

FOOD AND DRUG ADMINISTRATION  
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH  
CENTER FOR DRUG EVALUATION AND RESEARCH

STRATEGIES TO ADDRESS HEMOLYTIC COMPLICATIONS OF  
IMMUNE GLOBULIN INFUSIONS

Washington, D.C.

Tuesday, January 28, 2014

1 A G E N D A

2 Welcome:

3 PETER MARKS, M.D., Ph.D.  
4 Deputy Director CBER/FDA

5 MARY GUSTAFSON  
6 Vice President, Global Regulatory Policy  
7 PPTA

8 KEITH HOOTS, M.D.  
9 Director, Division of Blood Diseases  
10 Research NHLBI

11 Statement of Problem:

12 DOROTHY SCOTT, M.D.  
13 CBER/FDA

14 Session I: Pathogenesis and Epidemiology of IG  
15 Product-Related Hemolysis

16 Moderator:

17 JAMES BUSSEL, M.D.  
18 Cornell University

19 Panelists:

20 MARKUS FUNK, Ph.D.  
21 Paul Ehrlich Institute

22 ELAINE TAYLOR, M.D.  
Health Canada

SCOTT WINIECKI, M.D.  
CBER/FDA

ROGER BERG  
PPTA

## 1 A G E N D A

2 GEORGE GARRATTY, Ph.D., FRCP  
3 American Red Cross

4 RUTH PADMORE, Ph.D.  
5 University of Ottawa

6 JACOB PENDERGRAST, M.D.  
7 University of Toronto

8 Session II: Product Risk Factors

9 Moderator:

10 DOROTHY SCOTT, M.D.  
11 CBER/FDA

12 Panelists:

13 DONALD BRANCH, Ph.D.  
14 University of Toronto

15 SUSAN THORPE, Ph.D.  
16 National Institute for Biological  
17 Standards and Control

18 CAROLINE BELLAC, Ph.D.  
19 Swissmedic

20 CATHERINE DE COUPADE  
21 LFB Biomedicaments

22 JAMES ZIMRING, M.D., Ph.D.  
Puget Sound Blood Center

NAOMI LUBAN, M.D.  
Georgetown University

21 \* \* \* \* \*

22

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

2 (8:35 a.m.)

3 DR. SCOTT: I think we'll begin our  
4 workshop now that the time is upon us. I just  
5 first want to make a couple of announcements, one  
6 is, no eating or drinking here. The reason is,  
7 they're concerned about the equipment, but you  
8 should also know that they have a three strikes  
9 and you're out policy, which means if they catch  
10 three different occasions of people eating or  
11 drinking in the audience, they can close the  
12 workshop down.

13 I don't know if this is an empty threat,  
14 but I don't want to find out.

15 You have speaker biographies and you  
16 have questions in your agenda. I think that we  
17 will not go through the biographies in any detail  
18 now, but please refer to those, and without  
19 further ado, we'll get started with our  
20 introductions and first we have Dr. Peter Marks,  
21 who's the deputy director of CBER and who's taken  
22 a great interest in all of these sorts of issues

1       that we have, and I'm very grateful that he's here  
2       to say hello to you all.

3               DR. MARKS: I made it. Sorry. I always  
4       forget how traffic is out in front of NIH in the  
5       morning, but thank you very much for coming this  
6       somewhat cold, chilly morning here.

7               This is actually the first -- I've been  
8       down in DC for three winters -- this is the first  
9       winter it's actually been like where I came from  
10      in Boston and New Haven, so welcome.

11              I want to welcome you to this  
12      FDA-PPTA-NHLBI workshop on strategies to address  
13      hemolytic complications of immune globulin  
14      infusions. Since the agenda is pretty full, I'm  
15      not going to spend too much time with opening  
16      remarks.

17              Though hemolysis has been a potential  
18      known complication of the administration of immune  
19      globulin infusions for quite some time, the  
20      increased use of these products in a variety of  
21      different settings necessitates a reevaluation of  
22      the patient and product risk factors as well as

1 the potential ways that steps can be taken in  
2 manufacturing to mitigate this complication, which  
3 can be relatively severe in some cases and  
4 actually trigger the need for additional medical  
5 evaluation and intervention.

6 We hope that the next day and a half is  
7 filled with an animated discussion and dialogue on  
8 these products.

9 At this point before I close, I just  
10 want to thank all of you for taking the time to  
11 participate in and contribute to this workshop,  
12 whether you're an attendee, a steering committee  
13 member, presenter, speaker, or session chair. I  
14 also want to thank PPTA and NHLBI for co-  
15 sponsoring the workshop. And I'll also take the  
16 opportunity to thank Mary Gustafson of PPTA,  
17 Phyliss Mitchell of NHLBI, and Dr. Darcy Scott, of  
18 our own Office of Blood Research and Review at the  
19 Center for Biologics, Evaluation, and Research,  
20 for putting together such a stimulating program.

21 So, I very much look forward to the  
22 dialogue and I will wish you a great day today and

1 tomorrow. Thanks very much.

2 (Applause)

3 DR. SCOTT: This will be Mary Gustafson,  
4 vice-president of Global Regulatory Policy, PPTA,  
5 and she will give us a welcome.

6 MS. GUSTAFSON: Thank you, Dorothy. On  
7 behalf of the Plasma Protein Therapeutics  
8 Association and our member companies, I want to  
9 welcome you to this workshop on this extremely  
10 cold day.

11 PPTA global member companies are Baxter,  
12 Biotest, CSL Behring, Grifols, and Kedrion, each  
13 manufacturer's unique branded therapies within the  
14 immune globulin class. While this is a U.S.  
15 workshop, we appreciate the global involvement in  
16 the planning -- with representation on the  
17 planning committee -- broad representation, and  
18 also participation in the workshop by  
19 international regulators and other manufacturers.

20 The manufacture and use of Plasma  
21 Protein Therapies including the immune globulins,  
22 are global. Regulatory approaches need to be

1 harmonized as immune globulins are not defined by  
2 boundaries.

3 We are grateful to the FDA workshop  
4 committee for the logistics of this workshop. In  
5 spite of the prohibition against food and drinks  
6 in the auditorium, this is a wonderful venue for a  
7 workshop and it's also hard to secure, and so I  
8 thank our FDA colleagues for being tenacious in  
9 getting this auditorium.

10 We also thank all the speakers,  
11 especially those who have traveled from a distance  
12 to be part of this workshop, and we too look  
13 forward to a valuable exchange of information  
14 during today and tomorrow morning. Thank you.

15 (Applause)

16 DR. SCOTT: I'd also like to welcome Dr.  
17 Keith Hoots, who's the director of the Division of  
18 Blood Diseases Research in NHLBI and to thank  
19 NHLBI as well, for their co-sponsorship.

20 DR. HOOTS: Thank you, Dr. Scott, and on  
21 behalf of National Heart, Lung, and Blood  
22 Institute, and in particular, the Division of



1 Blood Diseases and Resources, we welcome you here  
2 to the NIH campus for this important FDA  
3 conference on intravenous gamma globulin.

4 In thinking about this conference, I  
5 kind of hearken back to my first experience with  
6 intravenous gamma globulin more than three decades  
7 ago as a junior faculty member, when a young  
8 teenager was sent to us from Panama with  
9 intractable ITP. She had failed splenectomy,  
10 azathioprine, prednisone, you name it, she'd  
11 failed it. And this was in -- literally months  
12 after FDA had licensed a drug for that indication.

13 And she, soon after arrival, developed a  
14 massive intracranial hemorrhage and, I think -- I  
15 don't know, but I think, and we actually did  
16 publish the experience, that because of  
17 intravenous gamma globulin, that young lady  
18 survived to go back to Panama.

19 So, it's obviously, since that time,  
20 gone through many iterations and many diseases,  
21 but it is an important biological product and I  
22 think anything that we can discover to make it

1       safer in that context is really important, hence  
2       our presence today and tomorrow and for that I'd  
3       like to thank all the speakers who agree to bring  
4       their expertise here to discuss this and  
5       particularly to FDA for putting this together and  
6       for Dr. Scott for organizing it on their behalf.  
7       And welcome again. Thank you.

8                       (Applause)

9               DR. SCOTT: All right, we'll get started  
10       with the first set of slides. Thank you.

11               Welcome to our workshop on strategies to  
12       address hemolytic complications of immune globulin  
13       infusions. We hope that this workshop will  
14       provide us all a better understanding and help us  
15       to develop better approaches to prevention and  
16       mitigation of this risk of immune globulin  
17       infusion.

18               So, that's the overall charge to you,  
19       the participants. I'm going to briefly review how  
20       we designed this workshop and I'm going to start  
21       off with some background.

22               As most of you know, hemolysis is a

1 long- recognized complication of immune globulin  
2 infusion and it's attributed to IgG isoagglutinins  
3 in the products that obviously co-purify with  
4 other IgG. Risk factors already known include  
5 high doses, recipient blood type A, B, or AB, and  
6 complications can include clinically comprising  
7 anemia, transfusion requirement, renal failure,  
8 and in rare cases, disseminated intravascular  
9 coagulation.

10           The specifications -- all products have  
11 to undergo this testing before release. For  
12 anti-A and anti-B titers in the EU and the U.S.  
13 are less than or equal to 1:64 by the direct  
14 agglutination test, which is validated by  
15 manufacturers.

16           Nevertheless, hemolysis occurs even  
17 though these products meet specifications. And so  
18 we do need to figure out additional risk  
19 mitigation strategies that could be useful in the  
20 clinic and also at the product side.

21           This is historical. It's still posted  
22 on the web, but I just wanted to make you aware

1       that FDA recommended some labeling for the  
2       complication of hemolysis, which essentially --  
3       and you don't need to read all of this -- states  
4       that immune globulins contain blood group  
5       antibodies, that these may cause hemolysis, and  
6       that IGIV recipients should be monitored for  
7       clinical signs and symptoms of hemolysis and they  
8       also mentioned appropriate confirmatory laboratory  
9       testing of hemolysis is suspected.

10               Since then, there's been a great deal of  
11       evolution in the package inserts and now we don't  
12       see entirely similar package inserts at all times,  
13       but we'd like to work towards something that's  
14       consistent and useful.

15               We published a safety communication in  
16       2012 mentioning our heightened awareness of the  
17       potential for hemolysis and also listing some of  
18       the risk factors that I've already mentioned for  
19       hemolysis, and this wasn't necessarily included in  
20       labeling.

21               The risk factors include non-O blood  
22       group type, people with underlying associated

1 inflammatory conditions -- I think we'll discuss  
2 this today and whether or not that can really be  
3 dissected from the high doses that most of these  
4 folks receive -- and people receiving high  
5 cumulative doses of immune globulins over the  
6 course of several days.

7 As already noted in the basic product  
8 labeling, patients receiving immune globulin  
9 products should be monitored for hemolysis  
10 particularly those at increased risk, and we  
11 listed in that communication some of the clinical  
12 symptoms, actually, and signs of hemolysis.

13 Now, this is just to give you the  
14 framework of where we are. We're actually a  
15 little farther than this. Some individual package  
16 inserts have a great deal more detail about  
17 hemolytic complications, and you'll see this in  
18 the newer package inserts.

19 Now, I'm just going to briefly go  
20 through the sessions and what I hope we get out of  
21 them. You might think of other things we will  
22 also get out of the sessions.

1           I think we have a great audience here  
2   and we expect a lively discussion.

3           Dr. Bussel is chairing the first session  
4   and this focuses on the epidemiology and  
5   descriptive analysis of patient risk factors and  
6   hemolysis outcomes. We have four talks here.  
7   Following that we will hear about eh pathogenesis  
8   of isoagglutinin-mediated hemolysis and IG  
9   product-mediated hemolysis.

10          The goals are to identify the most  
11   important risk factors and to refine information  
12   about known risk factors for hemolysis, so this is  
13   helpful when we're thinking not only about risk  
14   communication, but also prevention.

15          We'll ask the panel and the audience to  
16   list potential risk factors that need further  
17   study in addition to the risk factors that we  
18   already know about.

19          We are going to ask for discussion of  
20   the feasibility and possible predictive value of  
21   patient or patient/product testing prior to  
22   infusion of high doses in at-risk patients. And

1 if we have time, we'd like to discuss what might  
2 be an optimal definition of hemolysis for purposes  
3 of case identification in immune globulin infusion  
4 situations.

5 The second session, chaired by myself,  
6 will concern the origins and characteristics of  
7 anti-A and anti-B isoagglutinins, the  
8 specifications that we currently have for anti-A  
9 and Anti-B in IG products, and that includes the  
10 testing methodology.

11 We will see a survey of hemagglutinin  
12 titers across products. We'll hear about  
13 alternative test methods for anti-A and anti-B  
14 measurements that are being developed,  
15 essentially, and we'll also discuss product risk  
16 factors that may be present in addition to anti-A  
17 and anti-B.

18 The idea for Session II is to identify  
19 approaches and methods to determine whether  
20 there's a threshold dose of isoagglutinins that  
21 causes clinically significant hemolysis. This has  
22 turned out to be more difficult than you might

1 initially imagine, so there are going to be data  
2 gaps and methodological gaps and several of us in  
3 this workshop have tried to come up with a way to  
4 look at this question.

5 We also want to discuss anti-A and  
6 anti-B specifications as well as the current and  
7 potential test methodologies and to identify the  
8 product characteristics that may exist other than  
9 anti-A and anti-B that could contribute to  
10 hemolysis and how these might be further studied.

11 The third session is focused really on  
12 immune globulin manufacturing and whether some  
13 risk mitigation can be attained through  
14 manufacturing changes. The first several talks  
15 are surveys of donor plasma to look at the  
16 distribution of anti-A and anti-B titers with the  
17 real underlying question whether or not some of  
18 these donor plasmas with high titers could be  
19 feasibly excluded from the plasma pools used to  
20 make immunoglobulin products.

21 And the second set of talks is about  
22 immune globulin manufacturing beyond the plasma



1 pool and clearance of isoagglutinins. So, we'll  
2 hear some talks about manufacturing methods that  
3 already exist in licensed -- for licensed  
4 products, manufacturing steps that may decrease  
5 isoagglutinin levels. In other words, can they be  
6 partitioned out or are they partitioned out?

7 And we also have a couple of talks about  
8 potential ways to remove isoagglutinins using a  
9 specific manufacturing step designed for that  
10 purpose.

11 For this session we'd like to discuss  
12 the feasibility, scientific basis, and utility of  
13 lowering titers in products by excluding high  
14 titer isoagglutinin donations, by considering low  
15 titer products to be developed for high dose  
16 patients. I have to tell you right now that the  
17 regulatory pathway for such a thing is not  
18 established, because I know the question will come  
19 up, and removal of isoagglutinins by  
20 manufacturing.

21 After the workshop -- I should say,  
22 before I go to after the workshop, we do look

1 forward to a lot of audience participation. If  
2 you're not sitting on a panel and you have  
3 something good to say, we'd all like to hear it.

4 After the workshop we plan to continue  
5 discussions with PPTA, individual members of  
6 industry, and patient groups, as well as  
7 clinicians, on how to optimize risk communication  
8 and perhaps even how to guide monitoring and  
9 testing for high-risk patients.

10 We plan to evaluate proposed  
11 manufacturing changes intended to lower anti-A and  
12 anti-B in products, so this is a regulatory  
13 submission issue. And in the near future, we'd  
14 like to address with our regulatory colleagues and  
15 with industry, whether or not the current anti-A  
16 and anti-B specifications are optimal for  
17 high-risk patients, and what further research on  
18 patient and product-related risk factors for  
19 hemolysis would be fruitful?

20 Also, pragmatically after the workshop,  
21 transcripts will be posted on the FDA website. We  
22 hope that we'll be able to post the presentations

1 on the PPTA website with permission, and we would  
2 like to produce a publication of these  
3 proceedings.

4 I want to very much thank our  
5 co-sponsors, the Plasma Protein Therapeutics  
6 Association and the National Heart, Lung, and  
7 Blood institute, the many speakers who have  
8 traveled from near and far on this dreadful winter  
9 day to come and help us figure out the answers to  
10 our questions, the steering committee, who  
11 provided many of the ideas, as well as the  
12 speakers, for this workshop, and the core workshop  
13 planning group, our colleagues here who've -- and  
14 at PPTA, who've helped on the administrative side  
15 in putting together everything that you see and  
16 without whom we couldn't have this workshop.

17 I have the steering committee members  
18 here and I'd like to acknowledge their extremely  
19 expert contributions and also to thank the  
20 International Plasma Fractionation Association for  
21 their input to the agenda.

22 So, thank you, everybody, for your

1 participation and without further ado, I think we  
2 will begin our talks on the epidemiology and risk  
3 factors for IgG-related hemolysis. And we'll  
4 start with Dr. Funk from the Paul-Ehrlich  
5 Institute. Thank you very much.

6 DR. FUNK: Well, good morning, ladies  
7 and gentlemen. Thank you very much to give me the  
8 opportunity to present the European experiences on  
9 hemolytic complications of immunoglobulin  
10 administration.

11 My name is Marcus Funk, I'm working for  
12 the Paul- Ehrlich Institute and we are responsible  
13 for the safety of blood products and blood  
14 components, and this will be the focus of this  
15 presentation.

16 I would like to start with a case of a  
17 serious hemolytic reaction that was reported to  
18 the Paul-Ehrlich Institute in 2013. An adult,  
19 female patient was admitted to a German hospital  
20 with a diagnosis of polyradiculitis, which later  
21 on was changed to botulism intoxication.

22 The patient had blood group AB and

1 received, during a period of five days, a total  
2 amount of 2 gram immunoglobulin/kilogram body  
3 weight. After this treatment the hemoglobin  
4 concentration decreased and the bilirubin  
5 concentration increased and two days later the  
6 patient developed a disseminated intravascular  
7 coagulation.

8           The vascular surgeons then had to remove  
9 a long thrombosis, which was located in the v.  
10 iliaca and v. Femoralis, and among other  
11 medication, the patient received or needed four  
12 units of red blood cell concentrates and finally  
13 recovered.

14           We ask our French and Swiss colleagues  
15 to test the involved immunoglobulin batch and they  
16 found an increased anti-A titer and confirmed that  
17 the batch was out of specification. Therefore,  
18 the company voluntarily recalled the affected  
19 batch in May 2013.

20           These are the test results of the  
21 Swissmedic Laboratory in detail and you can see on  
22 this slide that in both test performance, the

1 anti-A titer of this involved batch was increased  
2 whereas a second batch, which was not involved,  
3 was within normal range and had a normal anti-A  
4 titer. And also a positive control was tested  
5 that reached the thresholds.

6 Now, meanwhile, there's a great number  
7 of articles dealing with hemolytic complications  
8 associated with immunoglobulins and some of the  
9 studies present results of the measurement of  
10 anti-A levels in different immunoglobulin  
11 preparations.

12 Very recently, Caroline Bellac presented  
13 results from the Swissmedic Laboratory, and as you  
14 can see, the group compared the median anti-A and  
15 anti-B titers of different products, and there was  
16 a great difference between these different  
17 products. I think Caroline will present these  
18 results in detail later on, though it's just to  
19 show you that there are differences.

20 So, because of an increased number of  
21 reported hemolytic reactions in Europe, but also  
22 in the United States and in Canada, the

1 Paul-Ehrlich Institute then initiated a  
2 retrospective analysis of seven products and all  
3 these products are covered in the (inaudible)  
4 procedure of our institute.

5 We used our database as well as the  
6 database of the European Medicines Agency and  
7 collected all reported hemolytic reactions  
8 worldwide over a period of five years. We then  
9 asked the companies to provide us with their data  
10 of their database and also to provide us with the  
11 worldwide sales figures. Well, in order to  
12 evaluate these hemolytic reactions, we tried to  
13 collect all relevant details regarding patient  
14 history, treatment, and of course, also laboratory  
15 data.

16 As mentioned before, immunoglobulin  
17 associated hemolytic reactions are defined as a  
18 new hemolytic process within ten days after the  
19 administration and to classify the cases as  
20 hemolytic reactions, certain laboratory criteria  
21 must be documented, as you can see, decrease of  
22 hemagglutinin and increase of bilirubin positive

1 direct anti-(inaudible) test, and so on.

2 Now, this is one of the results of this  
3 retrospective analysis, the frequency of the  
4 hemolytic reaction is expressed as a confirmed  
5 case per thousand kilograms immunoglobulin sold  
6 per year and when you are now looking at this  
7 frequency, there is a difference between  
8 immunoglobulin products, which -- with a  
9 concentrate of 5 percent, here marked in blue, and  
10 of immunoglobulin products with a concentrate of  
11 10 percent, marked in red.

12 And there is also a significant  
13 difference between the frequency of product A and  
14 B when compared with the other products. We've  
15 found a frequency of five to two cases per  
16 thousand kilogram per year in these post products  
17 A and B, and in the other products, one case or  
18 less per thousand kilogram.

19 Well, these and other data were  
20 discussed with the member states of the European  
21 Union, with the European Medicines Agency, and  
22 also with the involved companies, and it was



1       agreed to include serious hemolytic reaction as an  
2       identified risk in the risk management plan. And  
3       also to include a warning in the section 4.4 of  
4       the product information for product A and B.

5               Well, according to the company of  
6       product D, these rare cases of hemolytic reaction  
7       were caused by a batch consisting of a high  
8       percentage of plasma from donors of -- with blood  
9       group O, and therefore the company announced that  
10      in future, they will manufacture batches with a  
11      more balanced mixture of donors with all blood  
12      groups.

13             The company with the product A initiated  
14      or implemented risk minimization measures. Well,  
15      as you can see, the first step in January 2013 was  
16      a screening of the plasma donors in order to  
17      select plasma donors with a low anti-A titer, and  
18      as a second step, the manufacturer is planning to  
19      make a modification of the manufacturing process.

20             Now, the benefit of these risk  
21      minimization methods will analyze, in a  
22      post-authorization safety study -- this is the

1 next slide, sorry -- and the PASS protocol cover  
2 three periods and the company will collect data of  
3 each period. So, before the initiation of the  
4 risk minimization measures and then after the  
5 implementation of donor screening in the second --  
6 in the third period, after the implementation of  
7 the modification of the manufacturing process.

8 This is the wording of the special  
9 warning that was included in the product  
10 information of product A and E, and as you can  
11 see, it is said that the following risk factors  
12 are associated with the development of hemolysis,  
13 and as we know, this is high doses. This is in a  
14 patient with blood group A or B, and in patients  
15 with underlying inflammatory disease. And you may  
16 remember the situation of the first case I  
17 presented in the beginning and all these criteria  
18 are fulfilled in this patient.

19 Well, I'm coming to my last slide. In  
20 June 2013, the European OMCL network decided to  
21 implement an anti-A and anti-B hemagglutinin test  
22 to analyze the finished product of all intravenous

1 and subcutaneous preparations. For this batch  
2 release test, the component authorities should use  
3 a direct method and they should also use, in each  
4 test performance, a positive control.

5 And furthermore, there is an ongoing  
6 discussion in this network on the definition of  
7 the right thresholds. So, I can summarize it,  
8 from our point of view, hemolytic reactions are a  
9 rare complication of immunoglobulins, there are  
10 certain possibilities to reduce this risk, and at  
11 the moment, we are in this process, it's an  
12 ongoing discussion. And I thank you for your  
13 attention.

14 (Applause)

15 SPEAKER: I wanted to just clarify that  
16 last point -- is this working? There we go. So,  
17 are you saying that hemolysis is rare or are you  
18 saying that hemolysis is more frequent, but only  
19 rarely has clinical consequences?

20 DR. FUNK: Thank you for the question.  
21 I think we have to be aware that we are speaking  
22 about serious cases and these databases only

1 collect or mainly collect serious cases. I would  
2 say that serious hemolytic reactions, they are  
3 rare events, so I would say two to four cases per  
4 1000 kilo, this means 300 administration per  
5 kilogram -- no, sorry, 30 applications per 1  
6 kilogram.

7 DR. SCOTT: I think if there are no more  
8 questions, we'll go forward, but I believe that  
9 the current question is relevant, it's how often  
10 does clinically significant hemolysis really  
11 occur? There are ways of getting at that, but  
12 probably use of spontaneously generated reports is  
13 not one that will give us a very precise answer.  
14 So, we have some other approaches and you probably  
15 are considering other approaches as well, but  
16 getting -- the reporting rate has its flaws when  
17 you're using spontaneous adverse event reports.

18 DR. FUNK: We are only talking about  
19 reporting rate, that's true, which is only a small  
20 few on this problem.

21 DR. SCOTT: Right. We have a way of  
22 getting at that data possibly with some of the

1 large insurance databases and we'll be hearing a  
2 little bit about that, but I'd like to move on to  
3 Dr. Taylor and invite her to speak. Thank you,  
4 Dr. Taylor. She has also come a long way to see  
5 us from Health Canada, but at least it's probably  
6 not colder here than there.

7 DR. TAYLOR: Yes, I'm pretty sure it's  
8 even colder in Ottawa.

9 Good morning. I'm from Health Canada.  
10 I'll be discussing the distribution of intravenous  
11 immune globulin in Canada, the IVIG adverse event  
12 reports for hemolysis, I'll do a bit of analysis  
13 for those reports that we have, the  
14 product-specific reporting rates requested in one  
15 of the draft agendas, so we'll look a bit at that,  
16 although spontaneous reporting is not very useful,  
17 frequency of exclusion criteria and reports was  
18 really something I wanted to look at just in terms  
19 of looking at how the definition works when we're  
20 looking at this data, and then conclusions.

21 In Canada we have a growing use of  
22 intravenous immunoglobulin, growing from 100 grams

1 per thousand population to 125 grams for 1000  
2 population from 2006 to 2011.

3 We have two blood manufacturers, blood  
4 distributors in Canada, the Canadian Blood  
5 Services and Hema-Quebec. Hema-Quebec is for  
6 Quebec and the Canadian Blood Service provides for  
7 the rest of Canada. In both jurisdictions, the  
8 Gamunex/IGIVnex are used the most with Privigen as  
9 the second most common, and you can see that in  
10 Quebec there's somewhat less use of the Privigen.

11 Canadavigilance is the database for  
12 spontaneous reports for Canada and we did a search  
13 from January 1st 2006 to the end of 2012 using the  
14 standard MedDRA query of hemolytic disorders.

15 The Canadavigilance is spontaneous  
16 reporting. There is mandatory reporting of  
17 serious Canadian cases by manufacturers. The  
18 causality is assessed using the Transfusion  
19 Transmitted Injury Surveillance System. This is a  
20 system in Canada that has been developed by the  
21 Public Health Agency of Canada and traditionally  
22 this is what we use for causality assessment.

1                   So, in going through the cases there  
2       were 226 that were meeting at least possible  
3       using, we'll call it, TTISS definitions.

4                   This is the case definition that was  
5       developed by the IVIG Hemolysis Pharmacovigilance  
6       working group. It has different laboratory  
7       requirements for laboratory data than the TTISS1,  
8       and the TTISS cases included cases that could be  
9       explained by alternate causes as possible cases.

10                  So, applying this definition, the case  
11       definition to the TTSS cases of at least possible,  
12       we ended up with 69 that met the definition.

13                  Now, since 2006, there are increasing  
14       numbers of reports and there's a fluctuating  
15       number that were able to meet the definition.

16                  So, in doing the analysis, I'm doing it  
17       on the 69 of the case reports that met the  
18       definition, gender, slightly more male than  
19       female, mean age, 53, age range from a year and a  
20       half to 91, age groups, mainly adult with 11  
21       percent under 18.

22                  And the blood group, I've displayed both

1 the case definition and the TTISS at least  
2 possible. Certainly A dominates followed by AB  
3 and with some B, and not provided more so in the  
4 cases that are the TTISS cases.

5 The most common implicated indications  
6 in our series was ITP and Guillain-Barre Syndrome.  
7 You can see the other causes there.

8 In terms of the reports meeting the case  
9 definition, the mean dose was 177 grams with a  
10 wide range. The grams per kilogram dosage was  
11 provided in a bit over half of the cases with a  
12 mean of 2.3 grams per kilogram, a range of 1 to 6  
13 grams per kilogram.

14 The mean doses didn't differ by product  
15 when we looked at that and the mean range was  
16 mainly between 2.1 and 2.4 for the products.

17 The characteristics -- the onset of the  
18 hemolysis was mainly a delayed onset with very few  
19 acute. The mean drop in hemoglobin was similar  
20 for each of the products and there were a small  
21 number for the Gammagard liquid.

22 Characteristics -- again, the grade of



1 reaction, mainly, was a grade four. Thirty-six  
2 percent were treated with blood transfusion, and  
3 in a quarter of the cases, whether there was  
4 transfusion or not, was not provided in the  
5 report.

6 Just going back -- no, okay, that's  
7 fine. So, in terms of the outcome, the outcome  
8 was not provided in about a quarter of cases, but  
9 in the cases where outcome was provided, it was  
10 minor or no sequelae.

11 There was one death, but it was  
12 unrelated, and in fact occurred after the recovery  
13 from anemia had occurred.

14 We looked at the number of reports of  
15 hemolysis, meeting the definition, compared to the  
16 overall number of all adverse event reports for  
17 each product, and the rates were quite similar.  
18 And just before I get to the conclusions, the --  
19 wanted to look a little bit at the use of the  
20 definition. Because of spontaneous reporting,  
21 there tends to be a lack of data, so that that  
22 resulted in the elimination of quite a few cases.

1                   And in conclusion, we did not see any  
2   differences between products. There is under  
3   reporting, it's a spontaneous system, so that it's  
4   difficult to draw much in the way of conclusions.  
5   The case definition did result in an elimination  
6   of cases, so reporters would need to be better  
7   educated about what lab work to provide.

8                   And this analysis really just confirms  
9   the known risk factors and is not able to identify  
10  any additional risk factors.

11                  We have no denominator data for use of  
12  IVIG by indication so that it's very difficult to  
13  make any statements using spontaneous data.

14  Anyway, thank you.

15                               (Applause)

16                  SPEAKER: You listed the diseases in  
17  which you found the hemolysis. Do you have any  
18  idea how that worked out as a percentage of total  
19  cases of those diseases treated?

20                  DR. TAYLOR: No. No.

21                  SPEAKER: You mentioned that most of the  
22  cases were delayed. How did you define delay?

1 DR. TAYLOR: Greater than 24 hours and  
2 up to 10 days.

3 SPEAKER: Thank you.

4 SPEAKER: You say you have both  
5 spontaneous reports and then the severe cases are  
6 required reporting. Could you tell us what a  
7 severe case is defined as that people need to  
8 report and is that the same for Health Canada and  
9 Hema-Quebec?

10 DR. TAYLOR: They don't report to  
11 Hema-Quebec, they report to Health Canada, and the  
12 mandated reporting is only for manufacturers, it's  
13 not for the physicians in Canada.

14 SPEAKER: Do they have a definition of  
15 what a severe case is?

16 DR. TAYLOR: Yes, it would be requiring  
17 prolonged -- prolonging hospitalization, requiring  
18 hospitalization, medically significant event, life  
19 threatening, death. So, they have a standard  
20 definition.

21 SPEAKER: In terms of the complications,  
22 I mean, you were talking about hemolysis and the

1 target organ that could be damaged is the kidney,  
2 and then these people get anemic and they're  
3 getting blood transfusions and the immune globulin  
4 has a long half life, so you have another  
5 possibility of antibodies there causing more  
6 hemolysis. So, how carefully are you -- and this  
7 is also for subsequent speakers -- do we have any  
8 data on renal damage? And is some of this renal  
9 damage permanent?

10 DR. TAYLOR: In the case series that we  
11 looked at, we didn't see any cases. I know that  
12 it can happen. There are cases, but in terms of  
13 our spontaneous data, we don't have any in the  
14 Quel Canada database from 2006, so I think you'd  
15 have to look at other sources for more information  
16 on that.

17 DR. SCOTT: We'll move on. I did want  
18 to mention, please identify yourself by name  
19 before you ask a question if you can remember to  
20 do so. It would help with the transcripts.

21 So, Dr. Winiecki, I think is next.  
22 Scott is from the Office of Biostatistics and

1 Epidemiology at FDA. Can we have Dr. Winiecki's  
2 slides, please?

3 Ah, I think I know what the problem is.  
4 We will get the slides in just a minute. I think  
5 we should move on to the next speaker, Roger Berg,  
6 if you're ready, and I think we'll need to speak  
7 to someone in the AV section. We'll be back.

8 DR. BERG: Yes, good morning, ladies and  
9 gentlemen. On behalf of the Plasma Protein  
10 Therapeutics Association, I want to provide you  
11 with an overview of our analysis results in a  
12 qualitative fashion of adverse events of hemolysis  
13 received at individual manufacturers who are  
14 members of PPTA. And just to avoid any  
15 misunderstanding, I'm, to my best knowledge, still  
16 an employee of Baxter Innovations, and not as the  
17 agenda says, PPTA. So, if that is of importance.

18 What we did actually is when we  
19 participated in setting up this workshop is we  
20 discussed developing a protocol to examine the  
21 relationship between immunoglobulins and cases of  
22 hemolysis reported to members. And we did collect

1 all spontaneous reports of hemolysis reported to  
2 any of our member companies participating for a  
3 10-year period between 2003 and 2012 with a  
4 question to identify potential patient risk  
5 factors to analyze the profile of immunoglobulin  
6 indications, examined dose level, body weight, and  
7 total doses received in this population, and  
8 identify other possible risk factors for this type  
9 of event.

10 So, what we used, very similar to what  
11 we have been previously seeing from other  
12 speakers, we used the MedDRA SMQ "hemolytic  
13 disorders" to identify these cases and then  
14 subsequently manually reviewed all these AE  
15 reports and included those into our analysis that  
16 had sufficient information on the data items we  
17 wanted to look at.

18 So, this is a short overview of the  
19 participating companies -- Biotest, Baxter, CSL  
20 Behring, and Kedrion. Special thanks especially  
21 to George Schreiber and his team at PPTA who were  
22 extremely helpful in developing this.

1           If we look at the dataset, we did  
2   retrieve overall when looking into our company's  
3   safety database is a total of 325 cases with that  
4   SMQ. Now, we did exclude a couple of cases, some  
5   of them with insufficient information, that is  
6   unfortunately the downside of spontaneous  
7   reporting schemes that of all cases truly carry  
8   information that is of value to analyze these  
9   cases.

10           There were a couple of cases that  
11   actually did not represent hemolysis. This is  
12   partially due to how this SMQ is being set up, so  
13   if you think of the term "transfusion reaction"  
14   without any associated terms that would indicate  
15   there was an hemolysis, those were excluded and  
16   then some other cases as well, as you can see.

17           So, when we looked at these total of 263  
18   cases analyzed, you see here the distribution per  
19   region, so the majority of cases, 178 cases, are  
20   68 percent from North America, and then 32 percent  
21   from Europe. If you look at North America you see  
22   there's a striking difference between the number

1 of cases retrieved from Canada versus U.S.,  
2 especially if you put this into perspective of the  
3 different size of the population, and our  
4 conclusion -- and I think very similar to what  
5 others would conclude from this -- is that you see  
6 markedly different reporting habits throughout  
7 countries and regions. That is what we frequently  
8 observe, not only for IVIG, but also for other  
9 products.

10 So, I think I would just like to  
11 reiterate what others said before. From a  
12 reporting rate it is very difficult to deduct  
13 incidents, which is, I think, impossible given the  
14 limitations of this system and missing  
15 denominators.

16 Looking at our data set, very similar to  
17 what we saw before, about 56 percent of the  
18 patients affected were female. You see here also  
19 the age groups affected, broken up in ten-year  
20 steps of age, and you can appreciate that  
21 virtually all age groups are affected, and we  
22 don't clearly see a preponderance of one gender,



1       and I think the more you break down small case  
2       (inaudible), the more you come into results driven  
3       by chance.

4               So, roughly a slight preponderance of  
5       female, but not significant.

6               If we look at a population that is of  
7       special interest, when you speak about safety, I  
8       think it's worthwhile to look at those who are  
9       under the age of 18 and this also validates the  
10      data set we present. You see that we had a total  
11      of 44 cases for patients under the age of 18, the  
12      majority of these actually being treated for  
13      Kawasaki Disease and the second leading indication  
14      in this patient population was Graft Versus Host  
15      Disease and transplant related issues. All those  
16      other -- the big group, with just one mentioning  
17      of an indication.

18              The distribution of cases by AB0 blood  
19      group, very similar to what we've seen before, the  
20      vast majority of all cases that we found actually  
21      associated with blood group A, 110 patients or 72  
22      percent, then blood group AB, 18 percent, the

1     other two blood groups, not really affected with  
2     an overrepresentation compared to their  
3     occurrence.

