories and these participants were asked to carry out both the New Oxford and the Bethesda assays on these samples.

So here is a table just showing you, perhaps alarmingly, the large interlab variability we see when using both methods. You can see that, for the Bethesda, we see CVs between 38 and 78 percent compared to a range of 47 to 128 percent for the New Oxford method. Incidentally, the spontaneous antibody sample 4, again we see a relatively large CV there and for sample 7, which

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was, in fact a sample with a low inhibitor titer--and I haven't got the slide here, but, essentially the Bethesda assay, the majority of the labs showed some sort of detection when it came to sensitivity whereas the New Oxford method couldn't find any antibody at all. The majority of the labs couldn't find any antibodies.

So this study also looked at interlab variability. We can see that, again, although it is relatively large, 37 and 65 for the two methods, they were, in fact, much lower than the previous interlab variabilities.

Another study here which looked at the difficulties measuring the Factor VIII inhibitors. It was a study carried out in 2001, in fact, by Eric Preston and Tim Woods. This was a study, it is a UK-NEQAS study, which looked at essentially the Bethesda assays across a number of laboratories. In fact, 60 U.K. labs were involved and 18 international labs.

The samples that they included in the study, first of all, two samples, was the 110 sample which is essentially a plasma from a hemophilic patient with inhibitor which also, incidently, cross-reacted with

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porcine Factor VIII. Now, this sample was HCV-positive and was essentially only sent out to the U.K. laboratories.

The second sample, the 110A, was a plasma from a patient with an acquired inhibitor and this was sent out to both the international and the U.K. laboratories. Participants were asked to carry out a single assay on each sample, primarily by the Bethesda assay.

They were also asked to declare the sort of local limit of detection of these assays and they obtained quite a varied response. But, in general, the majority of the labs showed that the lowest limit of detection was around 0.5 Bethesda units.

So, looking at the actual human Factor VIII:C inhibitor assay, let's concentrate first primarily on the CVs. Again, we see a relatively large interlab variability. We see these around 47. The same sample was assayed in a porcine inhibitor assay. They obtained a slightly large CV of around 60, 60.5, percent.

On the second sample, 110A was assessed. This was the acquired inhibitor. Once again, we see large,

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relatively large, CVs for both the international and the U.K. labs varying from 68 to 86 percent.

Now I am going to talk about a study that was carried out at NRBSC which is a NIBS wet workshop. This was a controlled study to compare the Bethesda assay where a number of participants, 16 U.K. labs, in fact, where invited. This is an unpublished study in which a number of hemophilic plasma samples were included which had inhibitors and also plasma samples which didn't have inhibitors.

Now, these participants actually came with their own reagents and materials sufficient enough to carry out their own normal assay. The only thing that was provided was coagulation machines, coagulometers. Each participants were asked to carry out replica assays repeatedly over a three-day period in eight different sessions with different conditions.

The findings of the study are shown here. Essentially, the plasma samples without inhibitor were assessed. We obtained between 15 to 26 percent CVs which is the kind of range we would expect. However, when an

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inhibitor was present, the CVs jumped to between 53 to 80 percent.

Now, in the red, we have here, is a figure, 20 to 30 percent, which represents the same assay when repeated relatively to a reference standard.

Moving on, when the inhibitor samples were assessed, when an incubation state was standardized--i.e., for all the labs--one, group, actually, assessed the incubation state--the CVs actually dropped down to 33 to 43 percent. Once again, the presence of a standard, this dropped even further, between 13 to 34 percent.

When the same assays were repeated with both the incubation and Factor VIII assay, stage standardized--i.e., essentially the one-stage assay--the CVs dropped further, between 14 to 20 percent, this relative to the standard, came down even further to between 6 to 29 percent.

We also looked at the intraoperator or interlab CV compared to the interlab CV and this, once again, appeared to be much smaller than the interlab CV.

After these studies, it was decided by the ISTHSSC Factor VIII/Factor IX Subcommittee that we should

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carry out a collaborative study in the hope to possibly standardize Factor VIII inhibitor assay and, in particular, to develop a reference standard which may be useful in these assays.

This study was carried out in collaboration with Dr. Steve Kitchen at Royal Halamshire Hospital. In this study, we had two samples containing human anti-Factor VIII monoclonals, both Type 1 and Type 2, and one sample containing a rabbit antipolyclonal These were assayed in a multicenter study, 15 centers involved. 17 sets of data were collected using the local Bethesda method, some 5 percent of which were the Nijmegen modification, and variability of inhibitor assays were assessed.

We asked the question, could any of these reference materials assist in the standardization of this drug. Looking at the patient samples, once again, we obtained a relatively large interlab CV varying from 33 to 52 percent. For the individual reference standards, again, we obtained relatively large CVs when measuring the Bethesdas varying from 26 to 30 percent.

Interesting, we also looked at the difference between one stage in chromogenic assays. Here we see

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Bethesda titers right across the board with the patient samples on the reference preparations and we see that, generally, the chromogenic assays gave much lower Bethesda titers compared to the one-stage assays. This was significant for the patient 1, 3 and the rabbit polyclonal antibody.

Two points here, really. First of all, the chromogenic assay, you could speculate that it may have something to do with the larger dilution that has been used. Also, the one-stage assay tends to have, or can have, a longer lag phase in the dilution perhaps with a shorter incubation time and this could, then, perhaps, create a large Bethesda titer.

Secondly, across here, we see patient 1, 2, 3 and their other polyclonal antibodies are intact, polyclonal in nature, and it may not be surprising to see that they behave quite similarly.

This is just illustrating using stacking history. I am illustrating the effect of a particular standard. Here we have, first of all, in the top slide, we see the variability of inhibitor 1 without any standards in the presence of, in this case, the

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polyclonal antibody reference standard. We do see a better agreement.

This can be seen in a sort of correlation graph. Here, once again, we are looking at inhibitor 1 patient relative to the polyclonal antibody and we see you get a very good correlation in all the different types of assays used.

So the relationship in terms of correlation coefficients; really, we obtained a significant correlation primarily with the polyclonal antibody. We did get some correlation for patient 1 using one of the human monoclonals but, primarily, it was the polyclonal. That seemed to have good correlation with these inhibitor samples.

So, now looking at a table showing, first of all, the effects on CVs. Without a standard, we see that the CVs are generally large for the three patient samples and, when we look at the CVs relative to the standards, we do see a drop in CVs. But the best drop is primarily with the rabbit polyclonal antibody and dropping down to between 26 and 33 percent.

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The conclusion really here is the high interlab variability that we see is expected compared across the previous studies. The high interlab variabilities are actually much greater than normal Factor VIII assays. We do see some improvement in CVs between centers using standards reference particularly for the rabbit polyclonal.

So the next step really is, perhaps, to look at a large-scale production of one of these materials as a possible standard with a full multi-assay, multicenter-study assay. If we were to carry out, it would be nice to find what sort of level we should put into these standards which may be useful.

Possible advantages of a reference preparation would be, obviously, to reduce interlab CVs. It may also be useful to have QC material for labs for clinical studies and also as a common sample in evaluating a number of new methodologies that are currently being developed.

So the proposal is to seek out feedback from participants and the hemophiliac community in general before embarking on this study.

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Before I finish, I would just like to raise a number of points which I think we should consider. We know that there is a high interlab variability and this is directly due to the presence of inhibitor. We know that the intralab CVs are better than interlab CVs by a physician within a lab but it is difficult to get an agreement between lab and better CVs are obtained with standard; hence, the need for a standard.

Other points to consider. There is a mistake here. The chromogenic--Bethesda titers obtained using chromogenic assays are relatively better, smaller, compared to one stage. And this may be due to a plasma-matrix dilution effect due to the chromogenic assay, itself. We may need to look at modifying the actual assessment of the residual Factor VIII activity.

This could be, for example, looking at high dilution stage in a one-stage assay, for example, and, also, perhaps, we could look at the reduced or standardizing the actual incubation time of these assays.

Same points time for a different Factor VIII assay to complete and to vary and this will affect inhibitor time to neutralize Factor VIII. Finally,

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should we consider standardizing the different stages of the individual assays; for example, the incubation stage such as the antibody dilution step which is critical and, perhaps, we should look at Factor VIII assay stages, itself.

These points are difficult for labs to agree on but they may serve as useful guidelines.

I would just to finish. I would like to thank Drs. Jorgen Ingerslev and Marc Jacquemin for providing antibodies and the various participants, the various labs, that took part in these studies. Thank you.

(Applause.)

DR. LOZIER: We have time for a few questions. Once again, activate your microphone and please identify yourself and give your affiliation.

DR. KEY: Nigel Key from the University of Minnesota. These antibodies, do they have Type 1 or Type 2 kinetics and, if so, are they also standard--have you looked for the acquired antibody patients or mild inhibitor patients with antibodies?

DR. RAUT: The two monoclonals that we used as the reference standard, one was Type 1. The other was

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Type 2. But we haven't specifically looked at the antibodies, the spontaneous or the quiet inhibitor examples using this specifically.

DR. LOZIER: Any other questions?

DR. LAWLER: Pete Lawler, Emory University. The Bethesda assay is defined as the dilution of manamide that produces 50 percent inhibition. In practice, when you look at the original paper that described the assay, there is a line that is drawn between 25 and 75 percent inhibition for which there is no experimental basis for the slope of that line.

In practice, when you talk to technicians about how they define that 50 percent level, you get a variety of different answers because it is frequently just left up to the technician and there are no standardized protocols by which that 50 percent inhibition is found.

In our experience, we experimentally tried to find that point by doing a series of dilutions and then doing a regression to find that value and find that there is a fair amount of scatter associated with it, much higher than the precision of the assay.

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In your studies, how do you actually--or have you proposed to try to more rigorously define the 50 percent inhibition points to try to decrease interlab variation?

DR. RAUT: I think these are the points we are going to address in the next study which I forgot to mention. But essentially, you are right in what you are saying. Different labs effectively do different things when it comes down to the actual inhibitor assay. The stage you are talking about, the level between the 25 and 75 percent, they do tend to vary considerably.

These assays are carried out as one-point assays because primarily they are clinical labs and they are going through a huge number of assays. In a research lab, you always do varying degrees of dilution steps. to find your exact 50 percent level. So, yes; we would like to address this point in the next study without being too prescriptive. This kind of thing we could discuss with the Factor VIII-Factor IX Subcommittee when it comes down to actual putting together the protocol.

DR. LAWLER: I would also like to make a comment. I think, in principle, using a rabbit

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polyclonal standard is not a good idea because you are going to create an apples and oranges comparison because of the fact that there is a lot of heterogeneity among human samples in terms of what epitopes are being recognized and it is not really possible to define a reference standard for an inhibitory antibody, in my opinion, because of the polyclonal and heterogeneous functional properties of the different antibodies that we are looking at clinically.

DR. RAUT: You are probably right there. I think we are limited as to what we could use as a standard. It would be very, practically, impossible to get human plasmas as a reference standard at this level. So, realistically, do you propose monoclonals? We have access to both polyclonals and monoclonals. This, again, is something we could discuss before proceeding with the protocol.

DR. LOZIER: Do we have one more question? Dr. Kessler?

DR. KESSLER: From a practical standpoint, on all of the assays that you performed on the specimens, was there any difficulty in determining the difference

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between a high-titer inhibitor and a low-titer inhibitor or are you just talking about laboratory precision on your assay?

DR. RAUT: In our particular study, the last study, we were looking at the interlab variability and the precision within the lab should have been addressed but wasn't, which we would hope to do that for the next study. But, really, we are trying to compare the precision with the agreements between labs for these assays.

DR. LOZIER: Thanks very much.

DR. RAUT: Okay.

DR. LOZIER: We will move on to our next speaker, Dr. Bert Verbruggen of Nijmegen, the Netherlands, who has worked for many years on modifications of the Bethesda assay that people used to assay the Factor VIII inhibitors. His modifications to the Bethesda assay have been known commonly as the Nijmegen modifications and we are pleased to have him present his talk on innovations in the Factor VIII inhibitor assay.

Innovations in the Factor VIII Inhibitor Assay

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DR. VERBRUGGEN: Thank you, Jay.

First of all, I want to thank the organizers for inviting me to give this lecture here. I am going to talk about the specificity, accuracy, precision and interlab variation and sensitivity. I am going to limit myself to the Bethesda-based methods.

Concerning specificity, we have done some research on the normal pool plasma and the control sample. It is imidazole buffer in the classical Bethesda assay. Concerning the sensitivity, we have done some work on the patient for treatment of patient plasma and a mix of patient plasma and normal pool plasma. Concerning the accuracy, I show you some results on the study that we have done on Factor VIII activity assays, especially on the use Factor VIII deficient plasma and also the use of Factor VIII deficient plasma and the Nijmegen modification.

Specificity is the ability to obtain normal results in a normal situation. We have modified the classical Bethesda assay in two ways. We have changed the control sample and we have proposed to use Factor VIII deficient plasma instead of imidazole buffer to be

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sure that the protein content and the control mixture and the test mixture are equivalent and we have buffered the normal pool plasma at pH 7 to obtain a stable pH and a stable Factor VIII activity.

The modification has a better specificity. Allen Giles has done some research on it. I think in this afternoon's session, there will be more about this research. He found that in 887 samples of the same number of patients an increase in data of zero Nijmegen Bethesda units compared to the classical Bethesda units, an increase of 32 patients with an inhibitor activity of zero. Of course, he found a decrease in the number of samples with an inhibitor activity in the grey zone between zero and 0.5 Bethesda units and in the number of patients with higher Bethesda units.

So these data are not, per se, an indication for a better specificity because they should be correlated to a golden standard. But, at least, it is an indication that the Nijmegen Bethesda assay is more specific. Currently, in our laboratory, we are trying to correlate both the classical Bethesda assay and the Nijmegen assay to a gold standard like the kinetic measurements.

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Concerning the specificity, these are our data of the last year. We analyzed 79 patient samples. Some of the samples were from the same patient but, in the last year, we analyzed 79 patient samples for Factor VIII inhibitor activity and we found 74, less than 0.2 Bethesda units and Nijmegen--but, of course, we analyzed it with the Nijmegen Bethesda assay--we found 74 samples with less than 0.2 Nijmegen Bethesda units and we found five samples with an increased inhibitor activity. Two samples were from a patient with a lupus anticoagulant. One sample was from a patient with a history of inhibitor at the end of his eventual onset of therapy.

And there were two samples of one patient with an inhibitor titer of about 0.4 Nijmegen-Bethesda units. This patient did not have--these were recent data. This patient does not have clinical signs of the presence of an inhibitor and currently we are doing some kinetic experiments. So we are not sure whether this patient has, indeed, a Factor VIII inhibitor.

So our conclusions with respect to the specificity is that we figured false-positive inhibitor results have been eliminated in the Nijmegen-Bethesda

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assay by buffering the normal pool plasma and by replacing the imidazole buffer by Factor VIII deficient plasma.

Accuracy; accuracy is a measure of agreement between the estimates of a value and a true value. I think there at least two important theoretical items; that is, that the Factor VIII activity in normal pool plasma that is used as a Factor VIII source in the incubation mixture may have some influence on the accuracy; that means when you have normal pool plasma with varying Factor VIII activity, this also causes variations in the accuracy and also, of course, the variations in the Factor VIII:C activity assays will have an influence on the accuracy. But Dr. Raut has also showed you a lot of data on it.

We have done some research on the influence of the Factor VIII deficient plasmas that are used in the Factor VIII activity drug is used as substrate plasma in the Factor VIII deficient plasma that are used as a control sample.

These are the results. We measured some four or five different samples. These are the results of one

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typical sample. We analyzed this sample with all types of--with the four types of Factor VIII deficient plasma, a substrate plasma and a Factor VIII activity assay and with the same samples of the same Factor VIII deficient plasma as control samples in the mixture.

We saw that the highest inhibitor titers were in samples with control when we used chemical depleted plasma as a control sample and other deficient plasma as substrate plasma in the activity assay. The reason for these high titers was the presence of activated Factor V in the chemical depleted plasma.

The lowest data we received with the immuno-depleted plasma and especially with one type of the immuno-depleted plasma with a very low, or the absence, of von Willebrand's factor in this Factor VIII deficient plasma. In fact, intermediate data we received with an almost homogenous system with the chemical depleted plasma so can be depleted plasma used in the Factor VIII activity assay and used as control in the mixing studies and also, but a little bit higher, with congenital deficient plasma, in the homogeneous system with congenital deficient plasma.

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I think that the conclusion is that the accuracy of the Nijmegen-Bethesda assay is influenced by the type of Factor VIII deficient plasma. Of course, it is important before you can define the accuracy, you need a standard. Until now, there is no standard but Dr. Raut also has talked about that in the last talk so I won't go further on it.

Interlaboratory variation; I shall spend only a few words on it because Dr. Raut has shown these results already. But our conclusion is that there was, in the survey of Dr. Raut and Steven Kitchen, no difference in the interlaboratory CV between the Nijmegen assay and the Bethesda assay. So the Nijmegen modification does not influence the interlaboratory variation.

Sensitivity; the sensitivity is the ability to detect an abnormal situation. So when you use a golden standard which says whether an inhibitor is present or not, and you use a test to assay this inhibitor with a certain cutoff value, the sensitivity is defined as the number of true positives divided by the number of--sorry; the number of positives with the golden standard and

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inhibitor test divided by the total number of true positives.

But do we have a golden standard? Secondly, can we define a clear cutoff point in order to decide which test is positive and which is negative. I think the answer to both questions is no. I think the answer is no because Factor VIII kinetics after replacement and bleeding status are golden standards. But I think they are not 18 carat.

The cutoff value for the Nijmegen-Bethesda assay in the literature is 0.4 units per ml. However, this is not evidence based and it is only slightly better than in the classical Bethesda assay.

The sensitivity of an inhibitor assay depends on the ability to detect small changes in Factor VIII:C activity in the test mixture. But normally the coagulation assays only have limited precision to detect small differences in Factor VIII activity.

So, we tried to improve the sensitivity of the inhibitor assays and we looked at the patient plasma and at this mix of patient plasma and normal plasma. We developed a test which we called the Nijmegen low titer

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inhibitor assay. In short, in the test, the plasma of the patient and the control are concentrated by selective protein filtration by a centrifuge technique and the concentrate rate is about four times. It depends on the filter you use.

The inhibitor type is measured in the mixture with normal pool plasma that is stabilized at a pH of 7.4 in a 1 to 3 ratio, so three parts concentrate patient plasma and one part normal pool plasma. Any residual Factor VIII in the patient plasma which is disturbing this test is removed, destroyed, by incubation with EDTA.

We have got these results. When we spiked a normal hemophilic plasma with an antibody to inhibit to Factor VIII to a concentration of about 0.2 Bethesda units per ml, and we measured with the normal Nijmegen modification the inhibitor activity. Then we get a correlation between the spike concentration and the measured concentration that is not significant. So this method is not able to detect these low inhibitor activities.

When we analyzed the same samples with the low titer assay, and here are the inhibitor activities. We

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express it as a low titer inhibitor unit. Then we see a highly significant correlation between the spike concentrations and the measured concentration. Our conclusion is that, with the low-titer inhibitor assay, the detection limit is increased about 10 to 15 times so it is 10 to 15 times lower than in the normal Nijmegen-Bethesda assay.

But should low titers be of concern, the important question. We are only a small hemophilia-treating center and we have about 35 severe hemophiliacs in our center. At this moment, we only have two patients with an inhibitor, a low inhibitor.

I show you some results with these two patients. Patient 1 is a man born in '72 with severe hemophilia. Von Willebrand's factor is 40 percent or 0.4 per units per ml and genotype is an inversion intron 1. Because of his Factor VIII inhibitor--he is a long-standing Factor VIII inhibitor that started already in 1983. But, after all these years, the inhibitor titer became zero about three years ago and remained zero over the last three years.

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But there were some clinical problems with this patient. The patient needed more Factor VIII concentrate than could be expected and the patient has still, unless inhibitor activity was zero, still had a severe bleeding tendency so we performed a kinetic of Factor VIII concentrate and of Factor VIII von Willebrand's factor concentrate and we found that the half-life of Factor VIII in this patient was extremely low, 2.9 hours where the normal range was between 8 and 12 hours. The same was, in a lesser extent, true for the Factor VIII von Willebrand's concentrate.

Case 2 is a man born in 1942 also with severe hemophilia, with a normal von Willebrand's factor and the genotype inversion intron 22. This was a fast-growing inhibitor. It was a low response with the highest activity of about 2.1 and also this patient returned to activity of zero about two years ago and also remained zero until now, with the normal Nijmegen-Bethesda assay.

But also this patient has much higher need for Factor VIII than could be expected in a patient with a chance of an inhibitor. Also, these patients suffered from abnormal bleeding tendency. The inhibitor titer was

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zero. We also performed a half-life, a kinetic measurement, and we found the half-life of 4.1 hours. It is also very much decreased.

The clinical presentation in both patients suggest the presence of inhibitor because of the unusual bleeding tendency and because of the fast disappearance of the Factor VIII. But the Nijmegen-Bethesda assay was negative.

So we performed the low titer assay on a number of samples which we had in stock of these patients. At the time that we performed the kinetic studies when the inhibitor titer with the Nijmegen-Bethesda was zero, we found an increased inhibitor titer with the low titer assay. This sample we had in stock and we found a very high inhibitor titer. These are not Nijmegen-Bethesda units but these are some other units. We have to define it further. At this moment, these are the latest data which I got from the laboratory the day before yesterday. Still, this patient has an undetectable inhibitor with the Nijmegen-Bethesda but still these patients have an increased inhibitor activity with the low titer assay and, moreover, this patient

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still has a big need for Factor VIII and still suffers from abnormal bleedings.

Case 2, this patient reached the maximum level of 3.2 Nijmegen-Bethesda units and then the inhibitor rapidly decreased to zero. At this point, we performed the kinetic of Factor VIII and, at this point, we also analyzed the low titer inhibitor assay and found 1.2 low titer units.

At this moment, the patient has both with the Nijmegen-Bethesda assay and with the low titer assay, zero activity of inhibitor. At this moment, this patient doesn't have any abnormal bleeding and we are performing at this moment again the Factor VIII kinetics. But this patient does not need any more than normal Factor VIII.

So, again, the question should low titers be of concern. I think maybe yes. Low titers of Factor VIII:C inhibitors may be of clinical relevance but we only have two patients. So I think we have to cooperate with other big centers to get more insight in this problem and I think more data have to be gathered to get more evidence about this problem.

These are my coworkers. Thank you very much.

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(Applause.)

DR. LOZIER: We have some time for some questions. Please activate your microphone or go to the stand-up mike and identify yourself. I see Dr. Aledort.

DR. ALEDORT: Dr. Aledort, New York. Just a question. When the low titer was 7.0, what was the normal Bethesda unit without the Nijmegen?

DR. VERBRUGGEN: So what you are asking for is the normal--

DR. ALEDORT: The regular Bethesda unit without the Nimegen addition. When you were at 7.2 at the time you had a low Nimegen.

DR. VERBRUGGEN: Yes. At that time, I think the Nijmegen-Bethesda assay was 0.4. And so we think our experience is that the low titer assay is about 15 times more sensitive than the Nijmegen-Bethesda. So there is a good correlation between the Nijmegen-Bethesda and the low titer assay at that moment.

DR. ALEDORT: But what about the normal Bethesda unit without the Nimegen part.

DR. VERBRUGGEN: Oh; okay. I'm sorry; we did not perform that.

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DR. LOZIER: Dr. Lusher?

DR. LUSHER: Jeanne Lusher, Detroit. I have two questions. One is when would you do the original Nimegen assay in assaying patients? I mean, at what level of Bethesda unit would you do that? Second, with this low titer Nimegen assay which your results look very fascinating, currently all of the trials, clinical trials with new products, use a cutoff as part of the inclusion/exclusion criterion, a cutoff of 0.6 standard Bethesda units.

May we, by using that 0.6 standard Bethesda unit be missing people who really do have a tendency to form inhibitors because we are not doing this Low Titer Nimegen assay as an inclusion/exclusion criterion?

DR. VERBRUGGEN: Yes. We have only little experience. We only had two patients. I think it is too early to draw conclusions. But I think it should be possible that we are missing--we are also missing these two patients with our normal Nijmegen-Bethesda assay.

DR. LUSHER: But, just in the general population when you find--the hemophilia population when you are assaying for an inhibitor, when should we use the Nimegen

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versus a standard? I mean, just the ones below 5 Bethesda units or everybody? What would you suggest?

DR. VERBRUGGEN: I should say always. But I think that the classical Bethesda assay has some limitations regarding the standardization. The Nijmegen-Bethesda assay is more standardized. So I think, if you have the chance to choose between a good standardized method or a less standardized method, I think you should use the good standardized method.

I think we are able, we are more able, to define a good cutoff point with the Nijmegen-Bethesda assay compared to the classical Bethesda assay. But still there is no good definition. Also, not in the Nijmegen-Bethesda, say, of the cutoff.

DR. LOZIER: In the interest of time, let's try one more question. Keith Hoots has a question back there.

DR. HOOTS: Keith Hoots, Houston. There was unusual PK on Case 2. I mean, you gave them 25 per kilo and you jumped all the way up to 80 percent. So the area under the curve was kind of unusual to begin with. How do you account for that?

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DR. VERBRUGGEN: Excuse me? I did not understand your question.

DR. HOOTS: In Case 2, your in vivo recovery was 80 percent, at least according to the graph, after 25 per kilo, which is extraordinarily high in vivo recovery. Even though the half-life was short, the recovery was high, suggesting that this individual has unusual pharmacokinetics to begin with. Any explanation for that?

DR. VERBRUGGEN: No. I don't know. I don't have an explanation. I think, with 25 units per kilogram, it is normal to get a top activity of about 80 percent and, in this patient, there was a rapid decrease of Factor VIII activity because of the presence of an inhibitor.

DR. LOZIER: Before we take a break, I want to point out that we have some speaker handouts for this afternoon at the front and, in addition, we have a couple of this morning's speakers' handouts that are new and improved. So let's take a 15-minute break and return for business just a little bit before 10:45.

(Break.)

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DR. LOZIER: In the interest of time, I would like to reconvene the workshop here after the break. We will be resuming with Dr. Gilbert White who is Professor of Medicine at the University of North Carolina at Chapel Hill. He has been, as you all know, a very prominent hemophilia healthcare provider for many, many years and a researcher in bleeding disorders and very, very active in the International Society for Thrombosis and Hemostasis.

He will now present a discussion of the ISTH rationale of recommendations for use of previously treated patients in clinical trials.

ISTH Rationale of Recommendations

for Use in Previously Treated Patients

in Clinical Trials

DR. WHITE: I was sitting in the back before and having trouble hearing so can everybody hear me? I will try and stand close to the mike here.

I think everybody knows that the stimulus for the current products that we use in the treatment of hemophilia, the stimulus for their development dates back into the mid-1980s when various bloodborne infections got

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into the blood supply and into the products that patients with hemophilia were treated.

The first plasma concentrates were developed in the late 1960s. Hemophilia was recognized in the early 1970s but it was really the development of AIDS in patients with hemophilia that really was the stimulus to the development of new Factor VIII and Factor IX concentrates. Within two or three years, Factor VIII was cloned. Monoclonal Factor VIII first was used clinically a few years after that. Recombinant Factor VIII was introduced in 1987. Gene therapy followed and now we have second and third-generation products.

It was, I guess, hoped and probably expected that monoclonal-antibody purified plasma and recombinant concentrates of Factor VIII would be free of those bloodborne infectious agents but, of course, in the initial clinical trials, the challenge was to demonstrate that. So I think the first trials, the clinical evaluation, was carried out in two phases, a first phase to demonstrate that the new products made by new technologies were at least therapeutically equivalent to the old products, and then a second phase.

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That first phase was generally carried out in previously treated patients as previous had been done, but then there was second phase to demonstrate the viral safety of the new products and those generally had to be carried out in uninfected patients. So, often, there was a phase I study in previously treated patients and then the a phase II study that was carried out in previously uninfected patients.