4             What we did look at was the drop of  
5     hemoglobin directed related to the hemoglobin  
6     starting level. So, if you have a look at the  
7     X-axis, at the right you see a high hemoglobin  
8     starting level and then if you go to the  
9     individual cases, you see that there is a  
10    correlation between the starting level and also  
11    the drop, so that means that the higher the  
12    starting level, there was a tendency to express a  
13    higher drop in hemoglobin in this case series of  
14    hemolysis compared to those at the left where they  
15    started with low starting level and consequently  
16    also had a lower drop.

17            The mean and median fall closely  
18    together. The median drop that we saw was five,  
19    the range actually, one in ten, and in our dataset  
20    the starting levels known were roughly more than  
21    half of the cases analyzed.

22            If we look at transfusion requirements

1 by different steps of hemoglobin drop, you can see  
2 that virtually there is no difference in terms of  
3 percentage required of patients requiring  
4 transfusion and no transfusion, so that was  
5 equally across all these different drops. So, if  
6 you add up numbers, actually, you need to add up  
7 the blue ones and that should go to 100 and also  
8 the yellow ones, so you see that there is no  
9 difference in transfusion requirement in percent  
10 of cases if it was bigger than six or if it was  
11 equal or smaller than three.

12 Very similar, what we see is the  
13 transfusion requirement by hemoglobin starting  
14 level with the exception of those patients who see  
15 equal or smaller than 11 where only a small set of  
16 patients did not require transfusion, whereas for  
17 the higher starting levels, the transfusion  
18 requirements equaled out a little bit.

19 When we look at the question of dose, I  
20 think we always have to look at the indication, so  
21 because dose and indication, at least with respect  
22 to a recommendations closely go together, what we

1     see here is at the right pie chart, you see  
2     results of a 2011 marketing research on patient  
3     populations treated with IVIG and SCIG, and I'd  
4     just like to point you to two of the pieces of  
5     that pie chart, namely the blue one, 28 percent of  
6     patients in that research actually requiring  
7     immunoglobulin treatment for neurological  
8     disorders and then if you go to the left one, that  
9     is actually the breakdown of the cases, which we  
10    present here, you see that there is a bigger part  
11    of that case series actually within neurology, so  
12    there is an overrepresentation of neurological  
13    disorders in our case series of hemolysis and  
14    consequently when you have a look at, again, the  
15    right one, the red box with allergy and  
16    immunology, you see in our case series only very  
17    few of those.

18           And potentially staying with that a  
19    little bit, actually we saw only a small set of  
20    patients in our case series, for instance, treated  
21    for primary immunodeficiency, which still  
22    represents one of the big indications for

1 immunoglobulins.

2           Now, when we have a look at this patient  
3 population from a slightly different perspective,  
4 here we looked at patients with additional  
5 co-morbidity, so that means a disorder that was  
6 present at the time of treatment in addition to  
7 the indication for which immunoglobulin was used.  
8 So, you see that 12 percent of patients did not  
9 report any additional co-morbidity in addition to  
10 the indication for use, but there was a large part  
11 of patients that was multi-morbid, you see there  
12 is a 34 percent of patients with three  
13 co-morbidities and even a significant part with  
14 more than four, and actually the median calculate  
15 to three additional disorders in addition to the  
16 indication for IVIG.

17           Very similar, actually, then when you  
18 look at the concomitant medications. About 35  
19 percent of patients were reported without  
20 concomitant medication, but again, the remainder  
21 was reported with a mean of three and a median of  
22 two additional concomitant medication, so

1 additional drugs or other products used in  
2 addition to immunoglobulin use.

3 Now, having said that before when you  
4 look at indication, you should look at dose per  
5 weight in kilograms and visa versa, so, of course,  
6 we looked at this and we broke it up a little bit  
7 more fine tuned than what we regularly see where  
8 many publications just split up between lower than  
9 two and bigger than two, and I think this bar  
10 chart nicely shows that the overwhelming majority,  
11 I think, is connected to doses around two and  
12 higher, and as we've already heard before and  
13 seen, that PID patients, for instance, are much  
14 less affected than one would think. You  
15 consequently see that a very small part of  
16 patients, actually, is affected with dosing per  
17 body weight that goes below one gram. And I think  
18 this is reassuring for a large part of the  
19 population that is being treated with IBIG.

20 When we look at time to event in our  
21 case series, one-third of cases developed within  
22 24 hours. The remainder afterwards, we see only a

1        few outliers after 14 days, but I think still a  
2        significant part of the events, one-third was  
3        reported within or after three days until 14 days.  
4        Actually, due to the way we set up the protocol,  
5        unfortunately we couldn't break this number of  
6        patients down into other groups.

7                    What I found quite interesting,  
8        actually, when we tried to apply both the FDA and  
9        the Health Canada definitions upon our dataset, I  
10       was surprised that virtually all, I would say,  
11       cases that we analyzed could be classified under  
12       the FDA definition. And you see here they broke  
13       down into about one-third of cases as definite,  
14       then equal parts into probable/possible, and  
15       similarly, of the cases that could be classified  
16       with the FDA definition, 206 out of them could  
17       also be classified under the Canadian definition,  
18       as either a yes or no, and you can see that the  
19       results are pretty much in line between these two  
20       definitions.

21                   One thing I think we should look at, and  
22       that raises some questions also for future

1 research, especially if we kind of leave the area  
2 of spontaneous reporting and traditional  
3 pharmacovigilance methodology and go into claims  
4 data bases, is there is roughly one-third that we  
5 identified as definite and I think it's critical  
6 when we do research with novel strategies to  
7 understand, if we can apply definitions to cases  
8 to clearly sort out those where we are, I would  
9 say, pretty sure about potential causal role  
10 versus those which are of possible association,  
11 but which would still leave open the question that  
12 there may be other etiologies connected.

13 Now, another question that we looked at  
14 was actually the titer value of implicated lot, so  
15 these titer values actually refer to indirect  
16 method, and you can see that first of all, 181  
17 cases were referred to us, including the  
18 information on one or more lots actually  
19 administered to patients. So, I can always just  
20 encourage treaters to report not only events, but  
21 also lot numbers, which is important, especially  
22 for these case series.



1                   And you can see that the majority of  
2    cases, 117 actually, were reported for the 1:16  
3    titer, that's about 65 percent. Now, to avoid a  
4    misinterpretation of this slide, I just want to  
5    say, this slide also may be a direct function of  
6    the lots that were manufactured. So, if you  
7    manufacture the vast majority of your lots between  
8    1:8 and 1:16, that is information that is  
9    important to know, which we unfortunately didn't  
10   get in time prior to doing this.

11                  Looking at titers and patients who were  
12   transfused, you see here that, first of all,  
13   hemolysis occurs at every titer level, and that's,  
14   again, important to say, and that's what we saw on  
15   the previous slide as well, so it doesn't mean  
16   that in 1:4 you don't see this event or the event  
17   doesn't get reported, and the severity of the  
18   clinical condition taking transfusion as a kind of  
19   surrogate marker, you see that the transfusion  
20   requirement was equal across all these titer  
21   levels and there was no preponderance of  
22   transfusion requirement in high titers versus less

1 in low titers.

2 Now, this slide, I think, requires a  
3 little bit of explanation, but I think we will  
4 address that question a little bit later during  
5 this workshop. We tried to address the question  
6 of the function of the total dose administered x  
7 anti-A titer. So, if you look at the simple  
8 calculation to the right, what we essentially did  
9 as a kind of surrogate marker development, let's  
10 say that, if you have a patient, for instance,  
11 that is being administered 100 grams of  
12 immunoglobulin, with a lot that has the highest  
13 anti-A titer of, let's say, 1:8, you would  
14 multiply the 100 grams times eight and then that  
15 would result in 800, so then you can express this  
16 function of total dose by anti-A titer, for  
17 instance, breaking it up into distinct subgroups  
18 as we did below 1000, then 1000 to below 2000, and  
19 so on, until 3000+. And when we did this for our  
20 case series, actually, where we could perform this  
21 calculation for 50 percent of the cases available,  
22 you see that case reports equally distribute

1 across all these different groups and also again  
2 the severity connected to either of these  
3 hemolysis was the same.

4 So, we don't see any difference in  
5 either absolute number of occurrence nor  
6 transfusion requirement irrespective if that was,  
7 let's say, high dose/low titer, or low dose/high  
8 titer. There is virtually no statistical  
9 difference.

10 So, concluding this presentation, I  
11 think the presented analysis of spontaneous case  
12 reports, to my knowledge, at least until today,  
13 represented the largest dataset that we had looked  
14 at. We conclude that neither age nor gender  
15 appeared to represent true risk factors. It's  
16 important to recognize that hemolytic events are  
17 observed with anti-A titers at the low end, 1:4,  
18 as well as at the high-end 1:32 in this series.  
19 We think that risk factors, previously identified,  
20 include blood group A and AB.

21 Again, I would like to point out that  
22 from this case series, I think you cannot really

1 speak about high doses without speaking of the  
2 indication, because they are connected to each  
3 other, so I would like to say that high dose and  
4 indication, as we saw with the overrepresentation  
5 of neurology, for instance, in our case series,  
6 seemed to play a role. Also when you look at the  
7 affected population with multiple co-morbidities  
8 and concomitant medications, it's not that patient  
9 who receives IVIG, but then no other additional  
10 product, but there is a large group of patients  
11 who receive additional drugs for additional  
12 co-morbidities.

13 And then, interestingly, what we saw is  
14 that high hemoglobin starting levels are  
15 associated with larger hemoglobin drops. The  
16 question would be, does a dilution affect may  
17 provide an explanation for that, at least  
18 partially, and then what we saw is that likelihood  
19 of transfusion associated with lower initial  
20 hemoglobin levels was present, but not with  
21 hemoglobin drop.

22 One small slide at the very end to

1     propose labeling that has been mentioned, to help  
2     mitigate the risk of hemolysis, PPTA proposed at  
3     the FDA liaison meeting in September 2013 to  
4     standardize warnings and include patient- specific  
5     risk factors such as high doses, patients with  
6     non- O blood types, and other individual factors,  
7     such as underlying inflammation. And in January  
8     2014, PPTA actually submitted this proposed  
9     labeling to FDA.

10             And with that, I will conclude my  
11     presentation. Thank you.

12                     (Applause)

13             DR. KLEIN: So, yes, obviously what you  
14     say is very correct in terms of the high dose in  
15     the neurological cases, but there's another factor  
16     that I think is at play here that wasn't mentioned  
17     and maybe we can hear from you and other speakers  
18     about it is the frequency and the repeated dosing.  
19     So, if I understand it correctly, a condition like  
20     CIDP, you would give the immune globulin over a  
21     prolonged period of time; with some of the other  
22     inflammatory conditions it would be a shorter

1     period of time, so it's the high doses, repeated  
2     dosing, and I would expect that the repeated  
3     dosing gives you a higher steady state level.

4             So, one of the things that could be  
5     measured in some of these patients that are  
6     developing the problems is what is the level of  
7     immune globulin in the plasma? And does that  
8     correlate with what you would expect that it  
9     would? So, that would also improve our ability to  
10    monitor these patients.

11            DR. BERG: Right, I couldn't agree more.  
12    Just, this is pharmacovigilance cases, so we are  
13    kind of limited by the information that we  
14    actually receive from clinicians reporting this  
15    event into us and I recall from my own experience,  
16    so I'm looking at every case of immunoglobulin  
17    adverse events every day, that very often,  
18    actually, in practicing pharmacovigilance, we even  
19    have the issue that many clinicians don't report  
20    us when actually IVIG was started. So, we can't  
21    even very often tell, so again, spontaneous  
22    reporting by itself has limitations, and I think

1       there are some questions which we, from a database  
2       perspective, at our desks, simply can't answer.

3               MR. HUGHES:   Jonathan Hughes from Blood  
4       Source.   In terms of your pie chart comparing  
5       indication versus incidence of hemolysis, in the  
6       allergy and immunology group, do you have an idea  
7       of how much of those were a SCIG versus IVIG?

8               DR. BERG:    So, I think SCIG in the  
9       market research is a smaller part.   I don't know  
10      the exact number, we can follow up on that later.

11              The cases we presented actually were all  
12      IVIG, so there is no SCIG.   Just to, again, make a  
13      small but important distinction, it's not  
14      incidents, so our data are reporting rates, so  
15      what is reported may not equal incidents, and also  
16      the market research is the utilization pattern of  
17      the product.

18              DR. WATSON:   With respect to the  
19      relationship between hemoglobin drop and starting  
20      level, according to the data shown on slide nine,  
21      I wonder if you've done this analysis expressing  
22      the hemoglobin drop as the percentage drop from

1       where you started?

2               It seems that by doing some crude  
3       calculations at three points on the line, it looks  
4       like it's about 35 to 40 percent regardless of  
5       where you are on the line if you think about the  
6       percentage of the drop rather than the absolute  
7       levels.

8               DR. BERG:   Yes.

9               DR. WATSON:   So, have you done that  
10      analysis and did that change your --

11              DR. BERG:   I don't have those data in  
12      front of me and I'd have to follow up with our  
13      data analysis team on it, but I think the  
14      important point here is not that much that may be  
15      if you convert into percent that you roughly add  
16      up with the same, but if you think of many of the  
17      definitions that kind of speak about, so this is  
18      great, this and this, depending on hemoglobin drop  
19      in terms of these numbers, I think it's important  
20      to see that there is a different function also  
21      when you look at starting levels.

22              So, kind of focusing on one value may



1       actually mislead you in misinterpreting the whole  
2       case.

3               DR. SCOTT: I thank you for that  
4       enormous case series. I understand it was a lot  
5       of work to come up with all of that data and put  
6       it together.

7               We now have Dr. Winiecki and his slides.

8               DR. WINIECKI: I think it's interesting  
9       hearing all the other presentations and I think  
10      our data agree with it quite a bit, so that's  
11      somewhat reassuring to me.

12              So, three objectives, first, just to  
13      review our passive surveillance data, which comes  
14      from the FDA Adverse Event Reporting System,  
15      otherwise known as FAERS, second, to assess the  
16      feasibility of using Mini-Sentinel Quick Queries  
17      to evaluate hemolysis, and specifically looking at  
18      exposures, that is to say the immune globulin  
19      product, the outcomes, and looking at various  
20      indications, and then just to summarize our  
21      findings so that -- I think so that nothing is  
22      misunderstood here.

1                   So, for our FAERS search, we looked  
2    between July 2007 and December 2010. We looked at  
3    any nonspecific IG product, so we excluded the  
4    hyperimmunes and anything that was a non-human  
5    origin product, and it had to be reported as a  
6    suspect product.

7                   And we, again, just like the previous  
8    presentations, used the MedDRA SMQ "hemolytic  
9    events".

10                  For our case definition we based it on  
11   the Canadian definition, but just because there's  
12   so much missing information frequently in these  
13   reports, we modified it a bit for a little bit  
14   less minimum criteria, either a one gram drop in  
15   hemoglobin or a clinical diagnosis of anemia. So,  
16   if the report said "patient developed anemia  
17   following IVIG", we counted it, but they also had  
18   to have a positive Coombs test or some evidence of  
19   hemolysis.

20                  Cases were excluded if there was an  
21   alternative cause of hemolysis, if the Coombs was  
22   positive prior to the immune globulin

1 administration, or if there was simply  
2 insufficient information.

3 So, we ended up with a total of 140  
4 reports for that time period, ended up excluding  
5 36 percent, most commonly for insufficient  
6 information. So, 89 met our case definition and  
7 when we excluded it to U.S. cases, known U.S.  
8 Cases, so if there was another country reported or  
9 no country of origin reported, we excluded it, we  
10 ended up with 47 cases, and that's what the next  
11 few slides are looking at, those 47 U.S. cases.

12 Again, slight, slight female  
13 predominance, but nothing particularly strong,  
14 53/43 of the known cases. The mean drop in  
15 hemoglobin was 4.6 with a range of 1.6 to 6.5 and  
16 that was from 27 of those 47 cases. And we could  
17 see that the majority of the indications were  
18 high-dose indications -- transplant, Kawasakis --  
19 but we also had seven immune deficiency patients  
20 or 15 percent.

21 So, if we look at the risk factors that  
22 have already been discussed today, we can see that

1       in our cases, a majority had greater than two  
2       grams per kilo, although again, 15 of those 47  
3       cases we did not have dose data for. And if we  
4       look at blood type, we see that type A frequently  
5       represented very strongly in the cases for which  
6       there was a known blood type.

7               So, just consistent with what's been  
8       discussed today and what's been published, high  
9       doses and non-O blood type were commonly reported  
10      in our hemolysis cases.

11              So, for the rest of the talk I want to  
12      talk a bit about Sentinel and about using Sentinel  
13      queries, specifically Quick Queries, as a way of  
14      exploring data.

15              So, FAERS or any passive surveillance  
16      system has well known limitations -- the  
17      incomplete data, we know there's under reporting,  
18      and we know that we don't always get or almost  
19      never get all the data we would like to have. And  
20      we have nothing to compare it to; we have no  
21      denominator data.

22              Sentinel Quick Queries are a new tool

1     for FDA and give us some advantages. They're  
2     population based and there's 18 data partners that  
3     participate in Sentinel, and we have about 150  
4     million individuals in that database.

5             So, the advantages are that we have some  
6     available denominators, we can get fairly rapid  
7     results, about a month, and it helps us prioritize  
8     safety issues, so we can look at what needs  
9     further investigation and perhaps what doesn't  
10    need further investigation, which may be more  
11    important to eliminate false signals or false  
12    positives.

13            The disadvantages are these are based on  
14    electronic codes, they're based on billing data,  
15    so the data you get are only as good as the set of  
16    codes that you use, and I think that's one of the  
17    most important points here. If you choose the  
18    wrong codes, you're going to measure something  
19    different than what you intend to measure. So,  
20    code choice is really important and validating  
21    that is essential, I think, if you want to use  
22    this data for regulatory purposes.

1           There is some limited confounding  
2   control and this doesn't replace a study with -- a  
3   protocol-based study with medical chart review.  
4   But a study like this with medical chart review is  
5   quiet expensive and takes a long time, at least  
6   probably a year to develop and to conduct, so it's  
7   a very serious investment, both financially and in  
8   terms of time and resources, so this might be a  
9   way to say which safety issues warrant further  
10   investigation or protocol study, and which don't,  
11   but certainly doing a series of Quick Queries does  
12   not replace doing a protocol-based study.

13           So, this is what a basic Quick Query  
14   would look like. So, we have a cohort of people,  
15   whatever that is, it could be IG users, it could  
16   be all people over 50, it could be whatever we  
17   select. We have a product exposure, so here we're  
18   looking at immune globulin exposure, some risk  
19   window, and we're looking for some health outcome,  
20   in this case, hemolysis.

21           And we can add exclusion criteria, both  
22   to the exposures and the outcomes to try to

1       measure something more specific if that's what  
2       we're looking for.

3               So, our plan here is we want to say, is  
4       this a useful tool? What is the utility of using  
5       Quick Queries to look at hemolysis following IG?

6               So, again, to stress, we need specific  
7       codes to identify both exposure and outcome, and  
8       so for this particular question we have some  
9       disadvantages: Not all brands of IVIG have  
10      specific codes; nonspecific codes exist, so  
11      medical centers can do billing -- we know an IG  
12      was used, but we don't know which one; and there  
13      is no specific code for post-IVIG hemolysis, so it  
14      becomes a question of how does one best identify  
15      that outcome.

16              So, this is a several step process:  
17      Assess how IVIG codes are used in the Sentinel  
18      system, assess the surrogate codes to identify  
19      hemolysis, because, again, there is no specific  
20      code for that outcome, and try to assess how does  
21      this happen according to indication.

22              So, we did a few different things here

1 to try to look at our codes and are those codes  
2 the right ones, and are they effective at  
3 measuring what we want to measure. So, we  
4 compared it in an outcome where we knew there  
5 should be hemolysis and a positive control, if you  
6 will, we tried to assess expected complications  
7 and did we see those in patients who had hemolysis  
8 and tried to look at the relationship between  
9 hemolysis and dose.

10 So, this is the group of codes -- and,  
11 again, these will all be published and public and  
12 you have copies if you want to look at the  
13 specific codes, but the most important thing to  
14 see is there's a series of brand-specific codes in  
15 that -- under immune globulin, the very first row,  
16 but then if you look at all the rest, they're  
17 nonspecific, they could indicate intramuscular,  
18 they could just say lyophilized but not be  
19 brand-specific, or they could just say "other IV,  
20 no brand specified", and we used a series of  
21 different hemolysis codes trying to look at how  
22 would this be coded and could we pick it up, and



1     maybe casting a bit of a broad net, because are  
2     people going to call this a transfusion reaction,  
3     are they going to call it AVO incapability. I  
4     don't think we know.

5             So, what we're trying to do here is  
6     match a certain exposure to a certain outcome, so  
7     IVIG to hemolysis, and then say, can we see a  
8     relationship between the two? But again, this  
9     data is based on those codes and we don't actually  
10    know how good those codes are.

11            So, I don't want people to look at our  
12    graphs or look at our data and jump to conclusions  
13    based on those because we've not done chart review  
14    to see how good these things are.

15            So, looking at brand and  
16    non-brand-specific coding, first thing we did is  
17    just take all patients who had any of those codes  
18    for an IG, we divided it into that first category  
19    of brand-specific and then all the other  
20    categories, which are non-brand-specific, we  
21    looked at 72 hours following administration for  
22    the outcome of hemolysis. We excluded any patient

1       that had received greater than one category, so if  
2       a patient had received both a branded and  
3       non-branded, we excluded those, and also anyone  
4       that had a hemolysis code in the three months  
5       preceding the immune globulin exposure.

6               I think what we learned is that coding  
7       is often non-brand-specific and, again, important  
8       to stress here that medical record review is  
9       required for any sort of brand- specific  
10      comparison, in both looking at users, so all  
11      patients that received any IG products, and  
12      looking at hemolysis cases, a majority in both of  
13      those groups were coded non-specifically.

14             So, if you were to just look at the  
15      brand-specific data, you're missing somewhere  
16      between half and two-thirds of the data whether  
17      you're looking at users or cases.

18             So, the frequent use of  
19      non-brand-specific codes make Quick Query data  
20      unsuitable for any sort of brand- specific  
21      comparison.

22             Looking at our positive control

1 scenario, what we tried to do here is look at  
2 anti-D exposure versus other ITP therapy, knowing  
3 that hemolysis is known and very common in this  
4 population receiving anti-D. So, we looked at  
5 males with ITP just to eliminate the whole issue  
6 of pregnancy, and then we looked at four groups --  
7 patients receiving Prednisone, Dexamethasone,  
8 Romiplostim, or anti-D, again, 72 hours after  
9 administration and looking for hemolysis.

10 We excluded anybody who got any  
11 non-specific IG and also any patient that got  
12 anti-D that was in one of those first three  
13 categories, Prednisone, Dexamethasone, or  
14 Romiplostim.

15 And what we see here is that in our  
16 Quick Query data, we can identify patterns of use,  
17 steroids are often first line therapy, so we see  
18 that the other therapy group, much larger in terms  
19 of percent of patients, anti-D can only be given  
20 to -- it's a second line, at least, therapy and is  
21 only given to RH positive patients with a  
22 functional spleen, so we see that anti-D use was

1 substantially less than the other therapies.

2           And so I think that gives -- that's what  
3 we would expect. So, we say, okay, well, maybe  
4 with our exposure we're measuring what we think  
5 we're measuring. And then if you look at  
6 hemolysis events for 10,000 users, about 3.5 times  
7 higher in anti-D group versus the other three  
8 therapies combined. And, again, so what we're  
9 seeing here in our positive control is hemolysis  
10 being seen in that group where we expect it.

11           So, then we tried to look at transfusion  
12 and acute renal failure asking the question, do we  
13 see transfusions given more commonly to patients  
14 with hemolysis than those without after receiving  
15 IVIG? And do we see acute renal failure more  
16 commonly in patients that had hemolysis following  
17 IG and then patients who didn't? And, again,  
18 here's our list of codes just to stress --  
19 important how you choose those codes because  
20 that's where your data comes from.

21           So, looking at our cohort, it was all IG  
22 users. They received -- any of the IGs in that

1     early slide that we had codes for, looked at the  
2     first 72 hours, divided them into two groups,  
3     either those who had hemolysis or those who  
4     didn't. We excluded anybody with preexisting  
5     hemolysis or acute renal failure, and then we  
6     looked for our acute renal failure codes or  
7     transfusion.

8             And what we see is that complications  
9     are, in fact, more common in the IG patients with  
10    hemolysis than those without, so a little more  
11    than 10 percent in those with hemolysis and less  
12    than 2 percent in those without for renal failure,  
13    and then transfusions, about 7 percent of those  
14    who had hemolysis received transfusion and about 2  
15    percent in those who didn't have hemolysis got a  
16    transfusion.

17            But, again, this is just an attempt to  
18    look and say, are the codes that we picked  
19    measuring what we think we should measure? You  
20    can't interpret this data to say, this means that  
21    10 percent of patients who get an IG and have  
22    hemolysis are going to need a transfusion. This

1 is not chart validated data, so you can't  
2 interpret it that way.

3 We asked another question saying, well,  
4 with increasing days of therapy, do we see more  
5 hemolysis? If therapy is a marker for dose, you  
6 get more days of therapy, that's a higher dose, do  
7 those patients hemolize more often? So, we  
8 divided the IG users and the patients receiving  
9 one, two, three, four, or five days of therapy, so  
10 they had their immune globulin exposure followed  
11 three days after their last dose, and did they  
12 have hemolysis?

13 We excluded, again, those with  
14 preexisting hemolysis and any patient that had  
15 received IG in the 20 days prior to the exposure  
16 we're measuring.

17 What we actually saw was that hemolysis  
18 was most common after day one and less common with  
19 increasing days of therapy. So, one possibility  
20 is we simply -- you know, our codes aren't  
21 measuring what we think we're measuring. Other  
22 possibilities include that patients who are likely

1 to hemolize do so after the first dose and  
2 therefore therapy is discontinued. Maybe therapy  
3 -- days of therapy is a poor proxy for dose,  
4 perhaps dose isn't a strong risk factor or  
5 hemolysis is correlated with some other factor,  
6 but I think the take home point here is when you  
7 do these, sometimes you will get answers you don't  
8 necessarily expect and it can lead to additional  
9 questions, and maybe there are other ways that we  
10 could look at this data.

11 So, looking at the effect of dose on  
12 hemolysis rate, we tried to do that by -- in a  
13 different way by looking at indications, so we  
14 took six selected indications, divided them into  
15 high dose or low dose, the low dose were the  
16 primary immune deficiency patients, high dose were  
17 Kawasaki's disease, ITP, CIDP, Myasthenia, and  
18 GBS, looking at three days after their last dose  
19 for the outcome of hemolysis, and of course,  
20 excluding anyone with preexisting hemolysis.

21 Our demographic data, if we looked at  
22 that match, what is know, so Kawasaki's disease

1     being a pediatric disease primarily of children  
2     under five, we had only divided our patients into  
3     four age groups, but 99+ percent were zero to 18,  
4     and also the age trends, CIDP and GBS both  
5     increase with increasing age, which is what we see  
6     in this data, patients over 64 are  
7     underrepresented in the Sentinel database, so it's  
8     not surprising that the number of patients would  
9     decrease in that older age group, but certainly in  
10    the three earlier age groups was increasing with  
11    age.

12           We were also able to look at our data in  
13    terms of male/female distribution and those  
14    matched up pretty good. Kawasaki's disease  
15    published rates is like 1.5:1 for male/female and  
16    ours was 1.4:1. CIDP and GPS are both slightly  
17    male predominant, and we were able to measure that  
18    as well.

19           So, we would think that patients with  
20    high dose indications would hemolyze more often  
21    and that is what our data showed us. So, if you  
22    want to look at per user, on the left, you see



1       that the high dose patients hemolyze more often  
2       than low dose, or you could look at treatment  
3       episodes, depending on how you prefer to look at  
4       the data, but of course the PID patients are  
5       getting repeated doses, so I think that makes the  
6       contrast more stark.

7               The results of these Quick Queries will  
8       be published on the mini-Sentinel website, so the  
9       data, the codes, et cetera, will be available.

10              And in summary, I think the Quick Query  
11       data correlated well with known information and  
12       expected results regarding our hemolysis codes,  
13       and that these sorts of queries can be a useful  
14       tool in exploring complex adverse event issues and  
15       can complement passive surveillance data. They  
16       provide rapid population-base data, they can  
17       assist us in prioritizing safety signals, and help  
18       us determine whether a more complete  
19       protocol-based study with its more serious  
20       investment in both time and resources is feasible  
21       and necessary for a certain issue and help us weed  
22       out those other issues.

1           A number of people who were really  
2   important in this project, and I just wanted to  
3   acknowledge them, and would be happy to take any  
4   questions.

5                   (Applause)

6           DR. WATSON: Yes, I had a question about  
7   the data on slide 19. I wonder if you could  
8   clarify. So, on the column marked "one day",  
9   that's the rate in -- per 10,000 new users of  
10   hemolysis occurring on the first day of therapy?

11           DR. WINIECKI: Of patients who received  
12   one day of therapy. So, it's looking at --

13           DR. WATSON: Okay.

14           DR. WINIECKI: If you got one day of  
15   therapy where that yellow diamond would be, and  
16   then looking at the following three days --

17           DR. WATSON: Okay.

18           DR. WINIECKI: -- for any coding of  
19   hemolysis.

20           DR. WATSON: Okay.

21           DR. WINIECKI: And then two days,  
22   similarly patients who receive two consecutive

1 doses, so on Monday and Tuesday, and then looking  
2 at the following three days after that.

3 DR. WATSON: So, your conclusion that  
4 it's more common after day one is the sum of days  
5 two, three, or --

6 DR. WINIECKI: No, what I'm trying to  
7 say there is that when we measured it, it was most  
8 common in patients receiving a single day of  
9 therapy as opposed to patients who got two days or  
10 three days or four days.

11 DR. WATSON: Okay, I see.

12 DR. WINIECKI: So, some of those  
13 patients, for instance, could have had a planned  
14 five day course, which was then canceled after  
15 receiving one dose because hemolysis was detected  
16 and then they didn't get any subsequent doses.

17 DR. WATSON: Yeah, and do you have --  
18 I'm sorry, do you have any understanding of the  
19 distribution of days of therapy across the entire  
20 population of IVIG?

21 DR. WINIECKI: You mean, for instance,  
22 the percentage of patients that get one day versus

1 two day, three day, four day?

2 DR. WATSON: Right.

3 DR. WINIECKI: Yes, we have some sense  
4 of that, I think. I don't have the data here, but  
5 it substantially decreases starting at about three  
6 days and then much lower at four days, much lower  
7 at five days.

8 DR. WATSON: Okay.

9 DR. WINIECKI: So, the number of users  
10 certainly goes down as you get above, say, three  
11 days of therapy.

12 DR. WATSON: But you still think there's  
13 a relationship here with one day being the highest  
14 risk?

15 DR. WINIECKI: I don't know that we can  
16 say one day is the highest risk, we can only say  
17 that we have a number of patients who got one day  
18 of therapy and then had hemolysis. I don't know  
19 if that means that one day is at higher risk. It  
20 may simply be -- that may be true, or it could be  
21 a reflection of the coding that those two codes  
22 show up, you know, on consecutive days or on the

1 same day, so you would really need to do chart  
2 review and verify the timing, because remember  
3 what we're looking at here are billing codes and  
4 not patients.

5 So, without actually looking at the  
6 charts and looking at the time that the IG was  
7 given and when the laboratory work was done, I  
8 don't think we can say.

9 DR. WATSON: Yeah, thank you.

10 DR. SCOTT: Let's have just one more  
11 question because we have a break pending.

12 MR. PIERCE: Ross Pierce, FDA. Could I  
13 make -- a question? One other comment on the  
14 analysis of the relationship between the number of  
15 days of therapy and the risk of hemolysis, I  
16 gather that urinalysis didn't require that the  
17 subjects received the same dose. So, you know,  
18 for example, if you look at ITP, the two labeled  
19 dosage regimens, and some labels of IGIV are one  
20 gram per day for two days in a row versus 400  
21 mg/kilo per day for five days in a row, you know,  
22 the first one I meant to say 1 gram/kilo.

1                   So, in some cases, the longer number of  
2    days is actually associated with a shorter dose  
3    per day, so, it makes it more difficult to  
4    interpret, I think, the data.

5                   DR. WINIECKI: Yeah, I agree. Remember,  
6    this is just billing data and we can't get dose  
7    from the billing data. So, what we're trying to  
8    do here, right, is use days of therapy as a proxy,  
9    but it's entirely possible that some patients  
10   would get a lower dose per day for more days and  
11   that could add up to the same or even less than  
12   another patient getting a single large dose. All  
13   you can measure from the codes is days of therapy,  
14   but you can't -- it's not billed as grams/kilo of  
15   IG.

16                  So, we can only look at dates where it's  
17   given and can't look at dose. So, that's entirely  
18   possible.

19                  DR. SCOTT: Okay, I think we'll break  
20   now. We'll return in 15 minutes. We're running a  
21   little behind, but we can probably catch up.  
22   Thank you.

1 (Recess)

2 DR. SCOTT: As we're filing in because  
3 we're a little bit behind time, I think we'll  
4 begin with the pathogenesis talks. Today I want  
5 to give a special thanks to Dr. Flegel of the NIH  
6 Clinical Center. Dr. Garratty was unable to join  
7 us, unfortunately, due to illness, and at the very  
8 last minute, Dr. Flegel agreed to give this talk,  
9 and he and Dr. Garratty both contributed to the  
10 slides.

11 I think we'll begin with Pathogenesis of  
12 Hemolysis.

13 DR. FLEGEL: Thank you for inviting me.  
14 I received this message on Sunday night only, and  
15 thank you for inviting me Sunday night only so the  
16 weekend was fine. Yesterday was a little bit more  
17 difficult to get this done. Thankfully Dr.  
18 Garratty gave me his set of slides and I'm heavily  
19 -- the talk is heavily based on what Dr. Garratty  
20 would have presented and I hope I can do that  
21 faithfully.

22 This is a picture of Dr. Garratty in his

1 youth, and that's currently, so at the very  
2 minimum, the spot where he is right now north of  
3 Los Angeles is warmer than this one.

4 I will not talk about any off-label use.

5 I don't have a conflict of interest relevant to  
6 this presentation. This is Dr. Garratty's first  
7 slide and you probably know all these details.

8 The immune destruction of circulating blood cells  
9 has two components to it, one is the intravascular  
10 complement-mediated destruction usually initiated  
11 by antibodies, and typically it's the IgM, but not  
12 only. There is also evidence that it can be IgG.  
13 Obviously in IVIG, you don't have the IgM  
14 component in it, but the IgG could also be  
15 involved intravascular hemolysis. It certainly  
16 contributes to extravascular macrophage-mediated  
17 destruction of the white cells, that's the IgG or  
18 the IgA component, and the complement mediated --  
19 obviously the complement part will be contributed  
20 by the patient rather than by the IVIG blood  
21 product.

22 And this picture shows that



1       schematically IgM would bind to the red cell in  
2       the vascular system, activate the complement  
3       cascade, poke a hole into the red cell, and the  
4       red cell hemolyzes.

5               The other component is the extravascular  
6       approach by IgG that codes and marks the red cell,  
7       that then get destructed extravascularly,  
8       typically in the reticuloendothelial system,  
9       primarily the spleen or the liver or other organs.

10              Now, the big difference is the maximum  
11       rate of red blood cell with destruction that you  
12       have to be aware of. Extra cellularly, this is  
13       limited to the capacity of the spleen,  
14       essentially, so this calculation taken from a text  
15       book is that you can destroy about 500 ml RBC,  
16       which is still a lot, within 24 hours, but it's  
17       kind of slow and typically the system and the  
18       patient can cope with that and it's not  
19       immediately life threatening, although it probably  
20       does not contribute to the betterment of the  
21       disease situation of the patient if a system has  
22       to deal with such an enormous amount of cell

1       destruction and somehow cope with that while being  
2       busy with other disease going on.

3               The intravascular component, however,  
4       can destroy a lot of volume within a very short  
5       period of time such that a drop of hemoglobin by,  
6       let's say 5 g/dl is possible within hours, and  
7       that can be easily lethal if not coped with.

8               Obviously, if you have a hemoglobin of  
9       15 and it drops by 5 g/dl, you might not even need  
10      to transfuse. That's different if your starting  
11      hemoglobin is 7 g/dl and it would drop to 2, and I  
12      wonder a little bit if these drops that were  
13      discussed previously, whether they are corrected  
14      for the fact that the blood is transfused.

15              Obviously, having a hemoglobin of 10,  
16      there might not be transfusion, but you certainly  
17      will transfuse if it's getting below 7 or even  
18      below 5, and that can easily happen with IgM or  
19      IgG related intravascular hemolysis.

20              There is also a component, the problem  
21      will probably get more pronounced in the future  
22      since we are transfusing less, probably for good

1     reason, but if your starting hemoglobin is more  
2     like 7 rather than 10, then the time you can deal  
3     with intravascular hemolysis maybe much shorter.  
4     So, one has to be aware of the fact that the topic  
5     of this conference is very timely and it may  
6     actually get more important over time.

7             Now, the receptors on macrophages  
8     governing the extravascular hemolysis are shown  
9     here, and I'm certainly not the expert of it, I'm  
10    just giving here this slide with the Fc receptor  
11    binding the various immunoglobulins and then the  
12    various complement receptors 1, 3 and 4 are shown  
13    binding of the complement factor 3, and in the  
14    last line, with the complement factor 4, you see  
15    this C3b, how it degrades and ends up with the C3d  
16    component, and that's a product that sticks to the  
17    red cell surface for the life of the red cell if  
18    it's there and the red cell is not destroyed. It  
19    does actually not affect the survival of the red  
20    cell, so if the complement did not destroy the red  
21    cell and its left over component C3d is attached  
22    to it, then the red cell will survive the usual

1 time, which is a very sensitive, but not specific,  
2 detection for previously ongoing hemolysis or some  
3 activation of complement at the red cell level.

4 Well, once the red cell gets attached to  
5 the macrophage, there are essentially three  
6 components or ways that the red cell can be  
7 affected, one is the phagocytosis by the  
8 macrophage, the other is fragmentation by nibbling  
9 off a part of the red cell and leaving over the  
10 rest of it, which then may look like spherocytes  
11 on a smear, and the third component is the  
12 antibody-dependent cell-mediated cytotoxicity that  
13 can be affected by macrophages or often with NK  
14 cells when it comes to the red blood cells.

15 This shows the fragmentation results --  
16 that results in spherocytes in a nice picture.  
17 And you see that a part of the red cell gets lost  
18 whilst the remainder is still floating in the  
19 blood system.

20 Now, the factors that influence the  
21 patogenicity of red blood cell antibodies are well  
22 understood and there are quite a number of them,

1 the characteristics of the antibody is very  
2 relevant and it's not only the IgM, IgG part, but  
3 there's probably much more to it that could be  
4 characteristic even at the molecular level if you  
5 had the tools to do that and the money to test for  
6 these variations.

7           The quality of red cell-bound IgG with  
8 complement makes clearly a big difference and is  
9 an important component difficult to quantify. The  
10 characteristics of the target antigen in this  
11 particular case you have the A and B antigen, is  
12 of interest and importance. The type of  
13 complement that is provided by the patient in  
14 conjunction with the anti-A or anti-B will be  
15 relevant. And finally the activity of the RES is  
16 relevant and will be addressed by subsequent  
17 speakers.

18           Now, when it comes to the  
19 characteristics of the antibody, there's obviously  
20 class and subclasses, specificity and affinity,  
21 also the thermal range, there are lots of cold  
22 antibodies available in quite a number of donors,

1       within 1 or 2 percent of donors you would have to  
2       expect that. They typically are, but not always,  
3       IgM, and supposedly not clinically relevant, but  
4       there may be exception. The warm auto antibodies  
5       are rare. They differ, obviously, by the thermal  
6       range and optimal activity.

7               Complement of note is active with -- is  
8       not active in the cold, but requires a temperature  
9       of 25 degrees centigrade or above. And that  
10      typically causes that the cold antibodies that do  
11      bind in the cold but then dissociate from the red  
12      cells, do not activate a complement, whereas the  
13      IgG antibodies binding in the warmth may  
14      complement the activity to some degree.

15             The characteristic of the antigen is the  
16      other component, the chemistry of the target  
17      antigen, you want to be very familiar with the A  
18      and the B antigen, and it may not be as simple as  
19      it looks on first sight. The quantity of the  
20      antigens on the membrane makes a difference, it's  
21      in the 100,000s when it comes to A and B. There  
22      are proteins in the red cell membrane that the

1 more prevailing ones have also 100,000 copies per  
2 one red cell membrane. Most blood group antigens  
3 have a antigen density in the order of 10,000  
4 proteins per red cell membrane. That's quite a  
5 big number.

6           The distribution of the antigen can be  
7 even or it can sit in pocket, and typically there  
8 are complexes of protein, though it's not actually  
9 evenly distributed. And a very important  
10 component is that when it comes to A and B, the  
11 sugar antigens, that they are distributed in all  
12 tissues. The red cell actually is not  
13 particularly covered with the A and B antigens,  
14 there are other tissues that have a higher density  
15 of antigens on the surface, which affects the  
16 health of the recipient even if it's not affecting  
17 hemolysis.

18           And there is a competition due to the  
19 availability of these antigens in tissues or body  
20 fluids, and that affects all copper hydrate blood  
21 groups.

22           Now, this is a slide I inserted. When

1     it comes to or I think about this antibody and  
2     antigen characteristics, we can conclude that the  
3     antibody specificity, essentially the blood group,  
4     is very widely used for blood group testing and  
5     matching. This is very well understood. So, why  
6     aren't the other items more widely applied? One  
7     might think about that, it's, in the end, a  
8     multifactorial component, therefore it's difficult  
9     to correlate, and there are often no specific  
10    interventions available other than blood group  
11    matching, and in the end, we may not have robust  
12    assays or these characteristics are difficult to  
13    quantify. Assays are not widely available.

14           This could be changed, in particular  
15    with molecular testing one might be able to get a  
16    better understanding how these various variants  
17    interact with each other, and one doesn't  
18    necessarily have to do that with a wide net, one  
19    could focus on those handful or two handful of  
20    components that already are characterized. These  
21    genes come in different flavors as alleles and the  
22    rarer alleles may actually, if they match up and



1 correlate, may contribute to hemolysis and this is  
2 well within the reach of current technology,  
3 although not regularly explored.

4 Now, autoimmune hemolysis as a primary  
5 -- one of the primary targets, one could argue  
6 that it's relatively rare disease with few  
7 treatment options, IVIG is one of them, though new  
8 options may become available. Hopefully they're  
9 not used off label but specifically developed for  
10 treating autoimmune hemolysis. And it may also be  
11 worthwhile considering that perhaps several of --  
12 two or more -- of those treatment options would be  
13 combined as a first line. Obviously, this should  
14 be done in clinical studies.

15 I'd like to point out that these  
16 antigens in tissues and body fluids are also  
17 soluble and this applies to all sugar blood  
18 groups. This is the list -- the complete list --  
19 of the known sugar blood groups ABO as number one,  
20 obviously, the most important and relevant in this  
21 context, but there are several others known and  
22 they typically don't come with obligatory

1     antibodies in the donor plasma, but they can occur  
2     there, and if they do, then this might actually  
3     contribute to hemolysis. I would think it's the  
4     ABO thing, but in fact perhaps there was one donor  
5     of high titer, clinically relevant IgG in your  
6     pool and that causes the problem in certain  
7     patients who are predisposed to developing  
8     hemolysis.

9             When it comes to the A, B, AB and A1  
10    antigens, you want to know that they're actually  
11    structurally different antigens and this is very  
12    well known. So, if you want to absorb the anti-A  
13    or anti-B, you want to have the right antigen  
14    structure. Anti-A can be absorbed with an A1  
15    antigen, but the other way around, it would not  
16    work.

17            And these antibodies, you might think  
18    perhaps there is only a slight difference, why do  
19    they call it anti- A and B and anti-AB, but these  
20    are actually different antibodies and it's not a  
21    given that an anti-AB antibody can as easily  
22    extract it by an A antigen as an anti-A would be

1       extracted.

2               There are minutia that are very  
3       important to be considered if you want to  
4       manipulate your IVIG product.

5               The other thing is the complement that  
6       is provided by the patient and contributes,  
7       possibly, to the hemolysis, in particular,  
8       intravascular lysis, but then also the  
9       extravascular hemolysis. This could cause a  
10      permanent sequestration as shown here with  
11      subsequent phagocytosis and the red cell is lost,  
12      but it may also be only a temporary sequestration  
13      with a shortened or even normal survival and what  
14      also happens quite frequently is that the red  
15      cells kind of get involved in a complement  
16      activation process, but survive it, and afterwards  
17      they have essentially a normal red blood cell  
18      survival for the rest of the duration of the red  
19      cell's life.

20              This is the complete list of  
21      complement-related blood groups. Four of them are  
22      described today: The Chido or Rogers system

1        actually is the complement component C4, which is  
2        not produced on the red cell surfaced but absorbed  
3        to it and then constitute a blood group system;  
4        Chroma is CD55 (decay accelerating factor) and  
5        just a couple of weeks ago described by my  
6        laboratories this blood group HRF, which is  
7        probably not the name that it will finally get.  
8        It's CD59 and a variant was found and such it's  
9        defined as a blood group system.

10                What I want to say here is they come in  
11        different variants with alleles and the allele may  
12        or may not affect an interaction with an anti-A  
13        and B, how this complex, in the end, affects the  
14        red cells. And these variants can be tested today  
15        by molecular means and it might be worthwhile in  
16        exploring these variants and its affect on  
17        hemolysis.

18                You may also consider that there is the  
19        possibility of low frequency antigens typically  
20        defined as a prevalence in donors of less than 1  
21        percent. Luckily the antibodies are typically  
22        also rare, but they are not systematically

1 screened, so you will not be aware whether your  
2 donor or the patient has the antigen or the  
3 antibody. They are obviously more likely  
4 occurring in large donor pools. They can be of a  
5 high titer and clinical relevance, so if you have  
6 an inadvertent match of antibody with the antigen  
7 in your patient with the clinical consequences,  
8 this may easily be missed. You're investigating  
9 for the influence of the anti-A and B, and in fact  
10 there was something else going on, which can be  
11 tested, if it's done thoroughly. There are hints,  
12 like complement activation, antibody illusion, but  
13 it has to be pursued in a very sophisticated way,  
14 otherwise it would be easily missed.

15 Now then there is the patient side with  
16 macrophage activity, and I just want to show this  
17 slide because it will be explored in greater  
18 detail by subsequent presentations.

19 Dr. Garratty summarized his wisdom in  
20 this study and published ten years ago showing  
21 that a macrophage assay can actually indicate that  
22 seemingly incompatible blood can be transfused

1 without risk. There is a correlation. However,  
2 this assay is hardly used outside of very  
3 specialized laboratory, for example, here at NIH  
4 it's not used and there are whole countries where  
5 no one would provide this assay.

6 These could be explored at least in the  
7 context of improving blood product quality.

8 This shows that there are many  
9 transfusion reactions, most of them are  
10 serological and supposedly without clinical --  
11 obvious clinical consequence. One-third is  
12 hemolytic, which supposedly is then a clinical  
13 consequence. Most of these transfusion reactions  
14 the patient was alive. But again, they do not  
15 necessarily contribute to the improvement of the  
16 underlying disease and one has to consider that if  
17 this contributes to a lethal outcome, it may often  
18 not be attributed to the antibody.

19 So, this may be, then, the final item  
20 that turned the fate.

21 When it comes to ABO incompatible  
22 transfusions by error, then luckily, many of these

1 patients, one might be surprised, have no adverse  
2 affect or are asymptomatic about half of those  
3 cases are reported in that way and it's well known  
4 in other studies that only one out of -- only one  
5 out of 50, one out of 60 ABO incompatible  
6 transfusions is actually lethal for the patient.

7           However, those patients who survive, may  
8 well have suffered a permanent damage in some  
9 tissues and this was already addressed previously  
10 during this symposium, and that's actually a very  
11 important point. It doesn't really help that the  
12 patient survives, one wants also to have the  
13 patient surviving in a very good condition.

14           This slide shows that obviously the  
15 clinical outcome depends on the volume that you  
16 transfuse. Often ABO incompatible transfusions  
17 are early recognized because of the severe  
18 consequences and then you stop the transfusion  
19 after a couple of mL or less than 50 mL and in  
20 this case it's highly unlikely to be lethal. It  
21 may still have negative side effects for the  
22 patients.

1           If it's continuing to be transfused,  
2     like with anesthesia, then it can be lethal.

3           Despite the fact that very many of those  
4     transfusions are not lethal, I'd like to point  
5     here to this FDA summary of fatal transfusion  
6     reactions. Non-ABO hemolytic transfusion  
7     reactions are number two cause of lethality today.  
8     And the ABO-related, which supposedly could be  
9     completely eliminated, are number three. This is  
10    not of low importance, it's actually very relevant  
11    and one could argue that perhaps there is an  
12    underreporting of the specific item relative to  
13    TRALI, so it may well be, at some point, equal or  
14    higher than the TRALI given that TRALI is  
15    addressed, whereas non-ABO as well as the ABO  
16    aren't a major focus of today's transfusion  
17    medicine improvements.

18           Here this shows a slide where we  
19    actually went on to improve our transfusion  
20    practice with platelet products. We've seen  
21    passive hemolysis in platelet recipients,  
22    obviously IgM related although, we think and we



1 removed high titer antibodies and (inaudible)  
2 practical approach we said 1:250 is the titer in  
3 our assay that we moved about one-fourth of our O  
4 platelet donors and a little of the A and B  
5 platelet donors such that those donors of the  
6 platelets will not be transfused in a minor  
7 incompatible way. They're either then ABO  
8 identically transfused or washed.

9 And that's about the frequency one would  
10 see if one wants to remove this IgM component.

11 We then look to the transfusion  
12 recipients that were at the border with their --  
13 you know, the donor was below this titer of 150  
14 but weren't there, and we could not see in this  
15 very, very small study that this affected  
16 hemolysis, so we think it's a safe procedure.

17 But what we recognized, since we are  
18 following up this study in the past four years, is  
19 that whilst the IgM is removed, those donors may  
20 have quite high IgG components in it. It's a  
21 little bit perplexing and probably worthwhile  
22 following up.

1                   So, Dr. Garratty summarized in one of  
2   his presentations that was later published, his 30  
3   years of experience and I cite that from this  
4   paper, "Nevertheless, after more than 30 years  
5   researching this area, I am sometimes embarrassed  
6   to realize how much I cannot explain."

7                   And these are three slides on questions  
8   that still need to be answered. So, why do  
9   circulating red blood cells die after 120 days?  
10   What is the mechanism that this happens at this  
11   specific timeline? Why can some red blood cells  
12   that are strongly coated with IgG normally  
13   survive? Why do ABO incompatible transfusions  
14   sometimes or actually quite frequently not cause  
15   severe reactions as shown by the slides?

16                  Why are some auto and alloantibodies  
17   causing severe immune hemolytic anemia, which are  
18   not detected by routine techniques? This implies  
19   that there are low titer auto and alloantibodies  
20   sometimes causing enormous problems whereas you  
21   have an alloantibody with high titer and it does  
22   not cause severe hemolysis. What is the

1 underlying reason for that?

2 Are there other cells, the lymphocytes,  
3 NK cells, participate in this immune hemolysis?  
4 The answer is almost certainly yes, but how? And  
5 do differences in the clinical severity and  
6 response to treatment relate to relative  
7 efficiency of macrophage-induced phagocytosis  
8 versus cytotoxicity, and what are the mechanisms  
9 involved with that?

10 How does the NK cells and the cure  
11 antigens interact with that? Why do  
12 hyperhemolytic transfusion reactions occur in  
13 sickle cell disease? Many mechanisms are  
14 proposed, but no conclusive test can be used to  
15 predict that.

16 Can antibodies cause immune hemolytic  
17 anemia without activating complement or  
18 interacting with the Fc/receptor? How do we  
19 predict the clinical significance of red blood  
20 cell antibody? That's the catchall question. And  
21 we really would like to get a better understanding  
22 in that regard. And how do we define "clinical

1       significance"? (Inaudible) serologic transfusion  
2       reaction not clinically significant? Is a  
3       hemolytic transfusion reaction significant?  
4       Probably yes because it may damage the tissue  
5       quite a bit.

6               So, in this list Dr. Garratty gave  
7       possible novel mechanisms for immune destruction  
8       of red blood cells and platelets. I'm just  
9       listing this here. The antibody independent  
10      cell-mediated cytotoxicity by NK cells is  
11      certainly of interest with regards with HLA and  
12      the cure antigens as the cure actually -- HLA is  
13      not present on red cells, so the NK cells  
14      typically would see the red cell assay target.

15             And this final slide shows George's  
16      personal opinion, just citing this here. Most  
17      hemolytic transfusion reactions associated with  
18      IVIG are due to ABO alloantibodies, and although  
19      reducing the titer of the anti- A and B will help  
20      lower the number of cases with hemolytic anemia,  
21      there will still be a few cases associated with  
22      low titer antibodies that are present in the blood

1 product.

2 The only way to stop hemolytic  
3 transfusion reactions is to have no anti-A and B  
4 in the products. Maybe it does not stop even if  
5 there's no anti-A and B in it because there may be  
6 other antibodies in it and this may well be also  
7 worthwhile exploring.

8 I thank Dr. Garratty for providing all  
9 these slides, which make it much easier to give  
10 this presentation, and I wish that he will get  
11 better soon because he's very much missed. He's  
12 actually the individual who accepted most of my  
13 publications and inexplicably rejected some. So,  
14 thank you.

15 (Applause)

16 DR. SCOTT: Just one or two questions  
17 for the sake of time. Since we're a little behind  
18 on time, Dr. Padmore has agreed to talk fast, so I  
19 want you all to hang on to your hats, and we  
20 welcome her.

21 DR. PADMORE: Thank you very much for  
22 the invitation to speak today on possible

1 mechanisms for IG product-related hemolysis.

2 I have two objectives today, to explore  
3 possible mechanisms for IG product-related  
4 hemolysis using clues from the clinical  
5 characteristics of patients with IG product-  
6 related hemolysis, and to explore possible  
7 influences of macrophage receptor polymorphisms on  
8 hemolysis.

9 I have no conflict of interest to  
10 declare, even though a lot of this product use  
11 that's off label has nothing to do with me.

12 In our paper, in 2008 -- and I'd like to  
13 acknowledge all my coworkers, Zohra Daw was a  
14 transfusion medicine fellow with us and Doris,  
15 Nancy, Melanie and Diane, technologists in the  
16 lab, and Bernhard and Alan and Antonio, my  
17 (inaudible) pathology colleagues.

18 We had a series of 16 patients where we  
19 identified what we thought was IG product-related  
20 hemolysis. They're characterized by female  
21 gender, a slight predominance similar to what's  
22 been reported this morning, and then 15 out of the

1     16 patients had at least one of the three  
2     following features, which is an underlying  
3     inflammatory state, large dose of IVIG, and non-O  
4     blood group.

5             I'm just going to go through each of  
6     these clinical characteristics that we identified  
7     in our small series and make a few comments.

8             So, female gender, I'm just going to  
9     wander through a few odds and ends. Maybe they're  
10    -- could possibly be equated to a sensitized state  
11    because multiparous females can develop antibodies  
12    during pregnancies. For example, HLA antibodies,  
13    although HLA antibodies can occur naturally  
14    without alloimmunization, I'd like to draw your  
15    attention to some papers I looked at in the  
16    literature. Ravindranath from the Tarasaki Group  
17    reported the presence of anti-HLA antibodies in  
18    IVIG products, a very nice paper in Blood  
19    recently. If you look at the fine print on the  
20    paper it references 34-36. They also talk about  
21    the presence of soluble HLA class 1 antigens  
22    present within the IVIG prep.

1           Been some talk this morning about what's  
2   the incidence. We had a 1.6 incidents of  
3   hemolytic reactions in our series. Kahwaji  
4   reported a higher instance of hemolysis in their  
5   highly sensitized HLA-sensitized renal transplant  
6   candidates, although they did attribute some of  
7   this high rate to use of liquid IVIG preps, which  
8   seemed to have a higher titer at that point in  
9   time.

10           Just a bit more on female gender.  
11   Again, I'm just sort of free thinking here. HLA  
12   antibodies, the Bennett-Goodspeed can rarely be  
13   associated with hemolysis Bga, it's B7; Bgb, B17;  
14   Bgc, A28 with A2 cross reactivity. Occasionally  
15   there's some other HLA antibodies -- antigens  
16   expressed on red cells A10, B8, A9, B12, B15.

17           There was a curious paper I ran across  
18   where you can get increased B7 expression on the  
19   surface of red cells in inflammatory conditions  
20   such as infectious mono.

21           So, you can speculate, are there  
22   increased HLA antibodies present in the patients



1 with IG-related hemolysis? Are these reacting  
2 with the antigens -- soluble antigens present in  
3 IVIG? Just to sort of add to the, you know, AOB  
4 issues. And are specific HLA haplotypes -- is  
5 that a predisposition risk factor for IG  
6 hemolysis? I couldn't find any literature on that  
7 at all.

8 As far as the underlying inflammatory  
9 state, this was defined in our paper by, you know,  
10 elevation of inflammatory markers. This is really  
11 low tech, you know, elevated serum haptoglobin  
12 because with haptoglobin usually you expect it to  
13 be low, like a marker of hemolysis, but I had to  
14 remind everybody that it can be elevated  
15 inflammatory state, so if it's high it doesn't  
16 mean that hemolysis is not present, (inaudible)  
17 react to protein, ESR, et cetera.

18 Can you have more reactivity in an  
19 inflammatory state to high-molecular weight  
20 moieties in IVIG leading to more complement  
21 activation? If you have like IgG dimmers can they  
22 bind to the red blood cells via CR1 and lead to

1       increased phagocytosis? And again I mentioned the  
2       increased expression of HLA antigens in  
3       inflammatory conditions. And a couple of  
4       references that allude to that.

5               This is a little picture I drew using  
6       PowerPoint for the review article I wrote in  
7       Transfusion 2012. After there was a meeting in  
8       Zurich on innate -- the innate immune system and  
9       after the meeting we wrote some articles on it and  
10      I just wrote a short review on hemolysis upon  
11      intravenous immunoglobulin transfusion, and at the  
12      low part of the graph here, where it's the green  
13      part, this is the good stuff. You're using IVIG  
14      therapeutically, it's an excellent product, using  
15      it in a positive immuno modulatory fashion, so it  
16      diverts activated complement from the target  
17      cells, which are causing the disease, scavenges  
18      activated complement and blocks Fc receptors and  
19      actually hemolysis is actually inhibited.

20             If you go too far up the Y-axis there  
21      into the red zone with increasing inflammation or  
22      sensitization of the patient, and you're using a

1 high dose of IVIG, you can -- the anti-A and  
2 anti-B can bind to the red cells removed by  
3 splenic macrophages, fixed complement on the red  
4 cells with subsequent hemolysis, and here's a  
5 little picture showing the xerocytes and  
6 polycromasia associated with one of our patients  
7 with hemolysis.

8 Other characteristics have been well  
9 talked about this morning, it's the high dose of  
10 IVIG. In our series most of the patients were  
11 receiving around 200 grams IVIG and it can be  
12 defined as more than 2 g/kg as a single dose or  
13 over several days or 100 or more grams over two to  
14 four days. And it's sort of a vicious circle. In  
15 the low dose IVIG you use for immune replacement  
16 in immunodeficiency patients, you don't usually  
17 see hemolysis. When you're using a high dose for  
18 the immunomodulatory effect, these patients have  
19 an underlying immune inflamed state, so you're  
20 giving them high dose IVIG, which predisposes them  
21 to hemolysis and they're inflamed and have an  
22 inflammatory sensitized state, so they're

1 predisposed to hemolysis as well. So, it's sort  
2 of two plus two equals four in hemolysis.

3 Non-O blood group, I think that's been  
4 shown by most of the -- all of the series, and I  
5 won't talk further on that. In our series we were  
6 able to eluate anti-A or anti-B off the positive  
7 DAT, which I think most cases did confirm that  
8 that was the etiology, although you can't exclude  
9 HLA or, you know, antibody to low incident  
10 antigen.

11 Just a little bit about hemolysis, I  
12 think I'll just drop down to the bottom point  
13 where you can have hemolysis may be exacerbated by  
14 a cytokine storm induced by IgG antibodies and  
15 draw your attention to the work of Robson  
16 Davenport, who wrote a couple, two or three  
17 articles, on this topic in the 1990s and then ably  
18 picked up by Jacob Pendergrast, who's following me  
19 as a speaker, about the role of inflammation in  
20 this whole situation.

21 Dr. Flegel also mentioned the issue of  
22 ABO incompatible platelets. I just wanted to

1       reiterate it. When you're giving platelets, you  
2       can often have minor incompatibility where you  
3       have donor antibodies, anti-A or anti-B inside the  
4       platelet plasma and you're giving it to a  
5       recipient who is A or B+, and there are a series  
6       where repeat ABO-incompatible platelet  
7       transfusions led to really significant hemolytic  
8       transfusion reaction.

9               There's a paper by Karafin out of Johns  
10       Hopkins, that's the bottom point there on the  
11       slide, where the title of the paper, "ABO Titers  
12       Are Not Predictive of Hemolytic Transfusion  
13       Reactions Due to Plasma-Incompatible Platelet  
14       Transfusions" -- that's a bit -- I wanted to share  
15       with you the fine print in that paper because I'm  
16       not sure their patient population was entirely  
17       representative. They had no patients under the  
18       age of 18 years and no patients under the -- so,  
19       they excluded younger, smaller patients, and no  
20       adult patients under the weight of 40 kilograms.  
21       So, they excluded all their high-risk people where  
22       they could, by dose analogy, be getting a higher

1       dose of the anti-A or anti-B.

2                   They did have two febrile reactions with  
3       a positive DAT with anti-A or anti-B, which is an  
4       incidence of .15 percent, but did not have overt  
5       hemolysis from this or a drop in the hemoglobin, I  
6       think just again pointing to it's not just the  
7       anti-A and the anti-B. Patient factors are really  
8       important. So, I'm not sure I entirely agree with  
9       the title of their paper because of their  
10      pre-selection criteria excluding children and  
11      small adults, very small adults.

12                  Just a word on the secretor status,  
13      secretors Le(a-b+) versus non-secretors, Le(a+b-),  
14      non-secretors might be more prone to hemolysis,  
15      they have less A and B soluble substances  
16      available essentially to sop up the acquired  
17      anti-A or anti-B.

18                  We looked at this in our case series of  
19      16 patients, we had two secretors and one  
20      non-secretor, so we couldn't draw any conclusions,  
21      but I think Dr. Pendergrast's paper case report  
22      does show that this probably may play a role in at

1     least some patients.

2                   Finally, I just want to take a -- I was  
3     just reviewing the literature a bit on the topic  
4     and wanted to share with you some thoughts about  
5     macrophage receptor polymorphisms, just sort of in  
6     the area of personalized medicine and looking at  
7     patient factors, and I would agree with Dr. Flegel  
8     that maybe molecular studies of patient risk  
9     factors might be a very fruitful area, also  
10    polymorphisms and NK cells and inflammatory  
11    markers, but I'll just focus on complement  
12    receptors, CR1, CD35, binds sufficiently with C3b.  
13    There are a couple different restriction  
14    polymorphisms referenced by Liu and Niu, Hind III  
15    restriction length polymorphisms, and essentially  
16    it's correlating to copy number of CR1 cells, so  
17    essentially the more copy numbers maybe the more  
18    prone you might be to hemolysis.

19                   Another polymorphism, this interesting  
20    paper out of India where they showed that CR1  
21    polymorphisms are associated with low expression  
22    of CR1 and confer protection against

1 malaria-related complications. I would read into  
2 that less hemolysis.

3 And they are able to define their  
4 polymorphisms there. Complement receptors CR3,  
5 which is CD11b/18, and there is an association of  
6 the conversion of arginine to histidine at the  
7 position 77, which is associated with reduced  
8 adhesion in phagocytosis in monocytes and  
9 increased incidents of SLE.

10 You might argue that this might be a  
11 protective effect against hemolysis with reduced  
12 adhesion to the monocytes, but there's been no  
13 work on this, so it's just a possible area.

14 IgG receptors on macrophages, high  
15 affinity/low affinity. The low affinity  
16 polymorphism has been identified on CD32 where you  
17 have polymorphism H131 more D-rosette formation,  
18 which is the rosette formation in the presence of  
19 a D+ red cell with anti-D. And then the  
20 polymorphism of the 518 locus where you change the  
21 rate of removal of RBCs coated with IgG3, rate at  
22 which those red cells are removed from the



1       circulation.

2               So, I really do think there is patient  
3       factors here that may be detectable by molecular  
4       mechanisms.

5               So, in summary, I've explored the  
6       clinical clues that we had to come up with a few  
7       risk factors including underlying inflammatory  
8       state, and explored some of the macrophage  
9       receptor polymorphisms that have been linked in  
10      the literature, if perhaps somewhat tenuously, to  
11      removal of red cells from the circulation. And  
12      then I talked fast. And thank you very much.

13                       (Applause)

14               DR. SCOTT: I compliment your speed. I  
15      think we'll take questions during the panel  
16      discussion and welcome Dr. Pendergrast, another  
17      Canadian, so he's not too bothered by the weather,  
18      I hope, and thank you for coming.

19               DR. PENDERGRAST: Thank you. Thanks  
20      very much.

21               So, aside from disclosing that I don't  
22      actually know very much about inflammation, I do

1 have some research support for this project that  
2 I'll be presenting some preliminary results, from  
3 Grifols and from Canadian Blood Services.

4 And what I'm hoping to go over in this  
5 talk is just a quick review of the two-hit  
6 hypothesis of IVIG-mediated hemolysis that Dr.  
7 Padmore has proposed and which I think has been a  
8 very useful concept, spend some time discussing a  
9 Sentinel case report that was published recently  
10 of the case of IVIG-mediated hemolysis where we  
11 were able to dissect the mechanism by looking at  
12 the monolayer assay results and doing a  
13 (inaudible) profile, and then provide some  
14 preliminary results from a perspective  
15 surveillance protocol, which is currently  
16 underway, which I think is providing some clues as  
17 to what the mechanism might be for IVIG-mediated  
18 hemolysis.

19 So, just talking quickly about the  
20 two-hit model of IVIG-induced hemolysis, it's  
21 certainly not a new idea that hemolytic  
22 transfusion reactions due to serologic

1 incompatibility represent an inflammatory reaction  
2 and in an early study by Dr. Davenport from 1993  
3 did show that the exposure to monocytes of IgG  
4 opsonized red cells is sufficient to provoke  
5 secretion of inflammatory cytokines such as  
6 IL-1Beta, IL-1 receptor antagonist, IL-6, IL-8,  
7 TNF alpha, in other words, it did not require the  
8 presence of the adaptive immune system, it did not  
9 require the lysis of red cells, the exposure of  
10 intracellular contents or stroma, it was purely  
11 through binding of the immobilized IgG on the  
12 surface of the red cell to, in particular, the  
13 high affinity SCR 1 receptor that was sufficient  
14 to trigger Erythrophagocytosis and then those  
15 phagocytose monocytes in turn released the  
16 inflammatory cytokines.

17 Dr. Davenport has subsequently published  
18 a model that's -- of a hemolytic transfusion  
19 reaction as an inflammatory event, emphasizing the  
20 importance of complement activation as being an  
21 inflammatory process, not necessarily leading to  
22 the completion of the membrane attack (inaudible)

1 and intravascular lysis, but because the  
2 complement split products, particularly C5a and  
3 C3a, are themselves inflammatory by nature, that  
4 they have properties that attract and activate  
5 leukocytes and then those leukocytes in turn  
6 secrete the inflammatory mediators, particularly  
7 TNF alpha, which is responsible for the clinical  
8 manifestations of hemolysis, such as fever,  
9 leukocytosis, hypertension, and so on and so  
10 forth.

11 So, the idea that hemolysis is an  
12 inflammatory reaction certainly is well  
13 established. I think the question at hand is  
14 whether inflammation itself can provoke hemolysis,  
15 at least in the setting of IVIG recipients, and so  
16 the idea that a patient needs to be inflamed and  
17 then they hemolyze, rather than the other way  
18 around, comes from case series like what was  
19 presented by Dr. Padmore. So, this is the case  
20 series of the 16 patients from Ottawa who  
21 developed hemolysis after receiving IVIG and you  
22 can see that many of their diagnoses were

1 infectious, there's a lot of necrotizing  
2 fasciitis, sepsis, viral meningitis, HIV, even  
3 conditions like lupus, that did seem to mean these  
4 patients were inflamed, to some extent, when they  
5 got the IVIG. And if you look at various  
6 inflammatory markers, such as elevated  
7 haptoglobin, ferritin, fibrinogen, et cetera, at  
8 baseline, these were elevated. Again, it  
9 suggested that the fact that these patients were  
10 inflamed before they got the IVIG was why they  
11 hemolyzed.

12 And summarizing this, it looked like the  
13 presence of a positive inflammatory marker was as  
14 important in developing hemolysis as being  
15 non-blood group O or getting a high dose of IVIG,  
16 which is defined in this study as 100 grams or  
17 more over two to four days.

18 It is hard to pick apart the actual  
19 contribution of IVIG in these cases because they  
20 were so sick, because they had co-morbidities, one  
21 might try to read into the dynamics of hemolysis,  
22 the mechanism by which it's occurring, for example

1     if the hemolysis was immediate and very brisk,  
2     then as Dr. Flegel indicated, that might suggest  
3     that there was an intravascular component whereas  
4     a more delayed, gradual onset would suggest maybe  
5     less complements activation, more extravascular.

6             It's hard to tell in these kind of  
7     passive reports because there's so many other  
8     things going on and, in fact, even in this case  
9     series, a third of the cases could not be said for  
10    sure were actually due to the hemolysis or not.

11            Nonetheless, this has been a very useful  
12    concept. This graphic, I think, is incredibly  
13    useful and it is doomed to be copied and pasted  
14    into presentations for years to come because I  
15    think it's so useful, showing this idea that  
16    having -- being exposed to a high dose of IVIG and  
17    not being blood group O, in this model, is not  
18    sufficient. You also have to have some other hit  
19    in this model, either HLA sensitization or a  
20    degree of inflammation before the IVIG was given.

21            In terms of what that might mean, like  
22    how exactly inflammation would lead to a

1 predisposition to IVIG mediated hemolysis, one way  
2 that you could look at this is to look at the  
3 concept of monocyte polarization. This reflects  
4 the TH1 and TH2 immune responses, which are  
5 thought to underlie the different responses of the  
6 adaptive immune system. This reflects sort of a  
7 mirror image in the innate immune system whereby  
8 if you have a monocyte exposed to agonists like  
9 interferon gamma, which is secreted by NK cells in  
10 response to viral infections among other things,  
11 or tumors, or if you have binding to (inaudible)  
12 like receptors by things like lipopolysaccharide,  
13 then you polarize towards an M1 phenotype, and  
14 it's this M1 monocyte that secretes the  
15 inflammatory cytokines like TNF-alpha, IL-1 beta  
16 and IL-6, and this in turn leads to the production  
17 of the acute phase proteins, generally by  
18 hepatocytes, although also by other monocytes, and  
19 these are the things that you can measure in  
20 studies looking at pre-IVIG inflammation, high  
21 complement levels, high fibrinogen, high  
22 haptoglobin, high ferritin, so on and so forth.

1 That same polarization towards the M-1 phenotype  
2 may up regulate expression of the high affinity  
3 SCR-1 receptor, which was shown in that paper from  
4 Davenport, to be important in erithro phagocytosis  
5 of IgG opsonized red cells, i.e., if you have a  
6 patient who has these positive inflammatory  
7 markers, that may indicate that they have  
8 polarizer monocytes towards a phenotype that is  
9 more likely to hemolyze IgG bound red cells.

10 So, that is one possible mechanism. The  
11 other polarization towards the M2 would seem to be  
12 less likely to be associated with phagocytosis,  
13 this is more of your wound healing phenotype of a  
14 monocyte involved in regeneration of extracellular  
15 matrix, angiogenesis, that sort of thing. It  
16 expresses a low affinity receptor, but these low  
17 affinity receptors actually may be important in  
18 certain types of immune-mediated hemolysis. It  
19 may indicate that, you know, an autoimmune  
20 hemolytic anemia or ITP, there is almost sort of a  
21 scavenging or cleaning kind of process of  
22 macrophages as opposed to an inflammatory kind of



1 response.