Those studies, I think, we are all familiar with. One of the things that came out of those initial studies is that there appeared to be, at least in the studies in uninfected patients, a fairly high prevalence of inhibitors, much higher than we were used to seeing, the usual 15 percent that we were all used to seeing as clinicians. So, in the three initial recombinant Factor VIII studies, there were prevalences that were up in the 30 percent range, nearly twice what we were used to seeing. And so that raised questions in a lot of people's minds about these products and whether or not they had been immunologically altered, perhaps because they were made in animal cells, perhaps because they were purer than previous forms of Factor VIII.

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So there was considerable concern in the community about immunogenicity and the emphasis of those clinical trials really switched from there being questions about viral safety to there being questions about immunogenicity.

I think we now recognize, although there is still some debate about this--I think we now recognize that this high prevalence of antibodies, one, did not reflect true high response inhibitors, that it was both low response, transient and high response inhibitors, and that the prevalence of high response inhibitors in these studies really was not greatly different from what we had been used to seeing.

So the current thinking is that, at least in part, this increased occurrence of inhibitors is due to closer scrutiny of patients during these clinical trials. We certainly were looking for inhibitors much more closely than we had ever done before. I think, as a result of that, we began to see, really for the first time, what the natural history of inhibitors were. We began to see that inhibitors formed transiently and then go away, that some people do develop low-titer inhibitors

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that stay low-titer inhibitors and then the traditional high response inhibitors or high titer inhibitors that we had seen back in the 50s and 60s and 70s.

That notion that, perhaps, these products were not as immunologically altered as the PUP studies made us first wonder was the study in previously treated patients where the occurrence of inhibitors was very low, 2 percent, 3 percent, even 1 percent, often one or two patients in the clinical trials.

So, with that in mind, the idea developed that previously treated patients who had had a high number of exposure days to Factor VIII or Factor IX had really shown themselves to be tolerant to exogenous Factor VIII and Factor IX and, therefore, were considered to be at low risk to develop inhibitors.

A corollary of that, if you will, was that, if one then used previously treated patients in these studies to assess immunogenicity that any increase in inhibitor formation in previously treated patients would be consistent with the product being neoantigenic.

Examples of that that we will hear throughout this meeting are the Belgian and Dutch examples of Factor

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VIII concentrates that were somehow altered during their manufacture and became neoantigenic.

One of the critical aspects of this is the number of exposure days. This is the rate, or the occurrence, of inhibitors versus exposure days that was published by McMillan from the Cooperative Inhibitor Study a number of years ago. You can see that, although, as was previously mentioned, the rate of inhibitor formation is highest down here in young individuals, that inhibitor formation continues with exposure days so that, by 50 exposure days, maybe 75 to 80 percent of patients who are going to develop an inhibitor will have done so but 15 to 20 percent of patients who are going to develop an inhibitor will not have done so.

You really don't reach an asymptote with 100 percent until you get out here to close to 250 days. So there are differences, I think, between studies that are carried out with 150 exposure days as an eligibility criteria versus 250 exposure days. Certainly, when one gets down here to 50 exposure days as an eligibility criteria, the number of inhibitors that one might expect

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to see, even in a previously treated patient cohort, is not an insignificant number.

So based on these, in 1999, the Factor VIII and Factor IX Subcommittee of the SSC of the ISTH deliberated about this and came up with a recommendation that previously deliberated about this and came up with a recommendation that previously treated patients with greater than 250 exposure days should be used to assess the immunogenicity of new products and previously untreated patients, or newly--somebody remind me what NIPs are--noninfected; that is what it is, not newly--and noninfected patient should not be used to assess immunogenicity because, in all likelihood, their number of exposure days will be so small that the risk of inhibitor development would be increased.

I think that that has made some sense. I think the FDA and European agencies have recognized that and that has become a standard. That is certainly the rationale behind why we currently use PTPs to assess immunogenicity. The only real point that I would make is that this number here is not a trivial number. It is a number that has some experimental backing and is

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a number that needs to be looked at with great care when one is designing clinical trials.

The Factor VIII and Factor IX Subcommittee later, in 2001, also addressed, because of concerns about differences between the definition of inhibitors and concerns that that might muddy comparisons between studies, also decided to make an official recommendation about what was a high response and what was a low response inhibitor, and those definitions are shown here. A high response is greater than or equal to 5 Bethesda units and a low response inhibitor is less than 5 Bethesda units.

So my charge was to go over those recommendations. I think that they are still valid today. I think that previously treated patients are the right population to look at neoantigenicity of new product and, as we go to new generations of Factor VIII and Factor IX that are structurally altered in order to make better molecules, I think that this is going to continue to be an issue that we need to be cognizant of and work on in our clinical trials.

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So I will stop there, Jay, and if there is time for questions, I would be glad to answer questions.

(Applause.)

DR. LOZIER: We have a few minutes for questions. Again, state your affiliation and activate your microphone. Dr. Aledort.

DR. ALEDORT: Aledort, New York. Do you have any assessment from the literature, which I think is really very difficult to do, if you really looked at a population of greater than 150 versus a population of greater than 250, what incremental or likelihood of the number of inhibitors you might find given 100 patients.

The other question is, unfortunately, all those data do not, in any way, talk about the genetic predisposition to inhibitor in those previously treated patients in terms of how far out you go. I think you would have to know a little bit more about that to feel sanguine about the population that you are actually studying.

DR. WHITE: I think your latter point is a good point, Lou. Let me answer your question first and then I will comment. The data was from McMillan. You know the

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study. It was not a study--it was a prospective study, certainly a valid trial at the time, but it was a mixture of patients, PUPs and PTPs and so on. So I think that data is a little bit tainted based on what we know today.

If you actually look at the curve, and there are not many studies that have looked prospectively at inhibitor development as long as that study did. It was about seven to ten-years study, as I recall. It certainly was not a 25-year study, but it was a fairly long study.

If you look at the curve, the difference between 150 and 250 exposure days in 100 patient would probably be one or two patients, so very small. I think my real point is that 50 is a number that has been used in some studies and I think that is a significant difference from 250 and 150.

As far as the genetic data, you know what I believe about that. You know that I think that there are a lot of answers in the genetics that Joan talked about but I don't think we are at the point where we can predict individuals yet. I think maybe we are not too far from it, but we are not there yet.

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I do think genetics will play a role in when a person develops an inhibitor. I don't know that it will be as simple that nonsense mutations are going to develop in inhibitors if they are going to develop them in 50 days and deletion mutations are going to develop them in five days. I don't think it will wash out to be that simple.

But I think molecular defect and other genetic factors are going to play a role in when inhibitors develop upon exposure to Factor VIII, but I couldn't predict as to how it would affect that.

DR. LOZIER: I think one other point about the McMillan study, as I recall, the criterion was 2 percent as opposed to 1 percent for severe. So that is just another factor.

Could we have one more question? Okay. Then, at this point, we had expected to have three speakers talk about epidemiology of Factor VIII inhibitors, particularly from the U.K., Canada and the U.S. Unfortunately, Dr. Charlie Hay from England was unable to make due to a last-minute emergency so we will be proceeding on here, when we have our slides, with Dr.

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Carcao who will talk about the Canadian experience and Dr. Evatt with the U.S. experience.

Canadian Experience with Factor VIII Inhibitors During Conversion to Recombinant Products

DR. CARCAO: I would like to thank the sponsors for inviting me to present some of our Canadian experience with inhibitors. I also want to thank Dr. David Lillycrap who is here in the audience. You should also know that Dr. Lillycrap was actually invited to do this talk but he graciously asked me to do it on his behalf.

Part of the reason why I have been asked to do this is that I am the Chair of the Inhibitor Subcommittee of the Association of Hemophilia Clinic Directors. The other members of the Association are shown there and, as you can see, alphabetically, I am the first on the list and I think that is probably why they asked me to be the Chair.

A little bit about hemophilia in Canada. Canada is a country of about 30 million people so you can put that into context in terms of your own countries. In Canada, there are 2,561 hemophiliacs. This is as of July

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of this year. How accurate are those numbers? We believe that they are probably close to 98 or 99 percent accurate but we certainly can't claim 100 percent accuracy.

Of those numbers, 2063 of the patients are hemophilia-A patients and that represents 81 percent of all hemophilia in Canada. 498, or 19 percent, are hemophilia B patients. Virtually all of these patients are followed and, hence, registered in 25 hemophilia treatment centers that are scattered throughout Canada.

If you work out the numbers, given that the population is about 30 million people, you will see that the prevalence of hemophilia is about 1 in 5,740 males. In Canada, presently more than 90 percent of the patients receive recombinant factor concentrates.

Specifically, I was asked to address hemophilia A and so, from now forward, I will be speaking about hemophilia A. In Canada, we have 2,063 total hemophilia-A patients. Of these, the severes constitute 30 percent or 614 patients. Moderate represent 12 percent of the total and mild patients represent 58 percent of the total.

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If you look at inhibitors versus no inhibitors, and this is prevalence data not incidence data, currently there are, of the 614 severes, 72 patients currently having inhibitors representing a prevalence of 12 percent. For moderate, the number is 3 percent, the current prevalence. For mild, it is 0.3 percent. If you want to translate this into incidence--we certainly can't--but you can probably estimate that maybe the incidence might be three times the level of the prevalence.

So, in total, inhibitors in Canada, we have presently 83. This is as of July. That represents 4 percent of all hemophilia-A patients.

Canada is a very large country, as you can see over here. In comparison to the United States, we are actually a little bit bigger than the United States--I certainly had to put that in--but most of our population is certainly concentrated within about 100 miles of the United States so we like to be close to the U.S.

As you can see, most of the hemophilia treatment centers that are shown here in green are actually very close to the border. There is certainly a concentrate of

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the centers in Southern Ontario and Southern Quebec where some of the largest cities are.

So, overall, inhibitors in Canada. We have a current inhibitor prevalence of 4 percent for hemophilia A whereas, for hemophilia B, it is less than 1 percent, so patients currently with inhibitors, a total of 83 hemophilia A and four hemophilia B. As I said, inhibitor incidence currently is not known. How we are tracking this and why, and where these numbers all come from, is that the individual clinic-center data is entered into and analyzed within a data-management software program.

This is a program that is known as CHARMS. This is an acronym for Canadian Hemophilia Assessment and Research Management System, CHARMS. So CHARMS was a computer-software program that was designed for data-entry management and analysis by all 25 Canadian hemophilia clinics.

It was primarily a tool that was to be used for tracking the distribution of factor concentrates from clinics to patients and then the usage by patients. The data is entered at individual clinic sites but is

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available as aggregate national data. This system has been in place now for the last five years.

So this CHARMS is actually something relatively now. Now, what I am going to talk about is actually before CHARMS existed. I was specifically asked to talk about and address the Canadian experience with the switch from plasma-derived Factor VIII concentrates to recombinant Factor VIII concentrates.

This was work that was done certainly before me so I don't take any credit for any of this, and it was the Inhibitor Subcommittee which, at the time, was being chaired by Dr. Alan Giles that took on this I guess project.

During the early to mid-90s, Canada, as a country, really decided to switch en masse from plasma-derived Factor VIII concentrates to primarily recombinant Factor VIII concentrates. So, given that, there was a look--there was surveillance for Factor VIII inhibitor development in the Canadian hemophilia-A population following the widespread introduction of recombinant Factor VIII replacement therapy.

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The study was funded by the Canadian Blood Agency which is a government organization that is entrusted with the purchasing and distribution of all Factor VIII concentrates and all blood products. The population for this study was previously treated hemophilia A patients who were felt by individual clinics to be inhibitor-negative and who, then, converted from plasma-derived Factor VIII to recombinant Factor VIII.

Now, certainly, as we heard, I think just right before me, previously treated hemophilia-A patients encompasses a tremendous range. That would include minimally treated patients to very heavily treated patients, so the 250 exposure patients and the much less than 250 exposure patients.

So, during that time, 814 patients were converted to recombinant Factor VIII and 478 patients were registered on study and data was obtained on those patients. Those are the ones that I will be discussing.

The methods were relatively simple. Plasma samples were obtained pre-switching to the recombinant Factor VIII and then one year and two years post switching. All the testing was done in a single central

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reference laboratory, that being in Kingston, Ontario, which, at the time, was being run by Dr. Alan and currently is being run by Dr. David Lillycrap.

The testing method was the classical Bethesda assay which was used for the initial or the pre-sample as well as the one-year sample. But, by the time that the two-year sample was done, what happened is that the Nimegen-modified Bethesda assay was available and so both the classical as well as the Nimegen-modified Bethesda assay were done in parallel.

A positive test was defined as an inhibitor level of greater than 0.5 Bethesda units. Here are the results. For inhibitor-negative patients, these are patients who are shown to be inhibitor-negative at the time of switching, at one year, of the 478 patients, nine patients, or 1.9 percent, had developed an inhibitor. Then, at the two-year mark, there was data available for 339 patients and, of those, ten patients, or 3 percent, were now inhibitor-positive.

Of note, all inhibitors were low titer. Also, the inhibitor prevalence did not change following the introduction of recombinant Factor VIII so some

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inhibitors that had previously existed did disappear and some new ones arose.

So the incidence, over the two-year period of the study, of inhibitors in these previously treated patients, and, again, I would just sort of note that there were, again, minimally as well as extensively treated previously treated patients--so the incidence was certainly less than 2 to 3 percent and, furthermore, some of these inhibitors were transient.

There was no attempt, at the time, to correlate inhibitors with either patient age, patient genetics, the dose or the intensity of treatment.

I am going to go on to since 1998 when this study was reported. There have been several other ongoing efforts amongst the Canadian group to look at inhibitor epidemiology. So there is a study that will be actually presented at ASH which is a relatively recent study to look at prospectively the development of inhibitors in patients who switched to a Factor VIII formulated in sucrose. Obviously, what we are taking about is Kogenate to Kogenate FS. That is going to be

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presented later on this afternoon, I think, by Dr. Peter Larson of Bayer. So I am not going to present this.

There have also been, through CHARMS, some sporadic reports of inhibitor development in previously treated patients but I certainly don't have the number here for you. What there hasn't been is a systematic tracking and reporting mechanism for such patients.

So that is sort of the past and a little bit about the present, and now, I was also asked to do a little bit about the future and what we, as the Canadian group, sort of envision potentially for the future.

So, the future is really how to study current factor concentrates that are marketed and being used in terms of inhibitor development and also how to study future products and, in doing so, how to address the current limitations of postmarket surveillance.

So, certainly, to study the current factor concentrates, we have to rely on CHARMS but we have to refine CHARMS and we are doing so. We haven't been able to track the incidence of inhibitor development. The major reason we haven't been able to do so is that we

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haven't actually be asking for the date of when patients actually develop an inhibitor.

All we have asked for is, on a yearly basis, does a patient have an inhibitor or not. So we are now asking for that data and, hopefully, we will be able to present a little bit more incidence data into the future. Any type of system such as this where it is relying on individual clinics to enter data, the most important thing is actually the data entry. So we have to certainly encourage improved data entry by individual clinics.

There is a very strong potential role for a central reference laboratory to confirm all new inhibitors and, by confirming all new inhibitors, to document all new inhibitors.

So, into the future. How can we do postmarket inhibitor surveillance and, I guess, should we do it. I believe, and most of us, I think, believe, that we should. But how to do that? There are a number of different ways. One could be separate studies so a new product comes onto the market and that product becomes

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studied or you can have the more common inhibitor surveillance program for all Factor VIII concentrates.

For the separate individual studies, a study that is linked to the introduction of one and only product, there are certain advantages and disadvantages. Certainly, an advantage is it is a little bit easier to undertake such a study. You are only dealing with one product and it is easier to track the costs of such a study.

But there are disadvantages. If two or three different products come out into the market over a period of a year to two years, and you want to do individual studies for each of these, then you are going to be left with having to do repetitive and duplicative efforts to develop separate protocols, separate consent forms and separate data-collection forms. As well, if you don't study all the products, then you would be left with a lack of comparability data between the different factor concentrates.

I am actually going to skip that and then go back to it. This is this wrong order.

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There are certain advantages and disadvantages to a common inhibitor surveillance program. Here, I think that the advantages outweigh the disadvantages. So this would be a program where all products, and, hence, all companies that are marketing those products, would commit to inhibitor surveillance for those products. Because I am talking about Canada, I just put in Canada, but any other country, I think, could be substituted for that.

This would avoid the duplication of effort to develop separate surveillance studies. It would ensure that all companies contribute to, in the case of Canada, supporting a national inhibitor laboratory which would be used for confirmation of inhibitors. The disadvantages; it is logistically difficult to organize this and it is somewhat costly. It would require a large commitment from hemophilia treatment centers to ensure that data-collection samples were being sent to the national laboratory.

Now, I am going to go back. So the common national inhibitor surveillance program, how we sort of envision it, that the companies that are marketing Factor

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VIII as well as companies that have, I guess, a vested interest in studying inhibitors would provide fixed funds for the support of the Canadian National Inhibitor Laboratory. We have actually implemented that and we have had good cooperation from all the companies.

Then there would be a global protocol for inhibitor surveillance for all Factor VIII concentrates which would then be modified to the specific Factor VIII product. This would apply to all PUPs who would be beginning on a product, and that would include the current products, because they would not have been previously exposed to other products, by definition, and it would also apply to all previously treated patients who switched from one product to another. Hence, this would also cover current products because patients could switch from one current product to another, but it would also apply to all new products because, by definition, they wouldn't have had it before and, hence, they would have switched to it.

Then patients who would either be PUPs or PTP switching to a product would then be followed for three years post starting the new product and manufacturers

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would be billed a small amount for inhibitor testing for these patients who are receiving their particular Factor VIII product.

So a common national inhibitor surveillance program; there is certainly a need. I think that, without a systematic, rigorous program for inhibitor surveillance, most cases of inhibitors, at least through postmarketing surveillance, fail to be reported and the incidence, prevalence and risk factors for inhibitor development, as such, are sometimes inaccurate.

I am going to end there. Thank you.

(Applause.)

DR. LOZIER: Let me just use the moderator's prerogative and ask, if I recall correctly in the Giles study, not only some previously treated patients who went from plasma-derived products to recombinants gained inhibitors but there were a handful, a small number, that actually lost inhibitors that they had acquired or they had generated during treatment with plasma-derived material.

I don't know if you want to comment on that. It is sort of the flip side of the coin.

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DR. CARCAO: Yes; that is exactly the case. As a result, the prevalence didn't actually change. There were the same number of inhibitors before the switch as there were after the switch. Some of these were, as a result, transient inhibitors. They come and they go, it appears.

DR. LOZIER: Other questions from the audience? Dr. Lusher?

DR. LUSHER: In the current studies, the surveillance studies, that you commented on and the new ones that you were designing, how often to the local hemophilia centers do their inhibitor testing? Do they do it every three months, every six months, or just once a year?

DR. CARCAO: We, as the Inhibitor Subcommittee, can put out recommendations and we will recommend that, for severe hemophilia patients, the test can be done every six months and that, for milds and moderates, that it be done every one to two years. But those are recommendations.

In terms of are they being followed, most clinics, but certainly not all clinics, are doing this.

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So I do think that the limitations of any type of surveillance which is not done in a much more rigorous fashion is that you are missing many things and you come up on a podium and you present data, but you recognize that you are missing lots of that information.

So it would be very nice if we, in the ideal world, had all that information. But we don't.

DR. LOZIER: Thank you very much.

To close out the morning session, our last speaker will be Dr. Bruce Evatt of the Center for Disease Control in Atlanta. He and his colleagues have been studying various aspect of hemophilia therapy and epidemiology including the occurrence of inhibitors in American patients with hemophilia A and he will now present an occurrence of inhibitors among patients enrolled in the U.S. Hemophilia Universal Data Collection Project.

Occurrence of Inhibitors Among Patients Enrolled in the U.S. Hemophilia Universal Data Collection Project

DR. EVATT: Thank you very much. It is a pleasure to be here today. What I would like to do in

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the next twenty or twenty-five minutes is really explain some of the issues about inhibitors that we have been able to glean from the Universal Data Collection System.

Now, the UDC, which is the Universal Data Collection instrument we use in our 140 hemophilia treatment centers in the U.S. and Territories of Guam and Puerto Rico, really are designed to measure healthcare outcome for patients attending the centers. It was initiated in the mid-90s and has been collecting information primarily directed at the occurrence of infectious bloodborne infections among patients but also it was designed to collect a number of healthcare outcomes and complications of hemophilia among these patients.

It is a very important tool because it gives us a large amount of insight into what the complications of hemophilia are and where some of the program resources should go, where are some of the things that we should be concentrating in the future on just designing improvements in the system.

The data that goes into the system is really reviewed periodically by a data-collection task force

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which is made up of physicians from hemophilia treatment centers, the patients, themselves, as well as other individuals from the various kinds of subspecialties which work in the system with patients with hemophilia.

To date, the system is quite extensive. It collects not only information on hemophilia but on other patients seen in the centers as well, but we currently have approximately 10,200 patients with hemophilia. What I would like to do this morning is review the information we have on both the prevalence and the incidence of hemophilia from that system.

Now, what we collect on patients are the measurements of titers and when they occur and the type of treatment they have received in the six months prior to the visit to the hemophilia treatment center. This occurs during the comprehensive-care visit. Some of the patients visit every year. Some of the patients visit every other year and so forth. So the data I am going to show you will consist of two types.

First of all, the prevalence data will be cross-sectional area, data that will give you individuals who came to the clinics during the years 1999 to as far as we

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are in 2003. This data is not nearly as useful as the incidence data to answer the types of questions you are, but I think it will provide some insights into possible uses of the data.

The incidence data will be on a total of a little over 1,200 patients who have attended the clinics on at least four occasions for different years during this period of time. So we are picking up more and more individuals, that we have a larger increasing database with multiple ones, but we picked four because it will give the most conservative and most complete data in terms of being able to examine some of the year incidence data.

Then we will look at some of the incidence rates for select patient characteristics that we have. Dr. Mike Sousi is currently analyzing this data and so this is preliminary so there is still more analyses that have to be conducted and that is currently underway. But I think this will begin to give you some of the insight.

First of all, this slide really gives you the number of patients that were entered into the database during the years 1999 to 2003 in terms of mild, moderate

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and severe cases. They really represent cross-sections of the entire database. The total number, really, is over 10,000 but many of them were seen only once, twice or three or four times during this period of time.

This shows the prevalence of low titer which is defined as a half unit to 5 Bethesda units in males over that period of time with this really representing the prevalence of inhibitors for severes, and this is milds. As Dr. Carcao had said, you can see that the prevalence really ranges from about 4 to 6 percent during that period of time, nothing really to write home about. For milds, you can see it is down less than 1 percent of the patients really have a low-titer inhibitor.

If you look at the distribution, it is pretty much the same. These represent age distributions that were seen in these cross-sectional areas during this period of time, with children two to ten really representing around 20 to 30 percent of the 11 to 20, the 20 to 40. And the smallest group is really the individuals which are above the age of 41.

Currently, we don't enter children less than two into the UDC database but a protocol has been established

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and that process is beginning so that the database, in the future, will also include children under the age of two. That will give us more information in terms of the incidence of inhibitors in young children.

This represents just the age distribution. I can skip that.

This represents the prevalence of high-titer inhibitors in the population so that you can see that the prevalence on high-titer inhibitors during this period of time really range in the same neighborhood of about 4 to 6 percent. Again, this is the milds and the moderates were somewhere in between which is not unexpected.

Again, the kind of sampling that the process that took place with the low-titer kind of data is very much the same. The numbers at the bottom give you the numbers that were included in the database and the age distribution of inhibitors, this gives you the number of individuals that actually had the high titers.

I think that what is important to see is how many patients we actually miss information on that do not have inhibitor titers during this visit. It actually ranges in the neighborhood of about 20 to 30 percent.

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These are the various age ranges. The blue represents 2 to 10. The purple represents 11 to 20, 21 to 40 and 41-plus, so that we only miss, of the younger individuals, about 20 percent of the individuals that don't have inhibitor titers measured when they come into our HTCs whereas it may be as high as 40 percent, 30 to 40 percent, with the older individuals. That just gives you some idea of the missing data that would not be included in the database.

Now, I think, for this audience, what is most interesting for you would be the individuals that have been followed repeatedly. For this study, we selected those individuals that were two years and older with four or more visits to the hemophilia treatment center because this will give you the most complete dataset.

I think if you examine both the demographic and the clinical characteristics of the cohort, they are really similar and no different from all the characteristics of the overall patients entered in the UDC. I am not going to show you that data today. I think that, if we had persons with only one inhibitor titer measurement made, that person was excluded from

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this cohort. And if we had people who had an inhibitor in the past, those individuals were also excluded. So we are only excluding individuals without a previous inhibitor who were followed over this period of time with at least four more visits and inhibitor measurements.

Of that, then we found that there were 75 percent of our 1,224 cohort hemophilia who were really eligible to fit into the analysis. The overall prevalent cases in that cohort were 7.1 percent and, of course, those were excluded. Of the remaining 852 persons who had an average of 3.7 years of follow up so that, during the analysis, there was a total of about 3,186 person years that were available for analysis.

What was interesting is that the low titer occurrence during this period of time was about 12.9 cases per 1,000 person years. There were eight high-titer inhibitor cases that occurred for an overall rate of about 2.5 cases per 1,000 person years.

So what I am going to do now is switch to showing you some data in terms of the incidence--and these are based upon incidence rates so that they really

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are rates of occurrences which are much more important than prevalence figures and probably much more stable.

What you see as 1999, the incidence rate was about five or six per 1,000 person years. During this period, there appears to be a trend in the upward direction in terms of rates of inhibitors of low-titer occurrences. In the last two years, there were much larger rates. I think we will come back to that in just a moment.

If you look at the low-titer incidence rates by age, race and severity, you can see, as you expect, the rates among mild are quite low, among the severes, and run about 15 or 16 per 1,000 person years. The rates among the different age groups occur here and, certainly, there is some variation that you might expect. But what was interesting to us that we hadn't expected is that the low-titer rates among people over the age of 41 appeared to be the highest.

If you looked at the occurrence of low-titer inhibitors among racial differences, the whites and blacks appeared to be approximately the same, and that is

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quite different, as we will see in a minute, from the high-titer inhibitors that occur.

One of the interesting aspects that people wondered initially was were there differences in individuals on prophylaxis or were there people receiving other types of therapy. You can see here the rates were pretty much equivalent for both.

If you looked at product type, the recombinant--and we grouped all of the plasma-derived in one group for this analysis, primarily because I think that, if you break it out into the different subtypes, it really takes even a much larger cohort than the approximately 1,000 we had here because these occurrences are quite low, within a few years period of time.

So it takes an extremely large database to be able to sort out those individuals but certainly the plasma-derived and the low titer appeared to be a larger rate than the recombination if you look at those that received both.

What Mike did on this was really not only look at the material they had received in the last six months but went back prior to six months to their prior visit

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and so they examined both of those. If they had received two different kinds of products, recommendation with the plasma, then they really got into the mixed type. So these were individuals that had actually received, reported only either recombinant or plasma-derived.

Now, we do collect a specific type of product that individuals receive. What we don't do is collect the amount. This UDC was not designed initially to look at the inhibitors as an inhibitor study. Those questions could be added, but it was really designed to monitor gross outcome data. So specific kinds of questions that you might like to have answered, we wouldn't have the data on this amount of material. We would have to go back and add those questions in the data types of collection.

If you looked at the high-titer inhibitors by year of incidence, you can see that the incidence rates remained in the neighborhood of three to four. There is, in 203--it is hard to say what this means because this is data only on half a year and you have only got one patient with an inhibitor, although this is a rate. You have to realize these are the same group of patients

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being followed every year so that it is almost impossible to say anything about this individual in here.

What is interesting is you can see the incidence rate among the different age groups is approximately the same except for 11 to 20 which appears to be maybe a little lower. But here you have a tremendous difference in the white/black racial difference among the high titers. Then this represents where we are seeing the rates among the moderates and severe which are not statistically different on this slide.

I think that, if you, again, look at type of treatment and product type which is all Mike was able to do in the time we had for this presentation, you can see that prophylaxis and other rates are 1 to 3 percent and certainly recombinant and plasma-derived materials are essentially the same for the incidence rates in terms of the high titers.

Well, in summary, you can make some conclusions about the data we have here. There are a couple of numbers wrong here and I will point those out. First of all, the incidence of inhibitors is quite low which means you need large databases to really draw any conclusions

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in terms of monitoring outcomes, after-market product monitoring.