2           So, it could be the M2 polarization as  
3 well. These two tend to be competitive. M2s  
4 secrete or express IL-10, also known as cytokine  
5 synthesis inhibitory factors, so it actually  
6 blocks polarization towards the M1, if you have  
7 the M2, the whole thing is skewed by the presence  
8 of immune complexes, which tend to lead to more  
9 IL-10 secretions. So, it's certainly much more  
10 complicated than this would suggest, but this does  
11 give you an idea of how pre-inflammation might  
12 predispose to more hemolysis once you get the  
13 IVIG.

14           So, we tried to apply some of these  
15 ideas to a Sentinel case report that was published  
16 recently in Transfusion. It was a somewhat  
17 complicated patient in that she has post-stem cell  
18 transplant that had converted her from O to an A,  
19 and then she had GVHD, some interesting  
20 implications regarding her secretor status because  
21 of all that, which I won't get into.

22           In any case, she presented with ITP,

1 bone marrow confirmed, normal engraftments, no  
2 other dysplasia, and she was given IVIG at a dose  
3 of a gram/kilogram daily times two, which is a  
4 typical dose for ITP. This was a big woman, so  
5 that ended up being 200 grams of IVIG. And  
6 several hours afterwards she began experiencing  
7 signs and symptoms consistent with hemolysis.  
8 And, in fact, she was brought to the attention of  
9 us in the blood bank because her spun plasma was  
10 beet red, so it looked like there was quite a  
11 significant degree of free hemoglobin, and just  
12 looking at the dynamics of her hemolysis, it is  
13 interesting to note that the hemoglobin fell  
14 mostly during the acute phase and then continued  
15 to fall over the next few days, even as the  
16 hemolytic markers began to resolve.

17 That might be interpreted as saying that  
18 there was sort of a two-phased type of hemolysis  
19 here, one that was acute and rapid and another  
20 that was slower. Sort of the typical bad actors  
21 were identified here, positive DAT, IgG only,  
22 anti-A1. We actually picked up in the reverse

1     typing as well as in the eluate, lots of free  
2     hemoglobin in the plasma, she was unwell, refused  
3     a transfusion although she was offered one,  
4     (inaudible) atrial fibrillation. Did eventually  
5     recover. Notably, no DIC, no renal failure.

6             And because we were sort of getting  
7     ready for a larger study, we had everything in  
8     place to do some of this other analysis like the  
9     MMA and the cytokine profile.

10            So, for the monocyte, monolayer assay,  
11     this basically involved opsonizing red cells with  
12     IVIG, washing off any residual unbound IgG, and  
13     then incubating those with monocytes and measuring  
14     the degree of phagocytosis. And because you would  
15     expect anyone who gets IVIG, to a certain extent,  
16     to have some degree of phagocytosis, if they have  
17     a red cell that expresses a cognate antigen, it  
18     was felt to be useful to define what "excessive  
19     phagocytosis" was and so this was a study done by  
20     Don Branch, who's here in the audience here, and  
21     what he did was took 21 normal control monocytes,  
22     incubated them with IVIG, looked at the mean

1 phagocytic index, and then one standard deviation  
2 above that was defined as being "excessive  
3 phagocytosis", so that was sort of the way in  
4 which we tried to identify anyone who had an  
5 excessive response to IVIG.

6 And so, this initial study looking at  
7 different doses of IVIG did show that there's a  
8 dose response in terms of the phagocytosis. It  
9 did appear to be predominantly A1 cells that were  
10 vulnerable to this. So, this was sort of a proof  
11 of concept that the MMA was picking up what we  
12 thought was the mechanism of IVIG-mediated  
13 hemolysis, i.e., increasing doses of anti-A  
14 targeting a cognate antigen on the red cells.

15 When we did this assay again using the  
16 patient's own monocytes and using the implicated  
17 -- or one of the implicated lots of IVIG, the  
18 results were very dramatic, a little hard to  
19 interpret, but certainly very significant degree  
20 of phagocytosis, above that threshold of 17 that  
21 had been defined with the normal controls.

22 Interestingly, the patient's own

1       monocytes were much more active than the control  
2       monocytes. These were monocytes that were  
3       collected on day ten after the IVIG was given, so  
4       we don't know if they were activated at baseline  
5       or had been goosed by the hemolysis itself, but  
6       certainly it was a dramatic difference.

7               It did look like, very interestingly,  
8       that the patient's own red cells were much more  
9       prone to phagocytosis than referenced red cells of  
10      the same blood group. In fact, there didn't  
11      actually appear to be any kind of relationship  
12      between the A antigen expression on the referenced  
13      red cells and the degree of phagocytosis for  
14      reasons that we couldn't quite explain, but  
15      certainly the patient's own group A1 red cells  
16      were very vulnerable to hemolysis, possibly  
17      indicating that they had somehow been damaged  
18      while in circulation following the administration  
19      of IVIG and were therefore more prone to  
20      clearance, perhaps by these same scavenger  
21      receptors that are part of the M2 phenotype, we  
22      don't know.

1                   Looking at the actual lot of implicated  
2    IVIG versus random IVIG, we didn't see any  
3    difference there. It didn't look like there was  
4    anything in that lot of IVIG that was any  
5    different than randomly selected IVIG, despite the  
6    fact that that implicated lot had actually been  
7    associated with hemolysis in another patient.

8                   So, if there was something special in  
9    that lot of IVIG that caused hemolysis in this  
10   patient, we couldn't see it in the MMA.

11                  In terms of cytokine analysis, there was  
12   evidence of a preexisting inflammatory state in  
13   this patient before the IVIG was even given. IL-1  
14   beta was elevated, IL-1 receptor antagonist was  
15   extremely elevated and I think this reflects the  
16   fact that IL-1 receptor antagonist, although it  
17   can be secreted by activated monocytes, is also an  
18   acute phase protein secreted by hepatocytes, among  
19   other tissues, and it actually did not go any  
20   higher once the hemolysis set in, although the  
21   IL-1 beta certainly did increase significantly.  
22   So, that probably reflects the IL-1 beta -- the

1 monocyte activation suggesting possibly an M1  
2 polarization as underlying the hemolysis here.

3           Somewhat confusingly, neither the  
4 typical agonists of M1 or M2 polarization were  
5 elevated either before or after the IVIG. So, the  
6 interferon gamma, which is your M1 trigger, or  
7 IL-13, IL-4. Those are certainly not the only  
8 polarizing stimuli, but the ones that we checked  
9 it did not appear to be elevated throughout.

10           Looking at other inflammatory markers,  
11 the patient's ferritin did increase pre to post,  
12 and there was a very significant leukocytosis as  
13 well. Again, if these are acute phase responses,  
14 this would suggest more of an M1 polarization, but  
15 of course there were all sorts of other  
16 confounders here to make this hard to interpret.

17           Trying to sort through, with this one  
18 case, why it was this patient hemolyzed so badly,  
19 you know, it's difficult because it is only one  
20 case. Was it the lot of IVIG? There are  
21 arguments for and against. The dose certainly got  
22 our attention. This patient had a BMI of 40. We

1 know that IgG is lipophobic, and therefore you  
2 risk overdosing patients if you don't adjust for  
3 their ideal body weight, which is increasingly  
4 being recommended when you dose IVIG.

5 Had you done that for her, the dose that  
6 she received would have corresponded to almost 3  
7 g/kg. On the other hand, even lower doses than  
8 two have been associated with hemolysis, so maybe  
9 that wasn't why. She didn't have her infusion  
10 titrated up gradually, she got it all at once at a  
11 standard rate, and maybe that was a risk factor,  
12 although the standard rate was not itself all that  
13 high.

14 Her blood group would predispose her,  
15 but she was not -- she was a heterozygote for the  
16 A antigen. You know, you didn't really see any  
17 patten of anti-A in the MMA. It looked like there  
18 was a lot of complement activation, possibly  
19 intravascular hemolysis, but the presence of  
20 plasma-free hemoglobin and complement depletion do  
21 not in and of themselves mean that there was  
22 creation of the membrane attack complex. And



1       certainly we didn't see any complement on her red  
2       cells by the DAT. Her PNH assay was negative by  
3       FLAER analysis.

4               It may be that she was inflamed at  
5       baseline. We have some evidence from both her  
6       diagnosis of ITP, the fact that she wasn't getting  
7       steroids as concurrent medication with this, and  
8       the elevated cytokines at baseline. On the other  
9       hand, we didn't see any triggers of those elevated  
10      cytokines at baseline or afterwards.

11              The TNF alpha, we don't know was  
12      predisposing to the hemolysis or a reaction to it.  
13      Again, there's only so much you can say from a  
14      single case report, even when you dissect it as  
15      carefully as was attempted here.

16              So, I will give some preliminary results  
17      from a prospective protocol where we're trying to  
18      enroll every patient who gets IVIG at a high dose  
19      to see if we can discern what the risk factors  
20      were. Very briefly, anybody who got a gram per  
21      kilogram or more during a 28-day period, this was  
22      a dose adjusted, we checked them for hemolysis pre

1 and immediately after the IVIG, and at day five to  
2 ten, and are planning to do sort of more -- the  
3 same sort of cytokine and risk factor analysis,  
4 MMA, FCR receptor polymorphisms, IgG sub-typing  
5 and so on and so forth.

6 What we have seen -- so, just to quickly  
7 indicate here, we're trying to sort of control as  
8 many variables as possible, just a standardized  
9 dose calculator. Everybody gets their dose  
10 adjusted for their ideal body weight. And the  
11 infusion rates are all standardized as well in  
12 order to try to remove any other confounders.

13 Just as an example, if you had a  
14 100-kilogram who was 180 centimeters, using this  
15 calculator, which is really encouraged in Ontario  
16 and other places, the dose would actually be 85  
17 grams for a gram/kilogram dose. So, again, this  
18 is sort of trying to control as much as possible  
19 the fact that the IgG is lipophobic.

20 We use a typical definition of hemolysis  
21 in the study. We did have some patients who  
22 clearly hemolyzed but maintained their hemoglobin

1 with a (inaudible) reticulocytosis, we called  
2 those borderline patients. Grading is as per the  
3 Canada definition of the Pharmacovigilance  
4 Committee.

5 What we found so far, we've had 41  
6 patients enrolled, most of them were for neurology  
7 indications, most of them were previously treated  
8 -- 83 percent have previously been treated. Using  
9 this surveillance protocol we found 7.3 percent of  
10 them did actually meet the definition of  
11 hemolysis. If you included the borderline cases  
12 who are clearly hemolyzing but compensating, the  
13 rate of hemolysis was twice that.

14 Looking at the dynamics, we had a grade  
15 four case, two grade twos, all of them were  
16 getting 2 g/kg or higher adjusted for ideal body  
17 weight. Anti-A or anti-B was identified in both.

18 It is interesting in cases one and three  
19 that the hemolytic markers actually decreased  
20 initially as the hemoglobin fell and then  
21 increased later, and that may indicate a  
22 dilutional effect of the IVIG. It may also

1 indicate that the red cells were initially being  
2 sequestered in the spleen and only later were  
3 actually being phagocytosed.

4           We had some borderline cases as well, I  
5 won't go into these in as much detail, but they  
6 were clearly hemolyzing as well. But the  
7 hemoglobin did remain stable. We did have a  
8 number of patients who have had positive DATs but  
9 did not hemolyze. So, 14 percent of patients had  
10 a positive DAT with anti-Q or anti-B or both, but  
11 did not hemolyze, although the DATs became  
12 progressively weaker over time, so maybe a third  
13 to two-thirds of the positive DATs were negative  
14 at day five to ten, an important fact if you're  
15 trying to define hemolysis on the basis of a  
16 positive DAT. If you wait too long you might miss  
17 it even though the hemolysis itself might not  
18 manifest until the DAT is negative, so you may  
19 need to measure at both time points.

20           In terms of risk factors, it did look  
21 like nobody who got less than 2 g/kg adjusted body  
22 weight hemolyzed. If you didn't adjust the body

1 weight for the BMI, there was a bit more overlap  
2 between the hemolyzers and the non- hemolyzers,  
3 with the adjustment that was very clear, it was  
4 right at two. Nobody less than that did develop  
5 hemolysis.

6 In that sub-group, interestingly, the  
7 risk was 16 percent. Nobody who was group O  
8 hemolyzed. Again, if you restrict this to just  
9 the non-O patients, the risk of hemolysis was 11  
10 percent, again, twice that if you included the  
11 compensated hemolyzers. And when you limit this  
12 to the non-O patients who got 2 g/kg or higher, in  
13 this case the risk of hemolysis was 33 percent in  
14 that subgroup, and again, if you included the  
15 borderline cases where they maintained their  
16 hemoglobin, it was two-thirds of them hemolyzed.

17 In that subgroup, interestingly, there  
18 were more women, but their risk of hemolyzing was  
19 not any higher than men. So, I think that's an  
20 important thing to dissect out that women may be  
21 more likely to be treated with high doses of IVIG  
22 and not be blood group O, but their simply being

1 women, in this state anyway, just preliminary, did  
2 not appear to be a risk factor.

3 BMI did not differ between the  
4 hemolyzers and non- hemolyzers. The infusion rate  
5 was the same. Interestingly, the diagnostic  
6 indication did not appear to predispose. For  
7 every patient who was not blood group O who got 2  
8 g/kg of IVIG for myasthenia gravis, for example,  
9 you know, we had one patient that hemolyzed and  
10 many more who did not. Similar pattern for CIDP,  
11 ITP, so it did appear that the diagnosis in and of  
12 itself cannot define a risk factor for hemolysis,  
13 even with everything else being present, even with  
14 a high dose, even being non-blood group O.

15 There was no difference in terms of  
16 premedication, whether they were getting steroids  
17 or other immunosuppressants at baseline, that did  
18 not appear to have any effect on whether they  
19 hemolyzed or not. Premedication, very few of them  
20 got steroids as premedication, but again, there  
21 was no difference between those who hemolyzed and  
22 those who didn't hemolyze. Looking at signs of

1 inflammation, interestingly, there was no  
2 difference in adverse reaction between the  
3 hemolyzers and the non- hemolyzers, possible  
4 exception of headache. These are very, very small  
5 numbers, but it was notable that none of the  
6 patients who hemolyzed had fever or fatigue, they  
7 all reported a severe headache, what they  
8 considered the worst headache they'd ever had.  
9 Maybe that was a sign of whatever inflammatory  
10 reaction was driving the hemolysis, but certainly  
11 not fever or fatigue or malaise.

12           We did see C3 drop a little bit more  
13 among the hemolyzers than the non-hemolyzers. The  
14 drop was mild, though, we're looking at a drop of  
15 -.2 g/L for the hemolyzers versus -1.5 for the  
16 non-hemolyzers. Is that significant? Certainly  
17 the standard deviations overlap quite a bit here  
18 and it was reversed by day five to ten. No  
19 difference in a change in white counts, either at  
20 baseline or by day five to ten. It did not look  
21 like that was a marker of inflammation.

22           The ferritin, however, did spike

1 significantly, not right away, but by day five to  
2 ten it had increased significantly more amongst  
3 the hemolyzers than amongst the non-hemolyzers.

4 And, again, all of this is limited to  
5 the non-O, 2 g/kg or higher dosing.

6 Looking at the MMA results, this is very  
7 preliminary, we don't have data on all patients  
8 yet. It does look like having an MMA of -- a  
9 phagocytic index by MMA of less than 17, which was  
10 the threshold that Don defined, was effective at  
11 ruling out clinically apparent hemolysis, at least  
12 in this very small group. It would not rule it  
13 in, however, so this is one patient, patient  
14 number five, who had a very high phagocytic index,  
15 did not actually hemolyze clinically and it is  
16 notable that they only got a gram per kilogram of  
17 IVIG.

18 So, it looks like you need to have both  
19 those things.

20 The increase in ferritin appeared to be  
21 a more specific sign of inflammation, either  
22 indicating that acute phase response or the



1       trafficking of processed iron, I can't tell.  
2       Looking at the cytokine results, it was notable  
3       that there was no apparent difference at baseline  
4       in typical inflammatory markers like white count,  
5       platelet count, ferritin, haptoglobin, we had one  
6       patient with an extremely high baseline of  
7       ferritin who nonetheless did not have any sign of  
8       hemolysis, haptoglobin elevated at baseline and  
9       one patient as well, again, borderline, maybe,  
10      hemolysis, but the interleukin 1 receptor  
11      antagonist was dramatically increased in the  
12      patients who did hemolyze.

13               So, again, it was not sufficient. We  
14      had one patient with a very high interleukin 1  
15      receptor antagonist who did not hemolyze despite  
16      being blood group A, but they only got 1 g/kg.

17               So, it may be that you need all three  
18      things, you need to have the high dose, the  
19      non-blood group O, and inflammation, and in this  
20      study, at least, best defined by an elevated  
21      interleukin 1 receptor antagonist level.

22               So, this would suggest that inflammation

1 as an underlying condition is important for  
2 hemolysis. It does also, at least from the data  
3 so far, suggest that it's very hard to define  
4 inflammation by either the patient's diagnosis or  
5 commonly ordered laboratory tests. So, it may  
6 need to be something more esoteric.

7 Okay, and I hope I didn't talk too long.  
8 So, anyway, thank you very much for the  
9 invitation.

10 (Applause)

11 DR. SCOTT: I think we'll ask the  
12 panelists to come up to the table for the  
13 discussion. We have about 25 minutes, I believe,  
14 and we'll try to address the questions that we  
15 have on board, but it's also fair game to ask any  
16 burning questions that you may have about these  
17 presentations that you haven't had a chance to  
18 ask.

19 So, all of the folks that have already  
20 spoken, I'm afraid it is a big group, although I'm  
21 happy to say it's a big group, and we have a bit  
22 of ground to cover.

1                   Can we have the slides with the session  
2     questions? You can go ahead to the next slide,  
3     and actually we'll pull up a few more chairs if  
4     need be.

5                   MR. SCHLEIS: I guess I'll go first.  
6     Tom Schleis. Dr. Pendergrast, I really like the  
7     work that you've done and the information that you  
8     provided. It really mirrors the work that was in  
9     the Kahwaji article, which is Stan Jordan's group  
10    at Cedars-Sinai, and they had a very -- I mean,  
11    it's almost -- their experience is almost  
12    identical. They were using products where they  
13    had no hemolysis. The one product went off the  
14    market, they started to use other products, and  
15    they had about a 5.8 percent incidence of  
16    hemolysis.

17                  They measured the titers of all the  
18    products and found out that the products with high  
19    titers were the products giving them hemolysis.

20                  So, pre-transplant they used a product  
21    that was very low in anti-A and hemolysis went  
22    away, but it was a product they couldn't use

1 post-transplant. So, in post- transplant, they  
2 still give about 2+ g/kg, but what they did was to  
3 give 1 g/kg on day one, skip day two, and give the  
4 other 1 g/kg on day three. And their incidents of  
5 hemolysis with that same product that they had  
6 hemolysis with previously, pretty much vanished.

7 So, I think there really is a  
8 correlation between sort of loading these patients  
9 up with enough IG that it causes hemolysis.

10 DR. PENDERGRAST: No, I think I would  
11 agree with that, that the -- it's not simply the  
12 dose, but the way in which it's given, which puts  
13 you at some risk. I should mention that, you  
14 know, before we got started with this study, our  
15 institution had adapted a whole number of safety  
16 features to try to mitigate the risk of IVIG  
17 mediated hemolysis, so controlling the rate,  
18 adjusting the dose for the ideal body weight were  
19 two things. We also did not allow anyone to get  
20 more than 1 g/kg per day, or at least if they  
21 really wanted that, they had to really make their  
22 case and try to appeal to the blood bank medical

1 director.

2 And we actually thought, given those  
3 three things, we might not see any hemolysis at  
4 all, so we were actually perversely happy to see  
5 some, although it was not -- none of those cases  
6 were all that severe.

7 So, I think probably giving it all at  
8 once probably does increase your risk, but  
9 splitting it into two days, at least, did not  
10 appear to be completely protective either. So, it  
11 may be that you need several things lined up to --

12 MR. SCHLEIS: Right, and I think we're  
13 just -- people are just too ready to just cram the  
14 stuff in.

15 DR. PENDERGRAST: Yeah.

16 MR. SCHLEIS: And there's a real  
17 emphasis on giving it rapidly and giving it over a  
18 short period of time where I think that's a  
19 problem.

20 DR. PENDERGRAST: Right.

21 DR. BUSSEL: Could you follow up on that  
22 or anybody else in the panel about this 10 percent

1       IVIG give more hemolysis than 5 percent even if  
2       you're giving the same amount of IgG?

3               DR. PENDERGRAST: I couldn't comment.  
4       We only use percent at my institution, so I  
5       wouldn't know about the risk from the 5.

6               DR. BUSSEL: Well, if you look at the  
7       articles and information that's been published,  
8       and there's not a lot, but the 10 percent products  
9       or some of the 10 percent products have higher  
10      titers and so they've been more implicated than  
11      the 5 percent, but I don't know if it's because  
12      they're 10 percent versus 5 percent. I think it's  
13      the anti-A titers that seem to be the trigger.

14              DR. BRANCH: Don Branch, Toronto. I  
15      sort of want to direct my question to both Dr.  
16      Padmore and Dr. Flegel. In evidence-based  
17      medicine now, since group Os rarely, if ever,  
18      really show hemolysis after IVIG, why are you  
19      thinking that anti-HLA or antibodies to low  
20      frequency might in fact be involved? Wouldn't you  
21      expect more group Os to have problems if that was  
22      the case?

1 DR. PADMORE: I think it's the -- I'd  
2 like to change the two-hit hypothesis to a  
3 threshold hypothesis. It's evolving. And I think  
4 -- I wonder if -- I think ABO is certainly  
5 implicated in a lot of them and you can elute A  
6 and B -- anti-A and anti-B off the red cells in a  
7 positive DAT. But I wonder in, you know, most of  
8 the patients don't get it -- overt hemolysis -- I  
9 wonder if there is possibly an antibody to a low  
10 incident antigen like you referred to or does HLA  
11 antibody, antigen sensitization or titer in the  
12 product, et cetera, is that an additional push  
13 through the threshold into overt hemolysis.

14 So, I think it might be if you look for  
15 it, you might -- but it's so hard to tease this  
16 out, you know, what's a factor, what's an  
17 incidental finding. But that was my aim in  
18 touching on it that I thought maybe -- we haven't  
19 certainly excluded it playing a role, I think.

20 DR. BERG: The data are compatible with  
21 the conclusion that it's anti-A and B primarily,  
22 but there may be other factors. So, from the

1 practical perspective, obviously, you want to  
2 focus on anti-A and B first but we don't  
3 necessarily want to lose the other aspects out of  
4 sight.

5 DR. BRANCH: Just to follow up a bit. I  
6 was just wondering if somebody on the panel or  
7 somebody here could comment on if you have a  
8 single donor who has a super high titer antibody  
9 to low frequency antigen and then you're pooling  
10 60,000 donors or 20,000 donors, what's the  
11 dilution factor? And do you really think that  
12 that antibody would be high enough titer to do  
13 anything at all?

14 DR. BERG: It could. This is not -- you  
15 cannot -- it is very unlikely, but it is possible.  
16 These titers can be very high and they might  
17 actually -- you know, they might be  
18 multifunctional aspects that you have a acceptable  
19 anti-A titer, but with the other antibodies,  
20 suddenly you become something that is a toxic mix  
21 for the individual recipient, in certain cases.  
22 Something like that.



1 DR. KLEIN: Yeah, but to go back to Dr.  
2 Branch's evidence earlier that the O groups just  
3 don't seem to have a frequency of hemolytic  
4 events, that would argue against that.

5 MR. GOLDING: Can I ask about the  
6 inflammation part? You know, the idea is coming  
7 across that inflammation is a risk factor, but the  
8 patients that are having a high level of hemolysis  
9 are dropping four grams, five grams, whatever.  
10 I'm assuming, I guess most people would assume,  
11 that that's intravascular and I don't see how  
12 macrophages and NK cells or any of the other  
13 things could even play a role there.

14 So, if it's intravascular, I think the  
15 straightforward explanation is that it's the  
16 antibodies and complement and so on. What might  
17 be happening in the spleen or extravascular, you  
18 might get macrophage activation, but even in those  
19 cases, it seems to be somewhat the chicken and egg  
20 argument. You have conditions, which have  
21 underlying inflammation, you're giving them high  
22 doses of IVIG, and those are the ones that are

1       having higher rate of hemolysis, and the argument  
2       that's being made, well, the underlying  
3       inflammation plays a role.

4                   Well, it may or may not, but it maybe  
5       better data -- and I'm just asking if you have  
6       done it in this way -- you're looking at different  
7       markers and you show high levels of IL-1 receptor  
8       antagonist and high levels of IL-1-beta, but have  
9       you done some kind of regression analysis to show  
10      what the correlation is? Is it the people with  
11      the -- if you plot out the levels of IL-1 receptor  
12      alpha and the incidents of hemolysis, do you find  
13      a good correlation?

14                  MR. PIERCE: Yes, just following up on  
15      that, I didn't see a statistical presentation that  
16      -- or statistical evidence that inflammation is  
17      necessarily causal here. I didn't see an  
18      incidence in individuals given the same dose of  
19      IVIG who have these preexisting inflammatory  
20      markers at some predefined level compared to the  
21      incidence of hemolysis and the individuals getting  
22      the same dose of IVIG who did not have a

1 pre-defined levels of this large number of  
2 inflammatory markers, and from the last talk I  
3 took away that we don't have any ready commonly  
4 clinically available marker of inflammation that  
5 at this point we're ready to say, this individual  
6 is in fact at increased risk of hemolysis.

7 DR. PENDERGRAST: So, I think there were  
8 two questions in there, and I'll answer the second  
9 one because I remember it. But you're absolutely  
10 right. The numbers we have are far too small to  
11 do any kind of statistical analysis or regression  
12 analysis to prove this. This is purely  
13 descriptive at this point.

14 The study is ongoing. We are hoping to  
15 get 150 patients enrolled. We're up to around 50  
16 as of now, but at some point, you know, we'll have  
17 to start doing some power calculations to figure  
18 out how many more we would need to prove the  
19 association. But I think, you know, just given  
20 that, it is kind of interesting that when you  
21 control for everything else, you control for the  
22 dose, you control for the blood group, you control

1 for the underlying diagnosis, you control for the  
2 infusion rate, everything else, still some people  
3 hemolyze and some don't.

4 And it might not be inflammation. It  
5 quite possibly could be FC receptor polymorphisms,  
6 it could be antigen density, it could be the lot  
7 of IVIG. We don't know. But it is sort of  
8 striking that simply getting, you know, 2 g/kg  
9 over two days as a group A patient and having  
10 CIDP, that doesn't appear to be enough to guaranty  
11 that you're going to hemolyze. There does appear  
12 to be something else.

13 And I can't remember what the first  
14 question was. I'm sorry. Oh, yes, complements.  
15 Yes, and so I think the -- that paper, that sort  
16 of Sentinel case report that we wrote up, we were  
17 very careful not to use the term "intravascular  
18 hemolysis" lightly and I think if Dr. Garratty  
19 were here, he would be very quick to reprimand  
20 anyone who assumes that the presence of free  
21 hemoglobin in the plasma, even a mild degree of  
22 complement depletion, means intravascular

1     hemolysis if you define intravascular hemolysis as  
2     the completion of the membrane attack complex as  
3     the completion of the membrane attack complex.  
4     You know, and it's quite possible that if you have  
5     a really brisk and aggressive extravascular  
6     hemolysis, that you can simply overwhelm the  
7     reticuloendothelial system and some hemoglobin  
8     will leak.

9             I don't know exactly how you tell the  
10    difference, to be honest. It is notable that none  
11    of the cases in our series, and I believe only one  
12    case in Dr. Padmore's series, have complement  
13    detectable by their -- on their DAT. And it does  
14    raise the question of how important complement  
15    activation actually is. Certainly, some degree of  
16    complement activation can be inflammatory in  
17    itself, the split products are inflammatory, but  
18    whether you need that to hemolyze, again, the MMA  
19    environment is relatively complement poor. I will  
20    defer to Don on this because he's the expert on  
21    that assay, and yet we did see a dose response of  
22    IVIg particularly with the anti-A targeting.

1                   So, I'm not sure how much of this is  
2    intravascular, to be totally honest.

3                   DR. BRANCH: I would just like to add  
4    that -- and Dr. Flegel can comment on this as well  
5    -- but anti-D induced hemolytic episodes can be  
6    very severe and anti-D does not bind complement.  
7    So, I think that the idea of whether this is  
8    intravascular or extravascular is still a  
9    question.

10                  DR. ZIMRING: Jim Zimring, (inaudible)  
11    Blood Center. Can I ask a basic epidemiological  
12    question, back to the earlier talks? And so, in a  
13    situation such as this where it would seem to me  
14    that it's not been done or it can't be done, to  
15    take all the patients to whom you would like to  
16    give IVIG, withhold it from half of them, and then  
17    look at the rate in which hemolysis is a part of  
18    the normal history of their disease, how much of  
19    an observation bias are we worried about and is it  
20    -- how conclusive is it that the baseline  
21    phenomenology actually occurs that IVIG leads to  
22    higher hemolysis?

1 DR. PENDERGRAST: Well, I can tell you  
2 -- I mean, what we're trying to do in our study is  
3 enroll everybody who gets IVIG and monitor them  
4 for hemolysis regardless of what their blood group  
5 or what their dose is as a way of trying to  
6 control for that and I think, you know, many -- it  
7 is true that many of the diseases associated with  
8 this are not diseases that you associate with  
9 hemolysis, like Guillain- Barre Syndrome and CIDP  
10 and Myasthenia Gravis are not typically  
11 complicated by hemolysis.

12 But I think, you know, I guess the FDA  
13 data would suggest that there is a baseline level  
14 of hemolysis epidemiologically that you need to  
15 correct for. So, I can only comment on our study  
16 that we weren't seeing any.

17 DR. WINIECKI: Yeah, I would agree that  
18 there's no appropriate "control group" here. You  
19 can't withhold treatment. But again, you don't  
20 typically see those patients have hemolysis and  
21 also you're sort of -- at that point you'd have to  
22 speculate as to why only non-O blood group

1 patients with a certain disease have that outcome  
2 and it would be hard for me to come up with a  
3 biologically plausible explanation of why do  
4 groups of patients with various indications -- why  
5 do only the non-O group patients then hemolyze?  
6 That would just seem to be a mechanism that I  
7 can't explain. So, I think that sort of ties it  
8 in.

9 But you're right, there's no way to know  
10 for sure to study it as you would in say a  
11 clinical trial.

12 DR. ZIMRING: So, I'm not trying to be  
13 obnoxious, but if you look at the epidemiology of  
14 O, A, ABO blood groups with a panoply of diseases,  
15 you see that different ABO types correlate with  
16 different outcomes in atherosclerosis, they  
17 correlate with different outcomes in malaria, they  
18 correlate with different outcomes in a number of  
19 infectious diseases, and so just to be a skeptic  
20 here, the question comes back to the core base  
21 phenomenology that this whole conference is about.  
22 You have a patient population who has illness and



1     they have hemolysis and you may even be  
2     scrutinizing, when you're looking at patient  
3     populations, you're looking at laboratory values  
4     more than you would in a basic patient population.

5             And so, I'm not disbelieving the  
6     phenomena, but I'm asking how confident are we in  
7     the actual correlation between IVIG administration  
8     and hemolysis as a causal determinant?

9             DR. BUSSEL: Can I mention some data  
10    that may or may not bear on that? Depending on  
11    whether you think of pregnancy as a disease, we  
12    give a lot of IVIG in pregnancy to women to  
13    prevent severe fetal thrombocytopenia and in utero  
14    and perinatal intracranial hemorrhage. And when  
15    you do that, we're giving either 2 g/kg per week  
16    for multiple weeks or a g/kg per week in steroids.

17            When we give 2 g/kg per week to the  
18    non-blood group A patients, five of the 16 got  
19    anemic defined by hemolysis, defined as a  
20    hemoglobin less than 10 in contrast of the blood  
21    group A patients, 13 of 18 became anemic, which  
22    isn't surprising.

1           The other point I would make out of that  
2   data that's on a different area but goes to the  
3   inflammation issue, is the use of steroids. One  
4   of the groups when we only give 1 g/kg per week,  
5   which is still a lot of IVIG, we give prednisone  
6   with it, and in those groups, even in blood group  
7   A, only about a quarter or a third of those women  
8   become anemic during pregnancy.

9           So, just relative to some patient  
10   factors from the clinician perspective, I think  
11   steroids are potentially a very useful thing. We  
12   had a patient where because of concerns with  
13   WinRho, we give high dose steroids before all  
14   WinRhos now and we accidentally gave the patient  
15   -- it was a small infant -- ten times the dose,  
16   and the patient survived without any damage,  
17   though she became very anemic, got transfused, et  
18   cetera, so I'm not sure that isn't a useful  
19   approach also depending.

20           DR. ZIMRING: I mean, no data are  
21   perfect, but when you look through the literature  
22   and you cull together the various controls that

1       have been done where they give IVIG to healthy  
2       people, all due respect to a two- or three- hit  
3       hypothesis, I'm not aware of those healthy  
4       recipients becoming hemolytic even if they're  
5       blood group A.

6               DR. PENDERGRAST: I guess the question  
7       is, why would you give IVIG to a healthy person?

8               DR. ZIMRING: This was done in control  
9       -- in fact, one of the papers the earlier speaker  
10      referred to on the sequestration of immune complex  
11      is that a healthy group, as control patients,  
12      because they couldn't recruit sick people, and  
13      they gave them IVIG and regardless of their blood  
14      type no hemolysis was observed whatsoever.

15              So, again, all due respect to a second  
16      hit maybe being required, it does beg the question  
17      of -- you know, it's a complicated dataset that  
18      we're looking at.

19              DR. BUSSEL: I think it goes to Dr.  
20      Padmore's threshold hypothesis: when you give  
21      enough, you still get some hemolysis.

22              SPEAKER: I wonder if (inaudible) that

1       are circulating. (Inaudible) being one of them,  
2       mitochondrial DNA being another, that might be  
3       elevated in some patients who had (inaudible)  
4       something along those lines, which might be that  
5       second hit that you're looking for. Thank you.

6               DR. PENDERGRAST: We're not including  
7       that one in our panel, but it's an interesting  
8       idea.

9               DR. SMITH: Okay, so since it's almost  
10       lunch time, I'd like to run through these  
11       questions and get the panel's and audience's  
12       opinion. I think we've already answered question  
13       one, the most important patient risk factors, at  
14       least that we've been able to identify, and those  
15       would be AB blood type, dose of immunoglobulin,  
16       inflammation is really uncertain but at least we  
17       have some research that is working to address  
18       that.

19               Does anybody else have a list or  
20       something that you think should go on most  
21       important, at least that we have enough evidence  
22       for?

1                   We did hear a lot about additional risk  
2       factors that could be explored, including the --  
3       looking at genotypes and phenotypes, for example,  
4       FC-receptors, and a number of other possibilities.

5                   What we're asking here is, what  
6       additional risk factors do you think might bear  
7       fruit if explored? And perhaps I'll turn this to  
8       the panel first.

9                   DR. BUSSEL: I don't think there is much  
10      data, but I would think, as somebody said in the  
11      earlier discussions, ongoing hemolysis,  
12      preexisting, which I guess gets, to some extent,  
13      to Dr. Zimring's point, would be a risk factor,  
14      and the use of WinRho, at least a group of is in a  
15      letter in Blood recommended not treating patients  
16      with Rhaetic counts more than 3 percent.

17                  DR. SMITH: Anymore takers? We didn't  
18      perhaps get into all the information we might like  
19      to have about whether there is additional patient  
20      or patient plus product testing that could be  
21      useful to predict the likelihood of clinically  
22      significant hemolysis, but I want to ask the

1     hematologists and blood bankers here,  
2     particularly, if you had a patient who, let's say,  
3     you were talked into giving 2.5 g/kg to and they  
4     had an inflammatory disease and it was really  
5     rather urgent, there wasn't any other treatment  
6     that had worked, what would you do in terms of  
7     testing that patient in advance? And what would  
8     you do -- I'll ask everybody -- in terms of  
9     attempting to prophylax against hemolysis  
10    presuming that this is a very high-risk patient,  
11    let's say they're blood type AB?

12                 Okay, so one, pretesting -- predictive  
13    pretesting, would that be at all useful in any  
14    way? And if so, what test?

15                 So, for example, something similar to a  
16    hemolysin assay with a product.

17                 DR. PENDERGRAST: I think that's the big  
18    challenge, is how do you define a safe titer when  
19    we can't even define what the safe tittering  
20    protocol is and we've tried that once. We had a  
21    case of rip-roaring hemolysis and we tried  
22    tittering it using our usual tittering technique

1       that we use for pregnant patients and got a titer  
2       of like 1/2000 or something like this.