This is really about 1.2 percent per year for low titers and, again, this is about 0.4 percent. These numbers were typos that we missed when we reviewed the slides. The rates appear to be increasing over time in the low titers but I think that we will see what that means in the future; is that normal variation or does it have to do with some of the kinds of things we don't quite understand.

The low-titer incidence appears to be highest in the 41-year-olds and the high-titer incidence appears to be highest among blacks. There doesn't seem to be a difference in terms of whether or not--the recombinant products do not appear, at this point, to be producing a higher titer incidence.

When you go back and you break down--I think what is more important than the overall data which I have shown you, in terms of incidence over the five-year period, what is probably going to have to be much more important is the annual analysis of some of this data on a year-by-year basis. That hasn't been completed for

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anything except I can give you some preliminary data which we were not able to make a slide on that looks at the low titer--and I want to go back and point out what I am talking about.

This apparent jump in incidence rates during the year 2002 and 2002, and was this related to one product or another; essentially, on a preliminary analysis of this, it appears to be that both those rate jumps were related to both recombinant as well as plasma-derived products. So there doesn't appear to be one of the type or the other that appears to be standing out in that rate jump for those two years. So I think that we will do some further analysis of this kind of data.

I want to apologize for not having the handout available because what happened is we attempted, on at least four occasions, three or four occasions, to e-mail our handout to the FDA and the federal system has a number of filters in it which keep out all kinds of attacks and so forth. So I think that either our computer or the FDA computer figured that mine was a computer attack and so it didn't load in the slides. So

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we brought them on one of the little magic discs that they have today.

I think that gives you an overview and the state of where we are now with data that we have in the UDC. Certainly, it is an incredible value to the hemophilia community because it does tell where issues are and it is very useful in monitoring a number of things besides just to safety of the products in terms of viral safety.

Thank you very much.

(Applause.)

DR. LOZIER: I actually have a question that is not directly related to your talk but you might be able to provide some information. As I recall, you are certainly following life expectancy and those sorts of trends. Are you seeing differences between patients with hemophilia that have inhibitors and not as far as life expectancy? It used to be a much greater gap and I had the impression it was narrowing in some data I have seen. But if you could comment.

DR. EVATT: We haven't specifically looked at the role of inhibitors in terms of life expectancy. When we did life-expectancy calculations, we did this

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predominantly on the HHS data which was a cross-section of not only--it was different from the UDC because, in that data sample, we collected data on all patients living in six states. That was a sample of 3,500. We reviewed charts, death records and everything. It was an extremely intensive analysis.

In that, the life expectancy of individuals that were HIV infected was 39. The life expectancy of individuals who were not HIV infected was 64.7 years as compared to 73 years for the normal male.

The major difference between hemophilia and the normal male was probably the hepatitis C and other kinds of chronic liver disease which was probably accounting for that difference. We see life expectancy increasing and we expect it to approach normal because none of our children have been infected with hepatitis C now since 1990. So I think that is going to make a major difference in terms of life expectancy in the future.

We could go back and try to do some calculations and see whether or not inhibitors, what role they really make. But we haven't looked specifically at that.

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DR. LOZIER: Dr. Golding of the FDA has a question.

DR. GOLDING: In terms of the--this relates to what Joan Gill was talking about, looking at things that may be risk-associated for this group. I think she mentioned that there is some evidence, or at least indication, that the children who are making the inhibitors may be more TH2-like. Does your database--would it capture if the children have a high incidence of ectopic diseases such as asthma or any other TH2-like disease?

DR. EVATT: No. It doesn't currently. It does collect information on a large number of other kinds of diseases but it wasn't really designed to look at these issues. One thing that the database does do is, along with the database, we also get a plasma sample. Currently, we only use that plasma sample for testing for the various bloodborne infections.

Routinely, the new infections that come along, we will go into the database and pull those and examine for things like West Nile and things like parvovirus as

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well as the regular known ones to see if they are related to a large number of other conditions.

If our data task force reviews this and says this is an important issue for the database to include, we can include any of those questions that could collect that kind of information. We could also, if it were decided the database, we could also go in and measure, do such things as genotyping. Our laboratory is a high through-put laboratory which is capable of doing a thousand genes a day as well as a large number of coagulation tests.

Our laboratory currently has that capacity. We could do that kind of thing if that is what the task force asks us to do. They really are our advisors in terms of working with us of what are important issues based upon the kind of information and data that is coming out of this.

I think this kind of surveillance doesn't preclude the kind of individual studies that investigators need to be doing in terms of looking at the basic kind of mechanisms and so forth. We don't do that kind of study. But, in terms of large population

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studies, as I say, the database with 10,000 patients is probably not duplicated anywhere and so it has the--if it is used for the right things, it can be a powerful tool. But it is not something you are going to use for individual kind of investigator research kind of projects.

DR. LOZIER: Glenn Pierce, I think, had a question.

MR. PIERCE: Glenn Pierce, Avegen. I am wondering, Bruce, you have got this enormous database and yet you are still being challenged to be able to look for the power through the low incidence of inhibitors. Could you also be biasing yourself because you are only selecting patients who have four visits in these four years?

DR. EVATT: Yes. I mean, the characteristics are the same. Rates are rates. It is much less bias gets introduced into a rate than into a cross-sectional area. So the 10,000 is a cross section and it is a big sample. But the rates are much better because they give you--the only reason we limit it to those is because we had the same patients in that cohort over a period of

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five years. And so you knew that we were not introducing new patients into the cohort.

Since the characteristics were the same that we were seeing in the rest of our database, we said it is probably a good assessment. What we would like to have is 10,000 patients where we had repeated visits. I think that it is one of the things that we are trying to figure out how to get more resources for our hemophilia treatment centers so this would increase the probability that we would get those.

Our hemophilia treatment centers are really, these days, pushed hard to do everything. What they really need is a data-management person in these centers. We are trying to figure out how we can get the resources to put a data-management person in our hemophilia treatment centers so they could really not only help the center with their research studies but also help us with collecting the kind of data that would help the whole community.

DR. LOZIER: Dr. DiMichele.

DR. DiMICHELE: Bruce, very nice. I recognize the fact that this is actually pretty preliminary data

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analysis because I know that inhibitors haven't been the focus of UDC so far. I am assuming by the way you define low titer and high titer by the usual greater than or equal to 5 or less than 5--is that correct?

DR. EVATT: Yes.

DR. DiMICHELE: I had just a point of clarification. What I get out of the database--initially, when you are presenting the non-incidence data, the first thing I thought about in terms of the increase in low titer and decrease in high titer was possibly the effect of immune tolerance on those statistics because you do collect, I know, data as to whether the patient is on immune tolerance or not. That is what I would have almost assumed.

But then, when you went into the incidence data and actually showed the increasing incidence of low-titer inhibitors, there are a couple of things that, to me, would need to be clarified. The first is that, at once, low titers are increasing. They are in the youngest population except for the over-41. There is a blip in the over-41, but, in general, there is that peak in the 2 to 10s.

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Yet they are more likely associated with plasma-derived products as opposed to recombinant when that population is actually increasing being treated with recombinant in the United States. So I am a little bit confused by it and also the higher incidence in 2003 with actually fewer cases. So I guess the database, the denominator must be lower.

DR. EVATT: I think the 2003 case, you have to be very careful about. Again, it is low numbers. The 2002--we took it off the screen. If we could go back, because I put the actual numbers in the chart. The 2002 data, there were large numbers there and it had that high rate again. So there was a high rate.

If you looked at--Mike did the analysis actually. He is doing analysis now on some of this kind of information. When he looked at the analysis of plasma versus derived, one year it is here and one year it is there. They are pretty even. If you take the last two, 2003 and 2002, together and you average the rates between those two years, it is really 28 over the last two years for the recombinants and 35 for the plasma-derived.

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So there is not that much difference in terms of the jump. The recombinants jumped in 2002. The plasma-derived jumped in 2003. But, 2003 is not yet complete. But these are rates which are pretty good indications. So I think there may be something there I don't understand.

Now you bring up a very good question as to whether or not the drop in the prevalence is due to the fact that they are doing immune tolerance. These individuals that were included in the cohort have never had an inhibitor that we knew about so they were kind of virgin patients. They had to have one negative titer in order--so we knew that they had a negative titer than they have a positive titer where you can say, if it were really low, we could have missed it.

But it is a large database and I don't think so. But I think you bring up some very good questions. I think they are questions that we need to try to sort out in what we have.

DR. LOZIER: Is there one last quick question here in the back?

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AUDIENCE: You have heard formal comments. One issue is that you have a lot of missing values in your over-41.

DR. EVATT: Yes.

AUDIENCE: Indeed, overall your missing values would be unacceptable in a clinical trial because they were so high. Were the same people missing overall. That is one of the questions. The other thing is, related to that, is that there is a very clear longitudinal aspect of these data that you have gotten the titers over time and that would be something to consider looking at, of taking account of, the longitudinal aspect.

Finally, something about the times your inhibitor development, basically a survival analysis, from the time people entered the study and the endpoint, of course, would be development of inhibitors.

One other point that was mentioned; were all of these patients PTP or is there a mix of PUPs and PTPs?

DR. EVATT: All these patients were over the age of two. So, presumably, they had all been treated in the past. You bring up a good point about the missing data

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for people over 41. That was the data for the prevalence data not the incidence data. So the incidence data is quite different. We pulled out that group of individuals that were regular customers through the HTCs and seen.

There is always the possibility of introducing biases in any kind of sampling technique. We would like to get that, of course, up and we would like to get more complete data. And we would like to have the instrument actually designed a little bit or if that is a high priority, we would like to have the instrument a little bit better designed.

Also, we have the capacity to--we are getting the blood samples, the plasma samples; we could do the titers all in-house as a service function. All these titers were done by the individual HTCs. We are taking their word that it was 1 Bethesda unit, 2 Bethesda units or 6 Bethesda units.

But, for this kind of information, that is quite okay. We could get more complete titers, though, if we turned around, if it became a priority enough to do them. It just is how high a priority is that compared to all of the other priorities that need to be done. Certainly, it

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is not beyond the capacity of our laboratory to do that sort of thing.

DR. LOZIER: Before we break for lunch, I will mention that lunch is available downstairs in the cafeteria here in Lister Hill. I understand there is some renovation and the service may be a little bit limited. There is also food available in the Natcher Building which is Building 45, straight out the front door of this building.

Any speakers who have not brought their slides forth for the loading for the afternoon, please bring them up. We will reconvene with Dr. Weinstein moderating the afternoon at 1:00.

(Whereupon, at 12:00 p.m., the proceedings were recessed to be resumed at 1:00 p.m.)

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

(1:00 p.m.)

DR. WEINSTEIN: Good afternoon. Welcome to this afternoon's session of the meeting. My name is Mark Weinstein. I am in the Office of Blood Research and Review at CBER. This afternoon, we are going to have this portion of the meeting directed more toward regulatory questions.

Our first speaker, I am very pleased to announce, is Dr. Rainer Seitz. Dr. Seitz is the Head of the Department of Hematology and Transfusion Medicine at the Paul Ehrlich Institute in Langen, Germany. He will talk about the requirements of the European Medicines Evaluation Agency.

Dr. Seitz.

Requirements of the EMEA

DR. SEITZ: Good afternoon. Ladies, and gentlemen, dear Mark, dear Jay, first of all, thank you very much for inviting me to speak here. It is really a pleasure and an honor to be here.

I am working for the Paul Ehrlich Institute which is the licensing authority for blood products in

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Germany, so to say a little FDA for blood products in Germany. But I am also very much involved in the scientific committees of the EMEA in London and so I was asked to present about the requirements of the EMEA. I can say, also, on behalf of my colleagues in the EMEA, that we appreciate very much the opportunity to be here and to discuss with you our requirements.

With this conference, we have got a number of tough questions. I will try to answer these questions in my talk. The first question is what are the EU requirements regarding potential inhibitor formation induced by Factor VIII products for preclinical testing, clinical trials and postmarket surveillance.

The second question is what was the rationale for selecting clinical-trial parameters such as the number of patients enrolled. The third question is how does the EU assess the potential for inhibitor formation induced by Factor VIII products.

Let's come to the first question, preclinical testing. Of course, we would be very happy if we would have laboratory tests to assess the potential of new products to cause inhibitors. We think it is very

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important to have extensive characterization of new products, particularly for recombinant products and particularly in the case of modified products; so to say, if you have a product you have been marketing for some years and introduce a new virus-inactivation step or something like that.

But we had to learn that this characterization of the products, the biochemical characterize of products, is certainly not enough. In Europe, our thinking is very much influenced by an experience we had about clusters of inhibitors with certain products, particularly with one product which was a double-inactivated product with SD treatment and pasteurization. This experience has been mentioned already today as the Belgian experience. Strictly speaking, it was a Belgian, German and Portuguese experience.

It was a little surprising that the product really behaved differently in those countries. But the experience in Belgium was published and analyzed in a very nice way but there were some special things in this case.

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First of all, it was really a cluster of inhibitors and, in the case of Belgium, a high number of patients was switched to a new product at the same time. Then, after detecting the first bleeding patient with inhibitors, all the patients were tested for inhibitors and they found a lot of other inhibitors. It was altogether eight cases.

Then they switched again those patients to their old product and the inhibitors disappeared. Another important thing with these inhibitors was that most of them were type 2 inhibitors and most of them occurred in previously treated patients.

When we analyzed the case in the laboratory, there were two proposals for a possibly predictive test. Very important was the work of the NIBSC in this case, Trevor Barrowcliff and Tony Hubbard. They found a slower Factor VIII cleavage by thrombin--this finding has, by the way, been also found by other laboratories--a more rapid Factor X:A generation and an enhanced phospholipid binding.

The company, themselves, they found a 40 kiloDalton impurity in part of their batches and they

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tried to demonstrate that this impurity would have been causative for the inhibitor generation and this could serve somehow as a marker for neoantigenicity. But this hypothesis could not be substantiated at all. Also, the proposers of the NIBSC have so far not found their way to be established as predictive tests.

The next question is animal studies. Of course, normal animal studies are not very helpful due to species differences in the immune response. There have been some proposals to perform studies in non-human primates but we think also this would be very difficult and very costly and still there would be some uncertainty about the meaning of the results and whether you can really transfer the results to the human behavior.

We heard today, and there are also some reports in the literature, that there are new animal models developed with transgenic animals. Maybe I should be more general and say genetically modified animals. We are looking forward very much to these developments. They are very promising. But, for the time being, I have to say we do not really have already good animal models for assessing these questions.

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So, for the time being, the mainstay of the assessment of the inhibitor potential will be clinical trials. We have, at the EMEA, a scientific committee, the so-called Blood Products Working Group. This committee, this working group, is elaborating guidelines for clinical studies with blood products and, of course, for today, those two guidelines are of particular interest.

You can find these guidelines on the website of the EMEA and can download the text so you can have a closer look on that.

The group also is working core SPCs and also gives scientific advice to applicants and the industry utilizes this possibility increasingly.

A very important fact which was already mentioned today, also by Gill White, is that we, in Europe, do not any longer formally require PUP studies since several years. In the current guideline, it is said that, for the question of viral safety, it is no longer necessary to use PUP studies because for the enveloped viruses, the transmission is such a rare event that you really cannot assess that in clinical studies

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anymore. In the case of the non-enveloped viruses, it is currently difficult to do it in clinical studies.

So, for this reason, PUP studies are no more required and also, for inhibitors, we think you do not really need, in the first place, PUP studies. We heard already about the rationale for that and we totally agree with that. In previously untreated patients, patient-related factors appear to be much more important in the product, in our view. Again, what impressed us very much were the two outbreaks of inhibitors really occurring in clusters in previously tolerant patients who were switched to modified plasma products.

It was apparent from this experience that the risk of inhibitor formation related to an individual product can be best evaluated in PTPs. And so this guideline recommends to study PTPs and not PUPs in the first place.

So what do we require in the European guideline? We require a PTP study. We say at least 50 patients. I think this number of patients will certainly be discussed this afternoon and I comment only very shortly. These

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PTPs should be more than 12 years of age. They should have severe hemophilia.

This is defined in this case as Factor VIII below 2 percent of normal. They should be immunocompetent. This is also a point to discuss; they should have more than 150 exposure days. Gil White told us that this may even may be a number to be discussed, that we should go possibly further in that. These patients should be followed at least 50 exposure days or six months.

Another aspect which is new in the European guideline and may be of possible importance is the Factor VIII consumption and efficacy. We have noticed at least reports that a lack of efficacy and an increased consumption can be an early sign of development of inhibitors. We had several of these case reports where this was evident and also what we heard today about the low inhibitors by Dr. Verbruggen points in this direction.

Of course, also the pharmacokinetic is important in this respect and this is not listed on the slide. In the European guideline, it is required to repeat the

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pharmacokinetics after at least six months. Also this could help to identify early inhibitor formation.

The Factor VIII inhibitor titer should, of course, be determined at baseline and then every three months. Of course, another important question is the methodology of inhibitor testing. Here we have a reference. We have already heard today. This is the Nijmegen modification of the Bethesda assay. Also, this question is, of course, a point which has to be developed in the future and I think the results we heard today are very interesting and very important for further development of the guideline.

Of course, this sounds a little bit unnecessary to say that, but I can tell you that it is necessary. We have, in the meantime, GCP inspections, good clinical practice inspections, in several big studies on recombinant coagulation products and really had very unexpected and very unpleasant findings with these studies. So it is really necessary to say that.

I said that we are no more requiring PUP studies. But we still require studies with the treatment of children. Children may respond differently compared

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to adults--there is a lot of science telling that not only in pharmacokinetics but also in inhibitor development--and so the guideline, the European guideline, requires that a phase IV trial, post-licensing trial, should be performed with at least 20 children under the age of six years.

These children, of course, should be tested for inhibitors every three months and, if there is any clinical suspicion of inhibitor development--and, again, here the Factor VIII consumption has to be documented and monitored very closely. This trial should not be started until data are available on at least 20 PTP patients participating in the PTP trial.

I would like two special cases of treatment modalities. One of them is continuous infusion therapy. This is practiced very much in the clinical setting but, from the regulatory standpoint, in most of the cases, this is not licensed, this posology. So the guideline includes also some requirements concerning the continuous infusion.

These are the requirements. I do not have to read all in detail. The important point is that, during

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the last ISTHSSC Subcommittee meeting, there have been preliminary reports about enhanced inhibitor formation during continuous infusion, particularly in PTP. And this is, of course, a point to be taken into consideration.

In my view, these reports are not yet substantiated and I am not sure whether this is really a true problem. But I think we should keep an eye on that.

The other point I would like only to mention very briefly is the immune-tolerance induction. So far, most of the products which are licensed, in Europe, at least, do not really have clear clinical studies to show the efficacy in immune tolerance. You know that it is discussed at the moment that the efficacy of products may be very different concerning the immune-tolerance treatment.

Postmarketing phase; on top of the studies I already showed you, there is also a requirement to perform a postmarketing study to assess clinical efficacy, immunogenicity and safety. This study protocol should be submitted with the dossier. That means, at the

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time of licensing, the dossier should also be submitted and, of course, licensed by the authorities.

Besides that, of course, Factor VIII products are part of the regular postmarketing controls as other medicinal products, also. They are, of course, subject to the normal pharmacovigilance system which means collecting any information on suspected adverse reactions. They have also the obligation to submit period safety-update reports about their products listing all the problems at the intervals stated here.

Then, lastly, all the data from the clinical trials and from postmarketing experience have to be included in the product information of the product in the SPC. The incidence of inhibitors in PUPs; you may say there is no PTP study anymore. That is right. There is no formal requirement for PTP studies but, if you treat PTPs, then you should document this treatment and present all the experience you have with the PUPs also in your product information.

Coming to pharmacovigilance; this leads us really to the actual problem we have. We had data about PTP inhibitors first in 1995 prompted by this inhibitor

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cluster with this double inactivated product I already told. In 1995, there were no reports available with any hemophilia Factor VIII product at that time on the market.

In the period between 2000 and 2003, our pharmacovigilance department in the PEI got reports about ten cases of PTPs with plasma-derived products and, altogether, 62 cases with recombinant products. This is at the first glance a difference but we are not yet sure that it is really a true problem again, and we have discussed the reasons for that.

It is a question of the observation. You can, of course, speculate that the old plasma products are not so closely observed as the new recombinant products. It is a question of the reporting compliance of the doctors, and so on. But, at least it is a kind of signal that we should look a little bit closer into the problem and the CPMP decided that there should be a request for information.

This request for information has been sent out to the marketing authorization holders of recombinant Factor VIII products. Responses will be considered by

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the scientific committees and the CPMP in the next few months. I cannot yet tell you any details about this review, but I can show you the main questions to be addressed by the companies.

The review really is about the worldwide cumulative number and reports of inhibitor development in PUPs and PTPs, cumulative information on inhibitor patients, cumulative worldwide patient exposure to each recombinant Factor VIII product and the number of units distributed worldwide to set this in relation, and narrative information on individual cases of inhibitors in PTPs so that we can really assess also the single individual cases.

For the moment, I can only say that the questionnaires are coming back and that we are quite optimistic that we could have some results of this review in the spring next year. Of course, this data will be very interesting.

Now, coming to the other questions, and I can address them only very shortly. The rationale for selecting clinical-trial parameters; I think the focus on PTP is based on experience with the product-related

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inhibitor clusters. We have already heard, by Gill White, the rationale for selecting PTPs to study the product-associated immunogenicity.

According to our experience, we really are focusing on inhibitor clusters. I think this is the thing we have to sort out at the first place. It will be very difficult to find very fine alterations of inhibitor incidence, that a product has a little more inhibitors than other products. That is certainly something you will not find out very easily, but I think it is crucial that we identify very early, before licensing products which really cause inhibitor clusters like the experience we had already.

Of course, the Bethesda assay is important for that and this assay should, of course, detect also type 2 inhibitors. The number of 50 PTPs is, frankly speaking, a compromise. Of course, we know that with this patient number, you will not detect very subtle differences in inhibitor incidence.

Again, I have to say what we want to have is to detect inhibitor clusters. For instance, the inhibitor cluster in Germany was 12 out of 141 patients and also,

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in Belgium, it was in the range of 12 percent of the treated patients. On the other hand, these studies should be practical and also feasible and this was really a compromise coming out of the long discussions.

Of course, you also have to consider that we have a compulsory postmarketing study. We think whatever patient number you pick, you will never be sure to have the problem solved. The prelicensing; we think you need also postmarketing study and particularly we need, of course, a phase IV study in children since there is no more formal requirement for PUP studies.

Then, at the end, this is my last slide, assessment of the potential for inhibitor formation. So far, the EMEA did not implement nor identify any preclinical testing predicting neoantigenicity. But I hope that this situation will be improved in the near future.

The current guidelines for clinical assessment focus on the detection of inhibitors, and I should say clusters of inhibitors in PTP. These requirements will be kept under continuous review and therefore we are very grateful that I can be here today and discuss with you

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and learn about new thoughts how to improve the situation.

Currently, a review of existing information on occurrence of inhibitors is underway and I hope that, early next year, we can communicate about that.

Thank you very much.

(Applause.)]

DR. WEINSTEIN: Thank you, Rainer.

We have time for some questions. Dr. White?

DR. WHITE: I think you raise an interesting and important point in this slide that you still have up in your first point, the neoantigenicity. There really needs to be some discussion of potential models for that at some point in time, perhaps here, but perhaps at some other point in time, too.

There are some animal models, some mouse models, that have been used preclinically to test neoantigenicity. They make some sense, but I think we have potentially better models now than we used to have with transgenic hemophilic mice and, perhaps, there ought to be something devoted to that maybe at the ISTH, in the

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Factor VIII-IX Subcommittee, perhaps at one of these kinds of workshops.

But I do think increasingly that is going to be a very important area and needs some emphasis.

DR. SEITZ: As I said, there are a lot of very promising things out but, you know, before they can go into a regulatory guideline, there needs to be some validation of these methods and so on.

DR. WEINSTEIN: Dr. Chang?

DR. CHANG: Andrew Chang from FDA. You did point out that you have 65--I forgot exactly the number, but you have a high observation for the recombinant product as compared to plasma. I was just wondering whether you are ready to disclose whether or not the marketing share between the plasma-derived and the recombinant product in your--

DR. SEITZ: That is very hard to say. These figures were spontaneous reports coming over the company's pharmacovigilance to us. These are worldwide data. So it is not easy to say which share. The share is already very different throughout Europe. There are countries which have already almost 100 percent

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recombinant and others still have about 50:50. But these are really spontaneous reports and they come from all the world. So it is very hard to assess that.

DR. WEINSTEIN: Including the United States.

DR. SEITZ: Yes. Everything the companies have; yes.

DR. CHANG: Another question is that you did point out in your slides that, in Germany, you have a cluster of 12 out of 141 on the PTP patients. Can you elaborate a little bit? What does exactly inhibitor clusters mean.

DR. SEITZ: This was exactly low titer same, or at least the manufacturer is exactly the same way as the Belgian product. The problem was that this product was already on the market in Germany and these data came from postmarketing pharmacovigilance studies.

The data came more or less from one big center in Germany and it was very hard to scrutinize these cases and find out the right consequences and, really, to demonstrate that this was really a product-related problem. Unfortunately, this case has never been really published in a proper way. But it was really 141

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patients and clearly, 12 of them got inhibitors, most of them type 2. I think nine of 12 were type 2 inhibitors and there was one really life-threatening bleed. One patient really almost died from this bleeding.

Of course, it was very difficult because these type 2 inhibitors are very hard to detect clinically. Particularly in this patient, it took weeks until it was realized that he had an inhibitor because you could always measure a certain residual activity in this patient.

DR. WEINSTEIN: One brief question. Dr. Silverman?

DR. SILVERMAN: Very nice talk. You outlined the requirements in terms of patient numbers and what you are looking for, but you haven't outlined for us what you would do with that, how you would analyze the data.

DR. SEITZ: I have to say I am not a statistician and I am not the right person to elaborate on the statistics here. We did some considerations about the patient numbers. It is a question what you want to do. Therefore, I stress so much that we want to be able

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to detect clusters of inhibitors, really products which have a high incidence of patients.

If you want to detect some increase over the normal inhibitor incidence, then, in the first place, you have to know the normal inhibitor incidence with such products, something which we do not know. Then, of course, you would need much higher patient numbers.

DR. WEINSTEIN: Thank you, Rainer.

DR. SEITZ: Thank you.

DR. WEINSTEIN: Our next speaker is Dr. Nisha Jain. Dr. Jain is a clinical reviewer in the Clinical Review Branch in the Office of Blood Research at Review at CBER. Dr. Jain will talk about FDA recommendations for clinical trials of Factor VIII products, our current thinking.

Dr. Jain?

Federal Recommendations for Clinical Trials

DR. JAIN: Good afternoon, everyone. I know it is after lunch, but please bear with me as I go through our current thinking on the clinical trials, recommendations of clinical trials for licensure of

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Factor VIII products and how it evolved to the current thinking which we have presently.

Today, in my talk, I am going to outline the clinical design of the products approved to date by the FDA, the types of clinical trials for approval of the new product, what was FDA's past thinking and present thinking, and how our current thinking evolved with respect to clinical-trial designs to support efficacy, clinical-trial designs to support safety of the product in relation to the immunogenicity.

My talk will not cover on preclinical studies and all my talk will contain will be information which is in the public domain.

Going to the licensed products, we can easily categorize them into two broad headings; plasma-derived and recombinant. The recombinants can be further classified as full length and B domain deleted.

To briefly review the plasma-derived products licensed in the U.S. are Hemofil M, Monoclate P, Monarc M, Humate P and Alphanate. The recombinant products are Kogenate, Kogenate FS, Recombinate, ReFacto and Advate, Advate B, the newest one licensed in the United States.

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The clinical trials for the plasma-derived products; most of these products were licensed in 1960s. The clinical-trial design was very, very rudimentary in those years and the licensure was mostly based on the PK studies. All the products which were licensed during that time showed a half-life ranging from 14 to 16 hours.

In the 1980s, all the plasma-derived Factor VIII products underwent a major manufacturing change. These manufacturing changes were either an addition of a purification step or steps, a viral-inactivation step or steps. For the licensure of these plasma-derived products which underwent manufacturing change, a comparative PK against the old product and, in the form of safety studies for inhibitor formation, no prelicensure requirement or number of subjects or exposure days was required in those days. All the information on immunogenicity of these products was obtained postmarketing.