3               And of course we had no idea how to  
4       interpret that and the industry representatives  
5       came and said, please don't publish that because  
6       that's not what we're putting in our -- in terms  
7       of the European pharmacopeia-defined titting.

8               That's kind of a moving target right  
9       there, so I actually -- and I wouldn't even know  
10      about how to define a minor side cross match  
11      result or anything like that. To me, I mean, I'm  
12      speaking mostly as a clinician here, the key thing  
13      is that that dose of 2 g/kg, I think everyone  
14      realizes is completely arbitrary. It's not based  
15      on anything, and if you look at guidelines,  
16      particularly for the chronic IVIG users, the  
17      guidelines do say that you should titrate for  
18      clinical response, and if I had somebody who had  
19      massively hemolyzed and they got 2.5 g/kg, I might  
20      ask, why did they need to get that much? And I  
21      might suggest that the best risk mitigation  
22      strategy would be to give them less next time.

1                   But that's the only thing I could  
2 suggest.

3                   DR. BRANCH: One thing that maybe you  
4 could consider, although it would add a lot of  
5 cost, would be on product testing to include  
6 hemolysin testing, actually test for hemolysins,  
7 don't test for agglutinins, test for hemolysins,  
8 and also look for other allo antibodies and let us  
9 know what you find, because, you know, none of us  
10 are doing that and it'd be nice if the companies  
11 could actually do that kind of screening for us.

12                  DR. BERGER: I have no data -- in the  
13 absence of data, I would suggest that it's  
14 clinically prudent in the situation you've  
15 described, to divide the IgG into no more than 1  
16 g/kg per day and to look very carefully at the  
17 patient the second day before giving them the  
18 second increment.

19                  If the patient has a positive Coombs  
20 test, or has a drop in hemoglobin for -- first of  
21 all, it's important to get a CBC before the first  
22 dose. Second of all, I would look at the patient



1       carefully before proceeding with the next gram and  
2       I would consider giving steroids prophylactically.

3               In one of the published cases, after  
4       receiving 1 g/kg the first day, the patient came  
5       in and said they were short of breath, dizzy, and  
6       their urine appeared like Coca- Cola, and the IgG  
7       was started at the same rate as the first day.

8               DR. GOLDING:   Would it be helpful to --  
9       I'm asking the blood bankers, why not do a cross  
10      match with a product that's going to be used in  
11      the patient's red cells?   I mean, why wouldn't  
12      that be a reasonable approach if you have the --

13              DR. BRANCH:   Because they'll be  
14      incompatible.   You have to give us the conditions  
15      -- how to dilute the IVIG, et cetera, because if  
16      you just take neat IVIG, it will agglutinate  
17      everything in the test tube.

18              DR. GOLDING:   Well, I guess you've got  
19      to do a titration and figure -- you know, what I  
20      also saw evidence for in the earlier talks that  
21      some brands in a particular patient may be more  
22      likely to cause hemolysis than other brands.   So,

1       could one do some kind of differential testing  
2       comparing different IVIG products and do a  
3       dilution with the patient's red cell?

4               DR. SMITH: Well, it sounds like a good  
5       lunchtime question for discussion because --  
6       especially in terms of feasibility, and obviously  
7       some kind of a protocol that made sense and was  
8       actually predictive would have to be used for such  
9       testing.

10              I think that whether it's possible and  
11       helpful to have agreement on a definition of  
12       hemolysis for case identification purposes, it  
13       should at least be asked of this group, and really  
14       defining case identification purposes for  
15       immunoglobulin mediated hemolysis.

16              We can't ask right now what that case  
17       definition should be, but we do have several  
18       different ones and I wonder if the epidemiologists  
19       here can comment on how to make that approach and  
20       if it's worthy of making.

21              It certainly would make it easier for us  
22       to compare studies, for example, and to compare

1 data. You can say yes or no or yes, but --

2 DR. WINIECKI: I think having one  
3 uniform definition is useful and that way you're  
4 comparing the same thing so you know that the  
5 Canadian cases and the cases from PEI and the  
6 cases from FDA and PPTA are all looking at the  
7 same thing. I'm not sure that the specifics of  
8 that definition are as important as having a  
9 definition because if you look at all the  
10 definitions, they may differ somewhat in the  
11 details of how many laboratory values do you need  
12 as evidence for hemolysis, but everybody agrees  
13 there should be some drop in hemoglobin, everybody  
14 seems to want to have a positive DAT.

15 So, I think the specifics of the  
16 definition may not be quite as important as having  
17 general agreement so that we're all talking about  
18 the same kinds of patients.

19 DR. SMITH: And Dr. Funk agrees and our  
20 Canadian colleagues agree, then I think that's  
21 something that could be done, and also our  
22 regulatory colleagues, obviously, would have a lot

1 of input.

2 This is just an interesting question and  
3 I want to find out if people have particular  
4 comments, whether there's evidence for a link  
5 between hemolytic events and new indications for  
6 IG products, and I think as noted, a lot of the  
7 new indications do confer a higher dose and we  
8 also have the inflammation complication, whether  
9 or not that's involved.

10 So, is there anything that people would  
11 like to add to that?

12 DR. ZIMRING: Just to play devil's  
13 advocate for a minute, because of the mechanisms  
14 of IVIG have yet to be determined, and I know at  
15 least 12 competing hypotheses and there's good  
16 evidence to reject all of them, if the mechanisms  
17 of IVIG have anything to do with mimicking anti-  
18 D-like effects, is it inconceivable that a low  
19 level of hemolysis is part of the efficacy and by  
20 getting rid of the hemolytic component all  
21 together, you may have a very safe product that  
22 does nothing?

1 DR. BUSSEL: It might certainly do  
2 nothing in ITP.

3 SPEAKER: But just to note that IVIG is  
4 effective in type O blood group and ITP.

5 DR. SMITH: And I think we've already  
6 talked about possible risk mitigating measures, at  
7 least some in the first question, but I want to  
8 ask if folks have anything to add on currently  
9 available risk mitigating measures. We've talked  
10 about dose frequency, dose lowering, the  
11 possibility that steroids might be useful,  
12 weight-based dosing or lean body weight based  
13 dosing. Am I missing anything?

14 DR. FUNK: Maybe infusion rates.

15 DR. SMITH: And possibly infusion rates.  
16 I don't want to keep anybody away from lunch. And  
17 I think we will reconvene at -- I don't have my  
18 most recent agenda. We'll reconvene when it says  
19 in the agenda. Looks like 1:15. So, we're good.

20 Thank you very much.

21 (Applause)

22 (Recess)

1 DR. BRANCH: Well, I don't know if I was  
2 introduced, but anyway, I'll introduce myself,  
3 because I think it's time to start. I was busy  
4 talking to someone else -- about IV IG, by the  
5 way.

6 Anyway, I'm tasked with trying to  
7 present you with something that's fairly simply,  
8 what anti-A and anti-B do, what they are. That's  
9 something that was very difficult for me, but very  
10 interesting, which is the origins of anti-A and  
11 anti-B.

12 So, I'm surprised that it took until the  
13 afternoon for anybody to get up here and mention  
14 certain individuals that were responsible for this  
15 whole problem -- as Laurel would say to Hardy, or  
16 maybe it's vice-versa: Another fine mess you've  
17 gotten me into, Landsteiner.

18 But disclosures, first of all, from  
19 Grifols, and Canadian Pharmacovigilence Committee,  
20 and from CSL Behring, get some funding and some  
21 in-kind support from those people.

22 What I'm going to do today is mention

1 the founder of ABO, Dr. Karl Landsteiner, just  
2 spend a couple minutes on him, talk about the  
3 antigens in the ABO system, the antibody classes,  
4 the ABO antibodies; talk a bit about ABO  
5 hemolysins, which I think are absolutely critical  
6 for the subject that we're here for, and then the  
7 origins of the ABO antibodies.

8 And I'll point out that on the  
9 references, this is a really important reference  
10 when you're trying to find out, really, about the  
11 ABO blood group system, which is, Mollison, at  
12 least in this addition, it's still pretty much  
13 true to his earlier editions. But it gives you a  
14 lot of historical background, and lots of very  
15 specific information about anti-A and anti-B and  
16 other antibodies in the ABO system. So I highly  
17 recommend that for everyone to take a look at --  
18 not the newer versions so much, but the older  
19 ones.

20 All right, so Karl Landsteiner, pretty  
21 gruff looking individual, I was lucky enough to  
22 get to talk to Dr. Philip Levine, who worked with

1 Dr. Landsteiner, and he says, "What you see is  
2 what you get." Okay? So not to say any more on  
3 that.

4 But, as we all know, Karl Landsteiner is  
5 the founder of the ABO blood group system, which  
6 he won the Nobel Prize for in 1930, for his  
7 discovery of human blood groups. Those of us that  
8 know a little bit more about Landsteiner know that  
9 he was actually a very good immunologist. He was  
10 a virologist. Part of the discovery of polio  
11 virus was Landsteiner. He sorted out the hapten  
12 immunology, which he probably felt he should have  
13 gotten the Nobel for that. He never wanted the  
14 Nobel for blood groups.

15 He didn't think they were that  
16 important, compared to his other contributions.

17 So, I apologize for the blurriness, but  
18 this is actually a figure from the original paper  
19 in 1901, which Karl Landsteiner got the Nobel  
20 Prize for. And you can see that what he did was  
21 he just took everybody in his lab -- "You VILL  
22 help me. Put out your arm." And he just took



1     their blood, and mixed the blood together. He  
2     actually separated the serum and the red cells.  
3     And in this figure you can see three patterns of  
4     -- so here's one pattern, here's another, and  
5     here's the last pattern.

6             So, he originally called this A, this B,  
7     and this C. So it was the "ABC" system, which  
8     Michael Jackson actually took credit for, but -- I  
9     know, it's a horrible life. But this is probably  
10    more of what he was really looking at when he did  
11    the test.

12            So, here you have A, and you can see  
13    that when different blood group sera are mixed  
14    with a certain, in this case, Group A individual,  
15    that you get the expected agglutination. Same  
16    with B, and the same with AB, and then, of course,  
17    O.

18            Now, AB wasn't discovered by  
19    Landsteiner, that was discovered by one of his  
20    students, written in a paper, actually, the year  
21    after his ABC paper. But it was easy for him, he  
22    could call it "AB."

1                   So, before we can really talk about  
2   antigens, we'll talk a little bit, I'll give you a  
3   little background on the genes of the H and the  
4   ABO. It's really the ABH system. But there's two  
5   chromosomes that control ABO blood type. One is  
6   FUT1, or fucosyltransferase. It's on chromosome  
7   9, and it codes for a fucosyltransferase, which  
8   puts a fucose onto a lattice carbohydrate  
9   precursor. And we'll talk a little bit more about  
10   that. And then the A and the B transferases are  
11   located on chromosome 9. So we have the  
12   N-acetylgalactosaminetransferase, which is for the  
13   A antigen, and the N-acetyl D  
14   galactosaminetransferase, which is also an A  
15   antigen. Now, here it's called "A2," But,  
16   really, these are both A1. It's just that this  
17   transferase puts two A1-recognition units on the  
18   backbone, and the A2 is basically the same enzyme,  
19   with a mutation, so it's not as efficient. And it  
20   actually only puts one on. So they become A2  
21   cells -- and we'll talk a little bit about that.  
22   And then there's the galactosyltransferase, which

1 is the B.

2 And, actually, a lot of people don't  
3 realize, but the Group O, or transferase, there is  
4 one, it just doesn't code for any functional  
5 product. But you can detect the product.

6 So, let's talk first about the H blood  
7 group a bit. So, you have this backbone here of  
8 carbohydrates, and it was mentioned today, these  
9 are carbohydrate antigens, they're not protein  
10 antigens. They're made through protein enzymes,  
11 which are transferases. So, you have a common  
12 backbone. And for the H antigen, what you need is  
13 a precursor for the A and B, you have this FUT1  
14 transferase, which puts a fucose onto this  
15 structure here, this glucosamine,  
16 N-acetylglucosamine. And then you have H antigen.

17 You have individuals that either lack  
18 the transferase for fucose, or have a mutated  
19 version, so they either don't put on the fucose,  
20 or they put it on very inefficiently, or at very  
21 low frequency, so you get this little "h." The  
22 little-h, without any fucose, would be called

1       Bombay," and the one where there's a little bit of  
2       the fucosyltransferase activity, those are called  
3       "para- Bombay." And, if you want, you can join  
4       the club in India for the Bombay blood group donor  
5       meeting.

6                So, here's a little bit more of the  
7       chemistry. But for the A-antigen, for instance --  
8       well, let's start with the O. So you have the  
9       fucose that's been added by the fucosyltransferase  
10      and now, in order to get A, you also need the  
11      N-acetylglucosamine -- yes, glucosamine  
12      transferase, which then adds that onto  
13      galactosamine, onto the terminal structure here,  
14      so that you have the fucose, and you also have the  
15      determinant for the A blood group.

16              And, likewise, for the B, you get the  
17      galactosamine, the galactose added by the  
18      galactose transferase, and so you get B-antigen.  
19      I think there's one amino acid difference, or two  
20      amino acid differences between these two  
21      transferases, but they actually encode for very  
22      specific carbohydrate additions to the acceptor

1 molecules.

2 Now, Bombay, of course, you don't have  
3 the fucose, and you have the Bombay phenotype.

4 Just to sort of trump myself here, but  
5 the antigen site density here is quite high. It's  
6 at least a million A- sites per cell, and on B,  
7 it's about 7-1/2 times 10<sup>5</sup>. So it's not much  
8 different than A, but slightly less for B, for the  
9 antigen-site density.

10 And here's a more complete list. So,  
11 for A1, which have two A1 groups that are added by  
12 the A1 transferase, you have 10<sup>6</sup> antigen sites.  
13 For A2 -- which is still an A1, it just doesn't  
14 work with the typing reagent called "anti-A1," but  
15 it's still an A1, it's just low site density, 3  
16 times 10<sup>5</sup>. For B, it's 7-1/2, as you saw, for  
17 10<sup>5</sup>. For A1B, it's 8-1/2, almost a million. For  
18 A2B, it's only 10<sup>5</sup>. And for H, it's about a  
19 million, as well. And this all comes from  
20 Mollison's book, all done by radio- labeling. So  
21 it's a radioactive analysis of antigen-site  
22 density. Try to do that today.

1                   That brings us to Landsteiner's Rule.

2       It's something that transfusion-medicine people  
3       certainly know about, and I'm sure you all do, as  
4       well. If Landsteiner would have been Newton, this  
5       would have been one of Newton's Laws, but in  
6       Landsteiner, it's a "rule." And basically, it  
7       just says that if you have the A-antigen, then you  
8       will always have anti-B in your serum. If you  
9       have the B- antigen, you'll always have anti-A in  
10      your serum. If you have both antigens, you have  
11      no isoagglutinins. And if you have neither A or  
12      B, you're Group O, you have both anti-A and  
13      anti-B. So it's very simple.

14                But it's not as simple as one might  
15      think, because the antibodies that you have --  
16      this is just a schematic of different antibodies  
17      classes that one could have. Basically, the only  
18      antibody here that doesn't code for an ABO antigen  
19      would be IGE -- although maybe someone will  
20      discover that. But all of these other ones do.  
21      So you have IgG, you have IgM, as you all know.  
22      But you have IgA, which hasn't been talked about

1     today, that is also ABO hemagglutinin. And you  
2     have IgD, but -- actually, this one will show it a  
3     little bit better, for the IgM versus the IgG. So  
4     we all know IgM is a pentamer, and we all know  
5     that there's four classes of IgG -- 1, 2, 3, and  
6     4. What you might not have been aware of, some of  
7     you, I'm sure, is that there are C1Q binding sites  
8     on the antibody in the heavy- chain conserved  
9     region, and in order to activate complement, you  
10    have to have C1Q binding to two sites. So, for an  
11    IgG, for instance, to activate complement, you  
12    have to have an antigen site density that's such  
13    that two IgG molecules can get close enough  
14    together in angstrom units that C1Q can bind  
15    between them. If that doesn't happen, you get no  
16    complement activation.

17           Of course, with IgM, your antibodies are  
18    all arranged in such a way, and are flexible  
19    enough, that it's very easy for C1Q to bind  
20    between these two IgM components.

21           So, that's why some antibodies doesn't  
22    activate complement, like anti-D only has 10,000,

1        maybe 20,000 sites per cell. That's just not  
2        enough for the statistical coding of the antibody  
3        to be close enough, with enough of the antibodies  
4        to activate complement. But IgM, it's not an  
5        issue.

6                And, of course, with an antigen-site  
7        density of a million-per-cell, IgG anti-A is going  
8        to do just quite fine, in terms of activating  
9        complement.

10               So, now, I'm going to talk about, I  
11        think, it's five antibodies in the ABO system, all  
12        of which could be an IV IG prep -- not just anti-A  
13        and B, guys. Anti-A, of course, we all know, is  
14        produced by group O, B and the Bombay phenotype,  
15        which makes all the antibodies. It's actually a  
16        mix of anti-A and anti-A1. So you can take, if  
17        you can type and find an A1 cell, you can absorb  
18        anti-A from Group B people, and you're left with  
19        an antibody that still works with Group A, but  
20        it's not anti-A1. It won't work with A1 cells.

21               It can be IgM, we know, IgG. But it can  
22        also be IgA, and is usually a fairly high IgA



1 component.

2           They're agglutinating antibodies that  
3 work best at room temperature. Both IgM and IgG  
4 are agglutinating because of the antigen-site  
5 density, in particular for IgG.

6           But IgM, being such a bigger molecule  
7 and able to be more flexible, it actually  
8 agglutinates to a higher degree than does IgG.

9 But IgG works well in the indirect antiglobulin  
10 test, with anti human globulin sera. And you can  
11 get very high titers, in the thousands, if you do  
12 indirect antiglobulin tests for IgG anti-A.

13           As I said, it reacts with A1 -- anti-A  
14 reacts with A1 in almost all subgroups, with a  
15 notable exception of a subgroup called "Ax."  
16 Anti-A from Group B people does not react with Ax  
17 red cells.

18           The titers are variable, but they're  
19 usually less than 256, if you're doing IgM titers.  
20 And, of course, they can activate complement. And  
21 they are clinically significant, and can result in  
22 intravascular hemolysis.

1                   So, this is just an example of 100  
2   donors that were tested by the Canadian Blood  
3   Service, showing titers of anti-A in normal  
4   donors. And you can see that the mean titer is  
5   134. But, basically, what they were trying to do  
6   was trying to figure out a threshold where they  
7   could type for anti-A in donors to exclude  
8   high-titer donors, but not exclude their donor  
9   base. So a lot of people are facing that issue  
10   right now. So they're probably going to go with a  
11   128 titer. Anything above that, they probably  
12   will exclude. But you can see that they're not  
13   really extremely high.

14                  So what's anti-A1? Well, anti-A1 is  
15   found in Group O. It's found in Group B.  
16   Everywhere you find anti- A, you find anti-A1.  
17   But in A2 individuals, in 1 percent of them, they  
18   have an anti-A1. And in A2b individuals, they  
19   have, about 25 percent of them have anti-A1.

20                  It's reactive only with the Group A1  
21   cells. It's produced by group -- as you see, A2  
22   and other subgroups of A. In fact, there's other

1       subgroups that make it, like Ax. It's useful,  
2       it's been found to be useful to type 1 cells with  
3       a lectin called dolichos biflorus, which is, at a  
4       certain dilution, is specific for the A1 antigen.  
5       So cells can be typed for A1, and that's what we  
6       do in the blood bank when we're trying to  
7       understand what caused the hemolysis -- is it an  
8       anti-A, or is it an anti-A1? -- we can distinguish  
9       between those two.

10               It's not produced by Group A1 cells, so  
11       it follows Landsteiner's Rule. It's always an IgM  
12       cold reactive. Rarely IgG. And it's usually not  
13       clinically significant, so probably nothing that  
14       we want to worry about too much in the IV IG  
15       world. But it's there.

16               Then we have anti-B -- again, produced  
17       by Group A, O, and Bombay. It reacts with Group  
18       B, most subtypes of Group B. Again, IgM, IgG  
19       agglutinating, working best at room temperature,  
20       IgM versus IgG. Working well at indirect  
21       antiglobulin tests, with anti human globulin.

22               Titers vary, again, but they're also

1 usually less than 256. Can activate complement.  
2 And, clinically significant, but anecdotally, I  
3 would say, it's much less so than anti-A. I've  
4 worked in transfusion medicine for many years as a  
5 transfusion service, and we saw lots of ABO major  
6 mismatches with A into O. But when we saw B into  
7 O, we basically didn't see too much problem. So,  
8 I would say that it's not as significant, although  
9 it can cause hemolysis and intravascular.

10 Again, titers from the Canadian Blood  
11 Service, 100 donors, looking at anti-B. And  
12 they're lower. So the mean is about 77, but we  
13 would probably pick a titer of 64, or maybe they  
14 would pick 128 for both A and B. I'm not sure  
15 what they're going to do. But you can just get an  
16 idea that normal donors have titers that are not  
17 really extremely high.

18 What is anti-H? It's probably not  
19 really worth talking about, other than if you  
20 really want to know what the ABO blood group  
21 system is, you can't avoid it. It's produced by  
22 Bombay and para-Bombay, as I said previously. It

1 reacts with all Group A, B, and O donors. It can  
2 activate complement. It's a highly significant  
3 antibody. You cannot transfuse these people with  
4 anything but Bombay blood.

5           The reactivity is one of the ways --  
6 they also use electin -- but one of the ways that  
7 they've characterized the amount of H-antigen on  
8 the various blood groups -- so, O, because it has  
9 the fucose, which is the H-antigen without any  
10 masking by any of the other transferases, reacts  
11 at a higher level than does A2, than does A2B,  
12 than does B, than A1, and than A1B. So, you get  
13 degradation, a grade of reactivity, depending on  
14 whether or not the H fucose part of the antigen is  
15 being masked.

16           It's clinically significant -- highly  
17 clinically significant -- but, luckily, very rare.  
18 I doubt you get too many Bombays donating to your  
19 plasma pool.

20           Now, here's an antibody I want to spend  
21 a little more time on, because this one never gets  
22 talked about, but I think it's a highly important

1       antibody in the ABO system.

2               It's called, in our jargon, "anti-A,B."

3       It's produced only by Group O people in Bombay.

4       It's almost always IgG.  It's little, if any, IgM

5       component.  It's cross-reactive with both Group A

6       and Group B.  It reacts with most Group A

7       subtypes.  And the reason, one reason, we know

8       it's not the same as anti-A or anti-B is it works

9       with Ax, and anti-A doesn't.

10              So it's a unique antibody.  It reacts

11       with most Group B subtypes.  It is an agglutinin,

12       but it's a powerful hemolysin, and it's clinically

13       significant.  And probably, according to the

14       literature, it's probably the responsible antibody

15       for most ABO-HDN, hemolytic disease of the fetus

16       and newborn, because it's always IgG and it

17       crosses the placenta.  So, somebody could study

18       that more and prove that it is the antibody that's

19       causing those problems when they do occur.  So

20       it's an important antibody, and perhaps we've got

21       to think of ways of looking for it.

22              So what about ABO hemolysins?  Well, ABO

1     antibodies are IgM and IgG, and they're all  
2     capable of activity complement and causing  
3     hemolysis.

4             Group O antibodies can have more  
5     hemolysin activity. When I was in a study a long  
6     while back, looking at enzyme-converted O cells --  
7     so taking A cells, converting them to O, in other  
8     words, taking all the A-antigen off so they no  
9     longer worked with Group B serum, no, any  
10    activity -- a certain percentage, about 15 to 20  
11    percent of Group O sera still worked with those  
12    cells. And they hemolyzed them. So that was the  
13    A,B. And so I think it's an antibody that you've  
14    got to start thinking about. It might be one of  
15    the issues in trying to identify which IV IG preps  
16    might, in fact, be hemolytic.

17            Most A and B show hemolytic activity, as  
18    we said, in the test tube. Now, this is  
19    hemolysin, again, but in order to see most, or 100  
20    percent, you had to either use enzyme-treated  
21    cells, old cells, or you let red cells age before  
22    you do the hemolysin test, almost all of the Group

1 O serum and Group A serum, and B serum, will show  
2 hemolytic activity.

3 But if you use fresh cells, it's not so  
4 much. So when you do hemolysin testing, depending  
5 on what study you read, the highest percent I  
6 could find was 60 percent if you're not using  
7 special cells, 30 percent was the lowest. And  
8 there's only a few papers published. So people  
9 haven't really looked very carefully at hemolysin  
10 activity in ABO, and especially normal  
11 individuals, and certainly not in IV IG.

12 So, it is correlated, the hemolytic  
13 activity, with the titer, 64 percent having titers  
14 of greater than 64. This was all by looking IAT,  
15 by looking at the IgG component of the activity.

16 So what's the in vivo clinical  
17 significance? Some of that was talked about  
18 today. We all know that you can have major  
19 mismatched blood. It usually results in some  
20 sequelae, but it doesn't always result in death,  
21 or even sever hemolysis, for that matter, but  
22 usually some morbidity. But there's many examples



1 of, really, not much happening when you give A  
2 into O, B into O, in particular.

3 We all know about minor mismatched  
4 plasma infusions, very little evidence that, you  
5 know, you can predict which platelet transfusions,  
6 or plasma infusions are going cause problems when  
7 they're ABO mismatched. IV IG infusions into  
8 Group A or B result in very few reported  
9 hemolytic, at least clinically, hemolytic  
10 episodes.

11 But then again, there are reports of  
12 even anti-A1 at very low titer causing severe  
13 hemolysis, and high-titer anti-A not resulting in  
14 hemolysis -- many reports, a lot of them  
15 anecdotal, but nonetheless, when it does occur, it  
16 can be severe and life threatening. The problem  
17 is, to this day no one can really predict with  
18 accuracy which products will hemolyze and which  
19 ones won't, with certainty. So we heard that  
20 today, and that's obviously true.

21 So, what I want to do in the last couple  
22 of minutes, because it's controversial, and I'll

1       just throw some things out at you, is how do we  
2       even have ABO antibodies?

3               Well, we've got the chicken guy, Georg  
4       Springer, back in 1950s, was the first one to say  
5       that, in fact, the ABO isoagglutinins are produced  
6       because of bacteria. And he did this work in a  
7       sterile environment with chickens. They only made  
8       anti-B. He really couldn't get them to make  
9       anti-A, but he got a really nice paper, Journal of  
10      Experimental Medicine, and that was the only work  
11      that was out there at the time.

12             Now, interestingly enough, Landsteiner  
13      himself thought that these antibodies were  
14      naturally produced. He thought they didn't  
15      require immunization. So he was against  
16      Landsteiner at the time. And if you get a chance  
17      to read the original paper, it's really  
18      interesting. Just don't go on PubMed, because  
19      when you check it out on PubMed, it has this in  
20      parenthesis: "Article in undetermined language."  
21      Well, I can tell you it's in English. I don't  
22      know why that's on the PubMed thing. That must

1     have been Landsteiner, the last thing he did  
2     before he met his maker, was he made sure it said  
3     -- "Make sure they tell that article is in  
4     'undetermined language' so nobody will access it."

5             Anyway, ABO antibodies do require  
6     environmental stimulus. This is all from  
7     Springer's work. He published about three papers  
8     in a row. He showed that if you feed bacteria to  
9     chickens, that they will make, in a sterile  
10    environment, they will make anti-B. They don't  
11    necessarily make anti-A. And then he showed that,  
12    in fact, bacteria, quite a few of them, had A or  
13    B-like activity. He could neutralize human  
14    antibodies with bacteria.

15            He concluded that exposure to bacteria,  
16    particular feeding bacteria, induces immune  
17    responses for the production, and that's how we  
18    get the antibodies -- in other words, a normal  
19    immune response to exogenous antigens.

20            This has not been confirmed by anyone.  
21    So the mystery of the origin still is not  
22    resolved. So Landsteiner's Rule, he believed ABO

1     antibodies were produced without stimulation --  
2     natural selection, or natural antibodies. And  
3     there is evidence for this.

4             Despite what you might have read or  
5     heard, there's plenty of evidence in the  
6     literature published that cord blood, either cord  
7     blood, or within a week of birth, that the babies  
8     have non-maternal anti-A or anti-B. And I did a  
9     project with Fenwal many years ago -- that didn't  
10    win, by the way, not enough data, probably -- but  
11    we were clearly showing that cord bloods had  
12    anti-A or anti-B of IgM class that were not from  
13    the maternal source.

14            These are antibodies to carbohydrate  
15    epitopes, which means they're T-cell independent.  
16    So that's not a typical classical immune response,  
17    perhaps.

18            So, one of things that's really  
19    outstanding to me is Landsteiner's Rule. Because  
20    in Landsteiner's world, there's no non-responders.  
21    Everybody who is Group B makes anti-A, and  
22    everybody who's Group A makes anti-B, and

1 everybody who's group O makes both. And even  
2 people that are Group A2 will make anti-A1,  
3 because they don't have the A1-antigen.

4 So, that's just kind of intriguing to  
5 me. So, as you read more and more about classical  
6 immune responses, or whatever you know from  
7 experience, you know there are non responders in a  
8 classical immune response. Anti-D, for instance,  
9 in delivered immunizations, is only produced in  
10 about 70 percent to 80 percent of the Rh-negative  
11 people. There's 20 percent non-responders. This  
12 is unheard of in the ABO world.

13 In mice, plenty of non-responders. In  
14 fact, you can purchase mice that won't respond.  
15 It's based on their Class II genes.

16 In humans, when antibody is produced, it  
17 often goes away quickly, so you need boosters to  
18 boost that antibody. It doesn't stay up at a  
19 level like ABO antibodies. And antibodies in the  
20 classical immune response are produced by what's  
21 called "B2 B-cells." These are T- cell-dependent  
22 B cells, and that requires adaptive immunity.

1                   So they require T-cell help, they  
2     require antigen- presenting cells. And these are  
3     CD-5 negative B2 cells.

4                   We also have a mucosal immune system.  
5     So, what happens there is if you're ingesting  
6     antigens, such as in Springer's experiments,  
7     giving bacteria to chickens, that you produce  
8     mostly IgA antibodies. And that's because of the  
9     so-called gut-associated lymphoid tissue. You get  
10    IgA1, which can -- you get Peyer's patches that  
11    transport to the surface of the mucosa, and they  
12    can then interact with certain cells and get IgA1  
13    produced in the circulation. You get IgA2, and  
14    secreted IgA in the secretions.

15                  But we know that there's IgA anti-A and  
16    anti-B, so how did those come about? Well, they  
17    probably came about from ingested antigens,  
18    probably bacterial. And in Springer's  
19    experiments, he never distinguishes the class of  
20    antibody, so he might have been dealing all along  
21    with IgA antibodies in his chickens. But also,  
22    you have to deal with oral tolerance, which is

1 another story.

2 Now, what's the difference between the  
3 classical antibodies and the natural antibodies?  
4 Well, all the natural antibodies are to  
5 carbohydrate antigens for the most part, such as  
6 ABH. But there's others. They have a restricted  
7 germ line genome, so they have a common variable  
8 gene. There's no somatic mutation or antibody  
9 maturation. And they're almost always IgM.

10 They're produced by a special kind of  
11 B-cell that's called B1. It's a CD5 positive  
12 B-cell. It does not require any T-cell help, nor  
13 does it require immunization. And it's part of  
14 the innate immune system by many people's  
15 category. So you need to read up on that.

16 So, why do we think anti-A and B might  
17 be actually just produced by natural phenomena,  
18 some kind of genome, Darwinian thing to protect  
19 us? That's because the majority of neonate  
20 B-cells are B1s. They're not CD5-negative,  
21 they're not ready for adaptive immunity, they're  
22 CD5s. The natural anti-A and B, like all of the

1 natural antibodies, react best at 4 degrees, or  
2 IgM. And, of course, there's numerous articles  
3 published that show that we have natural anti-A  
4 and anti-B at birth, or either in the cord, or  
5 less than a week old. And, in fact, even in one  
6 of Springer's papers, where he shows the pre-level  
7 before he gives the antigen, they have levels of  
8 anti-A or anti-B. So, it's kind of crazy that he  
9 would go on and say that it's immune-mediated.

10 Okay, so in my opinion, what we have for  
11 the origin of these antibodies is both innate and  
12 adaptive, both. We have natural antibodies,  
13 spontaneously produced, part of the innate system.  
14 And then we have the humoral immune response,  
15 which is the adaptive system that comes through  
16 subsequent challenges to bacteria and  
17 transfusions, et cetera.

18 So my conclusions are that all of these  
19 antibodies in the ABO all can cause agglutination,  
20 hemolysis -- with hemolysis, in my opinion, it's  
21 going to be greater with anti-A,B than anti-A,  
22 then anti-B, and then anti-A1.



1                   Group O sera are probably more  
2   dangerous, as IgG antibodies are more common,  
3   especially the anti-A,B. And I predict, like n  
4   that study where they depleted the O donors and  
5   showed less hemolysis, that if you could have two  
6   arms of IV IG, one Group O donors only given to  
7   Group O, and one A and B only given to A and B,  
8   that you might eliminate the problem that way.

9                   Ability to cause hemolysis is related to  
10   titer -- I think we all agree with that. But you  
11   can't accurately predict. So, a low titer can  
12   still give hemolysis, and high titer may not. So,  
13   yeah, it's better to reduce the titer, but it's  
14   not going to solve the problem completely.

15                  And then, they're produced as part of  
16   the innate system -- CD5, B-cells, IgM -- and  
17   through the adaptive immune system.

18                  So -- thanks. (Applause.) A little over  
19   -- sorry.

20                  DR. BRANCH: Yes? Just jump in.

21                  DR. GOLDING: Interesting to me your  
22   presentation, because it raises some, I think,

1     basic immunological questions. The one is, you  
2     talk about IgG. We're talking about  
3     immunoglobulin intravenous, it's an IgG, it's not  
4     IgM.

5             We're not talking about innate immune  
6     responses, natural antibodies. We're talking  
7     about IgG, which is adaptive immune response.

8             But when you're talking about IgG and  
9     the subclasses, you don't go into that, and I'm  
10    wondering if we're missing something. What is  
11    known is that carbohydrates, in particular, induce  
12    IgG2, and protein antigens induce IgG1 and IgG3.  
13    IgG1 and IgG3 bind complement, and are very good  
14    at lysing and agglutinating. And IgG3, in  
15    particular, has a long hinge region.

16            So have you, or anybody, looked at when  
17    you do get immunoglobulin preparations that are  
18    inducing hemolysis, what is eluting off the red  
19    cells that are being hemolyzed.

20            What subclass is it? And that would be  
21    a very important information, because it could  
22    explain, for example, a high titer or low titer.

1       It might be low titer, general, but it might be a  
2       high titer antibody that's more like an IgG3,  
3       that's more likely to hemolyze.

4               And just one extra point is, if we knew  
5       that, we could do something about the  
6       manufacturing maybe to change the composition of  
7       the IgG so it would have less of the component  
8       that's more likely to cause hemolysis.

9               DR. BRANCH: That's an excellent point.  
10       And we don't look at that, but in this prospective  
11       study that Dr. Pendergrast discussed earlier,  
12       maybe one of the things we had discussed, but  
13       maybe we should add it, was when we elute off the  
14       antibody, anti-A, anti-B, we should subclass it,  
15       and see if it's any particular subclass. It might  
16       be. It might be something like -- you wouldn't  
17       expect IgG2, but it might be some subclass that's  
18       more important than any of the others -- IgG1,  
19       IgG3, IgG2 does cause hemolysis. But we haven't  
20       looked at that. IgG4, I think, is the only one  
21       that doesn't, or hasn't been shown to.

22               But, that would be something good to

1 include in a prospective study, I think. So  
2 thanks for that point.

3 But, again --

4 DR. ROMBERG: You showed your data on  
5 anti-A and anti-B levels on donors. You talked  
6 about a specification.

7 Are you just going to screen and reject  
8 donors? Or what is the thinking there?

9 DR. BRANCH: Yes.

10 DR. ROMBERG: So you'll reject donors?

11 DR. BRANCH: Yes, the purpose of that is  
12 to screen donors under that technique that they've  
13 employed, and exclude any donors that are above  
14 their cutoff, which hasn't been clearly decided  
15 yet.

16 DR. ROMBERG: Okay.

17 DR. BRANCH: But there will be some  
18 elimination of high titer for plasma infusions and  
19 platelet infusions.

20 Any other questions? All right, thank  
21 you very much.

22 DR. SCOTT: I guess I just would point

1       that, in terms of -- Dorothy Scott -- back to Dr.  
2       Golding's point, we do know that some of the  
3       products differ quite a bit with respect to their  
4       IgG3 concentration, which is another reason,  
5       perhaps, to look at this.