When a plasma-derived product underwent a heat treatment as an viral-inactivation step--this could be either the single viral-inactivation step or in addition to another existing one, in addition to the PK study,

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comparative PK study, with the old predecessor product, FDA required that a phase IV postmarketing study be conducted to assess the rate of new inhibitors in the PTP population.

This was primarily based on the data which was available and we have heard about that data in the morning and in the afternoon, the Belgian experience, suggesting that the heat treatment can compromise the integrity and that function of the Factor VIII protein.

The requirements which were primarily in the 1990s, or I could easily say 1997, at that time and based on the CPMP guidelines, the sample size required was of 50. The exposure duration days for these PTP patients were two years. The safety endpoint was based on the assumption that the maximum biannual rate of observed inhibitor incidence of 3 percent. Then the one-sided 95 percent of confidence interval for this incidence would be, with a sample size of 50, 0 to 7.3 percent.

So, with a sample size of 50 evaluable patients monitored for two years, if more than three patients developed inhibitors with a titer higher than 0.7 Bethesda units and that persisted a month, the incidence

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would be deemed higher than that of any licensed product at that time.

Again, this was the thinking in 1997 or prior to that when the plasma-derived product underwent heat treatment as a viral-inactivation step.

Coming to the recombinant products. The trials for the initial licensure for these recombinant products, and now we are talking about early 1990s, the licensure was based on a comparative PK study against a licensed plasma-derived product. The efficacy studies for the treatment of bleeding episodes and surgical prophylaxis were originally required in PUPs but were later modified to include PTPs

With the earlier product, and as the FDA thinking evolved, the safety data on immunogenicity was collected mostly postmarketing with minimum requirements premarketing. But this, later on, changed as the FDA thinking evolved. In the late 1990s, when any product underwent a major manufacturing change, for licensure of those products, a comparative PK with the predecessor product was required and efficacy studies for treatment

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of bleeding episodes and surgical prophylaxis was no required in both PUPs and PTPs.

The safety study was now only required in PTPs. The PUPs patients were not required to be studied for safety information. But these were replaced later on by pediatric studies. The information on immunogenicity of these products were to be available prior to licensure. We were no longer depending on postmarketing availability of these data, the safety data.

In terms of safety endpoints, the number of subjects which were to be treated would be at least previously treated patients following for no less than 50 exposure days for development of all types of inhibitors.

The reason we chose the sample size of 80 subjects and the evaluation for 50 exposure days was that, if 80 subjects are evaluated for 50 exposure days and none of them develops an inhibitor, that outcome enables one to rule out, with a 95 percent confidence, a frequency of true inhibitor rate of more than 4 percent.

However, if one patient, out of 80, developed an inhibitor, then it rules out, at the 95 percent confidence interval, a rate of 5.6. But if two patients,

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out of 80, developed an inhibitor, then the true inhibitor incidence--that is the upper bound of confidence interval--may be as high as 8.47, we felt, and we judged it clinically unacceptable.

This slide primarily contains the information which is publicly available. I see two typos in here. But this slide mainly covers the incidence of inhibitors in the IND studies for the recombinant products. Advate is the most recently licensed one. The number of patients with inhibitors over number of patients exposed to the product, in the Advate clinical-trial study, one out of 103 was reported, and the observed rate here being 0.9 percent.

The follow-up for the number of exposure days in this trial was more than 75 days. Based on this observed incidence rate, the two-sided confidence interval calculated was 0.02 percent to 5.29 percent. The type of inhibitor developed in this trial, in this one individual patient, was a low titer. The definition of positive titer in this trial was 1 Bethesda unit.

For the Refacto study, the number of patients who developed inhibitor was one out of the total number

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of exposed patients being 113, the true observed incidence rate being 0.9 percent. The follow-up period or number of exposure days for this trial was more than 50 days. The two-sided confidence interval calculated on the observe incidence rate was 0.02 percent, the upper bound being 4.83. The type of inhibitor developed in this patient was high. The definition of positive inhibitor in this trial was 0.6 Bethesda units.

This should be Recombinate, not Recombinant. Recombinate; no patients developed inhibitors in this trial, in this clinical trial, out of 142 patients. The number of exposure days they were followed was more than 70 days. So, based on that, the two-sided confidence interval is 0 and 2.56.

Unfortunately, the definition of positive inhibitor for this trial was not within any FOIable materials so I can't put it up here on the slide.

How our current thinking evolved over the last two years, actually. We were calling a new product any new molecular entity or manufacturing change of an existing licensed product. The indication sought for that Factor VIII product was to control and prevent

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hemorrhagic episodes in patients with hemophilia A or for surgical prophylaxis in patients with hemophilia A.

The trials which were needed to support licensing of these products were a comparative PK study. Unlike the past years, now we are requiring manufacturers to do a comparative PK with a licensed plasma-derived product against the new product, not what we have done in the past against the predecessor product.

The PK study would show that the 90 percent confidence interval for the ratio of the test product over the reference product for all primary PK parameters should fall with the interval of 0.80 and 1.25.

The recovery of the product before and after 50 exposure days for safety and efficacy study is also required.

The trials needed to support licensure in terms of efficacy studies were mainly required in PTP patients for treatment of bleeding episode and surgical prophylaxis. No PUPs study is requirement. The efficacy and PK studies in the pediatric population could be done post-licensure as a phase IV study but the protocol for

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this study has to be submitted and approved prior to licensure.

The trials to support safety were to be conducted in mainly PTP population. Actually, the PTP population, the definition of PTP being heavily treated, more than 150 exposure days--but maybe we will have to rethink this exposure days based on Dr. White's presentation this morning--the PTP population, having no previous history of inhibitors, they being immunocompetent, and the inhibitors to be detected in these, the definition of inhibitors in this PTP population is mainly the sponsor's responsibility.

The sponsor has to clearly define in the protocol what would be a low and a high inhibitor, what would be the cutoff value of a low inhibitor, what assay would be used to detect these inhibitors and what assays would be--and how will the confirmation of the positive inhibitors be done. But, overall, all inhibitors, high or low, will be analyzed as intent-to-treat for the primary safety analysis.

Now, the acceptable endpoint for the safety analysis was based on the sample size of 80 and a minimum

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of exposure days of 50 exposure days. The sample size of 80 would rule out a 6.8 percent as the upper bound of the two-sided 95 percent confidence interval for the rate of all inhibitor incidence by intent-to-treat analysis. Dr. Ng is going to go into the details of how we arrived at this.

The postmarketing studies could be done for additional indications. That would be continuous infusion or routine prophylaxis for each of these protocols to be submitted to the FDA for review. We would strongly recommend every manufacturer to set up a pharmacovigilance registry so that the safety data on inhibitors is made available to the FDA in a timely fashion to change the thinking.

(Applause.)]

DR. WEINSTEIN: Thank you very much, Nisha.

We are open for questions. Yes; Dr. Larson?

DR. LARSON: I am sorry to have to point out that Bayer has a plasma-derived product in the marketplace called Co-8 DVI. I noticed that the Kogenate studies were also missing from your slide.

DR. JAIN: I'm sorry; can you please repeat?

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DR. LARSON: I said Bayer has both a plasma-derived product in the U.S. marketplace as well as a recombinant product that was missing from both of your slides.

DR. WEINSTEIN: Co-8 and Kogenate FS, rather important.

DR. JAIN: No; I did put in Kogenate FS.

DR. WEINSTEIN: I think it might have been missing.

DR. ALEDORT: Dr. Aledort, New York. I am just a little concerned on the issues of getting something through an IRB in terms of ethics in this environment asking a patient who has been on recombinant material exclusively to now go into a study and take a plasma-derived. This is not a personal statement but it is a statement for those who really believe you should never switch. It will restrict, as one of the issues brought up by the previous speaker, the ability to recruit patients who have been only on recombinant.

DR. JAIN: FDA actually considers the plasma-derived products to be safe and effective at the present time.

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DR. ALEDORT: So do I.

DR. JAIN: And plus the plasma-derived products, the PK parameters of those, are considered as gold standards, being very, very clearly--you know, very close to the endogenous occurring Factor VIII products. So that is what went into our thinking introducing Factor VII plasma-derived product as a comparator for PK.

DR. WEINSTEIN: Dr. Feingold?

DR. FEINGOLD: With all due respect, as a treating physician, I don't think that it really matters whether the FDA regards the plasma-derived products as safe or not. It is what the patients think and what the community thinks. So, of course, you regard them as safe. Otherwise, they wouldn't be on the market. I think most people in this room would agree, but it doesn't really matter because it is what the patients and the treating physicians have to think.

DR. WEINSTEIN: Dr. Larson?

DR. LARSON: The other point I wanted to at least put forward in front of this audience is the concept of doing a comparative PK study with the confidence intervals that you have presented there

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because, hopefully, going forward in the future, we will have products that exhibit better behavior in terms of pharmacokinetics and that would certainly be a boon in terms of improvements in therapy. So we should probably think about how those parameters would be defined for a product like that.

DR. WEINSTEIN: Of course, there always may be questions about whether you are leading to a more thrombotic situation, too, longer half-life--

DR. JAIN: What I put was minimum, showing the minimum comparability between the two products.

DR. WEINSTEIN: Dr. Golding?

DR. GOLDING: I notice that there were quite a few differences between what the Europeans do and what we do. Just two of them that I would like to maybe address and others maybe can think about after the meeting. But one is that I thought that Dr. Seitz said that in Europe they have a second PK trial and that would detect inhibitors that are probably involved with clearance due to PK, clearance rather than actually inhibiting function. I would like to know what is the importance of that and is this something that we need to think about.

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DR. JAIN: Actually, that is our current thinking, also. We are also requiring now to do the recovery of the product after 50 exposure days so that if the inhibitors were not--if the interval between the inhibitor detection was not done maybe after 50 exposure days. If there is a change in the recovery, it tells you something about it.

DR. GOLDING: The other thing that I think is important, maybe not different but is important, is the pharmacovigilance idea and the postmarketing. Do you want to comment on what is our experience with postmarketing with a product that was approved and were we able to get the data that we wanted. How effective is the postmarketing strategy here?

DR. JAIN: Postmarketing, right now, is all voluntary. So what data we get is voluntarily based on who submits it. The postmarketing data becomes very important and more meaningful if every information, every information, is submitted to us. Then we have the numerator and we have thd denominator to assess what is the incidence of are we going in the wrong direction or are we going in the right direction. But, right now, it

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is all voluntary. If the manufacturer submits it, we have it. Otherwise, we don't have it.

DR. GOLDING: But is the track record that we get the information from those studies?

DR. JAIN: I don't know. I haven't gone through the track record as yet. But one thing which you actually pointed out the difference between EMEA and FDA is what I noticed was the EMEA requires a postmarketing follow-up study but, to date, only if they are changing, they want something additional like continuous infusion indication or a prophylaxis indication, then they are doing postmarketing. We do not require any manufacturer to do a postmarketing study unless their previous trial has not supported what they have asked for.

DR. WEINSTEIN: Dr. Pierce, did you have a question?

DR. PIERCE: Just a quick comment going back on the earlier point of IRBs being more hesitant in supporting trials that would compare a licensed plasma-derived product to an investigational new clotting factor just to emphasize again that you can't presume that which you don't formally already know. We have to remember

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that the purpose of these premarketing pivotal phase III studies is to evaluate what is formally an investigational product whose safety as well as efficacy is formally, technically, as yet unproven.

DR. WEINSTEIN: Dr. Lusher?

DR. LUSHER: To follow up on this, this concept of the comparative PK study, for patients who have been only on recommendation products, to have them enroll in it, have to go on a plasma-derived product, no matter what you think about it or what anybody else thinks about it, you are going to remove a substantial number of patients from participating because they just believe that the recombinant products are safer and they have not been exposed to anything else.

So you are really going to cut--it is hard enough now to enroll patients in studies when there are lots of recombinant products out there now on the market so they don't have the incentive they did when the first recombinant Factor VIII products came out.

So it is hard enough to enroll them but now to tell them they have to go on a plasma product, I know practically all of mine will say, "No way; I am not going

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to enroll in that." So I think that has to be considered.

DR. JAIN: Dr. Lusher, your point is well taken but, as far as the comparative PK is concerned, the sample size is very small. Only when they go on to the efficacy or safety study, then the sample size becomes very large. But that is another thing to think about.

DR. WEINSTEIN: We take your comments to heart, here. Dr. Gill?

DR. GILL: I would just like to echo again what Jeanne and others have said about requiring patients to be on a plasma product for a PK study. I really don't see why you can't choose one recombinant product and use that as a gold standard instead of the numerous plasma products that might have different PK values themselves.

DR. JAIN: Let me ask you, which one would you choose?

DR. GILL: I don't think it makes any difference. I think you just have to choose one and then you can use that product to compare the other products.

DR. WEINSTEIN: Yes?

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DR. REIPERT: Reipert, Baxter. We have heard several times today that we might have future products that have an altered structure and altered sequence in order to increase half-life. Have you already considered implementing additional test systems to sort of exclude safety issues or immunogenicity issues since I think, if you have altered structures, then we might have to think about new test systems.

DR. JAIN: We have not implemented anything but we are thinking towards when we see any--I mean, this is a good point and we have to think about it, to be specific.

DR. WEINSTEIN: Dr. Bergman.

DR. BERGMAN: Garrett Bergman, Philadelphia. Just one question. In looking at the requirements for licensure, 80 patients, 50 exposure days. It is not specified here if that could be met entirely by a prophylaxis regimen in all patients or how many actual episodes of bleeding do you have to demonstrate efficacy in?

DR. JAIN: In the safety study, patients on prophylaxis can be included because it is easier to get

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to the 50 exposure days, the minimum of 50 exposure days, by taking those patients, you know, if the trial is not too long.

DR. WEINSTEIN: Thank you, Dr. Jain.

DR. JAIN: Thank you.

DR. WEINSTEIN: Our next speaker is Dr. Tie-Hua Ng. Dr. Ng is a statistician in CBER's Office of Biostatistics and Epidemiology, Division of Biostatistics. He will talk about sample-size determination in the safety evaluation of Factor VIII products.

Statistical Considerations for Design of FDA Clinical Trials

DR. NG: When I make presentations at statistical conferences, I always require to put this disclaimer. To be safe, I have this disclaimer here, too. The title of my talk is different from but more specific than what is in the agenda.

As you know, the inhibitor formation is the major safety concern of the Factor VIII products. My talk today focuses on the sample-size determinations in

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the safety evaluations of the Factor VIII products, in particular the inhibitor formations.

I will not present any complicated formula. Everything will be very simple and straightforward. I promise. Read my lips. No complicated formulas.

Here is the big question. How do we determine the sample size? Actually, this is not that big.

This is really, really big.

As a statistician, I am often faced with this question. What sample size do I need? For these short questions, I have a simple answer. Here is my magic answer. It works for any study. Without knowing the study design and the study objective, this is the best I can do. So we need to talk about the study design and the study objective.

The studies to date have lacked concurrent controls so we need to rely on the historical data. In other words, we would compare the inhibitor-formation rate of the test product with an upper acceptable limit. The rate here refers to the proportions of subjects who develop inhibitors as opposed to the rate per unit time.

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The study objective; for safety evaluations, we want to show that the inhibitor-formation rate is low. In other words, we want to show that the true inhibitor-formation rate of the test product is less than an upper acceptable limit. I want to emphasize that this is the true inhibitor rate, not the observed inhibitor-formation rate. We don't know what the true rate is.

But, then, how could we make such comparisons if the true rate is not known? This is where the statistics comes into play. We could compute the upper confidence limit based on the observed rate and then compare the upper confidence limit with the upper acceptable limit.

The question is, how low is low; that is, what upper acceptable limit should we use. Should it be 1 percent? I think it is too low because it is almost impossible to show that the upper confidence limit is less than the upper acceptable limit.

30 percent? Well, I think it is too high because it is clinically unacceptable. So we need to make a cut somewhere in between 1 percent and 30 percent. Where should we make the cut? I will come back to these questions later.

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I want to note that if the upper confidence limit is less than the upper acceptable limit, then the true inhibitor-formation rate of the test product is most likely to be much lower than the upper acceptable limit. In other words, we have low chances of being successful.

We know that the sample size depends on the upper acceptable limit. A smaller upper acceptable limit will lead to a larger sample size. It also depends on the confidence level. If you want a higher confidence level, then we need a larger sample size. Finally, it also depends upon the number of inhibitors that are allowed to be considered a success for the trial. So, if you allow more inhibitors, then we need a larger sample size.

Has anyone heard about the rule of 3? It is a very simple and useful tool that could be used for sample-size calculations. For a large n, such as at least 20, if no inhibitors are observed, then the rule of 3 states that the one-sided 95 percent upper confidence limit is approximately 3/n. Note that this does not apply if one or more inhibitors are observed. It also works for the 95 percent confidence level only.

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If you consider 97.5 or 99 percent, then it doesn't work. Finally, it works for a large n. Let's see how we can use this rule of 3 in the example.

Suppose we set the upper acceptable limit to be 10 percent and the confidence level has to be 95 percent to use this rule. Recall that the rule of 3 said that the one-sided 95 percent upper confidence limit is approximately 3/n if you observe no inhibitor. So we set 3/n to be the upper acceptable limit which is 10 percent. Our n is 30. To be more accurate, n is 29.

I want to emphasize that if one inhibitor is observed out of 29 subjects, then the study fails because the upper confidence limit would exceed the upper acceptable limit. To allow for one inhibitor, we need more sample size. The next slide will show you how to come up with this sample size.

You have seen this 29. That corresponds to upper acceptable limit of 10 percent and the confidence level of 95 percent. So, if you observe one out of 29, then the upper confidence limit is 15 percent. So the study fails. To allow for one inhibitor, we need a sample size of 46 because, if you observe one out of 46,

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then the upper confidence limit will be just below 10 percent; that is, if you observe one out of 46, the upper limit is 9.9 percent. But if you observe one out of 45, then the upper confidence limit exceeds 10 percent. So that is why this is the minimum sample size.

Note that, if you increase the confidence level from 95 percent to 97.5 percent, then the sample size would increase from 29 to 36 here and from 46 to 54 here. Note also that, if you decrease the upper acceptable limit from 10 percent to 5 percent, then the sample size will be double.

Here is the FDA current thinking, as you have heard from Dr. Jain. The analyses will be intent-to-treat and the upper limit of the two-sided 95 percent confidence interval for the inhibitor-formation rate is less than 6.8 percent. So implicitly we use a one-sided 97.5 percent confidence level and the upper acceptable limit is 6.8 percent.

Why 6.8 percent? Essentially, it allows one inhibitor out of 80 subjects. I will come back to this question later.

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In setting the upper acceptable limit, we should realize that the inhibitor-formation rate depends upon many factors. For example, it depends upon whether the patients have or have not been previously treated. That is a PTP or PUP. I think FDA accepted the PTP. It also depends upon the definitions of inhibitor formation such as the lowest unacceptable inhibitor level.

It also depends upon the duration of exposure; that is, the number of exposure days. FDA recommended at least 50 exposure days. It also depends upon the assay for detecting inhibitor formation.

There could be other factors such as you have heard some of the speakers this morning; the disease type, the disease severity and the patient characteristics such as age, sex, and so on.

To set the upper acceptable limit, I think it should depend upon the inhibitor rate for the historical data. For example, if the historical data is 1 to 2 percent, then 6.8 percent may be too high. If the historical data is 3 to 4 percent, then 6.3 percent may be reasonable. So we need to ask the inhibitor-formation rate for the historical data.

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Why 6.8 percent? Where does 6.8 come from? I think it is driven by the data. Now, the upper limit of the two-sided 95 percent confidence interval for the inhibitor-formation rate for observing one inhibitor out of 80 subjects is 6.77 percent. To pass the outcome of one inhibitor out of 80 subjects, the upper acceptable limit is set at 6.8 percent.

Scientifically, we should determine the upper acceptable limit and then come up with the sample size. But, in practice, that is not always the case. As you have seen here, we have a sample size of 80 and we allow one inhibitor. So then we come up with the upper acceptable limit. So it is kind of like back calculations.

In summary, I have shown you how to determine the sample size for a given confidence level, upper acceptable limit and the number of inhibitors that are allowed. We need to estimate the inhibitor-formation rate for the historical data so that the upper acceptable limit can be determined.

Here are two interesting topics that were not covered in this talk. The power; well, I just want to

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say that the statistical power depends upon the upper acceptable limit, the confidence level, the sample size and the true inhibitor-formation rate of the test product.

Another topic is this. If the study fails--that is, the upper confidence limit is greater than the upper acceptable limit--can we enroll more patients? So these are the two interesting topics.

Maybe--just maybe--I would see you again next year.

Thank you.

(Applause.)

DR. WEINSTEIN: Thank you very much, Tie-Hua. We have very limited time here. We will have two questions.

DR. MAGUIRE: Thank you. I'm Bob Maguire from Wyeth. I really just have two things to raise. I think firstly, and I know there will be discussion of this later, we are assuming that an inhibitor is an inhibitor is an inhibitor and that we count them all. I don't think that is true and the clinicians in the audience ought to comment on whether a very transient low-titer

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inhibitor is the same as a high-titer inhibitor associated with bleeding.

But the real point I want to ask you statistically, and I am not a statistician, if you do an 80-patient trial, and you have made the comment that 6.8 might even be too high a number if 2 percent is the true incidence rate. Have you calculated that if 2 percent is the true rate, what the probability of exceeding that upper bound is in an 80-patient study because I know, or I suspect, it is very, very high if your true incidence is 2 percent, which I think drives the community towards a larger-sized trial. That is where I think this will go. If that is the number you want, we will have to do bigger trials.

Secondly, you are talking about intent-to-treat for sure and if you remain with that upper bound, I think it is going to make it a little more difficult to accrue to these studies. So I think we are going to do bigger studies. We are going to have to go out and make sure nobody has had an inhibitor, even low-titer, in the last, I don't know, ten years, maybe.

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It is going to be hard accrue to such studies and I think the risk of exceeding the upper bound is going to very high and I don't know who is going to take a chance with new products to really study them under these conditions.

Thanks.

AUDIENCE: I just want to make the comment--I just wanted to make the comment--I just wanted to emphasize on Dr. Jain's slide that in our experience of premarketing studies of recombinant Factor VIII products, we had the last three products that were approved had an inhibitor incidence, an observed inhibitor incidence, ranging from 0 to 0.9 percent, so all under 1 percent for the actual premarketing studies for recombinants.

DR. MAGUIRE: That may be intent-to-treat. It may not. But we have seen a large observational experience in Canada that was presented today. The number was 2 to 3 percent.

DR. NG: The reason I didn't present anything on the power is that--one thing, because of the time and for another, that is the sponsor's risk.

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DR. WEINSTEIN: I am afraid we will maybe take some questions later at the panel-discussion time because of our time limitations. So hold your questions. Keep them in mind. You will have a chance to address the speakers later on.

Dr. Aledort is our next speaker. He is the Chair Professor and Mt.l Sinai Hospital in New York. He will talk about the data monitoring board in clinical trials.

Role of the Safety Monitoring Board in Clinical Trials

DR. ALEDORT: Thanks. You have just heard a lot about the design of clinical trials. One has to be aware that it is now currently recommended that all clinical trials have a data safety monitoring board. The question is why. There are really three main reasons. One is to maintain the integrity of the study, to maintain the independence of the study and, by that, we also protect the integrity of the sponsor, and to maintain the blindness of the data so we eliminate bias.

Those are the main reasons to do that. If one looks at one of the responsibilities, one of the first

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responsibilities is to actually review the protocol. Why do we do that? Very much for the same reasons we just heard the statistician say and that is so that the design can actually, in all likelihood, reach the goals of the study. That means that the statisticians have to be involved and help you understand the power and the numbers that are involved.

But, in addition, now not only suggested but mandated, that a data safety monitoring board must develop a charter. What are the charters really about? The charter has got three main elements. It has got the element to guarantee safety so that they are made aware of all adverse events and the second is to guarantee the quality of the study which has a lot to do with compliance with the study parameters. Three, is to evaluate the endpoints.

I think the membership is really quite important. The most important, noninvestigator. An investigator in the study cannot serve and must not serve as a member of the data safety monitoring committee. In addition to statisticians that may be involved in the actual analysis of data, involved with the sponsor of the

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study, there must be an outside independent statistician to evaluate the study.

The question is, what is the role of the sponsor in a data safety monitoring committee. The guidelines and rules are pretty straight on that in that the sponsor may very well be able to be a voting member or a nonvoting member of the committee at the will of the sponsor. However, the confidentiality agreement which has to be done with every member of that committee, also must be applied to the sponsor meaning that a sponsor then can't go home and discuss with any other member of the sponsor's organization the findings that are blinded in the study.

The question that comes up is who does the data monitoring committee really report. They report to only the sponsor. The sponsor can be an industry company of any kind that sponsors the study but it can also be a government agency. This has been certainly witnessed by studies, clinical trials, that have been sponsored by FDA, CDC and HSA as well as NIH. That is important that the confidentiality issue be totally adhered to.

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They gave me a short amount of time because I don't think I need any more, but the most important part of the final piece of this is what do you tell the investigators. The investigators are kept out of the data monitoring committee because, if they are apprised of blinded data as they evolve, we may enter tremendous bias into the study in terms of withdrawing patients, not entering certain kinds of patients, changing regimens of patients as these things evolve.

But expected outcomes are perfectly acceptable to not detail to the audience at all, meaning the investigators, but unexpected, like adverse reactions or experiences are mandated so that the IRBs in your own institution are aware of the complications of the therapeutic intervention.

But, in terms of blinded data, meaning things that relate to the outcome, they are not to be disclosed to the investigators until the study is either over or the data safety monitoring committee has decided that the study has to stop because of either adverse reactions that are well beyond what they think is reasonable or

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because you have reached an endpoint long before the study was necessarily supposed to end.

Although this is a very short presentation, it is an important part of being sure that the issues that we just heard most of the morning talked about are really followed so that, at the end, whoever the sponsor is, can, without any difficulty, present these data to the appropriate agency.

So, with that, I thank you for your attention.

(Applause.)

DR. WEINSTEIN: I think we will go on. The next part of our program will be devoted to presentations by industry about the results of preclinical/clinical trials as well as postmarketing surveillance.

Our first speaker will be Dr. Bruce Ewenstein. Dr. Ewenstein is the Global Medical Director for Hemophilia for Baxter. He will talk today about studies of Advate recombinant.

Industry Perspectives

Baxter

DR. EWENSTEIN: I would also like to begin by thanking Jay and Mark and all of the organizers of this

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meeting. I think it is an extremely important topic that we have addressed today. I have learned a lot already and I am looking forward to the discussion as well.

We, like the other speakers, I think, were asked to answer some very specific questions and I will try to do that and also tell you a little bit about our pharmacovigilance an a little bit more detail that I think gets at something that we heard earlier.

First of all, as mentioned, we are going to dealing with two different products here and I am trying to make sure that I am clear about which one I am talking about. Our two recombinant products, Recombinate and Advate, I thought should be the topic of this short presentation. Obviously, there are older data on some of the plasma-derived products which are still on the market as well.

The two are easy to describe sort of side-by-side. They are both made in CHO and they both are transfected with full-length human Factor VIII and von Willebrand's factor cDNAs. Now, the difference comes, of course, in the culturing conditions. In Recombinate, there is the presence of bovine albumin, insulin and

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aprotinin whereas, in Advate, those animal and human-derived additives have been removed.

There has also been a difference in the cell-culture process. We went from a batch re-feed with Recombinate to a continuous or so-called hemostat perfusion with Advate. Then, the final formulation includes human albumin for Recombinate and mannitol and trehalose as bulking and stabilizing agents in Advate.

So the products, themselves, are both full-length Factor VIII molecules and they have comparable close translational modifications including N and O-linked glycosylation, sialic-acid content and tyrosine sulfational key elements in the function of Factor VIII.

I won't go through a lot of the detail, but they have also been shown to be comparable with respect to some of the tests we heard earlier such as a rate of thrombin activation binding to phospholipid binding to von Willebrand's factor and the like.

With respect to specific activity, it is probably best to think about Recombinate before the addition of the albumin in which case you can see the specific activities are just about the same.

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So I thought I would actually start with the Advate immunogenicity evaluation in the now-completed pivotal study and then jump back to Recombinate which would include the pharmacovigilance data as well.

So, for Advate, we had an eligibility requirement of 150 exposure days which has become the recommendations, as you have heard. That, of course, would include any other Factor VIII product. Patients were excluded with any history of an inhibitor greater than 1 BU. Of course, that came out of the medical record and included whatever test was available at that center.

Now, we did fairly frequent inhibitor testing during the study. This occurred at study initiation and then prior to the PK evaluations and then, after 15, 35, 45, 60 and 75 exposure days during a required prophylaxis period and then, again, at study termination.

The question was posed to us about sort of what limits we looked at with respect to the assays and what assays we used. We have heard a lot of very technical and, actually, very elegant discussion about these assays before but I would agree that, although our lab, our

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reference lab, says that the Nijmegen can go down to zero, we heard before that is probably not true perhaps with the very newest version. But we have taken, as a practical limit, something above that.

With the Bethesda assay, although I have seen it written as sort of technically able to go down to 0.4, I think 0.6 is probably more realistic.

We tried to divide the world of inhibitors into high, low and also transient. We didn't hear too much about transient during the discussion and maybe this can come up later using the now standard definitions of 5 BU, and these would have to be confirmed in the central laboratory. For low responders, we would say anything between 1 and 5.

Now, transient inhibitors would be a subclass of the lows, therefore, with a titer of less than 5 but no longer detectable at study termination and also with a recovery that would be greater than--that should be deciliter--1.5.

Now, if the inhibitor was less than 1 BU, the plasma samples would be retested. What I didn't write here was the fact that, at least in the initial study,

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the pivotal study, the Bethesda assay, itself, the original Bethesda assay, was used as the initial screener and the Nijmegen, as you can see here, sort of used as a confirmatory assay. That probably doesn't make that much sense, really, because the specificity and sensitivity would be just as good using the Nijmegen from the beginning and that is what we are doing currently for all the studies past the pivotal.

Now, an important thing, and I think this harkens back to what Dr. Aledort just said, we decided, a priori, about stopping rules and the need for the DSMB evaluation. So we would suspend the study if we saw one high-titer inhibitor or a greater than one high-titer inhibitor or greater than two low-titer inhibitors. These were actually based on some of the same considerations that you heard in the previous two FDA presentations.

In reality, in the Advate pivotal study, only one subject tested positive out of 108. This was a low-titer inhibitor followed 26 exposure days. There was no symptomatology. The patient actually, and this is a funny story that I have told before, happened to be my

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own patient before I joined Baxter and the only patient, of course, who developed an inhibitor on the whole study, which is something I have never been able to totally live down.

But he was not really made to be on a prophylaxis study and withdrew. Only later did I find out, in fact, that he had this inhibitor from the central lab. Our own hospital lab didn't see it. But eight weeks later, I brought him back and the inhibitor was undetectable and he had a normal recovery and abbreviated PK study half-life.

In the follow-on studies beyond the pivotal-study surgery, pediatric and continuation studies, we haven't detected any inhibitors to date.

Returning back to Recombinate, there were, and you have heard this presented before so I will go just quickly through it, in the PTP study, there were no de novo inhibitors but there was one subject with a history of an inhibitor who had a transient low-titer inhibitor of 0.8 BU but with a decreased recovery and normal half-life. But he had that prior history.

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In the PUP study, inhibitor testing here occurred every three months. The overall rate was 30.1 percent. You can see the breakdown of high responders, low responders and the subset of transient responders. As we have come to see, the median exposure day was pretty early for the appearance of inhibitors. Here you can see specifically what the inhibitor-free survival curve looks like with the probabilities of developing an inhibitor at 10, 20 and 40 days of 0.13, 0.24 and 0.3 respectively.

Inhibitor development is a pretty rare event. Like so many rare events, there really is a role for pharmacovigilance that I think cannot be accomplished in prospective studies alone. We, like all of the manufacturers, of course, have an active pharmacovigilance program where reports are solicited, both spontaneous reports from treating physicians and any of the healthcare workers involved with the patient and also from the literature.

So we took at look at a ten-year experience with Recombinate in which we noted there were 89 documented inhibitor cases that had been prospectively collected in

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this fashion since licensure. At that time, and I don't think I am giving away any secrets, there was a total distribution of about 6.5 billion units of Recombinate and also of Bioclate, which essentially is the same product from the point of view of inhibitor risk.

We then set out to try to establish what the inhibitor risk was both looking at it in the traditional way of events per million IU distributed but also, to make it a little more palpable, try to create a model that would allow you to sort to sort of think about this as a percentage of treated patients. Of course, to do this, we had to estimate how the product that was distributed was actually being used.

The details of this, actually, have been submitted for publication so I will just try to give you a quick flavor of the modeling. First of all, we tried, from the reports, to figure out the extent of the prior Factor VIII therapy. We divided this into three groups initially, 1 to 50, 50 to 150, and greater than 150.

These sometimes came from very precise reports that we would receive from many of you folks. But sometimes, we had to use a certain amount of clinical

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judgment. For example, a 50-year-old man who had been bleeding twenty times a year, we assumed he had exceeded 150 exposure days.

Based on this, we eventually came to divide the patients into two groups; PUPs, which I arbitrarily said would be less than 50 exposure days, and PTPs, which would be greater than 50 exposure days. Why 50? Well, if you look at, for example, the inhibitor risk-free survival that I presented, that accounts for about 95 percent of the risk period plus or minus a couple of percent looking at each study. So I think it is probably capturing most of the risk to look at it this way.

We also had to look at the prevalence and incidence of hemophilia in the population. For this, we went to the CDC database and figured out that the products were probably distributed about 3 percent to PUPs and about 97 percent to PTPs.

We also had to sort of estimate how much a PUP and PTP would use. These are the numbers we came up with, about 50,000 and 150,000. Then, from these, incidence rates were calculated for all inhibitors and also for the high-titer inhibitors and we then also

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attempted to look at the predictors of inhibitor development using a multivariate modeling system.

So, again, I can only present sort of the highlights. Here are the patient characteristics. I think there are not any huge surprises here. I think, apropos what Dr. Evatt presented, you can see about 50 percent of the risk is probably being missed in the UDC because they are occurring under two, at least in our reporting system, but we do see the other 50 percent are being captured in the over-two population.

As you might expect, about 80 percent are occurring in severe. But it is also important to look at the 20 percent that are occurring evenly divided between the mild and the moderate. About 80 percent of the patients appeared in what I am calling PUPs here, but there was also a significant number in the above-150 exposure days.

If you look at the titers, though, about 50 percent are low and 50 percent are high. But many of the low are really low at less than one. Again, these are all comers from the lab. It is not in our lab.

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So the overall incidence in this ten-year period was 0.317 percent and about half of that risk, as I showed you, was in high-titer inhibitors. There were no lot-related clusters which, of course, is important to a manufacturer in terms of any process defect.

If you break it down, and this maybe is the most relevant to the PUPs and the greater than 50 exposure-day population, you can see there is about a 100 to 1 risk factor where the PUPs are about a hundred times more likely to get an inhibitor but with an absolute number of 0.12 percent in the greater than 50.

Of course, one of the questions that comes up is what is the gearing system here; in other words, how many are we actually seeing compared to the real number. It is a hard number to establish but, because there have been some periodic spikes in more active surveillance and registries that have been established in a few countries--France, for example. Canada we heard about--it has been possible to sort of estimate what that might be and it is probably around 5 to 1. But this is an approximation.

So just to summarize this piece, Recombinate appears to be of low immunogenicity but I think, perhaps

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more importantly even in terms of going forward with future data collection, is the fact that the post-licensure surveillance results have been able to confirm, in broad outline, what we saw in the actual formal prospective trials.

The data from the Advate clinical trials were also encouraging. It was that single inhibitor that we talked about and no inhibitor so far in the postmarketing surveillance. But, of course, the product has only been licensed in the U.S. and we are only a few months into the postmarketing period.

In terms of perspectives, obviously, we will have a chance to discuss this. I just really chose one thing to sort of mention here and that is that I think there have been several speakers that have been harping on the issue of animal models. I think that, truly, this is going to be of critical importance. I think it is getting to the point where one can imagine--well, we already have one non-native Factor VIII molecule and I think we are getting to the point where there are other non-native Factor VIII molecules that may have enhanced biologic activity that may be very useful. But

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immunogenicity is likely to be the single greatest limiting factor.

It is really difficult to imagine having to do into clinical trials with all of these sorts of molecules without having some sort of animal model. I can just say, broadly speaking, that Baxter is working on such models, some similar to what you heard, some others as well, and that I think that these will prove really necessary if we are going to go forward in terms of future Factor VIII development.

So let me stop there and try to keep us still on time. Thank you for your attention.

(Applause.)

DR. WEINSTEIN: Thank you, Bruce. We could have a couple of questions. If not, we will go on to our next speaker, Dr. Peter Larson, from Bayer Corporation. He is the Global Medical Director and he will present information about Bayer's recombinant products.

Bayer

DR. LARSON: Thanks, Mark. While they are putting the slides up, I would like to thank Jay and Mark for inviting me to present Bayer's experience. What I

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hope to do is talk about our clinical experience over the past fifteen years with our Kogenate molecule.

First, what I am going to do is talk to you about the description, generically, of the molecule, a little bit about our preclinical testing and then a couple of neoantigenicity studies that were done to support the license applications for both Kogenate and Kogenate FS and then move into the clinical studies. I think there has been enough discussion and enough of the history behind the prospective studies for these recombinant products.

I will talk first about the Kogenate studies and the Kogenate FS studies, some postmarketing studies that we have done and, finally, I will conclude with our spontaneous reports on inhibitor formation.

Both Kogenate and Kogenate FS are full-length recombinant Factor VIII products. They are produced in an identical BHK cell line under identical fermentation procedure. The procedure is a continuous perfusion one that runs for greater than 100 days. The molecule is not co-expressed with von Willebrand's factor. Both are

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immunoaffinity purified by the same monoclonal antibody against a light chain.

The real difference between the two products is in formulation. The original product was formulated with albumin. Kogenate FS is formulated with sucrose.

When both products have been compared in thrombin-cleavage experiments, they have the same thrombin-cleavage sites as plasma-derived Factor VIII as determined by Western blotting. Both the N and C terminal-sequence analyses concur with the cDNA sequence that was used and trypsin digests separated by reverse-phase HPLC are similar to plasma-derived Factor VIII.

We have done extensive carbohydrate analysis which is published in the reference as cited below. Essentially the only differences between plasma-derived that were observed in these studies were the presence of blood-group antigens in plasma-derived that were not present in recombinant Factor VIII and the presence of a gal alpha 1 to 3 gal group linkage in the recombinant Factor VIII which is a linkage that is not seen in higher primates and humans. As a consequence of that, we did further studies to look at recoveries and showed no

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difference between molecules with higher concentrations of this side chain and plasma-derived.

The box on the side just shows some of the typical characterization that we do for the products.

As part of a general preclinical package, we did acute single-dose and subacute studies in toxicity. Obviously, these studies are limited by the number of doses since these animal models will make heterologous antibodies. They were performed in four species listed here with repeat doses up to five. There were no issues observed and supratherapeutic doses. These following studies were not done again because of the immune responses and all the excipients used in manufacture are generally regarded as safe.

Our preclinical group developed an assay about ten years ago to attempt to look at neoepitopes on the recombinant Factor VIII molecule. This was a rabbit immunization study in which antibodies were raised in rabbits using Florenz adjuvant to either plasma-derived or recombinant Factor VIII and hyperimmune sera were derived from that and immunoabsorbed on a column against plasma-derived Factor VIII.

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Through a number of experiments, what was shown is that the immunoreactivity, or absorption, against plasma-derived was decreased in the same fashion with original Kogenate as it was with plasma-derived Factor VIII. Several positive controls were used in this experiment including using a partially B-domain-deleted molecule which showed positive results. This is not the same molecule as the marketed ReFacto product.

The results were also confirmed in a competitive ELISA and the conclusion was that any antibodies formed in the rabbit species that reacted with plasma-derived also reacted with Kogenate. They were depleted in the same manner and there were no new antibody specificities detected in that assay.

With the licensure of Kogenate FS, the study was repeated again only this time using Kogenate 0 as the molecule that was on the column and, again, the same results were observed, the conclusion being that, at least within this limited model, that there was no suggestion of neoepitopes.

I will now move into the clinical studies. I am going to try to briefly go over fifteen years worth of

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clinical experience with these two molecules. I have tried to group them according to PTPs and PUPs with the different molecules. Some of these studies have been published. Not all of these data are in the public domain, but I was asked by the FDA to try to put as much of our data forward as possible. So that is what we have tried to do here.

The top studies in all of these charts will represent, essentially, the licensure studies. This is the original Kogenate study that enrolled 103 PTPs. I think, as has been pointed out by a number of speakers this morning, the definition of PTP has evolved since 1988. So this trial included many patients who, although they had previously been exposed to Factor VIII products, did not meet the definition of either greater than 100 or 150 exposure days. In fact, several of the patients on this trial actually had very few exposure days.

The overall median follow up for the cohort was 4.6 years. A total of over 17,000 infusions were done on this prospective, well-observed cohort. I think Gil mentioned this morning there were two inhibitors in the study. Actually, what was detected were three, one of

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whom was subsequently determined to have a preexisting inhibitor.

The other patient did have what we considered to be a de novo inhibitor. This was a low-titer inhibitor in what was felt to be a true PTP with greater than 100 exposure days. The third patient was a patient from Greece who was undergoing surgery and, by report, had been infrequently treated. Actually, the patient's report was that he had never been treated but he was hepatitis-C positive so it was presumed that he had received blood products in the past.

So our interpretation of these data is that there was one de novo inhibitor observed in this cohort of 103 patients.

We have subsequently sponsored or conducted several postmarketing studies, most of these being in Europe. One of those studies of 13 patients showed one inhibitor in an infrequently treated patient. We don't have the number of exposure days that that patient had previously. It was a low-titer transient inhibitor at 0.78 Bethesda units; so, for a total cohort of 205 PTPs observed in prospective studies with Kogenate, over

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24,000 infusions, two inhibitors that were considered to be de novo and one of those was transient.

Moving to the Kogenate FS studies, we conducted essentially one licensing trial that was done partly in Europe and partly in the United States. The data were merged for submission. They are listed as the first two studies on this table. The period of follow up was approximately four years, a total cohort of 73 patients and there were no de novo inhibitors observed in this cohort.

A single patient, who had been multiply transfused on the study, underwent surgery and developed a low-titer inhibitor up to a peak titer of 2.6 Bethesda units. When we went back and looked at his enrollment studies, he had actually had a detectable inhibitor that was below the threshold of 0.6 so he was eligible to enroll but had had a measured inhibitor in the past.

The last three studies on this are small pharmacokinetic studies that were conducted but, again, there were no reports of inhibitors in those studies and we did a 20 PTP study in Japan for licensure there with

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over 1500 infusions and no inhibitors observed in that cohort.

I think this slide doesn't need to really be reviewed with this group, the fact that both the Recombinate and the Kogenate PUP studies really were groundbreaking clinical studies and that these several points have been brought up before, that the studies were--there was more observation in these studies with inhibitors being measured at least every three months and it brought to light the phenomenon of transient and low-titer inhibitors.

Subsequently, review articles have essentially confirmed that the results of these studies are comparable to the inhibitor incidence or, as Dr. White likes to say, prevalence in these cohorts compared with plasma-derived studies.

So, moving to these PUP studies, the first Kogenate study enrolled 102 eligible patients. Out of those, 65 were severe and I think the meaningful number there is that 19 out 65 developed inhibitors in this cohort, so an incidence of 29 percent. Twelve of those

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were high. Nine were low. The threshold for high versus low was considered less than 10 Bethesda units.

In the early Kogenate studies, the inhibitor assays were done at the local institutions. The confirmation of positive inhibitors were done at a central laboratory at the University of California at Davis.

Three postmarketing PUP studies were conducted worldwide, in Japan and in Europe, giving a total of 185 PUPs that were observed prospectively over 20,000 infusions including the development study. The number of patients within all of these studies, and you can see I have got listed there as best as possible the numbers that were low titer and high titer, 47 all told. If you use the denominator that includes the whole 102 PUPs in the original PUP study, the overall incidence for inhibitors with the experience with Kogenate in these controlled studies was approximately 25 percent and, as expected, for any Factor VIII product.

The Kogenate FS studies have just recently completed. 61 patients were enrolled into that study, again split across Europe and North America. One of

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those patients was found to have a pre-existing inhibitor on his enrollment sample and was not considered for an inhibitor analysis. The follow up was around 2.5 years. A total of nine inhibitors were observed in the whole cohort. Four in the European study were all low titer. Five in the North American study were high titer. A total of nine inhibitors or an overall observed incidence in this cohort of 15 percent.

A number of postmarketing studies have been conducted. I have mentioned a couple of them in the earlier slide on PTPs but I want to point out that we have recently finished up a Kogenate PTP study in Japan. This group enrolled 123, again by their definition, previously treated patients and it was a mixed group of patients with varying degrees of previous exposure.

The assays for this particular study were performed in the local hospital laboratory. The data at present were unverified. There were five PTPs that were determined to have inhibitors measured in that cohort that were felt to be potentially de novo. One of those, it couldn't be determined whether the patient had had a negative inhibitor analysis in the past.

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I think you can see below that I have listed the inhibitor titers. With one exception, they are all below 1 Bethesda unit.

We have three postmarketing surveillance studies that the company is conducting ongoing right now with Kogenate FS. Two of these are in Europe and one if these is in Japan. The enrollment numbers are listed there.

Dr. Carcao went over, in detail, the results of the study that we worked with our Canadian colleagues to conduct once Kogenate was licensed in Canada. We have a particularly beneficial situation in Canada in that, by contract, for the first five-to-eight years of Kogenate licensure, the predominant product being used in Canada was Kogenate and we were able to conduct the study, the results of which he has presented to you earlier.

When Kogenate FS was introduced into the market, we worked with the group in Canada to do a similar study looking at change from Kogenate to Kogenate FS. There are some minor differences in the study design, but some initial results are now available and, again, these will be presented at ASH. There were 354 patients enrolled. All 354 had an initial or baseline pre-switch sample.

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221 have had a second sample and 116 have had a third sample. Those samples are spread out every six to 12 months and, in all of those patients, there has not been observed a new inhibitor.

I want to just quickly finish up by the way we have looked at the spontaneous reports into our global drug safety database. This is a little busy slide. I apologize. Over the course of the reporting period which has been 1992 through today, we have had a total of 58 inhibitors reported into the safety database. Of those 58, and I won't go through the math there, we consider 34 of those to potentially be PTPs.

Two of those 58 were confirmed to have had a prior history of inhibitor. 22 of those 58 could be definitely defined as having less than 20 exposure days. Seven we could confirm as having between 20 and 100 exposure days. Sixteen had unassessable exposure data based on the information that was provided. Eight we thought were possible PTPs and three were felt to represent true PTPs.

So, in the interest of being conservative, we have taken everything but the PUPs and the two patients

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with prior history and considered those to be PTPs. During the period of time 1994 to 2003, we have distributed over 4.7 billion units of recombinant Factor VIII. I have done two calculations here to get a denominator. I think a couple of people have pointed out the inability to interpret these spontaneous reports without trying to come to some denominator over which to consider them.

So the first calculation, what I have done there is try to be very liberal and say that, in a 70-kilogram adult being treated on prophylaxis with 25 IU per kilogram every other day would use approximately 212,000 international units per year. Obviously, this is a highly conservative estimate of patient years of exposure in that a significant proportion of recombinant Factor VIII is being used in the pediatric population and there is little penetrance of full prophylaxis in adult populations in Europe or North America.

But, given that, that comes out with 22,000 patient years of exposure. So 34 divided by that comes up with a rate of about 1.5 per thousand patient years of exposure.

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On the other extreme, or perhaps not extreme, assuming that an adult patient bleeds once a month and gets treated with 50 IU per kilogram per bleed, a calculated dose of 50,000 IUs per year. That computes into patient years of exposure 94,000 or a rate of 34 divided by that is 0.4 per thousand patient years.

What I have chosen to do here is compare that to a baseline used from the Rosendahl paper on the case-control retrospective analysis of the Dutch outbreak of approximately four new inhibitors in PTPs per thousand patient years on standard therapy.

So, understanding that reporting to these global drug safety databases is probably limited, that these inhibitors are underreported to these safety databases, we still come up with, either conservatively or less conservatively, a rate that is less than has been published by the Dutch group.

So, just to summarize, we have had over 15 years of clinical experience with the Kogenate molecule over the course of 12 prospective studies. In 300 PTPs, we have seen two de novo inhibitors. One of those was low titer and transient. Five prospective studies in over

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246 PUPs, and the inhibitor development rate was as is expected for a Factor VIII product. At least two large postmarketing studies in Canada that have been done to look at switching of products did not show a problem with inhibitor formation and that our spontaneous reporting is at or below what has been reported in the literature.

With that, I will finish up.

(Applause.)

DR. WEINSTEIN: Again, we have time for a few questions, if there are any.

DR. CARCAO: Manuel Carcao from Toronto. This question could equally have applied to your talk, Peter, or to Bruce's talk and that is the role of postmarketing surveillance that both companies have been doing. In both cases, you presented the data but it is very much hinging on how many of the cases have actually been reported to the company.

In both cases, I think, for Baxter as well as for Bayer, if you had to make a guess a guess as to what percent of all inhibitors have actually been reported to the company in these postmarketing surveillances, do you think that the range would be more than 50 percent, 20 to

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50 percent, 10 to 20, or are we talking about maybe you are only capturing less than 5 percent of the actual occurrences.

DR. LARSON: I think I want to first discriminate between the postmarketing surveillance work and the spontaneous reports to the global drug safety database. The studies that I have reported in postmarketing surveillance have all been surveys where treaters are filling out, on a prospective basis, observations with their patients.

So an inhibitor that is not picked up on every six-month or every annual testing, as in your study, would no the detected in that kind of a cohort. With respect to the drug safety database, I wouldn't venture a guess as to what percentage is being missed. Certainly, we have seen more as each year goes by, a reporting of more and more inhibitors as people become more aware of these mechanisms to report inhibitors.

I would say that, as I have looked at those data, fully 75 percent of the reports have been high-titer inhibitors suggesting to me that the more serious

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the inhibitor, the more likely it is to be reported into the safety database.

DR. WEINSTEIN: Dr.Aledort?

DR. ALEDORT: I think that is a good question that he asked and that is always facing FDA. But I would just like to tell you about the post-surveillance that the Recombinate Data Monitoring Committee did on every PUP in the original 73 PUPs in the Recombinate. 65 of the 73 patients provided data. 41 gave us four to five-years worth of data. 41 of the 65 stayed on Recombinate and the others only took recombinant materials.

Three of the five transient inhibitors had recurrence of their inhibitor during that five years and three new inhibitors were defined in that group. This was with annual follow up and surveillance so there is a big discrepancy between that kind of surveillance and waiting for people to call you.

I think that is important and I think that is what needs to be done if we are really going to do post-licensing surveillance is follow the patients that you put into the studies.

DR. WEINSTEIN: Thank you.

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Our next speaker will be Dr. Garrett Bergman. He is the Vice President of Research and Develop at Octagen Corporation and he will review studies performed on recombinant porcine Factor VIII.

Biomeasure/Octagen

DR. BERGMAN: Thank you, Dr. Weinstein, and thank you to the organizers for inviting me to speak.

Today, I am going to be speaking on a new product that I am involved in developing called a recombinant porcine Factor VIII product that we are calling OBI-1. It is a little bit different, what we are going to be doing is a little bit different than what we have been talking about. First of all, I am only going to be reporting on preclinical data and not any clinical data because we don't have any yet. Secondly, as you will see, or may already know, this product is intended to treat patients who already have developed antibodies to human Factor VIII.

Octagen Corporation is a small start-up development company located outside Philadelphia. We have entered into a partnership with Ipsen Limited, which is a division of a European company, to develop OBI-1 and

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other Factor VIII products. The technology derives primarily from the laboratories of Dr. Pete Lawler at Emory University.

Ipsen Limited manufacturers and distributes the porcine plasma-derived Factor VIII product Hyate:C. In recent years, the supplies of Hyate:C have been severely constrained because of concerns about the starting plasma.

Now, I am going to talk a little bit about characterize of our product before I get into some of the data because I was asked to do that so you can get some context. OBI-1, by the way, stands for Octagen-Beaufour-Ipsen, so it is not Obi-1-Kenobi. It is manufactured in serum-free medium using a well-characterized BHK cell line that is expressed as 170 kiloDalton glycosylated B-domain-deleted heterodimer, cleaved into a metal ion-linked heterodimer. The specific activity can be measured approximately 12,500 by the one-stage clotting assay calibrated against an NIBSC porcine standard.

Here are some of the characterizations of the heavy and light chains with regard to which domains are

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in which, the molecule weigh and the number of amino acids present in each.

This shows some of the characterize with regard to glycosylation, N-linked and O-linked, and the sulfated tyrosine residues and the number of disulfides.

As I just said in my introduction, porcine Factor VIII utility is used only to stop or prevent bleeding in patients who already have an anti-human Factor VIII inhibitor. This would include patients with congenital hemophilia who have developed an inhibitor and also patients who don't have hemophilia from birth but have an acquired autoimmune antibody to Factor VIII.

The reason for its utility is that it can bypass the inhibitor in many cases because the antigenic determinates are different on the porcine molecule.

I am going to talk about two kinds of studies that we have done in the preclinical development of the product that have some relevance to our concern today of immunogenicity. The first is I am going to talk about our studies in the knockout hemophilia-A mice and talk about what we found there. That study was designed specifically to compare the immunogenicity of the

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recombinant product with that of the plasma-derived Hyate:C.

But I am also going to talk about some of the data that came out of our 90-day toxicology study in primates. Some of the things that we monitored, some of the things that we measured, have some indicators of immunogenicity that we will talk about.

So, first, in the hemophilia knockout mice. We tested these in the E16 knockout mice. These are CRM negative, I believe. The mice were presensitized with human recombinant Factor VIII weekly for five weeks to simulate what the clinical setting would be. In other words, humans that have hemophilia don't get Hyate:C as their first exposure to a Factor VIII product so we had these mice also not get porcine Factor VIII as their first exposure. We first induced antibodies to Factor VIII in them and essentially all of them became sensitized in this regimen.

Then the groups received either 110 or 100 units per kilo either the plasma-derived Hyate:C or the recombinant OBI-1 weekly for four doses and then were tested two weeks later. The antibodies were tested both

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in the Bethesda assay and by ELISA. The ELISA was done by two methods, one a specific IgG ELISA and another that is an IgG specific for Factor VIII ELISA.

In this slide, this is the results of the ELISA testing. You can see from this slide, the left three columns are the 1, 10 and 100 dose exposures to the recombinant product and the right three columns are the exposure to the plasma-derived porcine Factor VIII product. You can see that, at 10 and at 100 units per kilo, there was a significant difference in the amount of antibody produced against the products by ELISA.

Now, you would say, well, you would expect that. We know that Hyate:C is an intermediate-purity product at best and has many extraneous proteins, but this graph, this slide that I picked, depicts only the Factor VIII specific IgG that was tested in the ELISA, not against any other proteins but Factor VIII.

Looking at the inhibitors, the inhibitory antibodies, of these same mice, we see that there was no difference. At 1, at 10 or at 100 there was no difference in inhibitor titers that were generated against the recombinant versus the plasma-derived

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product. At 10 units per kilo, it looked as though there might be a trend, but it certainly did not carry over to the higher dose.

So the conclusion here was that Hyate:C generated greater nonspecific as well as Factor VIII specific IgG when tested in an ELISA and the recombinant in plasma-derived products showed no difference in immunogenicity when you looked at the inhibitor formation by a Bethesda assay. Okay; that was very reassuring to us.

Then, as part of our preclinical development in order to be able to study this product in humans, we did a 90-day study in primates in which we gave them either just the vehicle for OBI-1 or Hyate or three different doses of OBI-1. A portion were necropsied at different intervals as indicated, but blood samples were also drawn at Days 7, 28, 56 and 90 for a variety of safety measures including some that we are going to focus on, specifically the preclinical aPPT levels and the Factor VIII levels at baseline before their next daily injections for up to 90 days in this toxicology study.