6               Because it may be that those differences  
7       have some correlation, whether it's fortuitous or  
8       not, to their propensity to cause hemolysis in the  
9       susceptible patients.

10              DR. BRANCH: I think that's a very good  
11       point, and we probably should include that in the  
12       prospective study, is to look at the subclasses  
13       that we get off of the cells.

14              DR. SCOTT: Thank you very much, that  
15       was a good presentation to have after lunch, I  
16       think. You kept us awake very successfully.

17              Dr. Thorpe, from National Institute for  
18       Biological Standards and Control, or NIBSC, will  
19       be speaking next. She has spent many years  
20       testing, and developing tests, for anti-A and  
21       anti-B. And I think we're very fortunate that she  
22       was able to come.

1 DR. THORPE: Good afternoon, and thank  
2 you for the introduction. I don't think my talk  
3 is going to be as amusing as the previous one.

4 Anyway, so I'm going to be talking about  
5 the specifications for anti-A and anti-B in IV IG,  
6 the history and rationale.

7 Okay, well, it's been known for over 70  
8 years now that transfusion of blood Group O, blood  
9 or plasma, to Group A or B recipients can cause  
10 hemolytic reactions. Although it was recognized  
11 that pooling plasma from Group O, A, B, and AB  
12 donors resulted in a variable degree of  
13 neutralization of the anti-A and anti-B content by  
14 A and B substances, at least in terms of a drop in  
15 saline titers, other early workers showed that  
16 such neutralization, in vitro, did not necessarily  
17 prevent hemolysis in vivo, because the so-called  
18 "dangerous" immune anti-A and anti-B antibodies  
19 were less readily neutralized by A and B  
20 substances than the natural anti-A and anti-B.

21 So, given the potential of plasma to  
22 cause hemolytic reactions, it was hardly

1 surprising that certain blood products -- notably  
2 factor VIII concentrates and IV IG -- were also  
3 associated with hemolysis in recipients. And,  
4 indeed, reports of hemolysis associated with IV IG  
5 regularly appear in the literature.

6 So, when members of Expert Group 6B sat  
7 down to draft the European Pharmacopeia monograph  
8 on IV IG, it was agreed to include a limit for  
9 anti-A and anti-B. But no one knew what the limit  
10 should be.

11 So the limit was chosen by the members  
12 based on their collective experience with IV IG  
13 products in their own countries. Unlike other  
14 tests and specifications, the test and  
15 specification for anti-A and anti-B were not  
16 subjected to a collaborative study. Members  
17 reported that the titers of anti-A and anti-B in  
18 products in their countries ranged from zero --  
19 which I find hard to believe -- to 64, from 5  
20 percent IgG.

21 They recognized that the limits had to  
22 relate to immunoglobulin concentration, and so, to

1 take account of all the different products on the  
2 market, they eventually agreed that the anti-A and  
3 anti-B titers must not exceed 32, using the  
4 indirect antiglobulin test.

5 So, basically, the limit was chosen  
6 purely to ensure that all, or virtually all,  
7 batches would pass. There was no clinical data,  
8 no other reasoning at all that went into it.

9 They decided to keep the existing  
10 Pharmacopeial method for the indirect antiglobulin  
11 test. And, interestingly, they decided that it  
12 was not necessary to include a specification for  
13 anti-D.

14 However, we found that the indirect  
15 antiglobulin is problematical with IV IG products,  
16 as the high IgG concentration neutralized the  
17 antiglobulin reagent. According to Mollison,  
18 neutralization of the antiglobulin reagent can  
19 occur in the presence of as little as 1  
20 microgram/mil of IgG. And he also states that  
21 neutralization is the most common cause of false  
22 negative results with the indirect antiglobulin



1 tests in blood group serology. And, remember, in  
2 serology, they're titrating plasma or serum with  
3 an IgG concentration ranging from around 5 to 15  
4 mcg/ml. The test, when it's intended for IV IG  
5 products, the starting concentration is 30 mcg/ml.

6 So, despite this well known problem with  
7 the indirect antiglobulin test, there's no mention  
8 in the European Pharmacopeia of the necessity of  
9 adding anti-D- sensitized R1r cells to negative  
10 tests to check that un- neutralized antiglobulin  
11 reagent is present, and therefore a positive  
12 reaction would have been theoretically possible.

13 And in our work, we found neutralization  
14 at least up to 1 in 8 dilution of 5 percent IV IG,  
15 and in many cases, up to 1 in 32 dilution of IV  
16 IG, unless the sensitized cells are washed four or  
17 five times, not three times as specified in the  
18 Pharmacopeia.

19 In addition, hemagglutination titrations  
20 are notoriously variable, but there was no  
21 reference preparation available to control the  
22 tests or define the limit.

1                   So when, in 2002, we were asked to  
2   investigate batches of IV IG associated with  
3   hemolysis in U.K. patients suspected to be due to  
4   anti-D, we developed a micro-titer plate based  
5   method of hemagglutination using papain-treated  
6   red cells. So, it's a direct method. There's no  
7   washing of sensitized cells, it's an immediate  
8   spin method. And we also found that it was less  
9   subjective reading the test results than the  
10   indirect antiglobulin test. It's allowed us to  
11   screen -- we screened over a hundred batches of IV  
12   IG, including the problem batches, very quickly.  
13   And we did indeed confirm that the batches  
14   associated with hemolysis contained high-titer  
15   anti-D.

16                  Based on our experience, we proposed the  
17   direct method as a new Pharmacopeial test for  
18   anti-D in IV IG. And we also proposed a maximum  
19   anti-D titer of 8, from 5 percent IV IG.

20                  Now, by this time, we were already  
21   collaborating with colleagues at CBER, FDA, namely  
22   Mei-ying Yu and Maria Luisa Virata-Theimer, on

1 harmonizing tests and specifications for IV IG.  
2 And to overcome variation in hemagglutination  
3 testing, and to facilitate implementation of the  
4 proposed specification, we prepared a lyophilized  
5 IV IG preparation containing the appropriate level  
6 of anti-D to act as a control for the test, and to  
7 define the Pharmacopeial limit. We also produced  
8 a negative control IV IG.

9           These preparations were subjected to a  
10 collaborative study. And, without going into any  
11 more detail, the new direct method for anti-D in  
12 IV IG, and the specification defined by the  
13 reference preparation, became effective in the  
14 European Pharmacopeia as from the first of January  
15 2006. The same specification and tests were also  
16 implemented by CBER, FDA.

17           However, we now had the rather  
18 unsatisfactory situation where there were major  
19 discrepancies in the Pharmacopeial methodology and  
20 specifications for IV IG. On the one hand, for  
21 anti-D, we had a reference method of direct  
22 hemagglutination, the titers all related to 5

1     percent IgG, because that's the concentration of  
2     most products on the market -- or multiples of 5.  
3     It was a controlled test, and the specification  
4     was defined by the reference reagent.

5             For anti-A and anti-B, the method, the  
6     reference method, was still the indirect  
7     antiglobulin test. The titers related to 3  
8     percent IgG. It was an uncontrolled test. And it  
9     had a specification of a maximum permissible titer  
10    of 32.

11            So, in another big collaboration with  
12    CBER, FDA, and EDQM, we set about to redefine the  
13    specifications and tests for anti-A and anti-B in  
14    IV IG. So we needed to assess the suitability of  
15    the direct method for anti-A and anti-B  
16    titrations, investigate the effect of titrating  
17    from 3 percent, or 3 percent IgG, and we had to  
18    determine an appropriate limit relating to 5  
19    percent IgG.

20            Now, we generated a lot of comparative  
21    data. This slide just shows a small amount of it.

22            So, on the left-hand side, we have

1 direct method titers, from 5 percent IgG, of two  
2 products, from different manufacturers. You can  
3 see that, in our hands, the anti-A and anti-B  
4 titers were very similar for these two products.

5 Most of the anti-A titers fell within a  
6 fourfold range of 8 to 32. The anti-B titers were  
7 approximately half, at 4 to 16.

8 On the right-hand side, I've plotted the  
9 indirect antiglobulin titers from 3 percent IgG,  
10 as reported by the manufacturers. So you can see,  
11 for Product 1, bearing in mind that these  
12 titrations are from 3 percent IgG -- i.e., about  
13 half what we were titrating from -- the agreement  
14 is actually quite good between their reported  
15 titers and the titers we got with the direct  
16 method. The European Pharmacopeial limit is  
17 indicated. And this data suggested that the  
18 direct method, using the papain-treated cells, and  
19 the indirect antiglobulin test, showed comparable  
20 sensitivity, and that 1 in 64 titer, using the  
21 direct method, would be an appropriate limit.

22 In the bottom panel you can see the

1 indirect antiglobulin titer for Product 2. Now,  
2 although we found the titers in these two products  
3 to be very similar, this manufacturer reported  
4 very much lower titers, possibly because of  
5 neutralization of the antiglobulin reagent, or  
6 maybe because of the subjective nature in reading  
7 hemagglutination tests.

8           So we thought that the direct method was  
9 possibly a more robust method, and would give  
10 titer, titration ranges.

11           The effect of titrating IV IG products  
12 from 3 percent and 5 percent IgG is shown here.  
13 About 80 percent of the titers from 5 percent were  
14 double those from 3 percent -- again suggesting  
15 that the specification of a maximum titer of 64  
16 from 5 percent IgG, using the direct method, would  
17 be comparable to the maximum titer of 32 from 3  
18 percent, using the indirect antiglobulin test.

19 So, this slide shows the anti-A and anti-B titers  
20 in over 1,000 batches of IV IG received at NIBSC  
21 over a five-year period. You can see that  
22 virtually all the anti-A titers fall within a

1 fourfold range of 8 to 32. The anti-B are about  
2 half, ranging from 4 to 16.

3 So, a proposed limit of 64 from 5  
4 percent IgG, using the direct method, would ensure  
5 that all batches would pass -- or all of these  
6 batches would have passed. So, that's applying  
7 the same sort of rationale as was applied when the  
8 limit was first set. And, according to this data,  
9 very few batches should actually come out on the  
10 limit.

11 So, ideally, we wanted to source a batch  
12 of IV IG with anti-A and anti-B titers of 64 to  
13 control the test and define the limits, but we  
14 couldn't source such a batch. But we did source  
15 an IV IG batch with quite high anti-A and anti-B  
16 titers. The anti-A titer was borderline between  
17 32 and 64. The anti-B titer was 32.

18 And if you look at our data again, you  
19 can see that it was very rare for any batches to  
20 have an anti-A titer above that -- of this  
21 positive control which is indicated by the arrow.  
22 And, also, we haven't encountered any batches with

1 anti-B titers higher than 32, which was the titer  
2 of the proposed positive control. So, this IV IG  
3 batch, we failed to produce the positive control,  
4 and coded it "07306."

5 Because all batches of IV IG contain  
6 some anti-A and anti-B, Baxter very kindly  
7 fractionated a negative control IV IG for us that  
8 was fractionated exclusively from AB donors, so  
9 there was no anti-A or anti-B in this at all.

10 And because we didn't consider the  
11 anti-A and anti-B titers of 07306 quite high  
12 enough to define the limit, so it was comparable  
13 to the old 1-in-32 limit using the indirect  
14 antiglobulin test, we spiked it with mass  
15 monoclonal anti-A and anti-B to raise the titers.

16 Okay, and I should just add here that  
17 data from Mei-ying and CBER, FDA, also showed that  
18 it was very unusual to have any batches of IV IG  
19 with titers above that of our positive control,  
20 07306.

21 So these three preparations were  
22 subjected to an international collaborative study.



1       The candidate reference reagents were dispatched  
2       to 23 laboratories in 13 countries.

3               Laboratories performed direct  
4       hemagglutination tests, which was the candidate  
5       reference method. According to detailed  
6       methodology provided, seven of these labs, plus  
7       two more labs, performed the indirect antiglobulin  
8       test.

9               The total number of tests resulting in a  
10       particular titer against A, B, and O cells was  
11       counted for each preparation. So, this slide  
12       shows the direct method titers for the positive  
13       control 07306 when titrated from 5 percent.

14              And you can see that the majority of the  
15       labs reported anti-A titers of 16 to 64, and  
16       anti-B of 8 to 64. Most of the labs found no  
17       reaction with Group O cells.

18              The results using the indirect  
19       antiglobulin tests were broadly comparable, but  
20       there was a slightly higher incidence of reactions  
21       with Group O red cells.

22              Using the direct method, the negative

1 control, which we coded "07308," was indeed  
2 negative in most of the tests. I should add that  
3 the starting dilution for testing using the direct  
4 method is a 1-in-2 dilution. Neat IV IG is too  
5 viscous to allow streaming of the negative cells.  
6 However, using the indirect antiglobulin test,  
7 there was a higher incidence of reactions with  
8 Group O and Group A and B cells.

9           So, this slide shows the number of tests  
10 resulting in a particular titer using the proposed  
11 preparation to define the Pharmacopeial limit.  
12 This preparation was coded "07310."

13           And you can see that most of the titers  
14 were over a twofold range, 32 to 64. And, bearing  
15 in mind that this was the first time most of the  
16 participating laboratories have performed the  
17 direct method for anti-A and anti-B titrations,  
18 the results are actually quite tight for  
19 hemagglutination tests. Most of the tests against  
20 O cells did not show agglutination.

21           In contrast, using the indirect  
22 antiglobulin test, the result is much less

1       satisfactory, there was much more spread of  
2       reported titers. And I think this slide shows  
3       just how meaningless it is to define the  
4       specification just in terms of a titer, which can  
5       vary by 16-fold or more. The specification has to  
6       relate to a reference preparation.

7               So, this slide summarizes the  
8       collaborative study results. So, for the positive  
9       control 07306, about three- quarters of the tests  
10      were within the 32 to 64 range for anti-A. For  
11      anti-B, most of the tests were in the 16 to 32  
12      range. And we recommend that these titers should  
13      be used as a guide for operators performing the  
14      test and setting it up.

15              And if they get titers that are wildly  
16      different from these, then they need to  
17      investigate their testing conditions.

18              For the proposed limit preparation  
19      07310, the majority of tests were within 32 to 64  
20      range for anti-A, and for anti-B. And the  
21      negative control preparation was negative in most  
22      of the tests.

1                   So, the upshot of all this was that the  
2   stocks of these preparations were shared between  
3   us for distribution as WHO reference reagents,  
4   EDQM, and CBER, for distribution as biological  
5   reference preparations.

6                   So, the positive and negative controls  
7   are intended to standardize the tests. But,  
8   because we didn't make very much of the limit  
9   preparation 07310, we recommend that only samples  
10   that have titers higher than the positive control  
11   07306 should be tested against the limited  
12   preparation 07310.

13                  So, the direct method was adopted as a  
14   Pharmacopeial test for anti-A and anti-B from July  
15   2011, with the limit preparation 07310 defining  
16   the new limits.

17                  Okay, so just when we thought that the  
18   issue of anti-A and anti-B in IV IG was done and  
19   dusted, we became aware of an increase in reports  
20   of hemolysis associated with IV IG, and we  
21   encountered some batches of products, from more  
22   than one manufacturer, that had unusually high

1       titers -- particularly of anti-A. And these  
2       included two batches of Privigen, one of which was  
3       associated with severe hemolysis in a U.K.  
4       patient. The patient actually suffered renal  
5       failure. This was at the John Radcliffe Hospital  
6       in Oxford.

7               When we tested the Privigen that was  
8       associated with renal failure, we found that it  
9       had an anti-A titer of 64, an anti-B titer of 16.  
10      So it wasn't actually out of specification. It  
11      did not exceed the Pharmacopeial limit. A second  
12      batch of Privigen which was not associated with  
13      hemolysis was the same. And also, for comparison,  
14      there are a number of other products.

15             Now, I should stress that all of these  
16      products came from the hospital pharmacy, and were  
17      provided by the clinician treating the patient.  
18      They were not submitted to NIBSC for batch-release  
19      purposes, so I'm not contravening any kind of  
20      confidentiality by showing you this data -- and it  
21      is, in fact, published.

22             Now, in Europe, batches of blood

1 produces and vaccines are tested independently by  
2 an official medicines control laboratory in what's  
3 called "official control authority batch release."  
4 This is to provide a better assurance of quality.  
5 Now, the OCABR testing, it doesn't cover all the  
6 tests that have to be performed by the  
7 manufacturer, but up until recently, there was no  
8 requirement for OMCLs to test batches of IV IG for  
9 anti-A and anti-B, only anti-D.

10 So, in view of our recent experience, we  
11 proposed that testing IV IG products for anti-A  
12 and anti-B should be implemented as a  
13 batch-release test, both to enforce the  
14 specification, and to collect data on titers in IV  
15 IG products, particularly the newer 10 percent  
16 products, which may be associated with a higher  
17 risk of hemolysis, because this data is needed  
18 before any decision, I think, is made as to  
19 whether the limit -- in particular, for the 10  
20 percent products -- needs to be lowered.

21 Of course, there's a balance between  
22 safety and compromising supply. There always

1       seems to be a shortage of IV IG. And even the  
2       high-titer batches can be used safely in about 50  
3       percent of the population. So this is a matter of  
4       debate as to whether the limit should be lowered.

5               So, finally, I just want to acknowledge  
6       my colleagues as NIBSC and CBER, FDA, EDQM, and  
7       the clinicians at the John Radcliffe Hospital.

8               Okay, thank you for your attention.

9       (Applause.)

10              DR. SCOTT: Time for one question.

11              DR. SCHLEIS: Tom Schleis. If you  
12       tested the 10 percent products undiluted, wouldn't  
13       it be one dilution higher, in terms of titer?

14              DR. THORPE: No, all the titrations  
15       relate to 5 percent.

16              DR. SCHLEIS: So, everything was diluted  
17       to 5 percent.

18              DR. THORPE: Yes, yes, yes.

19              DR. SCHLEIS: But if you were to not  
20       dilute it to percent --

21              DR. THORPE: Then there would be --

22              DR. SCHLEIS: They would be one dilution

1 higher --

2 DR. THORPE: I guess.

3 DR. SCHLEIS: -- in terms of titer.

4 DR. THORPE: Yeah.

5 DR. SCHLEIS: Okay.

6 DR. SCOTT: I think, in the interest,  
7 we'll move forward.

8 Dr. Bellac is here from Swissmedic. And  
9 it's a good follow-on for the talk you just heard,  
10 because she will be showing you titers for  
11 immunoglobulin products from a survey that they  
12 did of a number of immunoglobulin lots.

13 DR. BELLAC: Thank you very much for the  
14 invitation. I will present our large comparison  
15 of hemagglutinin titers across antiglobulin  
16 products.

17 So, we started our large market  
18 surveillance study in 2012, and there were mainly  
19 two reasons why we did that.

20 So, we saw an increase in report rates  
21 of severe hemolysis cases over the last years, and  
22 this was not only seen in Switzerland, but



1 globally. And then, in 2012, the Paul-  
2 Ehrlich-Institut published about this increase.  
3 And about the same time, under CSL and Health  
4 Canada, informed about the safety of Privigen in  
5 context with hemolysis.

6 And this table here is from the  
7 publication of the Paul-Ehrlich-Institut, showing  
8 the total number of hemolysis cases during the  
9 period from 2004 to 2012, and then comparing five  
10 different products, and including all the products  
11 and modern products. Some of them were only  
12 licensed in 2008. And you can see that there is  
13 clear difference between the different products.

14 And then the second reason was a more  
15 technical one. So as we have heard before, in  
16 2011, the official mechanism changed in the  
17 Pharmacopeia, in the European Pharmacopeia. So,  
18 the indirect method was replaced by a direct  
19 method, and we wanted to establish this new method  
20 in our lab.

21 And we observed early on that with the  
22 direct method, we produce higher titers than with

1 the indirect method. And also early on, we saw  
2 that several products showed really high anti-A  
3 titers. So we thought that it's best if we use  
4 exactly the same methods to compare the different  
5 IV IG products, and under the same conditions.

6 So, when we started to compare the  
7 products, this was in 2012, a lot of manufacturers  
8 were still using the indirect method. And I'm  
9 showing you here the results of four different  
10 products. And, in blue are the titers produced by  
11 the manufacturers, and in red, the titers we  
12 measured with the indirect -- with the direct  
13 method, and in green are titers for the positive  
14 control.

15 And you can see that with the direct  
16 method, we are producing one titer step higher  
17 titers than with the indirect method. And you can  
18 also see that these products differ in anti-A  
19 titers. Some of them are as high as the allowed  
20 positive control, and some of them are lower.

21 So, these are the results of a different  
22 product. And we analyzed around 170 batches.

1 And, in blue, again, are the results of the  
2 manufacturer, and the manufacturer was using the  
3 indirect method up to a certain time, and then  
4 switched to the direct method, as well. And you  
5 can see this jump in titers, as well.

6 But, importantly, for this product, more  
7 than 50 percent of the batches were as high as the  
8 positive control, and that's 1:64, so, the maximum  
9 allowed. And you can also see that even using the  
10 same direct method, we generated different  
11 absolute values, even with the direct method.

12 And we think that this is due to the  
13 time point of reading. So the time point is  
14 really critical, in terms of absolute values. It  
15 is defined in the Pharmacopeia with "read after 4  
16 to 5 minutes, or when the negative controls have  
17 streamed." And in our hands, it was after about 1  
18 minute. But this has a clear effect on the  
19 absolute values.

20 So, we were a bit concerned about this  
21 variability of the test. And when we started with  
22 the comparison, we also assessed alternative

1 methods. So, here we were comparing four  
2 different analysis methods with the official  
3 direct Pharmacopeian method. So we also evaluated  
4 a direct gelcard method, and then the indirect  
5 method according to the Pharmacopeia, and then  
6 indirect gelcard method.

7 And, as you can already see, the  
8 evaluation of the results is very different for  
9 the four different methods, with the direct  
10 method, a positive reaction, is seen -- or  
11 agglutination as seen as a cell button, whereas in  
12 the gelcard method, a positive reaction is seen as  
13 a red cloud or a red stripe. And then, with the  
14 indirect method, agglutination is seen as -- or,  
15 we defined it when four or more cells  
16 agglutinated, and you see that under a microscope.  
17 And then, with the indirect method, you see again  
18 a red stripe or a red cloud if the reaction is  
19 positive.

20 But to really compare the results  
21 generated by the four methods, we exactly -- or we  
22 diluted all the samples to exactly the same

1 concentration of 2.5 percent IgG, for a dilution  
2 of 1:2. And you can see that the experimental set  
3 up is different for the four different methods.  
4 And we then also generated different absolute  
5 values. So this was method-dependent.

6 But to really define which one was the  
7 most suitable method to do this comparison, we  
8 compared different criteria. And to make a long  
9 story short, we concluded that none of the four  
10 analysis methods can be considered ideal in every  
11 respect, and that the direct gelcard method could  
12 be a valid alternative to the Pharmacopeian  
13 method, based on efficiency and lower costs. But  
14 it could only serve as a limit test to define if a  
15 certain sample is above or below the positive  
16 control. And the official Pharmacopeian method  
17 still has the best precision, or the lowest  
18 variability of all these four methods so far.

19 So, we used these official methods to do  
20 the comparison of the products. And here are the  
21 results of this large study.

22 So, we compared 11 different

1 immunoglobulin products, also including  
2 subcutaneous product, Product K, and the anti-D  
3 IgG. Anti-A and anti-B are plotted side by side  
4 for each product, and you can also see that we  
5 analyzed different numbers of batches per product.

6 And we calculated the median titers for  
7 each product. The median anti-A titers are shown  
8 in red, and median anti-B titers are shown in  
9 blue. And you can clearly see that the different  
10 products do differ in terms of anti-A and anti-B  
11 titers.

12 So, for Product A, the median anti-A  
13 titer was at 1:64, so that's the maximum allowed,  
14 and followed by Product B and C, with a median  
15 titer of 1:32, and then the rest of the products,  
16 at around 1:16. And we can also see that anti-B  
17 titers are general 1 to 2 titer steps lower than  
18 the anti-A titers.

19 But, of all these batches analyzed, we  
20 had none out-of-specification batches. They were  
21 all within the Pharmacopeian limit.

22 To better understand why these products

1     differ in titers, we compared their manufacturing  
2     process. And here you can see all the products  
3     listed again, from A to K, with their IgG content.  
4     And you can also see the composition of the  
5     products.

6             And, instead of indicating the absolute  
7     values, we have calculated or expressed the titers  
8     relatively to the positive control. This allows  
9     for even better standardization or possibility to  
10    compare the different products, because, as you  
11    have heard before, for example, the positive  
12    control can be 1:32 to 1:64, and the same is true  
13    for the product. So, if you just take the  
14    relative difference between the sample and the  
15    positive control, you can compare better.

16            And you can also see that there is a  
17    clear difference between the modern products and  
18    the older products. So, older products are lower  
19    in anti-A titers and anti-B than the more modern  
20    ones. And the older products, the excipients of  
21    the older products are sugars, and the more modern  
22    products contain amino acids as excipients. So

1       you can see the difference between the modern and  
2       older products.

3               But, importantly, we think that the main  
4       difference between the older products and the  
5       newer products is this confractation Step 3.

6       Or it was obvious that products that contain this  
7       removal of fraction III precipitate are lower in  
8       anti-A and anti-B titers. So this might be an  
9       explanation why the modern products are higher in  
10      comparison to the older ones.

11             And then, also, the more modern  
12      products, they do contain a precipitation step  
13      with octanoic acid, which probably enriches the  
14      IgG -- yes, the IgG, but this also includes the  
15      isoagglutinins of type IgG. And, yes, we think  
16      that the absolute titers are linked to the  
17      manufacturing process.

18             But, as mentioned before, of all these  
19      batches analyzed, even of Product A, we only had  
20      -- or let's say, of Product A, of 320 batches  
21      analyzed, only four times we had to run the limit  
22      test when the sample was above the positive



1 control. But still, these batches were within  
2 specification -- in contrast to one time with IV  
3 IG-D, there was a clear out-of-specification  
4 batch. And usually, Product D showed low titers,  
5 with the exception of this batch. And as we've  
6 heard this morning, this batch was produced with  
7 90 percent of blood Group O plasma. So this could  
8 be an explanation for this high-titer batch.

9 And coming back to the table from the  
10 beginning, we have now added the titers to the  
11 total number of hemolysis cases, and we've  
12 calculated the reported cases per 1,000 kilo of  
13 sold IV IG. And anti-A and anti-B titers are  
14 again expressed as a relative difference to the  
15 positive control. So here, at least, it seems  
16 that if the titers are as high as the positive  
17 control, there is an increased risk, maybe, of  
18 hemolysis.

19 But so we were picking 20 batches of  
20 Product A that were involved in hemolysis, and  
21 looked at the titers of these batches. But then  
22 we saw that they were not different to regular

1        batches. They showed a median anti-A titer of  
2        1:64, so they were not higher than all the batches  
3        we have tested before.

4                    And in Europe, these are the common  
5        indications and doses for IV IG treatment. And we  
6        think, or we've discussed that before, that there  
7        is an increased hemolysis risk when a modern IV IG  
8        is given as high-dose treatment in patients with  
9        blood Group A or AB. And we defined other  
10       indications, then listed here, as off-label use.  
11       And IF IG treatment is administered quite often as  
12       off-label now, these days.

13                   Now, I'm showing you new results. So,  
14       these are the results of batches of 2013 from a  
15       product that was manufactured from screened  
16       plasma, in comparison to unscreened plasma. And  
17       we see that batches manufactured from screened  
18       plasma are one titer step lower than the ones from  
19       unscreened plasma, in terms of anti-A titer. We  
20       don't see an effect in terms of anti-B.

21                   So, to conclude our findings, we can say  
22       that the various products do differ in anti-A and

1 anti-B titers, and that the modern products show  
2 one to three titer steps higher anti-A titers than  
3 the older products, with the confraction removal  
4 step, and that the different test methods result  
5 in different absolute values, so it's important to  
6 include the reference material. And in our hands,  
7 the direct method was the most suitable for a  
8 comparison of the IV IG products, but that all IV  
9 IG batches were within the European specification  
10 of 1:64, with the exception of this  
11 out-of-specification batch that we analyzed for  
12 our German colleagues.

13 And that the median anti-A of batches  
14 that were involved in hemolysis was not different  
15 from regular batches of the same produce. And  
16 also, lots that were manufactured from screened  
17 plasma showed one titer step lower anti-A titers  
18 compared to those that were made from unscreened  
19 plasma. And that a modern IV IG, with the  
20 isoagglutinin depletion step shows a median anti-A  
21 titer two titer steps lower than the official  
22 reference material.

1                   And I put up one point for discussion:  
2     If it might help if the titers were indicated on  
3     the product label, or at least on the OMCL  
4     website.

5                   So, now I'd like to thank my colleague  
6     from Switzerland, and also my colleagues from the  
7     Paul-Ehrlich- Institute from France. And thank  
8     you for your attention.

9                   (Applause.)

10                  DR. SCOTT: Thank you very much for such  
11     a thorough presentation of this data. As far as I  
12     know, it's the most extensive data of this kind  
13     that we have about modern products. So we can  
14     talk about the differences among products some  
15     more, I think, in the panel, and what hypotheses  
16     there are.

17                  But -- thank you. And, next, we'll go  
18     to Catherine de Coupade, from LFB, and she will  
19     talk about alternative test methods for anti-A and  
20     anti-B measurement, and perspectives on the  
21     current specifications.

22                  MS. DE COUPADE: So, the objective of my

1 talk today is to present potential alternative  
2 methods for anti-A and anti-B measurement in  
3 intravenous immunoglobulin products.

4 So, as you know, there have been  
5 increasing incidence of immune hemolysis following  
6 high dose IV IG administration. And it's becoming  
7 great importance to map the critical steps in the  
8 process to improve clinical safety for the  
9 patient, including higher tolerance -- suggesting  
10 lowering IgA procoagulation factors, vasoactive  
11 substances and hemagglutinins -- also, to enhance  
12 biological safety, and secure product stability  
13 related to proteases. And still, of course,  
14 gaining efficacy with high purity of  
15 immunoglobulins and functional Ig integrity.

16 Okay, so in the European monograph for  
17 IV IG, there are around 15 assays that are  
18 required before IV IG release. And among them  
19 there is the anti-A and anti-B hemagglutinin  
20 assays. So, as I said at the beginning of my  
21 talk, infusion with high dose of IV IG is usually  
22 well tolerated, but still incidents can still

1 occur, incidents of severe hemolysis. And strong  
2 evidence suggests that these hemolyses are related  
3 to alloantibodies, and specifically to anti-A and  
4 anti-B antibodies.

5 So there was a request to limit the  
6 level of anti- A and anti-B antibodies. And each  
7 product of IV IG must be tested for the level of  
8 anti-A and anti-B by using the European  
9 Pharmacopeia assay that is described in Section 2-  
10 6.20, which is a direct method of agglutination.  
11 So, no agglutination should be observed when IV IG  
12 diluted at 25 grams/liter is (inaudible) more than  
13 1 in 64.

14 Despite the fact that all the IV IGs are  
15 compliant with the European guideline, we still  
16 observe some hemolysis events. A ratings  
17 equations whether or not the release method and  
18 all the dilution limits are enough reliable to  
19 avoid those adverse effects observed in patients  
20 following IV IG administration.

21 So, here you will recognize the graph  
22 that is just on top is just presented to you. So,

1     it's the collaborative study that was published in  
2     2009, with the objective to validate the direct  
3     hemagglutination method that is now described in  
4     the European Pharmacopeia. So, the monograph  
5     states that you can have a twofold difference,  
6     which is not considered as significant. So, even  
7     if the new direct method restricted the range of  
8     titer, you still observed a huge range of  
9     titrations of the anti-A and anti-B that can go up  
10    to between 8 and 64, for example, for the positive  
11    control, which is 5 percent IV IG, or even for the  
12    limited reference preparation, that can go from 8,  
13    titer 8, up to 128 -- which is quite a huge range  
14    of titers. So there is quite a high  
15    inter-laboratory variability still for the actual  
16    EP method.

17            So, this slide presents data that were  
18    published also in 2009 using the old method,  
19    comparing five different IV IG products. And  
20    you'll see that they were all anti-A and anti-B  
21    compliant with the European Pharmacopeia guideline  
22    -- so, actually, it's not 1 in 64, but 1 in 32 --

1 with two of them, the two last ones on the right  
2 side, that were not compliant for the anti-A,  
3 because it was 1 in 64.

4 But still, we can observe a variability  
5 in antibody titers in IV IG preparation, with  
6 higher titer noted in liquid, non-lyophilized  
7 products. And despite this compliance with the EP  
8 guideline, we still observe 18 cases of IV IG  
9 hemolytic anemia. And an author from the paper  
10 asks the question why we should not limit  
11 hemolysis risk by using low anti-A and anti-B  
12 titer products? So it seems that people are not  
13 fully, or totally, confident with titer, even if  
14 it's compliant with the EP guidelines.

15 So, we developed in our lab a flow  
16 cytometry method to specifically determine the  
17 anti-A and anti-B in IV IG. And we think it is a  
18 promising alternative method to the European  
19 Pharmacopeia one.

20 So, on this slide is presented the  
21 principle of this flow cytometry method. So,  
22 basically, you incubate A or B red blood cells,



1 usually RhD-negative cells, with increasing  
2 concentration of either your IV IG as a standard  
3 -- for example, the positive one -- you can find  
4 the DQM, or increasing concentration of your  
5 sample you want to test.

6           Then, after several washes, you reveal  
7 the immune complexes by using a fluorescent  
8 secondary antibody. And what you read is  
9 fluorescence, plotted here as mean fluorescent  
10 intensity, that you can plot against concentration  
11 of your IV IG. And you get linear dose- response  
12 function that you can plot for your standard,  
13 which is the positive one, and use, for example, a  
14 high quality control which contains fourfold more  
15 anti-A or anti-B antibody.

16           And we express results as the ratio  
17 between the sample line slope and the positive  
18 control IV IG line slope.

19           Then we have set some validation  
20 criteria -- like, for example, reproducibility --  
21 that we investigated by using our two quality  
22 controls, our two Qcs -- so the high and the low

1     quality control. And we express the results, as I  
2     told you, as a slope ratio between high and low  
3     Qcs. So, you have on the y-axis the slope ratio,  
4     and on the x-axis, the number of assays. And you  
5     see that you get quite reproducible results for  
6     independent flow cytometry assays, either for  
7     anti-A or for anti-B.

8             Here is presented part of our internal  
9     study that was performed at LFB. So, we compared  
10    five different IV IGs -- 1, 2, 3, 4, 5 -- using  
11    three different batches per product. And we  
12    tested either the direct hemagglutination, or the  
13    flow cytometry method. So, on the left panel you  
14    get the direct hemagglutination, and you get all  
15    individual results, like the three results for the  
16    three batches, for each product. And also, for  
17    the flow cytometry, you get the mean (inaudible).

18            And so what you can see is, even if you  
19    can see difference in terms of titer, like two  
20    different groups at the low titers and the high  
21    titers, it's kind of difficult to really get  
22    accurate information from a level of anti-A and

1 anti-B using the direct hemagglutination -- while  
2 when you're using the flow cytometry method, you  
3 can have higher precision in terms of difference  
4 between IV IG in anti-A or anti-B contents.

5 And we think that this can be really  
6 useful if you want either to compare different IV  
7 IG, but also if you want to do a fine tuning of  
8 your process optimization, for example, if you  
9 want to see what step can contribute efficiently  
10 to remove your anti-A or anti-B.

11 We wanted also to correlate the levels  
12 of anti-A and anti-B to the physiological effect  
13 of those antibodies, which is the hemolytic  
14 activity. So we set up a specific lysis assay of  
15 human A, B, or O, Rh D-negative red blood cells,  
16 by the five liquid IV IG products -- in the  
17 presence of complement, of course. And what we  
18 saw is that the IV IG that have the lowest level  
19 of anti-A or anti-B, which is IV IG 1 and IV IG 2,  
20 induced the lowest level of specific hemolysis.  
21 And you can really see the two groups that you've  
22 seen before here, in the flow cytometry. So you

1     have a good correlation between your anti-A and  
2     anti-B levels, and the physiological effect, which  
3     is hemolysis of your red blood cells. With  
4     O-negative, of course, you've got no hemolysis.

5             Okay, so as far as the conclusion, it  
6     seems that just the complex method of production,  
7     and various processes that can be used by  
8     manufacturers, you will have different IV IG that  
9     will exhibit meaningful differences in terms of  
10    safety. And it's of great importance to master  
11    critical steps in the process, to improve the  
12    clinical safety for the patient.