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The preclinical aPPT values and Factor VIII levels are a reflection of either excessive amounts of Factor VIII in the circulation, which you will see because the higher the Factor VIII level, the lower the aPPT, or cross-reactivity of antibodies generated against the porcine molecule are cross reacted with their endogenous Factor VIII. Keep in mind that these monkeys don't have hemophilia. They have their own normal amounts on Factor VIII on board and, after daily exposures for a certain length of time, they developed antibodies against porcine that cross-reacted with their own.

We also looked at Factor VIII recovery values at one hour and six hours post-infusion at specified time points to see whether we could identify and characterize the inhibitor antibodies developed in the groups against the injected porcine product that they got. These inhibitors, we measured both in the qualitative mixing study, yes/no, as well as measuring them by inhibitor titers.

In this slide, you have three bars representing the results from vehicle, Hyate:C and OBI-1--I think that

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this shows up very nicely with the colors--showing the aPTT values on Days 1, 7, 28, 56 and 90. Notice that, on Day 1, that is prior to the exposure to any product, that the three groups have essentially comparable aPTT values and, by Day 7, they are essentially unchanged.

But, by Day 28, both the monkeys exposed to Hyate and those exposed to the recombinant product have a prolonged aPTT and pretty much it stays at the same levels for the rest of the time that they were followed. This implied to us cross-reactivity of the antibody that was being formed after 28 days of exposure that cross-reacted with their endogenous Factor VIII.

Looking both by a chromogenic assay and a one-stage clotting assay, we looked at the baseline Factor VIII levels prior to their next dose on each of these days. So, on Day 1, you can see that there was a difference in the way the that products--this is before injection. So this shows you what the monkeys' endogenous Factor VIII levels were by one stage and chromogenic assays. The groups differed slightly but not statistically, obviously, Hyate being in red and OBI-1 in green, if you can't see the legend.

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You can see that by Day 7, the Factor VIII levels are slightly higher in both groups and that probably represents a little bit of carryover from day to day of their daily injections. But, by Day 28, the baseline Factor VIII levels are low in both, lower in the recombinant than in the plasma-derived group. By Day 90, the Factor VIII level in the recombinant stays low and, in the Hyate group, it starts to rise but doesn't come back up to baseline.

Now, that would indicate that there is a difference in the cross-reactivity that we observed against the monkey Factor VIII. You will see that was a little hard to demonstrate, however.

One of the things that we observed in looking at the post-infusion recovery values--again, these are by one-stage assay--was that there was a difference in the recovery values of the recombinant versus the plasma-derived product. On this slide, the recombinant product is on the left, the plasma-derived is on the right. The white bar is the one-hour and the clear bar is the six-hour value.

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So, prior to any exposure, the first two bars on the left of each graph, just represent the variability from monkey to monkey prior to--right after their first injection. So you notice that the recovery of OBI-1 is much greater than it is for Hyate. Actually, for OBI-1, it is about what would be expected whereas for Hyate, it is much less than one would expect and probably has to do with the milieu of mixing porcine Factor VIII into a monkey plasma with all the other porcine plasma proteins that are present.

You see that, by Day 7, the one-hour recoveries for both products are reduced, much more so for Hyate than for OBI-1, again implying maybe the antibody specifically against the porcine product infused was greater for the Hyate than it was for OBI-1, sort of conflicting with what we have seen prior. Then, on Days 28 and 90, we essentially got no recovery in either.

So, baseline Factor VIII levels were associated--the decrease in the baseline Factor VIII levels implying cross-reactivity with endogenous monkey Factor VIII was substantiated by the clinical findings in the monkeys of an acquired hemophilia-like picture with bleeding at

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venipuncture sites, joint and soft-tissue bleeding. This suggested that both groups that were exposed to porcine Factor VIII developed cross-reacting antibodies.

However, when we tried to identify them, when we tried to measure them, we could only identify them in very few of the monkeys and, even then, at very low titer.

In the qualitative screen, the mixing screen, for looking at the presence of antibodies against the porcine product that they received, you can see that, at each time point that we measured, there was no difference in the two groups, that, by Day 7, only one monkey developed a measurable antibody by the mixing test but, by Day 28, they all had.

This shows the Bethesda titers, when we measured Bethesda titers in the two groups. Again, in this slide, the Hyate:C is in red and the OBI-1 is the clear. You will notice that, by Day 28, the anti-porcine Factor VIII titer in the OBI group was higher than it was in the Hyate:C group, the Hyate:C group being in red, again. But, by Day 90, there is a reversal of that. By Day 90, the Hyate:C group has a higher titer antibody against the

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Hyate:C Factor VIII as opposed to the recombinant--the monkeys who received the recombinant porcine Factor VIII.

I will caution you, don't take the error bars to mean statistical significance because, as you recall, these are very small numbers in each group. But it is interesting to make these observations.

On the toxicological evaluation of the monkeys, I also want to point out that those monkeys that received Hyate:C had a significant pathological finding not see in the OBI groups; that is, they developed splenomegaly with lymphoid hyperplasia in the spleen. In some cases, the spleen was dramatically enlarged in those exposed to Hyate:C, particularly over the long term. Again, this was attributed to the extraneous plasma proteins present in the plasma-derived product.

So here is a sort of summary slide. Comparing what we saw, all these findings in the monkeys, trying to put together a conclusion. Day 1 incremental recovery value was almost two- to three-fold greater for OBI than it was for the Hyate. So, therefore, maybe the dose that we exposed the two groups to wasn't really comparable.

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The monkeys developed splenic lymphoid hyperplasia and splenomegaly, again, a nonspecific immune response to the contaminating proteins and, in some ways, suggesting there was some immunosuppression could have taken place in that group.

The cross-reactivity; both groups developed cross-reactivity to their endogenous Factor VIII and developed the acquired hemophilia-like picture.

So, in summary, the monkey studies suggested that maybe there were some indicators that the recombinant product was more immunogenic and, if so, that could be due to several possibilities. One is that the monkeys that were given the recombinant product, it is possible the product has an increased intrinsic immunogenicity. But it is also possible that the monkeys that received the OBI-1 actually received a much higher dose to their immune system because of the increased bioavailability.

It is also possible that the Hyate:C group, the group exposed to Hyate:C, actually has some immunosuppression and didn't mount the reaction that was normal and seen in the recombinant group. So it is not

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clear which of those and to what extent any of those are true.

In the mice, on the other hand, if you recall, there was no difference by Bethesda assay in the inhibitor titers that were developed and there was a greater anti-Factor VII specific IgG elicited by ELISA in the mice.

If we are trying to look at which models we can use, one of the things that we have an interest in is trying to make modifications in the Factor VIII molecule, itself, to make it less immunogenic. Many of you, or most of you, or all of you, know that this work is being done, again, by Pete Lawler at Emory University because they have published several papers in this area.

So, in guiding us in developing such a lower immunogenic product, we are taking the B-domain-deleted human Factor VIII, the A2 and the C2 domain are the two domains where most of the antibodies are directed. The theory is, or the hypothesis is, that, by making specific individual changes in those epitopes that we might make a molecule that is less immunogenic.

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Pete made a series of different constructs in which he substituted a single or two amino acids in one or both of the domains of interest. So you can see, here, there are a number of different constructs that have our A2, C2, epi1, A2, C2 epi2, just our name for the different constructs that he made. The amino-acid substitutions in some of the cases mimic the substitutions that are found in nature in other animal species.

We used the hemophilia knockout mice and exposed cohorts of mice to each of these constructs. I would like to point out, at the very center, HSQ is a B-domain-deleted human Factor VIII molecule and that group of mice developed a titer of 290 Bethesda units against the human. So the A2, C2, epi2, to its immediate left, really showed no difference in the inhibitor titer that was developed against it.

However, if you look at the one immediately to the right, the A2, C2, epi3, you will see that only less than a third of the monkeys developed any antibody, and inhibitor, and the average titer was 6.8. So, if we were to guess, we would think that that molecule might be

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something we would like to carry forth into additional studies as a potentially lower immunogenic Factor VIII product.

I believe that is it. Thank you.

(Applause.)]

DR. WEINSTEIN: Thank you very much, Garrett. Any questions?

Not seeing any, our next speaker will be Dr. Jay Feingold. He is the Senior Director of Global Medical Affairs for Wyeth. He will discuss data obtained in the development of ReFacto.

Wyeth

DR. FEINGOLD: Good afternoon. I would like to thank the FDA organizers for inviting me to speak on behalf of Wyeth Pharmaceuticals. Wyeth, as you all know, is the manufacturer and marketer of ReFacto, a B-domain-deleted recombinant Factor VIII molecule that has been on the market in the United States since 2001 and in Europe since 1999.

At the time of initial licensure, ReFacto had the largest clinical program and database for any factor concentrate. Wyeth regards postmarketing safety to be a

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natural extension of the careful safety monitoring that is part of all clinical trials. What has become abundantly clear today and what we think we knew previously is that there are no universal standards for collecting or interpreting postmarketing reports of inhibitor formation or even for interpreting reports and information from different clinical studies in different publications.

We recognize that hemophilia patients will remain on replacement products for their entire life and, in many cases, will receive thousands of infusions.

Inhibitors are one of the most important safety concerns for all hemophilia patients. But recombinant Factor VIII and plasma-derived Factor VIII products have a similar incidence of inhibitors in clinical trials, as you have seen earlier today. Recombinant Factor VIII and plasma-derived Factor VIII both have a low but real incidence of high-titer inhibitors and PTPs as well, and I think you have seen some information today and I will show you some more shortly that will substantiate that.

What the community needs for both patient safety and for better surveillance is to establish uniform

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standards of what is an inhibitor and what does a high-titer and a low-titer inhibitor mean. Wyeth believes, and I think Donna DiMichele will talk more about this later as well, that a global surveillance program should be implemented for all hemophilia A patients regardless of what product they are being treated with in an effort to better understand the incidence of inhibitor formation.

With that background, I would like to talk about the ReFacto molecule as well as the preclinical and clinical development programs. As you all know, ReFacto is a B-domain-deleted recombinant Factor VIII molecule which is produced through a genetically engineered Chinese-hamster ovary cell line. It is designed to correspond to the smallest of the multiple active forms of Factor VIII found in plasma-derived concentrates.

The complexity and heterogeneity have been greatly reduced through the elimination of the nonessential B domain which is very heterogeneous and is not necessary for hemostatic function.

This diagram shows a full-length Factor VIII molecule as well as ReFacto and, as you can see, the

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major difference is that the full length has the B domain and the ReFacto does not. However, after processing, the deactivated Factor VIII is the same heterodimer regardless of what type of molecule you start with.

ReFacto comparability of the full-length Factor VIII was accomplished in preclinical trials in which in vitro functional assessment of von Willebrand's factor binding, thrombin activation, inactivation by APC and its ability to act as cofactor in Factor X-A generation were all analyzed.

Additionally, primary protein structure, carbohydrate structure and other posttranslational modifications were consistent with what would be seen for a full-length factor molecule, or what was seen for a full-length factor molecule, produced through a CHO line as well. Pharmacokinetic studies in canine models of hemophilia A demonstrated comparability with regard to secondary critical bleeding-time correction and prolonged whole-blood and clotting-time correction using the same dose and schedule as with the full-length Factor VIII molecule. Additionally, single and repeated-dose toxicity studies demonstrated comparability in rat and

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monkey studies with a toxicity profile similar to that observed with the plasma-derived Factor VIII molecules.

Following completion of the preclinical analysis, an extensive clinical development program was designed. This was discussed and agreed to with regulatory authorities prior to its initiation. PK comparability was established with plasma-derived Factor VIII molecule in two cross-over PK studies. The safety and efficacy of ReFacto was established for bleeding control and prevention on PTPs and PUPs and surgery, routine prophylaxis and on-demand treatment.

The clinical trials demonstrated that ReFacto was both safe and efficacious in the treatment of hemophilia A.

As you can see, Factor VIII concentration over time is identical for ReFacto and plasma-derived Factor VIII. Additionally, the recovery and half-life data are essentially identical for both molecules as well.

The PUP and PTP trials were designed to demonstrate long-term safety and efficacy of prophylaxis and on-demand treatment. Both were open-label noncomparative trials and patients could be followed for

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up to six years. Patients has to have severe Factor VIII deficiency with less than 2 percent circulating Factor VIII at study entry.

To be considered for the PUP trial, patients had to have no prior transfusions with blood, blood-product derivatives or other Factor VIII concentrates. To be considered for the PTP trial, patients had to be greater than or equal to seven years of age, had to have a one-year history of previous prophylactic treatment or at least 30 exposure days per year, and they had to have no documented history either at the time of study entry or in the past of a Bethesda titer of greater than or equal to 0.6 BU.

101 patients were treated on the PUP study with a median age at entry of eight months. 113 patients were treated in the PTP study with a median age at entry of 26 years.

ReFacto efficacy was demonstrated for both and the duration of treatment is shown on this slide. As you can see, in the PTP trial, more than 75 percent of patients remained on the trial for four years and 40 percent, approximately, remained on the trial for six

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years. In the PUP trial, 54 percent of patients remained on the trial for four years and 29 percent for five years. This does not include the patients who develop inhibitors.

The median number of exposure days in the PTP trial was 313 and in the PUP trial it was 197. 47,649 infusions were given in the PTP trial and 32,442 in the PUP trial. In the PUP study, 85 percent of bleeding episodes during the on-demand period resolved with one to two infusions and, in the PTP trial, 88 percent of bleeding episodes resolved with one or two infusions. The excellent good ratings in both trials were 92 percent.

This slide is meant to show the difference between the mean number of bleeds during on-demand periods and prophylaxis periods in the PUP and PTP trial. As you can see, the mean number of bleeds decreased from 11.4 during the on-demand periods to 6.2 in the PUP trial and from 24.5 to 10.3 in the PTP trial.

Keep in mind that some of the patients in these trials received prophylaxis as infrequently as once a week. Others were treated twice a week, three times a

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week, and in the PTP trial, some even more than three times a week.

I would like to now turn to our extensive safety monitoring first in the clinical trials and later in the postmarketing setting. During the clinical trial, we extensively monitored--and I am going to focus really on inhibitors because that was the purpose of today's forum. During the clinical trial, extensive inhibitor monitoring was done. Patients were monitored at baseline, two weeks into the trial, one month into the trial and then every three months for the first three years.

In the PUP trial, they continued to be monitored every three months. In the PTP trial, they were monitored every six months. The precision of the method was within 11 percent and the limit of quantitation was 0.6 Bethesda units. A negative report of inhibitor or no inhibitor was considered to be present if the titer was less than 0.6.

Three independent Bethesda inhibitor assays were performed centrally, one against the normal human plasma test base, a second against the ReFacto test base. The Nijmegen inhibitor assay was used to confirm low-titer

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inhibitors. Additionally, all samples were tested in an ELISA assay against ReFacto.

In the PUP trial, 16 patients developed high-titer inhibitors and 16 low-titer inhibitors. This data was consistent with what had been seen in PUP trials for other Factor VIII products already approved. The median number of exposure days prior to developing inhibitor was 12. In 25 and 32 of these patients, the inhibitor resolved, meaning the titer returned to 0, 20 or 25 of whom had received ITT therapy or ITT, and five of seven who did not.

In the PTP trial, as previously mentioned, one of 113 patients developed an inhibitor, initially a low-titer inhibitor. This patient developed a high-titer inhibitor after an additional 18 months. The patient withdrew from the study but later clinical follow up revealed that he was doing well clinically.

I thin it is important to discuss the clinical data from other studies at this time because we have heard some information about this and I would like to give you some more. If you look at the Schwartz data from 1990 reported on a first-generation, full-length

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recombinant Factor VIII molecule, two of 86 patients developed high-titer de novo inhibitors. One of these did have a positive Western blot but all the inhibitor assays were negative. This gave an incidence of 2.3 percent and a confidence interval up to 8 percent.

White and colleagues in 1997 described another pivotal study for a different first-generation full-length recombinant Factor VIII. In this trial, two of 69 patients, for an incidence of 2.9 percent and a confidence interval up to 10 percent, had anamnestic inhibitors during the trial, one patient with a remote history of a previous low-titer inhibitor and one patient who had a low-titer inhibitor at baseline that became high titer during trial.

Abshire and his colleagues in Thrombosis and Hemostasis in 2000 reported on a second-generation full-length recombinant Factor VIII in which one of 71 patients developed an anamnestic response to a previously noted low-titer inhibitor. That was mentioned before by Dr. Larson. This gave an incidence of 1.4 percent with a confidence interval up to 7.6 percent.

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Courter and Bedrosian in 2001 in Seminars in Hematology reported on the ReFacto experience and they described the patient I had previously mentioned.

What about in the postmarketing setting? In 1998, MacMillan and his colleagues in Blood reported on the prospective observational trial that looked at 919 PTPs amongst the patients in the program. 3.2 percent of these patients developed inhibitors, 26 with documented greater than or equal to 25 exposure days. Fourteen PTPs, for 1.6 percent, developed high-titer inhibitors greater than 5. All of these patients, obviously, were treated with plasma-derived Factor VIII.

Giles, et al, as mentioned previously by Dr. Carcao and others, described in Transfusion Science in 1998 the large Canadian experience in switching PTPs from a plasma-derived product to a recombinant product and the fact that they saw a 3 percent incidence of new inhibitors developing at two years, all low titer.

Earlier this year, MASAC conducted a survey for the NHF in which hemophilia treatment centers in the United States were asked if they had seen an high-titer inhibitors in the past three years develop in patients

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that they knew had no history of previous inhibitor and had greater than or equal to 50 exposure days.

Forty-five centers responded representing approximately 3500 patients. Twelve PTPs, for an incidence of 0.35 percent, were found to have had greater than 50 exposure days and who developed high-titer inhibitors during that time period, ten more on recombinant product, two on plasma-derived product which probably reflects market share. Interestingly, six of 12 of these inhibitor patients have move than 250 exposure days.

If I can, just for one second, go back here to just mention one thing I forgot to say. If you look at these confidence intervals, based on the results here in an intent-to-treat analysis, none of these products, with the exception of ReFacto and Kogenate FS--no; sorry. Just ReFacto--would have been approved as well as, of course, currently Advate.

So the conclusions from the literature are that there is a broad range for inhibitor development PTPs of 0.2 to 3.2 percent. The reported range for high-titer inhibitors in the reports that I described range from 0

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to 2.3 percent. What is clear is that there are broad and overlapping confidence intervals and, if one looks at them in an intent-to-treat analysis, they clearly would not meet the guidelines being established.

What is also clear is that there are no good definitions out there for inhibitors with respect to what is a high-titer inhibitor and what is a low-titer inhibitor. Are only de novo inhibitors important or are anamnestic inhibitors just as important? In clinical trials, do we care about patients--well, of course we care about them, but do we care about results when a patient has a recurrent inhibitor or are we only interested in new inhibitors.

It is clear that we need a consistent standard for reporting inhibitors and collecting this information so that we can provide important safety information for patients and healthcare providers.

The Wyeth postmarketing surveillance for inhibitors is very extensive. Wyeth reports any spontaneous event of inhibitor development whether or not we receive supportive clinical or laboratory data. If we

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receive a report that says inhibitor, it goes into our database.

We do extensive follow-up data collection. Anybody who reports an inhibitor to us is asked to complete a specific questionnaire regarding inhibitor formation so that we can gather as much information as possible about the patient. If this information is not forthcoming or if there is still information we are lacking, we will follow up with telephone calls.

In order to better analyze these reports once they are received and we have the data, we do set up some definitions based on consensus from key opinion leaders as well as what is available in the literature. First, I would like to note that there is no central-laboratory testing performed on these postmarketing reports.

I should also mention that postmarketing reports includes both spontaneous reporting and phase IV studies. We regard a positive titer as any that is greater than or equal to 0.6 and a high titer is greater than or equal to 5.0. A positive history inhibitor is any documented report any time in the patient's life of greater than or equal to 0.6 Bethesda units. A patient is only

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considered de novo if we can document no prior history, meaning we cannot find any in the chart of greater than or equal to 0.6 Bethesda units on a BIA.

Our data looks like this through April 2003. We approximate that 5800 patients have been treated worldwide. We make that approximation based on number of units sold as well as some market research. Perhaps this is wrong, but we have assumed that we have a higher number of PUPs based on the number of patients that participated in our clinical trial and when our product became available in Europe and the United States.

We assume that 1450 PUPs and 4350 PTPs have been treated with ReFacto. We do not define PUPs as less than 50 exposure days. The PUP definition that we use is a little bit more stringent. We define it as a patient who has never received any blood product prior to going onto ReFacto.

We have received 83 reports of inhibitors in the postmarketing setting including the phase IV studies. Remember, this is all reports regardless of whether we have supportive clinical or laboratory information. 31 of these are in PUPs and I won't discuss them any

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further. Twelve have either unknown number of exposure days or less than 50 exposure days to all Factor VIII products.

Seven had a history of inhibitor prior to initiating ReFacto therapy. For four patients, we have been unable to get any additional medical information other than the report which didn't tell us much. In one patient, no titers were drawn. The diagnosis was based on increased factor consumption.

Therefore, we have 28 reports that we call de novo inhibitors in PTPs with greater than 50 exposure days to all Factor VIII products that developed their inhibitors in the postmarketing setting while on ReFacto. Twenty of these were low-titer and eight were high-titer inhibitors for a reporting rate of 0.5 percent and 0.2 percent respectively, keeping in mind that I have no idea how many exposure days any of these PTPs might have had.

Many initiatives in PTP inhibitor monitoring are ongoing. We heard today about the Canadian prospective inhibitor surveillance. I learned today that when you are not sure if somebody is going to show up, you shouldn't put them in your slide. But Charlie did

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present this data at the ISTH Factor VIII/Factor IX Subcommittee back in July and it did show that there was no product specificity and that the incidence appeared the same across a ten-year review of the U.K. database regardless of plasma-derived or recombinant products.

Interestingly enough, his data showed that, of course, most inhibitors occurred in the first decade of life and there was a very low level for the next several decades with a small peak again in the sixth and seventh decades.

Also I just gave you some data from the MASAC survey that high-titer inhibitors have been seen with both plasma-derived and recombinant Factor VIII. I think shortly we are going to hear from Donna that the ISTH has some interest in the global surveillance program. We, at Wyeth agree wholeheartedly with such a program.

My conclusions are that recombinant Factor VIII and plasma-derived Factor VIII products have a similar incidence of inhibitor formation and that both have a low but real incidence of high-titer inhibitors forming in multiply infused PTPs. Wyeth believes that a global perspective surveillance program is needed to assess the

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incidence of inhibitor development. This would allow for a defined period of patient observation, standardized data-collection techniques and definitions and the gathering of complete information including serial inhibitor testing, genotyping and other relevant data such as epitope mapping.

Standardized spontaneous data collection will lead to data-driven labeling which will provide accurate and important safety information to healthcare providers and patients.

I would like to thank, again, the FDA for inviting me to speak today.

(Applause.)]

DR. WEINSTEIN: Thanks, Jay. Again, we have time for a few questions. Ross?

AUDIENCE: (FDA.) The package insert for ReFacto in talking about the comparison that you showed in your slide of the frequency of bleeding episodes while on routing prophylaxis compared in the same patients to the frequency of bleeding episodes while on demand therapy, the package insert mentions that those data in that comparison should be made with caution because of

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the nonrandomized nature of that comparison. There was no systematic manner in which patients went on and off prophylaxis during that trial and I didn't hear you mention that.

The question that I wanted to ask is if you could show us the distribution of previous exposure days to other Factor VIII products in the MacMillan experience that you alluded to.

DR. FEINGOLD: I'm sorry. I am not sure I--

AUDIENCE: The 1988 MacMillan study with the 3.2 percent incidence of inhibitors, if you could talk about the distribution of previous exposure days as of when people began that observation period in that study.

DR. FEINGOLD: I think somebody mentioned earlier that 75 percent of the inhibitors in that particular evaluation occurred within the first 75 days of evaluation--no; within the first 50 days. All the inhibitors that they saw happened within the first 250 exposure days.

But I think this points out a larger issue which is obviously that was, A, a plasma-derived product. Second of all, none of those patients were on primary

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prophy. Probably virtually none were on secondary prophy. They were problem mostly treated with on-demand therapy.

The paradigm of treatment has shifted. I probably should have mentioned it as I described all these studies. Even from the time the first-generation recombinant Factor VIII was studied until now, the paradigm of treatment has shifted so far because, especially in kids, many more of them, in the United States, at least, are on primary prophylaxis. But that is a good point. In that particular study, all the patients developed their inhibitors by 250 exposure days.

DR. KEY: I am Nigel Key from the University of Minnesota. Just listening to your presentation and the Baxter and Bayer, do you have any data that continuous infusion is a problem for PTP patients, or is that something that we are going to forget about at this point?

DR. FEINGOLD: There were certainly some patients who were treated with continuous infusion for surgery during the pivotal trial, but not enough to make any conclusions nor to ask a regulatory agency to give us

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an approval for that indication. But it certainly has been done and effective.

DR. KEY: Do you think that this issue that came out of Germany is a non-issue? It may not be fair to ask you but I am just sort of listening to the various presentations on this. There were, I think, eleven or twelve cases that were reported at the ISTH and they were all in PTP patients. I think eleven out of the twelve patients had more than 50 exposure days. So I am just wondering whether continuous infusion is really a variable or not in the risk development.

DR. FEINGOLD: That is a really good question. I actually discussed that with Claude Negre because, as you may know, he is a big proponent of the continuous infusion in surgery and he doesn't have that experience. So I guess the answer I could give you is larger trials, more prospective data, would probably be the best way to look at it.]

DR. WEINSTEIN: Dr. White?

DR. WHITE: I wanted to show a slide. I just wanted to make a comment about postmarketing surveillance studies. I just wanted to take you through my talk

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again. I think we are going to have some discussion about postmarketing inhibitor surveillance. I think we may have a nomenclature issue here. What I am really talking about is the kind of--what I want to make some comments about are the kind of postmarketing licensing inhibitor surveillance that has been done to date.

I think what Donna is going to talk about is probably something quite different although I think she will call that a surveillance study. I think if you look at the studies that have been done, we heard about the MASAC, the Wyeth and the Baxter studies. The rate of inhibitor development, or the prevalence of inhibitor development, was all down here around anywhere from 0.3 to 1 percent.

We heard about the Canadian study which is up here around 3 percent and the MacMillan study which actually was a post-licensing prospective but surveillance-type study which is up here around 3.2 percent.

I think the point that this slide makes is that these are all probably underestimating, because of the volunteer nature of them, what is probably a more

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realistic prevalence of inhibitor formation. This, in particular, was a study that probably has a fair amount of accuracy to it, and I think this one did, too.

I think that these postmarketing surveillance studies are probably only capturing about 10 percent of what they should be capturing. That question came up earlier and I think this data speaks to that to some extent and says they are probably pretty low.

Trying to compare them, which has been done in the past, probably doesn't make a whole lot of sense when you get right down to it.]

DR. WEINSTEIN: I think we are ready now for a fifteen-minute break and we will reconvene at ten minutes after 4:00. Thank you.

(Break.)

DR. LOZIER: We would like to get started with our speakers and then proceed into our panel session which I think may be one of the more rewarding parts of the day.

First, I would like to thank my fellow members of the Workshop Planning Committee including Mark Weinstein, Andrew Chang, Nisha Jain, Tony Meyers Lewis

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and Mr. Joe Wilczek who has kept the computers running and worked very hard to keep this workshop operating smoothly.

As you have heard, this conference is cosponsored and supported by the IABS which is the International Association of Biologics which gave an unrestricted grant for this conference to proceed. We also are grateful for support from the Courtesy Associates that has helped defray some of the expenses in bringing our international guests here.

Now the proceedings, as far as a transcript, will be available on the web in about fifteen working days, we are led to believe. We would like to have comments from members of the audience or participants and we will leave that option available for let's say the next 30 days or so, and you can e-mail that either to me, which is lozier@cber.fda.gov which should be in your handout, and my contact information, or Joe Wilczek whose e-mail likewise is there.

So we will try to incorporate comments from the participants or the registrants for any proceedings that we may eventually publish.

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The issue has come up regarding slides. The policy of FDA is that we will be putting our slides out on our web page and, if you want to get slides from any of the other speakers, you can contact them directly through their e-mail to get them. It is not an issue of legal propriety or anything. It is simply a server space issue.

So, our next speaker is Donna DiMichele who is at Cornell Medical Center. She will be talking to us about preliminary ideas on a proposal for pharmacovigilance which is very, very important to what we are talking about. I would like to thank her for a lot of help in getting this organized and getting the proposals in good working order and getting things focused.

Donna?

Preliminary Ideas on Prospective International Studies of Product-Related Factor VIII Inhibitor Formation

DR. DiMICHELE: Thank you, Jay. I want to thank the FDA as well for inviting me to participate in what I think is a very important conference. Our hope is, once

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I finish my presentation, we are going to go straight into a panel discussion which I will introduce at that time. Our hope is that, after listening to the greater part of the day and the presentations and the wonderful information that we have received during the course of the day, that we can begin to make some very crucial decisions, or at least offer crucial input, into the decision-making process that will affect future studies, hopefully prelicensure and postlicensure.

But, before I do that, I was asked by the FDA to comment on a proposal for prospective pharmacosurveillance. I just want to say that this project has arisen primarily out of the Factor VIII/Factor IX Subcommittee which developed a previously treated patient inhibitor working group in response to the greater awareness of the problem of PTP inhibitors that was raised over the last year or so. Some of the input into this proposal has certainly come from that working group.

So, although these are some of my ideas and I only speak for myself, please know that there are a group of people who are really thinking about this.

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The major question that we ask, that remains to be asked, is even with the more perfect design of prelicensure clinical trials, will they ever have the power to ascertain the true--and I am sort of glad I used the word--the true PTP inhibitor incidence or will we arrive at that information only with further data, both from the standpoint of greater subjects and a longer observation period through a postlicensure program.

When we talk, and I am going to be coming back to this in the latter part of my talk--when we talk about true inhibitor incidence, I want to add that underlying that question remains another vital question that was asked this morning and that was, when we talk about the development of an inhibitor in a PTP, is it product or is it the host, or how much of it is product, how much of it is host, and not only host but host-treatment interaction. I use that rather than host-product interaction.

So I am going to be coming back to what I think is a very subtle difference and potentially how a pharmacosurveillance program ought to be structured keeping both of those in mind.

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Now, the issue is why pharmacosurveillance. The whole concept of postmarketing pharmacosurveillance has already been recognized by industry and regulatory organizations as important to the identification of not only ongoing safety and efficacy concerns but a constant refinement or redefinition of the risk/benefit ratio and that was published by more of a pharmacology group in the British Journal of Clinical Pharmacology in 1998.

So, certainly, the importance of pharmacosurveillance has been underscored in the literature and several times already today. Currently, the large part of that involves mandatory spontaneous adverse-event reporting of clinical safety and efficacy concerns and this heretofore remains the primary way that this surveillance has been conducted so far.

I would like to just, before going into the pharmacosurveillance program, sort of divert into this issue of spontaneous adverse-event reporting because the validity of spontaneous adverse-event reporting has, again, been discussed and questioned several times during the course of this conference.

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Again, there is a literature in the pharmacology literature, in the clinical therapeutics literature, addressing this of spontaneous adverse-event reporting. It is certainly recognized that it does serve an important function in terms of alerting both physicians and regulators as well as industry, itself, to potential early and very strong drug-event causal associations. It is in that function that spontaneous adverse-event reporting serves a very important function as well as to delineate very severe and/or unexpected adverse events.

Now, another important function, as, again, written in the literature, is to foster suspicions. In other words, adverse-event reporting can actually begin to give us pause, or give us cause, to question the potential safety and efficacy of a licensed product and prompt further warranted investigation. I think, in many ways, that is what has happened here and what has prompted this conference.

Of course, as has again been alluded to several times and this is, again, documented in some of the literature, spontaneous adverse-event reporting has its limitations. Indeed, estimated in the literature, is

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that less than 4 percent, and this was a question that was raised earlier, really, what percent of adverse events are actually reported.

The estimations in the literature are less than 4 percent of adverse events are reported and, very interestingly, potentially less than 10 percent of even severe adverse events are actually reported post-licensure

The other thing is that reporting appears not to be a constant event. In other words, it usually is greatest immediately post-licensure and that there is a precipitous decline in reporting after the second postmarketing year. Again, that is not necessarily for biologics. That is, indeed, for all of the drug industry.

More importantly, there are confounders and biases. The reporting environment and the importance of reporting within the medical communities very much affects how much is reported and, certainly, the quality of the data. Spontaneous adverse-event reporting has problems with respect to establishing not only a good numerator but also a denominator in assessing true risk.

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Frequently, it also lacks temporally associated clinical and laboratory data and challenge and rechallenge information is often missing as is the ultimate patient outcome.

So what are the alternatives? Once again, there are several different alternatives. One is that randomized clinical trials can continue post-licensure and, in some cases, they are. They are frequently industry sponsored and they usually involve a larger subject accrual than the pre-licensure study. But, if conducted to GCP specs, as has been suggested, they are certainly very expensive to do.

There has also been some history, again, outside of biologics, with postmarketing cohort studies that are primarily industry sponsored. They have really suffered from very slow recruitment and also, of course, the lack of a control arm which any kind of surveillance study has the potential of having.

Now, the other option, indeed, is long-term global pharmacosurveillance programs. These can either be industry sponsored or independent of but potentially supported by industry. As we begin to talk about the

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potential for global pharmacosurveillance, I just want to add, and I want to reiterate, that it would be greatly facilitated--if, indeed, we decide to go with this option, it would be greatly facilitated by regulatory harmonization. So I know that, as Dr. Seitz was mentioning, that there certainly is a direction in which the European regulatory agency appears to be going with respect to making decisions in this area but I believe that the ongoing communication between U.S. and international regulatory agencies would be very important in order for such a program to succeed.

Assuming that the answer to the first question is yes, and that a long-term pharmacosurveillance program is necessary, I think there are certain questions as to Project Scope, who should initiate such a surveillance. Should it be physician-initiated? What is the role of government agencies and, certainly, of industry? What type of data should be collected and how long should this occur for?

Most importantly, what is the clinical and laboratory data that should be collected? How will it be analyzed? How will it be reported? Should this be done

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on a national basis or an international basis and, once again, in terms of interpreting and reporting this data, what is the role of the various stakeholders? What is the role of physician organizations, government agencies and industry?

Of course, not to be left out, where is the funding for all of this going to come from?

So, in beginning to potentially put out some ideas for a global pharmacosurveillance program, I propose the following. With respect to participation, I think that one thing becomes very clear in hearing about the different programs and trying to compare data from one product to another appears to be not quite like comparing apples and oranges but certainly it doesn't appear to be like comparing, really, the same event for the same type of product.

So one of the things that I would propose is that the data-collection system be universal and that it occur for all Factor VIII products. Now, whether that be international or national really depends on the different products and whether they are globally distributed or whether they have a more limited distribution within some

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national markets. So, depending on the distribution, the distribution of the product should really dictate the database and the extent of the database.

One of the things that I would like to propose, however, is that the database, the prospective pharmacosurveillance, indeed be industry driven by really be driven by clinicians and by the hemophilia treaters. Actually, I am not the first one to propose this. This was actually proposed in a Lancet article shortly after--in terms of a comment on the recombinant inhibitor problem and the need for ongoing surveillance. This was a Lancet article and an editorial that was written by Drs. Vermylen and Briet in 1993.

Now, the other issue that is of critical importance becomes subject selection. Obviously, we are talking about PTPs and who should be included. Well, PTPs, all PTPs, as defined by prelicensure clinical trials. What I mean by "as defined by prelicensure clinical trials," and we are going to come to this in part of the panel discussion, the question is does the definition need to be revised. What is a PTP? Is it

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going to be someone who has had over 50 exposures, over 150 exposures, over 250 exposures?

I think, as a community, we are going to have to make that decision. Most importantly, how is an inhibitor-negative PTP defined as something that is going to bear, hopefully, a lot more discussion and I am going to come back to that in a little bit. But, regardless, the PTP and postmarketing surveillance should be defined in the same way as the PTP is defined in prelicensure clinical trials because it is data verification.

As I said before, I believe the PTPs on all Factor VIII products should be included whether they are plasma-derived, recombinant. As was mentioned, certainly any of the future further-modified products also ought to be included in this.

The observation period is up for discussion but appears to need definition primarily on the basis of cumulative Factor VIII exposure days and not necessarily time, although there may be some practicalities there.

What about the dataset? This is where I propose we really separate or try to separate issues related to product and issues related to host or host-treatment

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interaction. In that way, I have sort of divided this into what I think is a minimum dataset and a more comprehensive dataset. I would like to discuss the minimum dataset to begin with.

I believe that the minimum dataset is the dataset that will really focus on product immunogenicity; in other words, what role does the product play in PTP inhibitor development. Obviously, given that this is a very important issue for regulatory and industry perspective, indeed, this minimum dataset may have to be defined by regulatory agencies with industry input.

In trying to ascertain product immunogenicity, obviously, the focus is going to be need be on incidence and prevalence of both high-titer and low-titer inhibitors. I think that they are--I would beg to differ in that I think that high titer and low titer is defined. Inhibitor may not be defined, particular the low-titer inhibitor may bear some modified definition, but, certainly in terms of when you get to high titers, I think we are all in agreement about what we are talking about.

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Certainly, the goal of such a dataset would be to define and further define the at-risk PTP population and the risk period as well as incorporate the outcomes of patients who go on to develop inhibitors post-licensure. Obviously, the goal of this dataset is the ongoing reassessment of product risk-benefit ratio.

What are the tools, in my opinion, the are required for this minimum dataset? Obviously, and this is, again, something that is going to bear more discussion, is that these surveillance tools have to be adequately powered. The cohort size has to be adequately powered as does the observation period to really give us the kinds of answers we want.

Again, we have to refocus the goals of such a pharmacosurveillance program. Is it going to be to pick up inhibitor clustering that may be product related or are we really looking for the true inhibitor baseline. If we are looking at the true inhibitor baseline, then the pharmacosurveillance has to be powered for that rather than these rare severe events.

The database has to be reliable with respect to both numerator and denominator. In my opinion, the

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numerator will be very dependent on reporting and the universality of reporting, whereas the denominator, which may be otherwise very difficult to ascertain, may require input from industry with respect to factor distribution data. In fact, distribution data has been proposed in the pharmacologic literature as a way to ascertain denominator and we now see that Baxter has done that in the study that was reported by Bruce Ewenstein.

With respect to the dataset, there really need to be strict definitions. I think where there has been tremendous controversy, and I think we have heard it again today, is in basically how a positive inhibitor titer--basically how a PTP with a negative inhibitor has been defined in terms of the patient going on study and, certainly, whether the patient has developed an inhibitor or not has been the subject of a lot of debate in the post-study analysis. That is where we see all of these numbers kind of being constantly revised by industry in terms of whether this was truly an inhibitor or not.

As Jay said, the question is what do we include in here. Do we include absolute de novo inhibitors, at which the PTP, without a previous inhibitor, may need to

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be much more strictly defined or are we also interested in the patient who doesn't have a clinical inhibitor but who may have had more subclinical evidence of antibody who then has and develops a clinically relevant inhibitor on a new product. And that has to be further defined.

I think, in defining inhibitor, like I said, both high titer and low titer, we are going to have to make some decisions about the assay. I think that rediscussing the assay with respect to sensitivity and specificity and the ability to potentially now, with Dr. Verbruggen's data, to pick up even much more low-titer inhibitors, is this, again, going to important with respect to not only defining the cohort but also defining the outcomes.

Ultimately, are we going to have to include recovery and survival data in order to define our PTP inhibitor population both coming and, certainly, coming out. Again, facility of monitoring and defining outcomes is going to have to be crucial.

How could such a collection system actually be organized. Since, like I said, this dataset is important to regulators and industry, I think it has to occur under

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the umbrella of regulators or industries, but, as we have heard today, there are many well-established and emerging national databases that are run by hemophilia physicians in many, many different countries and the potential is certainly for establishing some of these in countries in countries in which they don't exist. With modification, these databases can provide wealth of information.

In the U.S., our database is not strictly physician run. It is with the CDC, but I know that the CDC and the Hemophilia and Thrombosis Research Society have been having discussions about potentially collaborating and creating a U.S. database that will actually give us a lot more information than we are currently getting and might certainly be able to well contribute to this with its structure, contribute to the information that we are seeking.

Now, obviously, this would involve national data collection and analysis. I propose that the clearing house for all of these national databases actually be the ISTH Factor VIII/Factor IX Subcommittee and a working group that is established to really take in all of the national data and to report it as an international data

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analysis and report it not only globally but specifically also report it to industry and report it to regulatory agencies so that they can make decisions.

Obviously, the question is who is going to fund this. Even the existing databases--if we really are going to have universality of data collection, even the existing databases are going to have to be revised and that is going to take funding. Any of the analysis work is going to take funding. Obviously, the funding is probably going to need to come from a combination of resources, not the least of which I am sure will be industry.

Now, I think, however, that there is other data that we need to collect. I refer to this as the comprehensive dataset. Here is where I think we will have to opportunity to really focus on the role of the host and the host-treatment interaction and PTP inhibitor formation.

This is where, I believe, this ancillary dataset needs to be defined more so by clinical investigators and scientists and needs to collect and focus on, in sort of answering this question, the host with respect to

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hemophilia and immunologic genotype and phenotype, pertinent non-product-related inhibitor risk factors, hemorrhage and treatment specifics as well as anti-Factor VIII antibody characterization.

The data-collection analysis and reporting for such a comprehensive database obviously needs to interface with the national databases but can actually occur through independent study or independent study designs proposed by clinician-scientist-research teams and, hopefully, would yield an expanded analysis combined with a sample repository to help answer some of these vexing problems.

I believe that certainly some of these organizations are already being formed. This is scientific data that, again, I believe, should be reported through the ISTH and the scientific symposia. Once again, if these are independent studies, there might be more reliance on private and public research grants to fund these ancillary studies but they would have to work hand-in-hand with the national databases which would be involved with minimum dataset development as well.

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This is all I have to say at this point and I do think that this is a unique opportunity to move forward and to really develop a system that is going to give us the answers rather than our continuing to raise the questions. So, with that, I would like to move on to the panel discussion and, hopefully, the crucial decisions that need to be made.

Thank you very much.

(Applause.)

I guess I will take one or two questions but then I think we should really move on to the panel discussion. Mark?

MR. SKINNER: Mark Skinner, Washington, D.C. I think it is interesting proposal that you put forward. That are a couple of very important dynamics which are missing from there not the least of which is the informed-consent process for the consumer for their data to go into this process, something with which we are very familiar with UDC and creating another process will be very difficult to persuade consumers to participate in the broader perspective.

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Certainly, on the back end of the process, the reporting back, the linkage is back to--the consumers who do get good data and good reports out of the data we are missing on the back end.

Then I guess just my other observation on the proposal; it seems highly complicated and vast for the problem that we are trying to solve here. I wonder if we are not trying to create a structure either to compete with the existing research mechanisms or, if there isn't some other design for this process down the road. So I am not sure why it needs to be this elaborate to at least our questions in the short run.

DR. DiMICHELE: It may not need to be this elaborate. Maybe I am making it more complicated than it needs to be, but I thank you for your comments from the consumer perspective. Certainly, there is no place like the U.S. in terms of where patient consent and HIPAA regulations really will interface on this data collection. That is why I do propose to use this UDC in collaboration with the hemophilia treaters group to really establish an expanded database as well as the

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mechanism for analysis and also reporting into a larger organization.

So I think, from that perspective, I agree with you. From the perspective of how complicated does it need to be, I think this is the issue that we faced in doing the immunetolerance study. We are dealing with a low incidence disease, a low incidence complication, now even a lower incidence complication when we were dealing with inhibitors and PUPs.

So, what needs to happen, as you can see, even with large databases like the UDC, we may not have the power to answer the questions and, obviously, at this point in time, the community thinks that the questions are important. So, unfortunately, I think the scope of this is probably going to have to be a large scope. But it may need to be broken down into individual pieces. I think that is where sort of the feeding in of individual databases comes in. That was my concept but it certainly may be much more--it may need to be much less complicated than that, I'm sure.

I will take one more question and then we will go on to the panel discussion.

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AUDIENCE: (Queens) I really like your presentation very much. I think, unfortunately, it needs to be global and it needs to involve the complexity that you have shown us because I think that the inherent heterogeneity of this problem, both on the host side and the product side, means that this interaction between the databases and the clinician-scientist research groups is absolutely necessary if we are going to understand why some of these events occur.

Not all hosts are equally susceptible and not all exposure days are equally likely to lead to this sort of problem. So, unless we look at those things very carefully, we will never understand this.

DR. DiMICHELE: Yes.

AUDIENCE: (Queens) So I like your proposal very much, indeed.

DR. DiMICHELE: Thank you.

Panel Discussion

DR. DiMICHELE: I guess we would like to move on. Just so everybody in the audience understands, in moderating this discussion, the panel discussion, you see here before you the regulators from the FDA and EMEA.

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Because this is a regulatory conference, we have decided that the regulators really need the first crack at asking the very difficult questions and getting the answers that they are going to need to make regulatory decisions. So we will begin by having the regulators prioritize the questions with respect to both preclinical trials and postlicensure surveillance and then we would like to open it up for greater discussion. They will be posing their questions primarily to the speakers, but we will definitely greater audience input after that.

So, with that, I was wondering if anybody on the panel would like to begin. You can either group your questions or make it a free-for-all, whichever you would like.

DR. LOZIER: I thought, during the workshop, that there were basically three areas of discussion, one being the assay for the inhibitor, the next issue being the patients involved in the trials that are involved with testing these products and, finally, how we assess the trials. I thought we could ask questions maybe first with regard to the factor assays. I had a couple that I could start with and others could jump in and then maybe

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we can move into the bigger questions of the patients and the trials, themselves.

One question or point I guess I would make about lab testing is we always have a problem, in every trial where there is a discrepancy between the local lab and the central lab. I wonder if either Dr. Raut or Dr. Verbruggen could comment as to whether or not you have looked at all at the issues of, let's say, shipping samples, moving them across continents or across countries, any possibility that there could be any issues or problems with how these samples are stored.

I know Dr. Seitz has commented on finding some problems with good lab practice in certain central laboratories and that sort of thing.

DR. DiMICHELE: Dr. Raut, do you want to take that question to begin with? You can come up to the microphone here.

DR. RAUT: With respect to the samples, I should have pointed out in my study that the materials that were lyophilized and were actually concentrate, we found that these materials behaved slightly better than the samples, the patient samples, that were sent out. I suspect we

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could similarly ampule these samples for tests, and we have very good degradation studies on these samples, if that was the concern.

DR. DiMICHELE: Dr. Verbruggen, do you have any comments?

DR. VERBRUGGEN: I think we don't have very much experience with transporter samples, of course. I think you are the experienced one in this audience. But, when you are talking about samples, frozen samples, then samples have to be transported at minus-80 degrees, I think. That is an absolute requirement and under GLP or GMP conditions.

DR. DiMICHELE: Jay, will you take a comment with respect to your question from the audience at this point?

DR. LOZIER: Yes.

DR. DiMICHELE: David?

AUDIENCE: It was just about, actually, the complexity of sending the samples. In Canada, the samples that come to Kingston, I know that, A, they have to come by courier because of the frozen issue and because many of them are biohazardous. The documentation

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and what the courier services will accept is sometimes also a bit of a challenge.

So I think there are some issues actually even within a country let alone across borders moving samples around.

DR. DiMICHELE: Dr. Seitz, the next question.

DR. SEITZ: I think, of course, the question of assay is very important. But my question is what do we want to have at the end or what are we doing all this for. I think what we want to have is to protect the patients from risk that they have. So I think the question of assay immediately relates back to the clinician severity of all this.

I learned in this conference, again, that the definitions are still not clear. It is not yet clear what is a positive inhibitor. It is defined with a value of a test and we have learned that this test is not at all reliable, that the values are not really very solid information.

So my question is to the treaters and also to the ISTH. Is there a possibility to define the clinical severity of inhibitors a little bit better than just by

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Bethesda values. Also the question, low responders, high responders, the frontier is 5 Bethesda units but, when we see the results of collaborative trials, the variations are so high that one laboratory may measure 4, the other laboratory may measure 6.

I wonder whether it is really so important to have the exact accurate value. I wouldn't care. I think it is much more important to have a feeling of the clinical severity and how could we define this better. That is my question.

DR. DiMICHELE: We are going to start with some of the speaker panel first and then we will get some comments from the audience, but--I guess you are a speaker. But, actually, in terms of phrasing the question, I think Dr. Seitz brings up a very good point. The question is, what are we interested in as clinicians. What is the problem here? I think understanding what the problem is here, I think, will beg the definition.

The question is, what is our threshold for concern. Maybe I would like Gil to comment on this first and then we will kind of go through some of the clinicians who are on the panel.

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DR. WHITE: I think that is a good question. I do believe that inhibitors have been defined based on what we, as clinicians, think is important. The attempt to distinguish between the high and low-titer inhibitor relates directly to treatment. If it is a low titer, you have got a chance of treating with Factor VIII. If it is a high titer, you don't.

I think that is why, when we tried to arrive at some definition, that we were trying to put clinical parameters on that definition.

For a study, it is quite different. For a study, you want reproducibility and something that transfers from one lab to another. I think the clinical definitions there are probably less important than just having something that you get similar results from one lab to another or from one lab to a central lab.

So I think the issues are a little bit different. I think the clinician parameters are reasonably well defined. Most of us have a good feeling for what will respond to Factor VIII and what won't. What we don't have is a good feeling for how my laboratory result compares with Donna's laboratory result

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and how both of us compare with a central lab and exactly what do you do when you are writing a paper when I get 2 Bethesda units and Donna gets 5 Bethesda units and the central lab gets 8 Bethesda units. Do we call it a low titer or a high titer or a low responder or a high responder?

I think you have hit a nail on the head. The other important problem is on the low end. What do we call an inhibitor? To me, that is harder to define than even what do you do when you have got a 4 and a 6. I can deal with a 4 and a 6. I can make some arbitrary rule and say that is the way we are going to do it for a study. But the sensitivity of your assay really does reflect your numerator and really does have palpable effect on a study.

DR. DiMICHELE: Dr. Carcao?

DR. CARCAO: I think that is where we need to work is on the sensitivity of the assay and making sure we have got something that we are all happy with. I certainly don't have much more to add to that, but I think, certainly, the number is not that important, I

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think, if somebody has 50 Bethesda units or 70. Nobody cares.

It is really the clinical impact and, as Dr. White was saying, it is how does the presence of that inhibitor cause us, as treaters, to treat that patient differently. Certainly, in pediatrics, it causes us to not put them on prophylaxis and that has a tremendous impact on their getting bleeds or not and then how do you treat those bleeds.

So I think when you talk about clinical severity, the most that we can say is that it is high titer and, hence, clinically severe or it is, for now low titer and hence not very clinically severe if that particular patient we continue doing what we were doing, meaning we continue to prophylax him and we continue to treat bleeds as we were previously.

DR. DiMICHELE: Bruce?

DR. EWENSTEIN: I think Gil is right about the level being the practical level above which you can't treat but I think we have sort of lost the other piece of it, when we went from low responder just to low titer, because I think that is important both for treatment as

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well as something biologic because I really consider the low responder to have two components, not just being below 5, but not being subject to anamnesis.

That actually has both implications. It is telling us something about the immune system but it is also telling us whether we want to treat a low-titer patient with Factor VIII or not because if it is going to turn into a 20, then that is going to be a problem.

DR. DiMICHELE: Although the question of anamnesis actually might need to be broadened because we may be assuming, then, these PTPs who are entered on study as not having had an inhibitor and then go on to have an inhibitor on the product and then they are disqualified from the final analysis because, well, maybe they did have a low-titer inhibitor and here they are anamnesing.

So I agree. I think the issue of response is an important one and is it important enough to really try to define even the lowest common denominator up front.

DR. EWENSTEIN: I would just maybe say you could look at the low responders into subcategories. The other question I had was more technical, especially as we get

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down into the sub-1 inhibitor titers, and that has to do with do we know that they are all directed against Factor VIII. When you get down to 0.1 Nijmegen units or Bethesda units, do we know that these are not antiphospholipid protein types of antibodies. I guess this is really just sort of a technical question.

Another technical question I have, although it might have a lot of implication, is we talk about the gold standard. We haven't really talked about the role of non-neutralizing antibodies. I wonder if we are sort of just touching the tip of the iceberg here. We know there are all these antibodies that don't show up in these assays and, when we are talking about an immune response, we are missing, like, three-quarters of the response just because we are sort of thinking like Gil is saying over what affects treatment, although it may affect treatment in terms of a half-life.

But if we are trying to understand the immune response, maybe the person who has one of these kinds of antibodies and then develops a very low titer, now of neutralizing antibody, maybe he hasn't done anything but

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modulate his immune response a little bit. The somatic mutation has changed just a little bit now.

DR. DiMICHELE: Dr. Seitz; did you get the answers to your questions? Okay. Dr. White? Maybe, Dr. Verbruggen, if you want to comment on maintaining specificity while increasing sensitivity in terms of the assays.

DR. WHITE: I neglected to say something else when I was talking about clinical importance and that is I don't know what the clinical importance of the low-titer inhibitors are. I am impressed that an inhibitor that was low enough not to be detected by the Nijmegen or Bethesda assay affected half-life.

I remember when we were doing the BeneFIX study, that Bonnie Rupp developed a very sensitive ELISA for antibodies to Factor IX. I don't know that we ever looked carefully enough at what the half-life of Factor IX was in those individuals who had inhibitors that could only be detected by her ELISA assay.

But I think what we have now are assays that are so sensitive that we may begin to detect the antibodies that all of us are supposed to have. Clearly, that is

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noise. That is not important. But somewhere, as your antibody titer goes up, it becomes of some importance in that it affects the half-life but you can give Factor VIII clinically more often and certainly do what you need to do clinically.

So, as a clinician, that doesn't bother me too much. The ones that really start to bother me are the ones where I can't get measurable levels of Factor VIII or Factor IX in them and, therefore, I can't do what I need to do in them.

So, clinically, there is a big spectrum here that I think we have some clues as to what they are clinically, but we still need more information about them and we still need to study them and we still need to know what the half-life is in those antibodies that are way down at the bottom level of detection.

DR. DiMICHELE: Can I just say, Bert, before you comment--I just want to add, Gil, we know that the assays that we have currently are have not sensitive. We know, very well, when we do immunetolerance that we can have zero inhibitor titers for a year before we actually reestablish a normal recovery and survival so that there

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isn't antibody that we are measuring even with the Nijmegen assay.

I think that is what Berg was presenting is that there may be a reason for these decreased recoveries and survivals that go beyond what we can measure currently and we may need, unless we want to keep doing recovery and survival studies which are very onerous, we might need a more sensitive assay that really does correspond to normal recovery and survival. Wouldn't you agree with that?

DR. WHITE: I do. And I think, actually, the ELISA assay that Bonnie developed for Factor IX that we used in the BeneFIX study was exquisitely sensitive.

DR. DiMICHELE: Bert?

DR. VERBRUGGEN: I want to remember that we only evaluated two patients and, in these two patients, we saw an increased inhibitor titer with the low-titer assay in combination with a decreased half-life time. That is what sensitivity--so, in these two patients, the assay was very sensitive and we think clinically relevant.

This morning, I forgot to tell that we also, of course, looked at the specificity of the assay and we

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have carried out the assay in a number of hemophilic, severe hemophilic, patients, without any sign of an inhibitor for the last six months or so. Always, we found inhibitor activity in this low-titer assay of 0, exactly 0, not 0.2. So I think the assay is-- the specificity of the assay is correct.

DR. JAIN: The other thing which we should keep in mind is how often are the inhibitor assays looked at, whether it is three months, six months. They are transient inhibitors which might disappear. This is all, again, going back to what the true rate would be if you had periods when you were looking at this.

DR. WEINSTEIN: Bert, just a question about the assay. You not only looked at patients with hemophilia. You looked at a large cohort of normal individuals? Was that done?