13            One of our goals is also to meet  
14    expectations of manufacturers and regulatory  
15    authorities, and also to implement alternative  
16    methods for safety and quality assessment of IV IG  
17    -- either by replacing old, historical methods, or  
18    by updating them if we think that they are not  
19    appropriate.

20            And we think that the flow cytometry  
21    method can fulfill all three points just described  
22    above, because it's reproducible, robust, and

1 accurate, especially compared to the non-automated  
2 hemagglutination method that was just described in  
3 the European Pharmacopeia.

4 So, the question that is open is: Do we  
5 need a revision of the current European  
6 Pharmacopeia? And what would be the position of  
7 regulators and fractionators on this question?

8 Finally, I wanted to present one of our  
9 ongoing projects at LFB. We are currently working  
10 on preparation of a reference standard of  
11 hemagglutination in anti-A or B. We are in the  
12 process of (inaudible) them and characterize them  
13 as specific anti-A and anti-B or allow  
14 quantification of hemagglutinin in IV IG  
15 preparation. But we still need to get good  
16 correlation between the current European  
17 Pharmacopeia and the cytometry method by using,  
18 for example, the positive control, the negative  
19 control, and the limit reference preparation that  
20 we can make up with human anti-A and anti-B spiked  
21 directly in the positive control, instead of using  
22 (inaudible) anti-A and anti-B as it is now.

1                   And, finally, this new method should be  
2     applicable to different products -- 5 percent, 10  
3     percent -- and be accessible to all users.

4                   Okay, and I just wanted to thank the LFB  
5     team who was involved in this work.

6                   Okay, thank you. (Applause.)

7                   DR. KLEIN: Klein, Bethesda. Did you  
8     have the opportunity to compare any of the  
9     products that caused hemolysis with those that did  
10    not, using your technique?

11                  MS. DE COUPADE: Actually, not, not  
12    really. No. But it will be a (inaudible). The  
13    thing is, it's kind of difficult to get those  
14    high-titer IV IGs.

15                  DR. BRANCH: Don Branch, from Toronto.  
16    You're using red cells in all of your assays, is  
17    this correct?

18                  MS. DE COUPADE: Sorry?

19                  DR. BRANCH: Red cells? You're using  
20    red cells?

21                  MS. DE COUPADE: Yes.

22                  DR. BRANCH: So, have you thought about

1       using synthetic A or B antigens, and doing an  
2       ELISA, just a simple ELISA, to quantify your  
3       antibodies? Because that is available quite  
4       readily, the synthetic molecules, and you should  
5       be able to just set up a simple ELISA. It won't  
6       be as technically challenging as somebody doing  
7       FACS. Have you thought about that?

8               MS. DE COUPADE: No, we didn't try a  
9       simple ELISA for that. Yes.

10              DR. SHEBL: Amgad Shebl. Just a  
11       question, or maybe a remark on slide number 11,  
12       because in most of the presentations we had, and  
13       most of the data sets, we have seen that anti-B  
14       titers are usually one step lower than anti-A  
15       titers. And here, I see that on the left side, I  
16       think most of the -- more than 50 percent were  
17       higher with anti-B titer. And once you made it by  
18       flow cytometry, it's even reversed.

19              So, do you have any explanation for  
20       that?

21              MS. DE COUPADE: Right now, no. Maybe  
22       I'll have to check this. But -- yes.

1 DR. GOLDING: You used pooled red blood  
2 cells, is that correct?

3 MS. DE COUPADE: Yes.

4 DR. GOLDING: Do you also treat them  
5 with proteolytic enzyme like papain?

6 MS. DE COUPADE: Papain? Yes. Yes,  
7 papain- treated cells.

8 DR. GOLDING: So it's like what we saw  
9 with the direct --

10 MS. DE COUPADE: Exactly. Yes.

11 DR. GOLDING: -- antibody test.

12 MS. DE COUPADE: Yes.

13 DR. GOLDING: So, in other words, it is  
14 somewhat of an artificial system. And, you know,  
15 I just wonder if it teaches us something, that in  
16 order to get agglutination, or even hemolysis, you  
17 need to treat the red cells to remove something  
18 that's interfering with the antibody.

19 So, it suggests that there's something  
20 else on the red cell membrane. And, you know, I  
21 wonder if that should be pursued.

22 But, a more direct question would be,



1     now you have, you and the others that have  
2     presented in this session, have the tools to test  
3     the red cell membranes, why not test, try and get  
4     red cells from patients that underwent hemolysis  
5     to see if there's something peculiar about the red  
6     cell membranes. Plus, if you could just do a  
7     population test, testing many individuals, do you  
8     find -- you, or any of the others -- look to see  
9     if the titers are very different using different  
10    red cells? When you use pooled red cells, you're  
11    just normalizing it. And what we know is, only a  
12    few people with certain batches are going to  
13    undergo hemolysis.

14           MS. DE COUPADE: Yes, I think that's a  
15    very good point. I mean, we actually did, I mean,  
16    both kind of assays by using papain-treated cells  
17    or non-papain-treated cells. The results were  
18    quite the same, except that it seemed that when  
19    you're treating cells with papain, I don't know if  
20    you remove something or if you expose better the  
21    antigen, so you get a much higher response. But  
22    the response is pretty much the same, it's just

1 the level of intensity that is quite different.

2 But you do have a response when you are  
3 not treating cells with papain, yes.

4 DR. SCOTT: One more question?

5 DR. SCHLEIS: Did you dilute the samples  
6 before testing? Tom Schleis.

7 MS. DE COUPADE: For the flow cytometry  
8 method? Yes, we do increasing concentrations of  
9 the IV IG.

10 DR. SCHLEIS: But you start with  
11 undiluted samples.

12 MS. DE COUPADE: No, we diluted them. I  
13 have to check -- it depends if it's 5 or 10  
14 percent, but you have to (inaudible) into the  
15 range of the linearity of your curve. Yes, but  
16 it's -- I don't remember exactly when you start,  
17 at what dilution you start, but you diluted it,  
18 for sure. It's not undiluted samples you are  
19 using.

20 DR. SCHLEIS: Okay.

21 DR. SCOTT: Thank you for an interesting  
22 and provocative talk -- or at least provocative

1 questions to regulators and industry.

2 And I think we'll go ahead and break for  
3 a snack and a stretch. And we'll see you back  
4 here at 3:25.

5 (Recess)

6 DR. SCOTT: All right, I think we'll get  
7 started. Dr. Zimring has come here from the Puget  
8 Sound Blood Center, and he's going to talk about  
9 possible product risks related to the presence of  
10 things other than anti-A or anti-B, or at least  
11 things that might accentuate or accelerate  
12 hemolysis. And we look forward to this  
13 interesting presentation to help us think about  
14 potential co-factors.

15 DR. ZIMRING: No one said it was  
16 supposed to be interesting. Okay.

17 After roughly 20 years of marriage, my  
18 wife came to me a few weeks ago and instructed me  
19 to grow a beard. So this is my first public  
20 speaking with hair on my face. And thank you.

21 (Applause.) I have no doubt that is the only  
22 applause I will receive for the rest of the

1 lecture.

2           So, I did take the liberty of being a  
3 little bit self-reflective, of modifying this  
4 introductory slide after the morning session,  
5 which I found to be very informative. And I would  
6 like to posit that if we're going to construct a  
7 (inaudible) syllogism around the conversation that  
8 we're having in a kind of simplistic and  
9 Aristotelian view, we have two premises based on  
10 empirical observation. The first is that IV IG  
11 contains anti-A and anti-B IgG. This is clear and  
12 empirically demonstrable. Also, I will concede,  
13 despite my skepticism this morning, that IV IG  
14 causes hemolysis.

15           So, if these are our two premises, the  
16 conclusion -- which is the hypothesis, and we  
17 didn't necessarily arrive at this in this order --  
18 the hypothesis going forward is that is the anti-A  
19 and the anti-B in the IV IG that are responsible  
20 for the hemolysis which is induced.

21           This hypothesis, from a  
22 hypothetical-deductive standpoint, gives rise to

1     certain predictions which one can obviously then  
2     test experimentally. One prediction, if this is  
3     the case, is that no hemolysis should be seen in O  
4     patients. And I think that what I heard this  
5     morning is, "no" is an exaggeration -- so,  
6     hemolysis is seen in O patients. Now, if Karl  
7     Popper were standing here he'd say, "Rejects the  
8     hypothesis. We're done. Thank you." Clearly  
9     such is not the case. But the fact that O  
10    hemolysis is seen does challenges the simplistic  
11    notion that it's simply due anti-A or anti-B.

12           One would predict that hemolysis would  
13    correlate to anti-A titer, or at least,  
14    stoichiometrically, to the total anti-A level.  
15    And Dr. Berg this morning showed data that within  
16    the 1:64 range, at least, such is not the case,  
17    that the hemolysis, by that assay, did not  
18    correlate with the titer.

19           A and AB would both hemolyze similarly,  
20    because they both have A. Forget for a minute  
21    levels on the red cells -- we'll get to that. But  
22    one might predict that in a very simplistic case,

1       and such is not the case. A is hemolyzed more.

2               The MMA would show RBC consumption in  
3       vitro with IV IG. And we heard this morning that,  
4       in fact, that is the case, but only sometimes.

5               And finally, I would posit that, given  
6       what we know about hemolysis, if you gave IV IG,  
7       and there was just anti-A and anti-B causing  
8       hemolysis, it would happen fairly quickly. And  
9       yet we heard this morning -- I think, also from  
10      Dr. Berg -- that 24 percent of the hemolysis  
11      happens starting after three days.

12              So I would posit to you that, in the  
13      simple syllogistic construct of 'There's anti-A  
14      and anti-B. We know IV IG causes hemolysis.  
15      Ergo, that's what causes it.'

16              The empirical data that have been  
17      described do not support that hypothesis.

18              However, we are scientists. We don't  
19      like to reject hypotheses, especially when they're  
20      grounded in our experience. So what one does, of  
21      course, as we all do, is we generate auxiliary  
22      hypotheses to rescue the main hypothesis, to whit:

1 We may say, "Well, yes, but inflammation of the  
2 recipient is important, and genetics of the  
3 recipient are important, and that's why the titer  
4 of anti-A doesn't always correlate." And, the  
5 density of A on the red cell surface is going to  
6 be different on As than A and Bs. And that's why  
7 A hemolyze.

8 I have no idea why it would happen so  
9 slowly. I don't have an auxiliary hypothesis for  
10 that.

11 Since my charge was to talk about maybe  
12 mechanisms other than anti-A and anti-B, I'd also  
13 like to point out that, from my point of view --  
14 which may not be fully informed on all the  
15 clinical data -- excluding DAT-negative patients a  
16 priori as those who are not hemolytic because they  
17 don't have DATs, may represent a confirmation bias  
18 towards the antibody hypothesis. If you basically  
19 say, well, anyone who doesn't have a positive DAT  
20 clearly didn't experience IV IG-induced hemolysis.  
21 You're basically saying, I'm unwilling to accept  
22 into my data set of those who hemolyze, those who

1       don't have demonstratable antibodies -- which is a  
2       bit tautological, from my point of view.

3               So, I'd like to turn back to this  
4       figure, which I somewhat nepotistically stole from  
5       a chapter that Steve Spitalnik and I wrote in the  
6       AABB technical manual, and just to revisit some of  
7       the main players we've heard about today.

8               And, regrettably, this pointer doesn't  
9       work. If I point to the screen, you won't see it.  
10      But here -- oh, here. Can you see that? Great.

11              So, antibodies have Fc domains, okay?  
12      And the Fc domains bind to Fc receptors, as we've  
13      head. And this is one route of opsonization.  
14      These antibodies, if juxtaposed in the right way,  
15      can also fix complement -- C3b -- and then there  
16      are complement receptors. And it has been  
17      demonstrated that Fc receptors and complement  
18      receptors can work together and in concert,  
19      somewhat synergistically, to cause a phagocytosis  
20      of red cells, or extravascular hemolysis, as some  
21      might call it.

22              Should this fail to occur, and the C3b



1 is in the right environment, it can promote C5  
2 deposition, membrane attack-complex formation, and  
3 then intravascular lysis.

4 It is this here, though, antibody and  
5 complement, that we seem to be focusing on in this  
6 context. And regrettably, this notion of it here,  
7 as we all know, is painfully and somewhat  
8 tragically over-simplistic.

9 So, to begin with, there are different  
10 types of immunoglobulins, as we've discussed. And  
11 even within the IgG component, there are four  
12 different types. In humans, IgG aptly named 1,  
13 2, 3 and 4. And they have different activities as  
14 far as opsonization -- so, 1, 3, and 4 opsonize to  
15 -- that asterisk to indicate it opsonizes if you  
16 have 1 Fc receptor-polymorphism, but not another.  
17 So we'll get to that a little later -- recognized  
18 by NK cells, activating complement. They're  
19 clearly different, IgG4 has essentially no  
20 complement activity. And George Garrity who,  
21 sadly, is not here, in his textbook, which you can  
22 get on Amazon for \$400 a pop because they're out

1 of print now, demonstrated, reported very nicely,  
2 that autoantibody patients, people who show up to  
3 the blood bank with positive auto controls, that  
4 are purely IgG4 -- essentially, they don't  
5 hemolyze. And I don't know the name, but one of  
6 our co-attendees earlier this morning brought up  
7 this issue of, well, what about the IgG1, 2, 3,  
8 and 4, we're not characterizing that. And that  
9 that is a composition we're blind to in our IV IG  
10 preps. No matter how much anti-IgA is there,  
11 right now, at least, the data I've seen, we don't  
12 know what the IgG subtype composition is, which  
13 may vary widely batch to batch, and give some of  
14 the unexplained variation we've been observing.

15 This is a table of the known human Fc  
16 receptors. And there are high affinity receptors  
17 which no one really knows entirely what they do,  
18 because they tend to bind monomeric  
19 immunoglobulin, which is around all the time. And  
20 the lower-affinity activating receptors -- 3a and  
21 2a, which tend to promote phagocytosis -- and then  
22 inhibitory receptors. And I don't know, I didn't

1 put it here -- each of the IgG subclasses have  
2 different affinities for these receptors. So the  
3 complexity now compounds itself. We have  
4 different IgG subclasses maybe in every prep.  
5 They have different affinities for these  
6 receptors. And these receptors are variant in the  
7 human population, as I showed in the previous  
8 slide, some polymorphisms will cause clearance  
9 with some antibodies, and some won't.

10 To make things even more complicated,  
11 it's now been demonstrated fairly clearly that the  
12 post-translational modification of the  
13 immunoglobulins -- glycosylation, desylization --  
14 will change their affinity for the Fc receptors.  
15 And so that's another parameter we're not  
16 measuring in our IV IG preps, is the  
17 post-translational modification of the IgG  
18 subclasses which were not measured.

19 Complement is not a simple matter,  
20 either in its composition or in its receptor  
21 diversity. So, if this is C3, it gets converted  
22 to C3b on the cell surface, C3a floats off and

1 acts as an anaphylotoxin. And the C3B binds to --  
2 there's four complement receptors, really five, in  
3 a way -- Cr1 and CrIg. But then, rapidly, it's  
4 converted to IC3b, which still binds these  
5 receptors, and then to C3, and then to C3dg, which  
6 binds Cr2. Cr2 is not on phagocytes. It's almost  
7 exclusively on B cells in humans.

8 And so saying that there's complement on  
9 the surface means that your opsonized here, but  
10 not here. And not only is this not an opsonin,  
11 but this covers spots that other C3 would attach  
12 to, and can act as a negative feedback to inhibit  
13 opsonization.

14 So, deeper than, "Did I see C3 on the  
15 surface (inaudible)?" The complexity of the C3  
16 that's on the surface, and what state it's in, is  
17 also going to very much affect the outcome.

18 So, I want to tell you about the immune  
19 transfer reaction, which I love, because it's one  
20 of these weird things in human biology which is  
21 just sort of cool.

22 If you imagine an immune complex --

1     which I'll get to in a minute, because that was my  
2     assigned topic -- this immune complex would be  
3     something, you know, that points to being  
4     something nasty, a virus, something horrible. And  
5     it's got immunoglobulins all around the surface.  
6     This is now ready to be cleared by the system, and  
7     it would be neutralized if it was a hepatitis A,  
8     for instance. And it fixes C3b all over the  
9     surface.

10           This, in itself, does not get eaten by a  
11     macrophage. That doesn't happen. What happens  
12     is, a red blood cell comes along and has CR1 on  
13     the surface, which is known to the blood-bankers  
14     as the Knops antigen. And CR1 then binds to the  
15     C3b on the particle surface, and brings it into  
16     the liver, a little bit the spleen, where mostly  
17     Kupffer cells in the liver then bind to the Fc  
18     receptors, with their Fc receptors, to the  
19     immunoglobulin. And they do this really cool  
20     thing. My graphics are crude, forgive me, but  
21     they eat the thing. And then, the red cell, which  
22     has this Knops antigen hanging off, pops off

1 uninjured. This is not a spherocyte, this is not  
2 an acanthocyte, this is a healthy, biconcave  
3 disk.

4           It is unclear, conflicting data, whether  
5 it releases the C3b, or whether the Knops get  
6 proteolyzed or extruded. But, it is a well know  
7 fact amongst immuno- hematologists that patients  
8 with immune complex diseases such as lupus, who  
9 are Knops-positive to start, become  
10 Knops-negative. They phenotype Knops-negative.  
11 And then once their disease resolves, they go back  
12 to being Knops- positive again, because all the  
13 immune complexes we're giving them are stripping  
14 the Knops, or the CR1 off of their red cells.

15           And there is this thinking that the way  
16 you get immune complex deposition in your tissues  
17 is that you exceed the capacity of this immune  
18 transfer system to clear immune complexes. So it  
19 would not be an inconceivable maneuver, in the  
20 context of IV IG therapy, to measure the Knops  
21 levels on the patient's circulating red cells, to  
22 see, with repeat administration, are we exceeding

1 the capacity of this system to manage whatever  
2 complexes the IV IG may introduce. And, if so, at  
3 that point are we having untoward effects on  
4 tissues -- which may not even present as  
5 hemolysis. It was raised earlier that not  
6 hemolyzing, but going into acute renal failure is  
7 not a particularly good deal either.

8 Okay. So what is an immune complex?  
9 You could say I have an immune complex, I think  
10 that's fair.

11 But, biochemically, an immune complex  
12 is, we start with an antigen -- and this is meant  
13 to be a polyvalent antigen. It's got spots all  
14 over it. And we have our canonical little  
15 Y-shaped antibodies. And, basically, a number of  
16 things could happen stoichiometrically. You could  
17 have so much antigen around that every antibody  
18 just binds one. And you can have so much antibody  
19 around that there's only one hanging on.

20 Or, you can have a zone of equivalence  
21 where this thing forms. This is really famous in,  
22 you know, RPR testing, where someone comes to you

1     and says, "Doc, you know, I went and I fornicated  
2     with a prostitute, and I got this chancre, but it  
3     went away, and I wasn't worried about it. Now  
4     I've got this rash on my hands and feet. What do  
5     you think it is?" And you do their RPR and it's  
6     negative. And you go, oh, my God, what's going  
7     on? And then you call the lab, and they dilute it  
8     out, and it turns positive. So this is present in  
9     all agglutination-based assays, and it's present  
10    inside people.

11               And so the reason I bring this up is,  
12    when you're pushing IV IG into an individual, and  
13    there is something that the antibodies are  
14    recognizing, if you get those antibodies high  
15    enough, they may do nothing at first because  
16    they'll be in a postzone state. And then as  
17    they're slowly cleared, they'll enter a zone of  
18    equivalence and then do something.

19               And that's the only explanation that  
20    come to me about why you may have hemolysis three,  
21    four, five days after giving IV IG. That's wild  
22    speculation with no data to support it. Thank



1       you.

2                   So, this is a -- but there's no data to  
3       refute it, either. There are just no data.

4                   This is an immune complex as I would  
5       conceive of it. And what I want to impress upon  
6       everybody is that this is not a symphony of  
7       biology, this is a cacophony of the immune system.  
8       Because you have all different types of IgGs -- 1,  
9       2, 4, 3 -- in different post-translational states,  
10      in glycosylation, fucosylation -- with C3 on them  
11      in different states, some of it C3b, some iC3b,  
12      some C3dg -- which is now inhibitory. That's what  
13      we're talking about when we're talking about  
14      immune complexes and immune complex disease.

15                  So this is known as Type III  
16      Hypersensitivity. The immune complex is deposited  
17      in your tissue. And I've already talked about the  
18      Knops. In the interest of time, I'll kind of keep  
19      moving along.

20                  This may occur if the nature of immune  
21      complex prevents clearing by normal methods,  
22      either by exceeding the capacity or by damaging

1     it, or by altering it -- as we've heard about with  
2     inflammatory pathologies. And it can affect  
3     different tissues very differently -- again, as  
4     IgG subtype composition.

5             So, this is a figure adapted from a  
6     review article in 2007, with some provocative data  
7     that, based on the IgG subtype, there's a  
8     correlation with the target tissue that's  
9     affected. And that's just an empirical  
10    observation.

11            How do we detect immune complexes if  
12    we're concerned that they might be in our  
13    products, and thereby causing a problem  
14    independent of anti-A and anti-B?

15            Well, the immuno-histology -- so, this  
16    is a well established technique, were you take  
17    secondary antibodies and stain either frozen or  
18    fixed tissues. Circulating insoluble immune  
19    complexes can be detected -- we're very familiar  
20    with cryoglobulins -- but also, you can detect  
21    them in situ, and you can use C1Q binding  
22    activity. I think Don Branch was introducing that

1 the action-end of the molecule hits C1Q when it  
2 first activates complement. C1Q binding activity  
3 may be a bit more functionally related.

4 The effector function of the IC is,  
5 well, they activate complement. And there's 25  
6 different proteins and membrane proteins. They  
7 can attract phagocytes, as I already talked about,  
8 either through complement receptors or Fc-gamma  
9 receptors.

10 They can cause all kinds of things of  
11 those macrophages, monocytes. They can eat them.  
12 They can sit there. They can kind of burp up  
13 proteases on them. They can be induced to  
14 undergoing ptosis. And immune complexes can  
15 regulate cytokine networks as a function of their  
16 presence. So -- either way.

17 So, again, I don't mean to be, like,  
18 ahh, anything can happen, but there are  
19 descriptions of a lot of different outcomes of  
20 immune complexes, probably because, as I indicated  
21 in an earlier slide, they are a complex mixture of  
22 all sorts of things.

1           Chronic immune complex exposure can  
2    cause blood platelets to become thrombocytopenic.  
3    And, certainly, HIT is a related pathology.

4           Classically -- and, here, I can tell you  
5    we don't have tons of immune complexes in our IV  
6    IG, because serum sickness, and the Arthus  
7    reaction, which were first described in 1903 by  
8    repeatedly given a rabbit, you know, a horse  
9    serum, and it would get sick. These things are  
10   well described vasculitides which don't happen to  
11   people to whom we give IV IG -- at least I'm not  
12   aware of anyone reporting them -- in which case I  
13   think we can conclude that what we're putting into  
14   people is not just some big immune complex  
15   mixture.

16           There is a model of acute lung injury  
17   that involves immune complexes in animals. But it  
18   necessitates aspiration of immune complexes  
19   intratrachially. So, by raise of hand, who gives  
20   IV IG through the lung? C'est bon.

21           All right. So, I want to call your  
22   attention to a couple papers which might catch

1     your eye on PubMed, if you were investigating this  
2     topic. And the first one is "Immune complex-like  
3     moieties in immunoglobulin for intravenous use, IV  
4     IG, bind complement and enhance phagocytosis of  
5     human erythrocytes." And so this figure here,  
6     which hopefully is visible to you, is taking  
7     basically red cells and incubating them with IV  
8     IG, and then looking at their phagocytosis, and  
9     looking at their complement -- or just giving them  
10    complement, or giving them immune complexes.  
11    These are tetanus-tetanus toxoid artificial immune  
12    complexes. But if you combine the IV IG with the  
13    complement, or the artificial immune complex with  
14    the complement, not only do you greatly increase  
15    the deposition of complement on the erythrocytes  
16    in vitro, you also increase the phagocytosis by a  
17    monocytic line sitting in the culture.

18           So this paper, which was published in  
19    '98, gave rise to the speculation that the IV IG  
20    preps themselves have immune -- you know, we're  
21    taking immunoglobulin, which is normally a soluble  
22    protein and making it into a cake. And so,

1        basically, they complex themselves. And then when  
2        we infuse them, those immune complexes can cause  
3        erythrocytic damage independent of any antigen  
4        specificity for a target on the red cell itself.

5                Now, the follow-up paper, which has a  
6        very provocative title: "In vivo administration  
7        of intravenous immunoglobulin can lead to enhanced  
8        erythrocyte sequestration." These authors -- and  
9        to their credit, English is not their first  
10       language -- I think they're using "sequestration"  
11       here differently than we might use it. They don't  
12       mean hanging out in the spleen and coming back.  
13       They mean, as best I can tell, just going away.

14               And despite their title, I have to tell  
15       you I'm pretty skeptical of the description of  
16       their own data. So this is just a size exclusion  
17       fraction column, and so the bigger things come off  
18       first. And so when they put their IV IG prep on  
19       their they saw this percentage of the fraction  
20       they're calling, you know, immune complexes, or  
21       heavier stuff. This would be the more monomeric  
22       forms. And when they took these two fractions and

1 did that same assay I just described to you, sure  
2 enough, this heavy fraction has the majority of  
3 the complement-fixing activity, compared to the  
4 light fraction. Okay that's fine.

5 But then they took their patient  
6 population. And I owe an apology to the panel  
7 this morning. I was mistaken.

8 I went back and read this paper at  
9 lunch. They did not give IV IG to healthy  
10 volunteers. They took red cells from healthy  
11 volunteers and exposed the IV IG in their in vitro  
12 phagocytosis assay, and so no phagocytosis in  
13 those patients.

14 But if you look at the patients here,  
15 what you're looking at -- these are their age,  
16 gender, their blood group, their hematocrit or  
17 hemoglobin prior to IV IG, their post-IV IG  
18 expected hematocrit or expected hemoglobin, based  
19 upon hemodilution of the volume of the IV IG, and  
20 then what was actually observed.

21 So their statement in the paper was,  
22 four of the seven patients examined, P1, 4, 5, and

1       6, showed marked red cell sequestration subsequent  
2       to IV IG treatment.

3               Well, okay, when I look at -- we'll do  
4       hematocrit, if no one minds -- when I look at P1,  
5       their expected versus their observed, P4, their  
6       expected versus their observed, P5, P6, there may  
7       be a subtle effect here. I think "marked" is a  
8       grotesque exaggeration. And I don't know that  
9       this isn't within noise of what they'd be seeing  
10      in their other patients. There is no correlation  
11      here to ABO type.

12              So, I'd like to posit that this paper is  
13      misleading, unless I'm misreading it. I read it  
14      multiple times, and I couldn't really find any  
15      other explanation. And that, at least in this  
16      context of what they are proposing, I don't see  
17      any in vivo evidence of this occurring in the  
18      literature. In vitro, in the macrophage system,  
19      it clearly does occur, and they've reported that  
20      multiple times.

21              So, I'd like to finish up with a brief  
22      story about a phenomenon that happens in



1 incompatible transfusion, which has been called  
2 "antigen suppression," or "weakened antigenicity,"  
3 or "antigen loss," or "antigen modulation" --  
4 because it resembles the immune transfer reaction,  
5 and it's a very interesting biology.

6           So this is a case -- and I'll go through  
7 it quickly, because I want to stay on time -- a  
8 34-year-old male presented with severe ITP and  
9 pronounced bleeding, resulting in a severe  
10 decrease in hemoglobin. This is was in British  
11 hospital, the spelling, you know. No evidence of  
12 hemolysis was found, and the patient's DAT was  
13 negative was negative for IgG.

14           So, due to the symptomatic anemia, they  
15 transfused. All units were cross-matched and  
16 compatible at that time. And when they typed the  
17 sera, they found an antibody against a high  
18 incidence Kell antigen. And the patient typed as  
19 K, Kell-negative, Cellano negative, Kp a and b  
20 negative, which would be consistent with a  
21 Kell-null phenotype. Hadn't been exposed to  
22 blood, had made a broad (inaudible), right? No

1       problem.  It's rare, but that's predictable.

2               So, they got cryopreserved stocks from  
3       the national reserves, and they gave them to him.  
4       These were Kell-null stocks, with steroids, IV IG,  
5       vincristine -- not being subtle.  And the patient  
6       recovered and entered a full remission.  And his  
7       anti-Kell went away.

8               So they said, great, we'll collect his  
9       blood to put back into storage in case he has a  
10      relapse.  But at that time, the Kell typed, the  
11      patient typed Cellano-positive, Ku-positive, Kp b  
12      positive.  Eh?

13              A year later, the patient returned again  
14      with severe thrombocytopenia and hematuria,  
15      resulting in symptomatic anemia.  No hemolysis  
16      here, mind you, just urinating out the blood.  And  
17      there was no evidence of hemolysis at all.  And he  
18      had erythroid hyperplasia in his marrow, as you  
19      might expect.

20              So they took his old units that they had  
21      frozen previously, which were now cross-match  
22      incompatible with him.  The antibody was back.  It

1        recognized his old units from his relapse. But  
2        having nothing else to do, they pushed all four  
3        units in. And he did great. There was no  
4        hemolysis. He did wonderfully well.

5                    And so what you're seeing here is an  
6        auto-antibody causing the loss of ability to  
7        detect an antigen on the surface -- in this case,  
8        Kell -- very much like the CR1 story I told you,  
9        with the Knops antigen. And so this appears to be  
10       a basic red cell phenomenon that happens in this  
11       context of immune complexes.

12                   They had the good sense to collect  
13       specimens for Western Blotting while they were  
14       going. And I won't belabor the point, but if you  
15       look at an N-terminal, when he was relapsed, the  
16       whole protein is undetectable. When he was in  
17       remission, it's there. So it's unclear what's  
18       happening to it, but it's not just capping with  
19       immunoglobulins and changing in a way.

20                   This has happened in multiple blood  
21       group systems. This is a review article from  
22       2009, by Kell, Kidd, Rh C ande, Gerbich, Cromer.

1       So, basically, this is an underappreciated  
2       phenomenon that occurs when antibodies bind to red  
3       cells.

4                   And this is my sort of narcissistic  
5       slide. We stumbled onto an animal model of this  
6       some years ago, and these are a list of  
7       publications -- which, obviously, you're all going  
8       to write down in their entirety -- based upon  
9       mechanistic work we did in the animal model, which  
10      indicates that the mechanism of modulation here is  
11      very similar to the Knops story. It requires  
12      multiple antibodies' binding and cross-linking,  
13      and ligating an Fc receptor in a similar way --  
14      and complement (inaudible) was involved.

15                  So -- conclusions. So, immune complexes  
16      are a diverse population of multimolecular  
17      aggregations. And it's crazy, right, that slide I  
18      showed, they change in composition over time. The  
19      IgG components of them change, the fucosylation's  
20      going to change, the status of the C3 is going to  
21      change. And all of this in the context of patient  
22      polymorphisms, so complement receptors, patient

1 polymorphisms of Fc receptors.

2           There are repots of altered efficacy of  
3 IV IG. And, by the way, (inaudible) complexes are  
4 part of the efficacy, and altered toxicity based  
5 upon this composition. But I find them personally  
6 unconvincing, and perhaps we can discuss that.

7           And so the effect of immune complexes  
8 remains superficially analyzed and/or absent in IV  
9 IG preparation.

10           Sorry for speaking so quickly. Thank  
11 you for your attention. (Applause.)

12           DR. BUSSEL: So, are you rejecting the  
13 hypothesis completely that anti-A and anti-B have  
14 anything to do with this?

15           DR. ZIMRING: Of course. Of course not.  
16 I think that what I am saying -- first of all, the  
17 fact that they may have an effect at high titer  
18 that you may mitigate or get rid of when you start  
19 to get down to the 1:64, doesn't remove the  
20 possibility that within that range there's  
21 something else going down.

22           All I am saying is that if you accept

1     that hypothesis as your premise, deducible  
2     outcomes that one would predict are not observed.  
3     Now, that may be because the assay is flawed, and  
4     so you're actually, you're not observing what you  
5     think you're observing. Or it may be that there's  
6     another thing going on in addition to it, to wit,  
7     inflammation in the recipient, or polymorphisms,  
8     or whatnot -- or the base hypothesis is wrong.

9             But I think it is fair to say that if  
10    you line up a thousand A people -- and let us  
11    assume for a minute that those A people all have  
12    roughly the same amount of A on their red cells,  
13    within a range. And you give them all the same  
14    lot of IV IG, some are going to hemolyze and some  
15    aren't. Well, if you're keeping those two  
16    variables constant, then there either is a third  
17    thing going on, or that ain't it.

18            It may be -- my colleague next to me,  
19    his name I'm blanking on, I'm sorry -- suggested  
20    that the rates of administration vary, how it's  
21    given varies, all kinds of things vary. But I  
22    would submit to you that the simple hypothesis

1 "It's anti-A, and that causes hemolysis," doesn't  
2 hold with the data we have. That's what I was  
3 (inaudible).

4 DR. GOLDING: Can I just clarify? You  
5 know, you obviously look carefully at the immune  
6 complexes, and possible complement activation.  
7 But, in reviewing the literature, and in hearing  
8 the talks that you heard today, I didn't hear much  
9 -- I was surprised, I didn't hear any evidence  
10 that there's a clear indication that the  
11 complement system is being activated in the cases  
12 of hemolysis that have been observed.

13 And does that imply that very little or  
14 no intravascular hemolysis is occurring? That all  
15 of the hemolysis is extravascular?

16 DR. ZIMRING: Well, first of all, the  
17 complement system can promote either intravascular  
18 hemolysis through the membrane attack complex, or  
19 extravascular hemolysis through a complement  
20 receptor ligation. And so the presence of the  
21 complement is not demonstrative of either pathway.

22 The main assay used here is C3 staining

1       on the erythrocyte. And it appears to be very  
2       present in some cases, if I'm not mistaken. But I  
3       would posit the following statement to you, which  
4       I think gets back to something Don was talking  
5       about earlier: You cannot measure the events that  
6       lead to a red cell's clearance by analyzing  
7       circulating blood. Because if it's still  
8       circulating, it hasn't cleared yet.

9                So, the absence of complement is equally  
10       consistent with complement not being involved, or  
11       the complement-coated cells' being the ones that  
12       are cleared, and therefore you haven't observed  
13       them -- right? I mean, so if the observation is  
14       equally compatible with incompatible hypotheses, I  
15       don't know exactly where to take that, if that's  
16       your only assay.

17               DR. GOLDING: But if the complement  
18       system was very important here, there are soluble  
19       factors that get released when complement gets  
20       activated --

21               DR. ZIMRING: Yes.

22               DR. GOLDING: -- and you should be able



1 to measure something in the plasma that shows  
2 that. And apparently people are not finding it.

3 DR. ZIMRING: I have not seen people  
4 looking. And maybe someone would care to correct  
5 me, but I'm unsure -- I don't know of anyone  
6 testing complement-released peptides or factors in  
7 this context. Anyone? Yes?

8 DR. PENDERGRAST: In our prospective  
9 study we were looking at just, you know, straight  
10 C3 and C4 levels, before or after IV IG  
11 administration, and comparing between the ones who  
12 clearly hemolyzed and the ones who didn't. And C3  
13 dropped a little bit more in the ones who  
14 hemolyzed than the ones who did not hemolyze. But  
15 it was a very small amount.

16 It was a difference -.2 grams per liter  
17 versus -.15 grams per liter, between the  
18 hemolizers and the non-hemolizers.

19 So, I don't know if that tells you  
20 anything.

21 DR. ZIMRING: Can't C3 also be an  
22 acute-phase reactant in a way? And so if you get

1       --

2                   DR. PENDERGRAST:  Absolutely.

3                   DR. ZIMRING:  Right.  So, if you  
4   activate, and it drops, and it rises -- a  
5   steady-state snapshot, it's hard to tell.

6                   DR. PENDERGRAST:  Yes, yes.  Fair  
7   enough, fair enough.

8                   DR. BUSSEL:  Just one more question  
9   about the delay.  I think it's fairly clear,  
10   though I don't know if you would disagree with  
11   this also, that an ITP, if you give IV IG, you  
12   slow the clearance of antibody-mediated platelets.

13                   I would more than agree that IV IG, as a  
14   treatment of autoimmune hemolytic anemia, is much  
15   less effective.  But in some of the cases of  
16   delayed hemolysis, don't you think one of those  
17   mechanisms -- up-regulation of inhibitory Fc  
18   receptor -- something might be occurring that  
19   causes the hemolysis to be delayed?