DR. VERBRUGGEN: Not yet; no. We are planning to do that, but we had some problems with samples that contained residual Factor VIII and, of course, with normal plasma samples, because of the residual Factor VIII in the plasma was disturbing our low-titer assay. But I think we have resolved, now, this problem so we can

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go on now with measuring the inhibitor activity in the normal population.

I, once again, want to stress the fact that we have to validate both assays, not only the low-titer assay but also the Nijmegen-Bethesda assay because there is no evidence-based reason for the 0.4 cutoff value. But we need the support of other people, of other institutes, because we have two less patients in our institute.

At this moment, currently, we are performing this evaluation of these tests and we are now assaying patients, severe hemophiliacs, without inhibitor to set the normal value for the kinetic studies. But, in a later phase, we need patients, we need more patients, with low inhibitor titers.

DR. DiMICHELE: But, based on the comments you made, I think it is very important that we also consider not only the absolute definition but the absolute definition over time. I just wanted to tell you that this issue of transient or, as some people refer to it as "disappearing," inhibitors has been discussed previously. Trying to arrive at a definition has not been easy

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heretofore because there is a lot of controversy. But we need work on that one.

Andrew?

DR. CHANG: I have a feeling that, at least I get from this particular workshop, that there is a general consensus that inhibitor titer and clinical significance should be related and that we should look at titer based upon clinical significance. We also heard conflicting opinions on the low titer, whether or not that is significant to the clinical aspect.

Some people felt it is significant and the majority probably felt it is not significant. So my personal suggestion is that I don't know whether or not clinicians in this field can discuss this area and then come up with some kind of a position paper or recommendation in this area that will be very helpful, in my view, to help the regulator.

DR. DiMICHELE: If I could make a comment on that, Andrew. I think that one of the things that we might keep in mind is that, with a global pharmacovigilance problem, we might be able to collect both pieces of data at the same time. We may want to

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collect the lowest common denominator in terms of what we define as an inhibitor but, at the same time, in collecting treatment data and bleeding data, come up, after the fact, with a definition of what we believe to be clinically significant if, indeed, it will differ from what the gestalt is, but maybe defining it in a much more quantitative way.

So it is very possible that, like I said, with an adequate study in terms of pharmacosurveillance over a long period of time that we can collect both pieces of data without having to define a clinically relevant inhibitor up front. I don't know what any of the other participants think, but--

DR. JAIN: This leads me onto the next question, what should be the definition of PTPs. We have heard various rates depending on 50 exposure days, 100 exposure days, 150, 250. I think that is the important definition which we should all have.

DR. DiMICHELE: I will open that to any of the speakers. Jay, you would like to start with that?

DR. FEINGOLD: I think that is a crucial question, but I am, if I can, going to add one to that

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which is--and even if we define what a PTP is, because what you are trying to do is define a PTP to figure out at what point it becomes less likely that somebody is going to get an inhibitor.

But, given what we know about the demographics of patients these days, you are not likely to be able to have the entire patient chart available so you are not likely to know, even if you can document the last 500 exposure days, whether they got in the 5,000 before that and whether there was ever an inhibitor, a low-titer inhibitor, anything like that.

So, while I believe that the definition of what is a PTP is important, I think we also have to determine how important an anamnestic response is when evaluating what happens to a patient when they switch between products and things like that as well. So you can pick a point but, with that point, whether it is 150 or 250 days, I think the corollary to that is that you have to decide whether you are only going to accept patients where you can document the history back to Day 1 exposure or whether you are going to accept the fact that you--because testing is intermittent at best any, you have

missed the possibility that they have had a transient low-titer inhibitor at some point, and transient only in terms of being able to find it, because I don't think that the immune system forgets something it has formed an inhibitor to before.

DR. DiMICHELE: Good point. Actually, Dr. Bergman, you have your light on. Did you want to make a comment?

Could people who want to make a comment turn their microphone lights on so I will kind of know who you are.

DR. BERGMAN: In response to the issue of defining PTP based on the number of exposures, would it not be possible to just have a mathematical correction or weighting if you know what percentage had 100, what percentage had 250, what percentage--and so forth.

DR. DiMICHELE: In other words, an extrapolation?

DR. BERGMAN: No, not an extrapolation.

DR. LOZIER: Maybe a stratification. The question I would have along the same the same lines, not only for degree of previously treatedness--it is

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axiomatic in clinical-trial design if you have a big enough n, in randomization, all this comes out in the wash. But we are not doing TPA trials with 10,000 in each arm. We are an orphan disease. We are looking at trials where we have 58, 80, 100. Maybe some stratification might be useful in regards to that.

The other issue relating to the patient is whether, Dr. Gil, do you think we are going go be, at some point--I know you don't think we are now, but are we going to be at a point where we should be stratifying for genetic factors like HLA, that sort of thing?

DR. DiMICHELE: Dr. White, Dr. Larson, Dr. Golding.

DR. WHITE: Peter has his light on before I did, but I just want to rephrase your question because I am afraid there is a little ambiguity in it. PTP is defined. It is anybody who has gotten a single treatment or more. What you are asking is what are the critical number of exposure days that a PTP should have for a study.

DR. DiMICHELE: Right.

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DR. WHITE: So I want to make sure we don't try to redefine what a PTP is; anybody who has gotten a single dose of factor of any form is the PTP.

DR. DiMICHELE: Sorry about that. Dr. Larson?

DR. LARSON: All these points are kind of coming together in one thought in my mind and that is looking forward, again, as we talk about modifying the Factor VIII molecule, there may be some level of changes in antibody that will be acceptable in order to get the much better benefit from a different molecule.

You have just brought up a point that I made in my talk about a patient who had an inhibitor detected on the study and, when you went back and looked really hard, you could find there was evidence of an inhibitor in the past. So it just challenged people to think about what sorts of patients like that would not factor into these elegant calculations that were defined by the FDA earlier in terms of what is going to be acceptable and not acceptable.

I think we are going to need to think about that going forward.

DR. DiMICHELE: Dr. Golding.

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DR. GOLDING: I have a question and the question is could the studies be designed somewhat differently. In other words, instead of taking PTPs and starting with a new drug, with a new Factor VIII, immediately, what stops you from having a period of time, whether it is a week, a month, or whatever the experts say is required, to establish that this person is not making inhibitors to the previous product, to the old product, and then switching them over so that there would be a period of time before you change it.

But I would like to make a comment related to some of the previous discussion. When it comes to antibodies and what their importance is, clearly, you can make antibodies to Factor VIII and some of them are going to neutralize, some of them are going to have an effect on PK, and that you can establish. It sounds like it is easy to establish that clinically while the PK studies are maybe a little bit more complicated.

But, in terms of high titers and which levels of inhibitors are going to have a clinical effect, you can see very quickly in your patients. So those are the endpoints that are much more important than looking at

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Bethesda units and whether it is 0.5 or 0.6 is an important cutoff. So the cutoff should be determined by the clinical outcome and not by the number of Bethesda units and the trials should be designed to look at that rather than looking at assays and variability of assays between labs.

DR. DiMICHELE: Important comment. With respect to this definition of the PTP, or the not at risk, or the presumably not-at-risk PTP, I am wondering if the regulators would mind if we open this up to some comment from the audience because I think this is a very important question. Does anybody out there have any other comments with respect to how one would define the not-at-risk or the presumably not-at-risk PTP. Dr. Lawler?

DR. LAWLER: It seems like the prospective trials of actuarial incidence of inhibitor development that have been done are fairly consistent in the number of exposure days at which you start to see a plateau. So if you arbitrarily said that--I don't remember the numbers, but after ten exposure days, 90 percent of the patients that are going to develop inhibitor will develop

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inhibitor. Then, if you build some safety number on top of that, then you could use that as the definition of PTPs who are not at risk.

All that good data is out there, the ReFacto data, the Kogenate data and the Recombinate data.

DR. DiMICHELE: It is there. I think the question is in the interpretation. As we have seen people present these studies, even today, they will come up with the variable numbers as the analysis differs. I think a lot of it has to do with the definition up front which is why I think, in my opinion, the up-front definition is very important.

But you are right. There is certainly some data there that can be looked at.

DR. LAWLER: No; I was talking about the PUP trials that show--

DR. DiMICHELE: Oh; I'm sorry. The PUP trials.

DR. LAWLER: After you get exposed, things level off where, then, the incidence of inhibitor development becomes very low.

DR. DiMICHELE: Right.

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DR. LAWLER: It seems like 50 exposure days is very generous if you just look at that data alone.

DR. DiMICHELE: I think the biggest issue there is that there are gaps because they are only followed for a certain period of time and then there are major gaps in that follow up. I think it that interim follow-up period that we are--after the major risk period that I think we really don't have a lot of the data for, at least, certainly, incomplete data. But I think your point is a good one.

I believe Dr. Hoots?

DR. HOOTS: I am not sure we ever reach a point where it is truly asymptotic. I think that is what the latest data would suggest, both the survey data and the data that came from the retro look at the MacMillan paper. So I think what Pete said is probably right. There is a point beyond which you have a very high risk.

But I think in terms of designing the impact of treatment prospectively, I wonder if we don't need to think of it slightly differently. Instead of statistically setting a risk threshold, why not look at it the way that it was presented in the MacMillan data,

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the way it has been presented in the Lusher Recombinate data, as Kaplan-Meiers over longer periods of time because we have forgotten certain confounders that we know, in terms of taking this into consideration.

Exposure days don't define time. You said that, Donna. You said that you thought exposure days, and I agree, that is probably a very relevant thing. But time may be important as well. If you follow a group for four years, the risk that they will have an inflammatory up-regulation is probably four-fold greater than if you follow them for one year. Those inflammatory up-regulations may be very important onto whether they have a predisposition to inhibitors.

If this were a cancer trial, you would do a prospective Kaplan-Meier and you wouldn't worry if you got too sudden inhibitors but if it leveled off for the next five years. But the way it is set up now, that is not the way it is designed. I would make a strong plea that at least the statisticians look at possibly defining it in that way.

It means they may have to follow patients longer before they can make their decision but better that than

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prematurely closing out a potentially good product for reasons of early inhibitor development.

DR. DiMICHELE: If I could just make a comment on that before we go to Dr. Carcao. I think you make a very good point and I am going to have to rescind something I said in terms of my schema because I think there are two issues, then. One becomes the issue of, in a short-term trial, how do you define a PTP, like in prelicensure trials. But, let's say, in the postlicensure pharmacosurveillance, maybe, indeed, the PTP shouldn't be defined as it is in a preclinical trial but rather actually the surveillance should pick up where the prelicensure clinical trials left off and, you are right, establish this--as Pete was suggesting, try to figure out where the prelicensure data ends and let's pick that up so we can ascertain the risk, after 50 exposure days, never mind after 150 or 250 exposure days, in terms of the long-term and for a greater number of patients. That is a good point.

Dr. Carcao.

DR. CARCAO: My comment was really, I guess, sort of simple. Just looking at the nomenclature of PTP,

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I think, as Dr. White said, anybody who has had one exposure is no longer a PUP because that patient is now a PTP, if you strictly go by the English language, because it not a useful term anymore. We know that, if you are within your first ten exposures, you are totally different than if you are between your 250th and your 260th exposure.

So, perhaps, we should either abandon the term and maybe substitute it with minimally treated patients and extensively treated patients or simply just look at the number of exposure days that you have had.

DR. DiMICHELE: I think that is a good point. I think if you really think about the way the definition arose, previously untreated patients meant totally previously untreated but, in many ways, we may have patients who were virgin to treatment, to any treatment at all. Then we have patients who may be previously untreated with respect to a particular product, or previously treated with respect to a particular product, so there are many layers to that definition. But you are right.

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DR. JAIN: Let me just clarify from the regulatory standpoint. The PTPs, what we are talking about here are previously heavily treated. The reason we are using this, in a very small trial, any patient which, not due to the previous treatment, develops inhibitor and develops in that 50 exposure days, these are very small trials.

You have to start somewhere. That was the reason we had this at least treated for 150 exposure days.

DR. DiMICHELE: Understandably, I think what you are saying is you need an immunogenicity model. You need a prelicensure immunogenicity model.

DR. JAIN: Right.

DR. DiMICHELE: I think the other question, though, is how do we test out the definition that you may or may not continue to use at this point. I think that is with some longitudinal studies that maybe avoid that definition altogether. We may begin to talking about apples and oranges.

I guess there is a comment from Dr. Ewenstein. Dr. Chang, do you have a question?

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DR. EWENSTEIN: I was going to second what Keith said. I think it is just obvious. If you try to immunize someone against hepatitis A or B, you can't give all the doses in the first three days and say, I got my three doses in. We know that is not the optimal way and I think we sort of forget sometimes that 50 exposure days in 50 days and 50 exposure days over four years is probably immunologically not identical. But I think we have gotten a little bit too hung up on just the EDs and forgotten the Disease.

He is right. I think the other point that he is right about and maybe this needs to be built into the trial designs prospectively are these other risk factors. Obviously, the folks up front and everyone else, really, are interested in product-specific effects. So we, right off the bat, talk about a product-related AE and an unrelated AE and we assume that certain things don't matter, like getting hit by a bus--it matters to the patient but not to the product evaluation--whereas other things are clearly, at least potentially, product related.

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If we were smarter and could think ahead of time--it has to be a priori, obviously--what sort of high-risk events might happen to a patient that would not necessarily count against the product. What we heard today is really having a pretty strict definition of failure in the study where only a couple of patients might actually end in an unsuccessful trial.

But if we could think ahead of time of what special circumstances might--it may be premature, and this may take additional research, but I think it is going to be necessary, if we are really going to try to understand what is product-related and what you called host/treated-related. That could include sever infection, surgery, that kind of think.

DR. DiMICHELE: I think the question is, Bruce, and maybe you will give some thought to this, is that the question is whether that can actually be studied in a product-specific, even a product-specific, pharmacosurveillance, in terms of there being enough numbers to really tease out, in a multifactorial way, what is going on here.

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That is where, again, a global pharmacosurveillance program that is looking at all products and looking at all of these host factors and these specific treatment factors in a much more global population might be able to tease out that information.

DR. EWENSTEIN: I agree. It is probably premature, but what I was thinking about is in terms of a goal of what you were proposing, was to have enough of a feeling about what those what we call environmental factors but that are non-product-related, that it would really be helpful, then, when you evaluate a product, to sort of know what the other risk factors were and not just assume it was all the product.

DR. DiMICHELE: Does anybody else on the panel want to ask a question at this point? Go ahead, Mark.

DR. WEINSTEIN: I think an important element of our discussion here, in thinking of the future, was one of the ones that were raised here by Sanj about the notion of a standard, something to help us standardize our assay, something that could be used by industry and regulators and so forth. I was just wondering if there was further discussion about--in other words, a question

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about whether polyclonal rabbit antibody is reasonable, or monoclonal antibody, what steps do we see that are necessary to try to promote this, or, in fact, do other folks see this as one of the critical issues in standardizing the assay.

Is there any question that that isn't the proper way to go forward. So there are two parts here; should we do it and what should we do if we decide to do it.

DR. RAUT: So, with respect to standardization, if we agree that a standard would help in these assays, the question really is what should we put into our standard. A number of people have expressed various opinions as to what we could put in.

Obviously, we have the rabbit polyclonal and certain humanized monoclonals, but the suggestion was why can't we put in patients', inhibitor patients', samples if it is possible to collect them.

Really, the clinicians here could help us answer if it is practically possible to, perhaps, pool together inhibitor-patient samples. Would that be useful? Perhaps people could comment on that

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The second question; what sort of level may be useful for the clinician to get a clinically relevant answer from these assays.

DR. DiMICHELE: Do any of the speakers--we will give it to the speakers first. Do any of the speakers want to answer that question? Do you want to answer that question?

AUDIENCE: Yes. I guess I just want to clarify the question about whether you are talking about a standard or a control that will be used to cross all laboratories because, if you are really talking about reading Bethesda units off of a standard curve, that really can't be done because the different patient samples won't have the same potency.

So, if you tested two different concentrates, you will get two different numbers. But I think having some controls that you can compare across laboratories to see how different the laboratories perform would be useful. However, I think that that has already kind of been done by sending out samples that multiple laboratories have tested and shown to get different numbers.

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So I think that what you are really talking about is not necessarily having a standard, being able to resolve the problem across laboratory differences, but laboratories have to sort of standardize the way they are actually running the assay and that can include things like really understanding how different lots of reagents compare, and so on.

So I think it is a lot more complex than just saying if we had a standard, all the assays would perform the same and the numbers would be the same.

DR. DiMICHELE: Dr. Raut, do you want to answer that? Are we talking about controls or standards? Do you have a response?

DR. RAUT: Obviously, a control would be important. I am not saying here that standard is the only--a reference preparation isn't an only issue here. I think, in my talk, I sort of alluded to the fact that there is a whole number of reasons why we may get variability. These also would need to be addressed at some point. But we need to possibly start somewhere in terms of getting--we may have it within sort of lab position, but, in order to clinically say one clinical

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lab has X and the other Y, certainly these collaborative studies have shown that we do get a slightly better result, and perhaps we should have a discussion on the potential guidelines as to these inhibitor assays, itself.

Essentially, we are measuring residual Factor VIII activity and relating to a particular unit, in this case, Bethesda units. So, really, we should be looking at those sort of factors. And I agree with you, that there are other factors like dilution steps, activation steps, of Factor VIII assays.

The Factor VIII assay, on its own, is an area, a mine-field area, really, in terms of interlab variability, or certainly can be. So if labs are actually having problems with just measuring residual Factor VIII activity, perhaps we should look at that, also.

DR. DiMICHELE: Dr. Chang? Maybe you can ask the last question. We are coming to the end of the time.

DR. CHANG: Actually, I just want to make a comment. I think you already have data to demonstrate the standardization of the method will reduce the

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variation. But the real question that whether or not you are going to have a standardized assay method that is clinically relevant.

You may standardize it, but it has drifted out from the clinical relevance. That is not a proffered outcome. So then you come to the reference standard. I think the reference standard probably can link to the clinical outcome in some degree But using the rabbit antibody, that probably is not the best choice. But you raised several times, what are the other ways to get the materials.

So I have no idea whether there is a possibility to create several monoclonal antibodies by using the human gene, then combining them together. But it is not a small project to do.

DR. DiMICHELE: I think the assay becomes a very complicated question. Dr. Lawler?

DR. LAWLER: I have two comments. One is one of my colleagues at Ipsen who had to leave made a suggestion about pooling plasma. I don't know if that is what you are alluding to but, ideally, if you had 25 or 30 inhibitor patients that were basically randomly selected

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and then pooled that plasma, that would, to me, be the best standard one could come up with. It would be very similar to a pooled plasma that is used to define the Factor VIII standard.

The second comment I would make regards the inherent imprecision in the assay. In our laboratory, on a research-grade basis, we have three different assays which we call the Volkswagen assay, the Cadillac method and the Rolls Royce method. The Volkswagen method is defined one datapoint that falls in that 25 to 70 percent range. We call that Bethesda unit which is the published method.

The way we typically do it, which is the Cadillac method, is we get three points that are between 40 and 60 percent which means the technician spends a lot of time trying to hit the target. And then, the more rigorous way, is we try to get at least ten points between 90 and 10 percent and then we do a regression line, which I mentioned earlier this morning.

I am not saying one should do that, but what you find, in our hands, anyway, with an experienced technician doing it, is that the scatter is scary. The

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regression coefficients that you get in that, when you do that, are typically about 0.7 or 0.8. which, to me, means that there is an inherent imprecision in the assay, the way it is done, which means that the coefficient of variation is going to be pretty high regardless of whether you have a standard or not.

I don't know if anyone else has that experience. I was just reading a paper the other night that said that the coefficient of variation on the Bethesda assays was 7 percent. I don't think that is possible. I think there is too much noise inherent in the antibody, in the way the antibodies work in the the assay.

DR. DiMICHELE: Thank you for your comments. A reference pooled, sort of a pooled patient plasma reference?

DR. RAUT: I think your comment on that respect is a good one. The only practical question is are we able to get hold of such material. I think it was suggested that, perhaps, 20 inhibitor patients or so could perhaps donate a liter or so of blood towards this.

If that is possible, and this is really where we need comments from clinicians here, can we extract that

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amount of, perhaps, 40, in order to pool together. And then are we looking at the question of low titer or high titer? This is a decision just to be arrived together with some sort of a body and needs backing before we could carry out.

AUDIENCE: The good news is that I would probably recommend having a plasma that has a Bethesda titer of about 10 or so would be what you would define as a high-titer plasma. You could actually go in and characterize it in terms of heavy-chain and light-chain specificity to make sure that it is typical with the population.

Dorothea Skandela's group defined heavy-chain and light-chain specificity. You could do that. One of the good things about working with the inhibitor plasma as opposed to the patient plasma as you do to define Factor VIII standards is that, if you have a ten-unit per ml Bethesda titer, that means you are diluting it substantially to get it on scale so that you would need less plasma than when you are trying to get your pooled plasma to define Factor VIII standards.

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DR. RAUT: Point taken. I think the second point, with respect to--sorry; the second point you were making about the assays, itself?

AUDIENCE: The fundamental limits to the precision or the--

DR. RAUT: Right; of course. Sorry. I mean, you are probably aware, but there are currently a number of assays being developed which are getting away from looking at Bethesda titers as such but for looking at the kinetics of these inhibitors, whether it is type 1, type 2, and that has been defined as type 2 and type 2.

We may need help here from clinicians, from inhibitor samples, to look at, in more detail; for example, how a type 1, whatever type of inhibitor we have, how it actually behaves kinetically and should we--I mean, there are assays. I am basically saying that there are assays being developed which are able to look at these factors fairly simply. The idea is to get these assays on board as well, but this is something that we haven't really discussed so far, which may be very relevant within the next two years.

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DR. DiMICHELE: Bert, did you have a comment? I was just going to have you make one very brief comment before we have Dr. Jain ask the last question of the session.

DR. VERBRUGGEN: Thank you. I want to comment on the standards. I think we need a standard which is about 1 Bethesda unit or Nijmegen-Bethesda, if you wish, because when you have a higher standard, then you also introduce dilution problems.

I would like to give an example. When you analyze an inhibitor and you get the data of 0.8 or 1.2, where you have diluted the plasma ten times, then you have a tremendous difference between these results. They vary from 8 Bethesda units to 12 Bethesda units. So it is really important, also in the lower range, to have a very reproducible and standardized method.

I think, whether you need a Volkswagen or a Cadillac or a Rolls Royce, it depends on what you want to know and where you come from. So, if you want to monitor a patient, you need an assay which is also specific in the higher region. When you want to diagnose a patient for the first time, you want to know whether this patient

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has an inhibitor or not. When you want to do some research on the reliability of low inhibitors, then you need a very sensitive method.

DR. DiMICHELE: Good point.

Dr. Jain, your last question to the audience.

DR. JAIN: One last question, and I promise it is the last one. I thought I had to ask this question before I leave this room. This question is for all the treaters present in the room here. The question is, today we have seen different observed inhibitor rates in--let's consider it only in PTPs--based on clinical trials, on pharmacovigilance data, and we have heard different ways, right now, for assessing true or observed inhibitor rates in the future.

Now, the question to the treaters is what would you expect, or accept, as for any new product as your safety acceptable rate of inhibitors for any new product?

DR. BERGMAN: I don't want to even guess at answering it, but I do want to ask you to just specify, when you say the rate of inhibitor in a PTP, do you mean per year, per exposure or during the course of a study?

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DR. JAIN: Let's start with at least a minimum of 50 exposure days, PTPs. We put out some numbers here based on our experience in the clinical trials. The other numbers we have put on for other speakers. Industry had--you put on their numbers based on their pharmacovigilance data.

I think we should now have--for any product, new product, what should be the safety limit which we are looking for as treater?

DR. BERGMAN: The question, just a follow up, what is the historical data on the rate of inhibitor in PTP for acceptable products on a per-exposure day basis?

DR. DiMICHELE: Or I guess--well, go ahead, Dr. Jain. You define it.

DR. JAIN: We have heard that; right? We have heard all this, what was the historical rate, the observed rate, the true rate or whatever we call it as the incidence rate here. I am not talking about the prevalence at all. I am talking about the incidence rate.

Any new product coming on the market, we have set our limits as 6.8 we said we would accept as--out of

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this sample size, we would accept that as our safety limit. As a treater, what do you think you would allow for a new product?

DR. DiMICHELE: Dr. White?

DR. WHITE: I think you have the answer to that already. I think we have done studies and you know the number of inhibitors that have occurred. I think you need to take that as your road map. I don't see any reason to necessarily change it. As Jay said, if you use the number that you are currently proposing, you would not have approved a couple of products. So I think you have to be careful with that number.

I think, as we get to know some of the risk factors for inhibitor--and whatever number you do pick, you have got to build into that number enough wobble so that if one study happens to have 50 percent significant deletion mutations, and 35 percent African American, that that study at least has a chance to give you a result that could be an approvable result compared to a study that has 95 percent Caucasians and 75 percent missense mutations.

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Those two studies are going to have a big difference in the risk of inhibitor formation and, until we know enough about real risk of inhibitor formation that we can start stratifying these studies, I think you are going to have to allow some wobble in the populations that go into making up those studies. Does that make sense?

DR. JAIN: That is one good suggestion. It is not basically relying on numbers. You would look at other stratification and come up with some sort of a list here.

DR. WHITE: I think you have to use your good sense, as you always have. You have to look at the results that you get and you have to say, this is bothersome to me. I am seeing too many inhibitors in this study. Let's look very carefully at the next couple of patients that are studied.

DR. JAIN: But, see, now we are coming back--in a clinical study, we are coming back to retrospective analysis now. Either you define it prospectively that these are--

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DR. WHITE: I am not going to retrospective. What I am saying applies to a prospective study. You just look at the data as it comes in and, if you see two inhibitors in the first five patients that you study, you have got to look at those patients. You have got to see what their risk factors were. You have got to see if they had prior inhibitors and you have to make a decision.

DR. DiMICHELE: Dr. Evatt?

DR. EVATT: I have to agree with Gil. You don't really have enough data to set this and, if you set it at the 6.8 at 95 percentile, you are going to reject 1 out of 20 products that are good because you are going to kill the study immediately when that second patient occurs. It is based upon assumptions that the data we are putting on the board is really truth. We don't have any idea whether it is truth or not.

They are observations. The data is real, but it is our interpretations that may not be correct at this point. I think it really needs some really good close examination before the limits are defined exactly and I would have to agree with Gil on that.

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DR. JAIN: See, that is the point of my question. We have, from a previous experience, seen this. So that means we now have to go along and change some criteria which you have set in a previous experience. That is the real question here.

DR. DiMICHELE: Dr. Jain, maybe that is why the pharmacovigilance is going to be of critical importance because it may be the pharmacovigilance that may define, up front, the prelicensure criteria that we need to use in terms of really seeing problems as they develop later on and leading it back to the prelicensure data.

DR. JAIN: Exactly.

DR. DiMICHELE: Let me give Dr. Feingold one last comment and then I am going to turn this over to Dr. Lozier for closing comments.

DR. FEINGOLD: I think that, in some ways, your fundamental assumptions that you made in your talk are incorrect because you spoke about an intent-to-treat analysis but you reviewed the data from the clinical studies that you showed without an intent-to-treat analysis. You can't have it both ways.

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If it is intent-to-treat, then every product up there except for one would not have been licensed according to your guidelines. If it is de novo inhibitors or inhibitors that occur after, with no history, then you are talking about a different group of patients. So I think you can't have it both ways. You are either going to look back at the previous studies and say you are going to use an intent-to-treat, in which case you better change your confidence interval, or you are going to say that it is based, in the study, with patients that were appropriate to the study.

DR. JAIN: I think that our statisticians can answer that question. But, as far as I know, I think all our studies have been licensed on intent-to-treat. As a regulatory standard, we use intent-to-treat. That is what our regulatory standard is.

Closing Comments

DR. LOZIER: I think, at this point, we will go ahead and close the conference. We are over time, but I think profitably so. Thank you all for coming. Please send in comments. We will give, let's say, 30 days of time for people to comment written or e-mail, as per your

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handout. If you can turn in evaluations as you leave, I would thank you.

Thank you very much.

(Applause.)

(Whereupon, at 5:44 p.m., the meeting was adjourned.)

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