20                   DR. ZIMRING:  Absolutely.  And thank you  
21   for that point.  That would be one of these  
22   additional variables I am suggesting we have to

1     invoke.  Because, you know, WinRho, for instance  
2     -- right?  So if you give WinRho to someone, they  
3     can have a hemolytic sequelae of injecting WinRho.  
4     How often to see that five, six, seven, eight,  
5     nine days after you gave the injection?

6             DR. BUSSEL:  You don't see it acutely,  
7     but when you give WinRho, you're basically still  
8     seeing a steady hemolysis a week later.  There's  
9     data on that.  And the only reason the hemoglobin  
10    isn't still falling is that you (inaudible) retic.

11            DR. ZIMRING:  In the context of the IV  
12    IG, the kinetics seem to be different to me.  But,  
13    you're right, it could be (inaudible).

14            DR. WATSON:  I think, in the case that  
15    was described this morning, where they were  
16    talking about hemolysis occurring three days  
17    later, I suspect what they were really talking  
18    about was somebody who was getting IV IG for four  
19    days in a row.  And they were saying there was no  
20    hemolysis after the first fraction of the IV IG  
21    dose, but as they gave the three-fourths rest of  
22    the dose, then the accumulating IgG tipped it

1 over.

2 It's not something that was given on day  
3 one --

4 DR. ZIMRING: Okay.

5 DR. WATSON: -- and hemolysis was  
6 observed on day four. It was given on day one,  
7 two, three, and four -- I suspect.

8 DR. ZIMRING: You're probably right --  
9 up to 10 days, even. The range I saw was up to 10  
10 days, that they were counting hemolysis up to 10  
11 days as a sequelae of IV IG administration.

12 Is Dr. Berg here? Would you care to  
13 comment on that? Because I think those were in  
14 your presentation?

15 DR. BERG: Yes, actually our data  
16 referred to "latency." So, between the last  
17 administration of IV IG and onset of the  
18 hemolysis. So that was seen either -- in a couple  
19 of cases, within 24 hours. But then, as you  
20 correctly said, in a large part of the series,  
21 between 3 days and 14 days.

22 DR. ZIMRING: After the last

1 administration.

2 DR. BERG: After the last  
3 administration.

4 DR. ZIMRING: Yes, so that was the basis  
5 of my concern. Yes.

6 SPEAKER: You have to know how often  
7 those patients were tested for hemolysis.

8 DR. ZIMRING: How frequently were they  
9 tested?

10 DR. BERG: I think the difficulty here  
11 is that spontaneous report. So --

12 DR. ZIMRING: Okay. So we don't --

13 DR. BERG: We don't really have that  
14 clear data.

15 DR. ZIMRING: -- that's a very good  
16 point.

17 DR. BERG: It's not a clinical trial.

18 DR. ZIMRING: Right.

19 DR. SCOTT: I'd like to introduce Dr.  
20 Luban, who's going to speak next, and is the last  
21 speaker before our panel discussion, unless --  
22 well, we may have one more slide actually coming

1 from somebody else.

2 But I want to thank you for coming here  
3 to talk about your clinical cases and insights  
4 that they may or may not shed on the underlying  
5 cause of hemolysis.

6 DR. LUBAN: So, I'm going to take you  
7 all now away from spontaneous reporting, insurance  
8 information, and take you right into a hospital  
9 setting, where you can see the nitty-gritty of  
10 daily serial evaluation of hemolysis in a group of  
11 children.

12 All of these children suffered from  
13 something called Kawasaki's Disease. You've seen  
14 that reported on almost all of the first series of  
15 slides today. And you may not know what it is.

16 It is an idiopathic, multi-system  
17 disease which is characterized by vasculitis, not  
18 only of arteries, but also of veins and  
19 capillaries. It has an incidence of 10 to 15 per  
20 100,000 children, all under the age of five years.  
21 That's part of the classification of Kawasaki's.

22 It's etiology is unknown. It is thought

1 to have something to do with an abnormal immune  
2 system activation. And it may be triggered by  
3 either viruses, bacteria, toxins, super antigens,  
4 or other infectious agents. However no specific  
5 infectious agent has ever been clearly  
6 demonstrated.

7 It does have both seasonality and  
8 epidemic types of presentation, which has led some  
9 people to compare it to Parvo B19. It also has  
10 substantive geographic clustering.

11 In very few studies, maybe only two,  
12 there has been thought to be a genetic  
13 predisposition through the ITPKC pathway, which  
14 results in aberrant T-cell proliferation and  
15 cytokine overproduction.

16 Most critically, 20 percent of KD  
17 patients develop coronary artery aneurysms,  
18 myocarditis, and significant other types of  
19 cardiac dysfunction. And, in fact, this is the  
20 only cause of myocardial infarction in infants and  
21 children. And it's the leading cause of acquired  
22 heart disease in childhood.

1           There are very established diagnostic  
2   criteria for classic KD, and they are included  
3   here. One of the hallmarks of the disease is  
4   extraordinarily high fever, with four out of five  
5   additional clinical features. There's also a  
6   phenomenon of incomplete or atypical KD, and this  
7   is more common in the younger children, ages two  
8   or younger, where the incidence of aneurysm and  
9   long-term adverse outcome is significantly worse.

10           Here are some photos of children with  
11   KD. There is, as you can see here, conjunctival  
12   injection, something called the "strawberry  
13   tongue." There is erythema of the soles. There's  
14   desquamation. A very, very typical rash, unusual  
15   swelling of the hands and of the feet. And a  
16   swelling also of the lips.

17           And so for those of you in adult  
18   medicine, you might compare this to, for example,  
19   Stevens-Johnson-like syndromes, or other kinds of  
20   vasculitides. And also, a very, very typical,  
21   very bizarre and unusual rash, which is the  
22   hallmark of the disease.



1           There are very limited hematologic  
2   findings in KD, usually a leukocytosis with a left  
3   shift. Spherocytes, but no frank microangiopathic  
4   findings. Thrombocytosis. Anemia is not  
5   classically reported, although there are a few  
6   cases of what has been called "autoimmune  
7   hemolytic anemia" in KD that date from the '90s.

8           Acute management is very interesting,  
9   particularly in view of what we've been discussing  
10  here today. Obviously, taking care of the heart  
11  is primary. And then high-dose aspirin -- and I  
12  would just point out to you, this is  
13  extraordinarily high dose aspirin. This is 80 to  
14  100 mg/kg per day. The purpose of the aspirin is  
15  to reduce fever, joint inflammation, and to treat  
16  pain. Some have argued it also inhibits platelet  
17  adhesion. And usually you then reduce the dose to  
18  six to eight weeks if there's no aneurysm, or the  
19  children are on aspirin indefinitely if cardiac  
20  abnormalities persist.

21           More pertinent to our discussion today  
22  is the use of IV IG. Recommended are 1 to 2 grams

1 per kilo per day, and the rationale for this is  
2 based on a series of theoretical actions of IV IG  
3 -- very few of which have ever been fully  
4 evaluated in patients. Most of this is from  
5 animal work.

6 Presumably the IV IG decreases  
7 TNF-alpha, IL1a, and IL6, downregulates  
8 chemokines, neutralizes super antigens should that  
9 be the triggering agent, and inhibits platelet  
10 adhesion. Others have suggested that there are  
11 other anti-apoptotic responses, but the exact  
12 nature of those are very poorly studied.

13 Probably the most critical thing for  
14 this group to recognize is that if the fever does  
15 not defervesce, then a second dose of 2 grams --  
16 and that's a typo -- 2 grams per kilo is often  
17 administered. And this occurs approximately 20  
18 percent of the time. There is some so-called "IV  
19 IG resistance" that's been reported in a few  
20 patients.

21 Unfortunately, there is no specific  
22 inflammatory marker profile that one can use and

1     apply to see if you need to re-treat. And so it's  
2     all done on the clinical basis of fever  
3     defervescence or not.

4             So, now I'm going to go through in  
5     chronologic order a series of patients that we've  
6     had at the Children's Hospital. And I think one  
7     of the things that you'll note as I go through  
8     these case reports is some of the information that  
9     you found lacking when you were trying to evaluate  
10    certain elements of the hemolysis, that you could  
11    not get because you didn't have specific hospital  
12    records.

13            So, this 16-year-old girl with suspected  
14    KD was admitted from an outside hospital after  
15    prolonged fever and cardiac dysfunction. She was  
16    given IV IG twice, three days apart. The first  
17    dose was 2 grams per kilo, the second dose 1 gram  
18    per kilo. And she had a weight of 58 kilo. The  
19    product brand and lot numbers are not known. They  
20    were not available from the outside hospital.

21            Our hematology team was consulted to a 4  
22    gram per DL drop in hemoglobin over 48 hours. To

1     see that plotted out, you can see here, I think,  
2     her first dose of IV IG, her second dose of IV IG,  
3     and you'll notice that her hemoglobin fell to  
4     approximately 5-1/2 grams, and she was transfused.

5             You'll also notice, initially a  
6     low-grade reticulocytosis, followed by a brisk  
7     reticulocytosis over four days, which were  
8     approximately six days following the first  
9     administration.

10            Her blood bank evaluation revealed that  
11     she was AB-positive, 2+ IGG, C3-negative. And we  
12     did, for a number of reasons, do an antibody panel  
13     for cold agglutinin, which was negative. Her  
14     antibody screen was negative, and she did not have  
15     another antibody in her plasma.

16            The second patient, a four-year-old girl  
17     with suspected KD was admitted from an outside  
18     hospital for management of hemolysis. She was  
19     given IV IG twice, one day apart. Her first dose  
20     was much less than what's recommended, it was 0.6  
21     grams per kilo, and then a second dose  
22     administered one day later. Here, Privigen was

1 the product that was given at the outside  
2 hospital.

3 Hematology again was consulted due to a  
4 drop in hemoglobin in the setting of IV IG  
5 infusion. Here, once again, we can see the IV IG  
6 administered, and then a dramatic drop in  
7 hemoglobin, again, from approximately 8.9 all the  
8 way down to I think it was about 6.2. And  
9 recrudescence of her hemoglobin following the  
10 transfusion. And, once again, a reticulocytosis  
11 which is somewhat delayed by about six to seven  
12 days.

13 Throughout all of these cases we  
14 actively looking for hyperbilirubinemia -- in many  
15 cases, LDH haptoglobin and hemoglobinuria, and did  
16 not find it.

17 And so one of the statements that I  
18 would like to make is I believe, at least in the  
19 setting of KD, we are not dealing with  
20 intravascular hemolysis but, rather, extravascular  
21 hemolysis.

22 Blood bank evaluation, similar to the

1 previous patient. You'll also notice that these  
2 two patients came in at about the same time, from  
3 two entirely different hospitals. Once again,  
4 DAT, IgG 3-plus positive, C3- negative. And this  
5 patient was Group A1.

6 Third patient, a little bit of a more  
7 unusual case, seven-year-old with Moyamoya Disease  
8 and several strokes, not a sickle-cell patient,  
9 with a history of unexplained vasculitis, and to  
10 rule out KD. She presented with severe abdominal  
11 pain, rule out acute pancreatitis. And I would  
12 also note that gallbladder hydrops is a  
13 complication of KD. And that was one of the  
14 rationales for the administration of IV IG -- in  
15 this case, Gammagard.

16 Once again, you can see here IV IG  
17 administered, substantive drop in hemoglobin, red  
18 cell transfusion when the hemoglobin gets to be  
19 around 6 or 7. Luckily, a good response to  
20 transfusion and maintenance. Here again, you can  
21 see a reticulocytosis.

22 Again, IgG 3, C3-negative. Eluate had

1 anti-A1, and she was Group A1.

2 Our fourth patient is our saddest  
3 patient, a three-month-old with suspected KD,  
4 presented at the hospital after five days of high  
5 fever, reaching 104 degrees. IV IG was given  
6 twice over 24 hours, the first dose 2 grams, the  
7 second dose 2 grams -- in this case, Gamunex.

8 Hematology service is consulted this  
9 time due to progressive anemia and thrombocytosis.  
10 You'll note here the IV IG is administered, and  
11 then you see a transfusion, a consistent fall,  
12 multiple red cell transfusions, down to a  
13 hemoglobin of 5 in some cases. And once again, a  
14 reticulocytosis.

15 Her blood back evaluation was quite  
16 complicated, and I won't go into all of this,  
17 except to say that this is the only patient that  
18 we had that also had C3 binding. You'll also  
19 notice, strangely enough, some anti-D there -- and  
20 where did we get the anti-D? We're not really  
21 sure. She did present with the anti-D, so it's  
22 not from the IV IG, per se.

1                   She was transfused seven times with red  
2   cells, one time with FFP for coagulopathy. She  
3   had multiple bone marrow aspirates and biopsies  
4   which were non-diagnostic. Viral, bacterial,  
5   protozoal, and a full immunologic workout looking  
6   for HLH, primary immune deficiency disease, and  
7   infantile lupus. All of those disorders were  
8   ruled out by virtue of the evaluation.

9                   She developed progressive  
10   hyperbilirubinemia, hyperferritinemia,  
11   multi-system organ failure, and she died in  
12   February. An autopsy was performed and  
13   demonstrated brisk erythrophagocytosis. This is  
14   the kind of thing that you see in hemophagocytic  
15   lymphohistiocytosis. She did not have HLH. There  
16   is genetic, as well as immunologic assays for  
17   that, and those were all performed and were all  
18   negative.

19                  This is her autopsy, and her autopsy  
20   looked like hemophagocytic lymphohistiocytosis by  
21   virtue of -- and I can show this to you -- again,  
22   marked areas of ingestion, macrophage ingestion of



1 red cells. And I think you can see many of those,  
2 in particular, right here.

3 And the last patient is a 10-month-old  
4 boy admitted after four days of fever. Again, two  
5 doses of IV IG -- in this case, Gamanex.

6 Hematology consulted this time for high white  
7 blood cell count and anemia.

8 A very, very similar story, many fewer  
9 retics done on this patient. Again, a  
10 transfusion. IgG 2-plus positive, anti-A1 eluted,  
11 and an A1 type.

12 In summary, what we see is very, very  
13 much more than a 1 or 2 gram drop in hemoglobin.  
14 I think you can see those here. In some cases, a  
15 4 gram drop in hemoglobin.

16 We see almost all of the patients being  
17 AB or A. We see elution of anti-A, anti-A1,  
18 except for our one strange case here, who will  
19 remain strange forever.

20 All of these cases required transfusion.  
21 Some had brisk spherocytes on smear, some did not.  
22 All except one patient had an extraordinarily high

1     reticulocyte count. And, as I mentioned, data not  
2     shown, no evidence of intravascular hemolysis.

3             So, I think our case series adds to a  
4     small published literature in the field. WE are  
5     presenting here dose-dependent hemolysis caused by  
6     IV IG, with severe anemia requiring transfusion.  
7     With most of our patients being A, A1, or AB, with  
8     extravascular hemolysis, frank  
9     erythrophagocytosis, and documented secondary  
10    hemophagocytic syndrome, and death in one case.

11            I would like the group to recognize that  
12    this is an under recognized complication by  
13    pediatricians and cardiologists, who are the  
14    primary treaters of KD. Without a hemovigilence  
15    system in the U.S., I'm sure that we are  
16    dramatically under-reporting these cases.

17            I would also point out that the warnings  
18    about hemolysis are not listed in the American  
19    Academy of Pediatric Red Book, which has an  
20    extensive section on Kawasaki's Disease -- and  
21    that is one of the bibles that pediatricians go  
22    to.

1           Case report publications, such as they  
2   are in the literature -- and there are about six  
3   of them -- are all in sub-specialty journals.  
4   They are not in pediatric journals, and they are  
5   not in cardiology journals.

6           Firm recommendations for serial  
7   hemoglobin and hematocrit measurements, especially  
8   after multiple dosing, is not widely disseminated.  
9   And, in my opinion, doing something like a  
10   black-box warning, without adequate education,  
11   will not bring to the fore more information on  
12   this disorder.

13           So, we'd like to acknowledge one of our  
14   PATH residents who did most of the digging on the  
15   case reports, Edward Wong, Phil Pary, our children  
16   and their families.

17           Thank you.   (Applause.)

18           DR. BRANCH:   Question -- on the patient  
19   with the mysterious anti-D, that was an  
20   Rh-negative patient?

21           DR. LUBAN:   That was an Rh-positive  
22   patient.

1 DR. BRANCH: So this could have been the  
2 beginnings of an autoimmune hemolytic anemia.

3 DR. LUBAN: Well, the mom was  
4 Rh-positive. So we thought initially that perhaps  
5 this could have been a case of maternal transfer  
6 -- although three months is quite late for  
7 maternal transfer of anti-D. And our other  
8 thought was perhaps this was somebody with a  
9 primary immune deficiency disease, like IPEX  
10 disease, where you do see an autoimmune component  
11 to the complex of IPEX. But we were never able to  
12 document that.

13 DR. BRANCH: So, the mother had anti-D,  
14 right? From the get-go?

15 DR. LUBAN: Correct.

16 DR. BRANCH: Titer probably not  
17 important.

18 DR. LUBAN: No, not titer. Mother  
19 breast-fed.

20 DR. BRANCH: Yes. I just have one other  
21 question. Presumably you gave whole red cells to  
22 the patient.

1 DR. LUBAN: Correct.

2 DR. BRANCH: But the hemoglobin kept  
3 dropping.

4 DR. LUBAN: Right.

5 DR. BRANCH: Did you rule out PCH?

6 DR. LUBAN: PCH, in a three-month-old,  
7 I've never seen -- and, no, we did not.

8 DR. BRANCH: So you didn't look for an  
9 IgG hemolysis.

10 DR. LUBAN: No.

11 SPEAKER: 27:20: What was the time,  
12 diagnosis of anemia after the administration of IG  
13 in those cases?

14 DR. LUBAN: So, when we re-do our table  
15 for publication, which is in process, I believe  
16 what you'll see is that it's three to five days  
17 following the administration when you get the  
18 nadir hemoglobin. So it goes along with much of  
19 what has been discussed here in the last few days.

20 It is not immediate -- for sure, it is  
21 not immediate. I think what I'd like this group  
22 to recognize is that you're starting, with most

1 kids, at a lower hemoglobin.

2           You're going to have hemodilution from  
3 the IV IG, which is going to bring it lower. In  
4 KD you are also going to have a component of an  
5 inflammatory-induced anemia. And that is just a  
6 very, very bad combination, in particular, if you  
7 have carotid artery aneurysms.

8           So the tendency is to transfuse these  
9 kids at around 6-/12 to 7, and not let them drop  
10 any lower than that.

11           DR. BUSSEL: How do you know that the  
12 erythrophagocytosis was secondary, and that it  
13 wasn't HLH as the primary underlying disease?

14           DR. LUBAN: I mean, HLH was our first  
15 diagnosis in this child. And so, she had a quite  
16 substantive evaluation, with T-reg, every test  
17 available at the University of Cincinnati,  
18 Cincinnati Children's Immunology, and she had  
19 genetic testing, all of which was negative.

20           I might point out that that child was  
21 Egyptian, and her parents were second cousins.

22           DR. GOLDING: Can I ask a quick question

1 before you go?

2 So, I mean, the drops in hemoglobin were  
3 pretty dramatic, but am I right in saying you said  
4 that there was no evidence of intravascular  
5 hemolysis, there was no drop in haptoglobin, there  
6 was no hemoglobinemia, no hemoglobinuria, and  
7 there was no evidence of renal disorder?

8 DR. LUBAN: There was no frank  
9 intravascular hemolysis. The only case that had  
10 dramatically elevated bilirubins was the case of  
11 the erythrophagocytosis, and that patient had a  
12 gall bladder hydrops. Her bilirubin went up to  
13 about 35. So that case is a very discrepant case  
14 among our case series, but I put her in there  
15 mostly because of the high fever, the presumptive  
16 KD, and the treatment with IV IG for the  
17 presumptive diagnosis.

18 But hemoglobinuria, frank  
19 hemoglobinuria, none of these kids had frank  
20 hemoglobinuria.

21 DR. SCOTT: I'd like to invite the  
22 panelists, as well as Dr. Virata and Dr. Lynch up

1       so that we can address the questions that we have  
2       for this session -- and others.

3               So, speaker questions that you held back  
4       on, I think it would be reasonable to ask some of  
5       those. We have about half an hour, should it take  
6       so long.

7               But it's certainly daunting, the  
8       complexity of these cases, and the bits of  
9       information that we have are very difficult to put  
10      together. Nevertheless, we do have some questions  
11      here. And if folks think of other, more  
12      interesting questions, do feel free to ask them.

13              Could we have the question set, please?  
14      The session questions.

15              Well, first, I want to find out if  
16      anyone in the audience has additional questions  
17      for the speakers -- and we have one out there.

18      "Other Product Risk Factors"

19              SPEAKER: This is more a comment than a  
20      question (inaudible) between older generation,  
21      newer generation.

22              DR. BELLAC: Yes, at this point it's



1       just speculation. We don't have any proof for  
2       that. But, the difference between the older  
3       products and the newer are quite striking, so  
4       there must be a difference between this, we think.

5               DR. SCOTT: Well, tomorrow, of course,  
6       we'll be discussing the manufacturing and the  
7       setting of anti-A and anti-B titers also, which  
8       might be a factor. I hesitate now to say that  
9       anything will be a factor, but I think that we  
10      have to consider the manufacturing of the old and  
11      the new products, the methods, as was mentioned,  
12      Fraction 3, and how that's removed, or its  
13      equivalent, how that's removed.

14             We also have differences in excipients,  
15      for example, differences in amounts of aggregates.  
16      There are quite a few differences across the  
17      products -- lyophilized versus non lyophilized --  
18      and other treatments that they may undergo which  
19      would potentially attenuate the strength or the  
20      amount of antibodies.

21             Does anyone on the panel have any  
22      additional questions or observations?

1 DR. LYNCH: Well, if I could, I'd like  
2 to follow up on that point. There are many  
3 differences among products, and I think that has  
4 not been -- that is not clearly understood, but  
5 it's the basis for the clinical preference that  
6 one physician may have for a particular product  
7 for a particular patient.

8 And I think it must go beyond the kinds  
9 of things you mentioned, Dot, that we measure,  
10 that certainly can make a difference in some  
11 settings, but don't write the whole story.

12 So, for example, the newer products  
13 versus the older products, we know they have many  
14 benefits -- you know, lower IgA, IgM contaminants,  
15 lower, you know, complement activity. Whether or  
16 not the old versus new, whether it's the  
17 isoagglutinin levels that are making the  
18 difference. And what we see observationally is  
19 kind of an assumption. It's one variable out of  
20 many that could be a contributing factor.

21 So, you know, what we heard today just  
22 highlights the complexity of the whole issue, and

1       assuming that it's one particular characteristic  
2       and not others is probably something we should be  
3       cautious about.

4               DR. BELLAC:   So, maybe something to add.  
5       There's also a clear difference in terms of dosage  
6       between the old products and the newer ones,  
7       because you wouldn't probably administer a 5  
8       percent immunoglobulin product for a dose of 2  
9       grams per kilogram, because this would result in a  
10      huge volume.   That's why you probably see more  
11      Yes, sir.   With the 10 percent products, because  
12      they are given at the higher dose, compared to the  
13      5 percent product.

14             DR. LYNCH:   Can I disagree with that?   I  
15      just think we administer the dose, and it's nicer  
16      to have less volume.   But I think we, in the past,  
17      before the 10 percents were available,  
18      administered the same doses.

19             I'm not sure exactly when the 2  
20      grams-per-kilo came in for Kawasaki's, but I would  
21      think it was before 10 percent was available.

22             DR. SCOTT:   I believe that would have

1       been for possibility Gammimune, I'm not sure.

2       Definitely for IVGam, which is a product that's  
3       not here anymore, and Gammagard SD.

4               DR. LYNCH:   Yes, it's very, very  
5       difficult.

6               DR. SCOTT:   So, that 2 gram-per-kilo  
7       dose was around.

8               DR. LYNCH:   Yes, it's very, very  
9       difficult to compare rates across different  
10      products, because the utilization may not be  
11      constant.   And certainly, the one thing that we  
12      saw that's unequivocally a risk factor is the  
13      dose, right?   So, if one product is preferred in  
14      these high- dose indications, well, you're going  
15      to see more of these events.

16              We had the opportunity for about a  
17      five-year period to market both an old 5 percent  
18      versus a new 10 percent product in Canada.  
19      Utilization was very similar.   And in that period  
20      of time, when there was heightened hemovigilance  
21      -- I can't see our Canadian colleagues -- but in  
22      that time limit a great increase in importing

1 rates, we didn't see a difference between those  
2 two products in the relative risk.

3 And I think that, you know, you just  
4 have to sort of due that. That's a very unique  
5 situation, because it's also contemporaneous.

6 DR. GOLDING: So, Dot, can I ask -- you  
7 know, the idea that the hemolysis is probably the  
8 Fc part of the antibody that's operating, and  
9 whether it's complement or Fc-receptor binding.

10 And what I would ask is do the different  
11 manufacturing steps have differential effects on  
12 the Fc receptor? And what I would guess, and it's  
13 purely speculation, is that the older  
14 manufacturing may have been a little bit harsher  
15 in terms of especially -- I think Dr. Zimring  
16 mentioned the fucosylation and the sialylation --  
17 that those may be different in the Fc part, and  
18 may interfere with Fc function.

19 As far as I know, we don't, FDA doesn't  
20 look at Fc function as a request for licensure.  
21 But I know the Europeans, some of the Europeans  
22 do.

1                   And I wonder if the European colleagues  
2     have looked at Fc function, and if they've found  
3     any clues in terms of comparing (inaudible)  
4     manufacture versus newer forms of manufacture with  
5     chromatographic methods?

6                   DR. BELLAC: We haven't done studies on  
7     the (inaudible) function.

8                   DR. SCOTT: All right.

9                   DR. ZIMRING: The other variable that  
10    may or not be at play there is that there are --  
11    you know, we talk about polymorphisms, but there  
12    are genetic variants in the Fc portion of  
13    antibodies, called allotypes, that vary with  
14    demographics. And there are variances in the  
15    glucosyltransferases, glycosyltransferases.

16                  So I don't know how collecting products  
17    in different demographic donor populations might  
18    affect their composition, irrespective of the  
19    method of purification.

20                  DR. GOLDING: Well, a lot of the product  
21    that's manufactured is made from U.S. plasma, even  
22    products that are made in Europe. So there may be

1 an ability to use the same source plasma and  
2 compare them across.

3 MR. ROMBERG: Dr. Golding -- Val  
4 Romberg, from CSL -- regarding Fc function,  
5 comparing older products to newer products, we've  
6 done those assays, and we don't see a difference  
7 between old products and new products.

8 DR. SCOTT: Well, I think we'll go ahead  
9 and progress to the questions. But that was  
10 interesting.

11 I thought I understood that the European  
12 for Fc is actually Fc-binding, but not necessarily  
13 function. So, it may not be the most refined  
14 assay for asking this question, so we'd be  
15 interested in seeing what CSL has come up with or,  
16 actually, what assay they used -- at some later  
17 time.

18 So, I thought these were going to be  
19 simple questions, but my head is spinning. And I  
20 actually don't know what kinds of answers we're  
21 about to get.

22 But the first question is: Does

1       hemagglutinin titer times dose predict the  
2       likelihood of hemolysis?

3               And if Dr. Berg is here, I think, his  
4       data is probably the closest we have so far to  
5       addressing this question. And that presentation  
6       was in the first session.

7               MR. BERG: Yes, and I think I can just  
8       say what we said before, I think we don't see a  
9       clear correlation between (inaudible) -- or  
10      rather, we don't see the association in our data.

11              DR. SCOTT: I guess I would ask what you  
12      plan to do, if anything, to further look at his  
13      dose question? Or what does anybody have any  
14      plans to do to look at the dose question?

15              And the reason is because it seems  
16      clear, irrespective of what you think the cause  
17      is, that people who get higher doses are more  
18      likely to get hemolysis. And I don't say any  
19      information to contradict that.

20              But maybe there is. So, Dr. Zimring,  
21      you know, you could tell us if there is.

22              DR. ZIMRING: When you say "high dose,"



1       you mean more IV IG.

2                   DR. SCOTT:   Of immunoglobulin.

3                   DR. ZIMRING:   Yes.

4                   MR. ROMBERG:   Dr. Scott, I think Dr.  
5   Bellac earlier showed evidence that comparing  
6   across products -- all right? -- so we talked  
7   about dose within a single product.   But when you  
8   compare a product, across products, clearly those  
9   products that have a higher average iso level have  
10   a higher risk.   Products that have a lower average  
11   iso level seem to have a lower risk.

12                   Dr. Bellac, is that right?

13                   DR. BELLAC:   Well, that's just what we  
14   observe.   But if it's really positive, the link,  
15   we just (inaudible).

16                   MR. BERG:   I would like to comment on  
17   that, because I think here we make a dangerous  
18   assumption.

19                   You basically show that there is a  
20   certain titer value, and there is a certain  
21   number, absolute number of reports, and the  
22   reporting rate.   And you infer that that they are

1 causally associated.

2 But what we showed in our data was that,  
3 depending on which country you go to, and which  
4 distribution channels you have, largely influences  
5 also the absolute number of cases you have. So I  
6 think, from a global data set, I would say that  
7 it's very clean scientifically for  
8 pharmacovigilence data interpretation.

9 So if you would show me the data for one  
10 country, and say that -- that is U.S., or Canada,  
11 I think that would be more credible.

12 DR. THORPE: Also, does the data take  
13 into account how much usage there is of that  
14 particular product? Because if a product is very,  
15 very, very widely used, then you would expect to  
16 see more cases of the hemolysis perhaps associated  
17 with it.

18 Does that data exist? Do some of these  
19 products have a disproportionate number of cases  
20 of hemolysis?

21 And also, if there is an association  
22 with, perhaps, the new generation, 10 percent

1 products, are there other factors in those  
2 products which are exacerbating the situation?

3 It's very easy to pick on anti-A and  
4 anti-B titers, but there may be other issues, as  
5 well.

6 DR BELLAC: Well, clearly, we have to do  
7 more research on that, or to consider all the  
8 factors. It's just -- yes, maybe we can just  
9 speak for Switzerland.

10 DR. ZIMRING: Dr. Berg, can I ask, for  
11 those assays that you did, what was the precision  
12 and accuracy? So, the coefficient of variation of  
13 doing the same sample over and over again? What's  
14 the statistical range of the variation you might  
15 see in your tests?

16 MR. BERG: We didn't do that. I think  
17 we have a descriptive sample, but no statistics on  
18 that.

19 DR. ZIMRING: So -- okay, so with the  
20 differences, you're going the 1:64, 1:32, there's  
21 no statistical determination you can make of the  
22 confidence of those determinations, for example?

1                   MR. ROMBERG: It's a European  
2   Pharmacopeial assay, so Dr. Thorpe described the  
3   variability in the assay in her presentation.

4                   MR. SILLIAS: I think the data I've seen  
5   with the assays, with the direct test, is about  
6   plus-or-minus 1 dilution.

7                   DR. ZIMRING: Because, I mean, if I  
8   remember the data, you know, it started to go up,  
9   but at 16, or at 32 it peaked, and at 64 it came  
10   back down again. I mean, there was like a reverse  
11   trend, Dr. Berg, in your data this morning. So,  
12   even if there was some uncertainty around that  
13   middle point, you wouldn't predict a reverse trend  
14   if it was just 1 dilution off that was causing the  
15   problem, at least the way I would think through  
16   that.

17                  DR. LYNCH: Just one more comment  
18   following up on the -- just following up on a  
19   comment made earlier about dose versus titer,  
20   which is something we should distinguish  
21   carefully.

22                  Dr. Bellac showed, yes, there are

1 differences in product, but when you look at the  
2 implicated dose within one product, there doesn't  
3 seem to be any difference in the implicated lots  
4 versus the general lots that are distributed. And  
5 the PPTA data that Dr. Berg presented this morning  
6 suggested the same lack of association.

7 We looked specifically at the  
8 distribution of titers of lots implicated in these  
9 reports, and they were not different at all from  
10 the general distribution of titers in the lots  
11 that are released and not implicated.

12 So, there's this question about "level  
13 of unsafety." There's clearly a dose component.  
14 How the titer contributes, I think, is not so  
15 easy. It's not a straightforward, as you  
16 mentioned, it's not just A times dose equals risk.

17 DR. ZIMRING: I'd like to come back --  
18 unlike transfusion, where like 3 percent of people  
19 get aluminized, but every unit's different.

20 And I come back to this issue that if  
21 you're taking the same, presumably the same drug,  
22 and you're administering it to people who are

1       roughly the same amount of A on their red cells,  
2       and some hemolyze, a few hemolyze, and the rest  
3       don't. Then either it's the way you're giving it,  
4       or another modifying factor, or it's not the  
5       anti-A. And that latter idea seems fairly  
6       distasteful.

7               But to the question you're asking, to my  
8       way of thinking, unless and until you can  
9       demonstrate a correlation between titer and  
10      hemolysis, then there's no rational basis for  
11      setting a preference to lower titer within the  
12      range that you've currently got.

13             DR. SCOTT: I think, then, the question  
14      that you're -- well, the question I'd like to ask  
15      is: Are there ways to improve the way that we're  
16      looking at that titer aspect?

17             Now, we don't have, obviously, chart  
18      review on the patients that we've heard about.  
19      There's a lot we don't know about the patients.  
20      And the titer is semi precise, I mean, within a  
21      certain range.

22             So, how would you ever look at that? I

1       suppose I should ask the whole audience. It's not  
2       fair to ask Dr. Zimring only. Although he may  
3       have an answer.

4               DR. ZIMRING: I'll speculate, of course.  
5       And how would I know? I'd cram it all into a  
6       mouse and see what happens, that's what I would  
7       do.

8               I think that, you know, if no  
9       correlation is observed between titer and  
10      hemolysis -- and maybe that necessitates more  
11      investigation, since there seem to be variant  
12      dispositions. But if that's the case, I think  
13      widening your gaze to other independent variables  
14      that are not currently within your capacity to  
15      observe would be a necessary step -- to wit: We  
16      know that there's IgG 1, 2, 3, and 4. And so the  
17      flow cytometry assay, you know, or even a  
18      solid-phase assay, just by using IG-specific anti-  
19      sera, would be a very straightforward way of  
20      assessing lot- to-lot variation.

21              Because if the data hold, a lot that is  
22      predominantly IgG 4 will not only be

1 non-hemolytic, it would actually protect against a  
2 lot that was hemolytic. So that seems to be an  
3 obvious variable and known biology that we're not  
4 currently assessing in the products.

5           Getting to the post-translational  
6 modification, there you need a peptide mass-spec  
7 analysis, and it's a bit more sophisticated, but  
8 it's simply within the purview of the current, you  
9 know, mass-spec metabolomic groups that are  
10 springing up all over the place. But it would  
11 require a little more finagling.

12           DR. THORPE: I'd just like to make a  
13 comment. I think it's important to look at the  
14 manufacturing processes, and why these new  
15 products appear to have higher titers than the old  
16 ones? What -- you know, what's going on? Is  
17 there something that could be remedied in the  
18 manufacturing process?

19           But until that's identified, you know,  
20 it's difficult to do anything.

21           DR. ZIMRING: I think there's a  
22 part-and-parcel -- and I'd like to bring up a



1 possible laboratory issue.

2 So, the manufacturing processes may --  
3 and I don't know how they're done. They may  
4 change the composition of the IgG subtypes.

5 The secondary antibodies that are being  
6 used for the assays, are these monoclonal  
7 antibodies, for the flow cytometry assay, for the  
8 solid-phase assay? Are these monoclonal, anti-IgG  
9 reagents?

10 DR. SCOTT: I can't answer that  
11 question, but I think there are people here who  
12 are working on those.

13 DR. BELLAC: So, in the indirect tests,  
14 and (inaudible) --

15 DR. ZIMRING: It's polyclonal anti-IgG  
16 that's being used.

17 DR. BELLAC: Mm-hmm.

18 DR. ZIMRING: Okay. And they were  
19 raised -- the reason I raise this is that if the  
20 secondary antibody has any preference for IgG  
21 subtype, then you may actually observe an increase  
22 in signal which is not actually an increase in

1       titer, from a stoichiometric standpoint.

2                   And so I think this gets into the same  
3       issue, how the composition might be changing,  
4       either based upon donor or purification.

5                   DR. SCOTT: All right, so why don't I  
6       answer some of these questions -- right? -- based  
7       on the discussion.

8                   Does hemagglutinin titer times dose  
9       predict the likelihood of hemolysis? I would say  
10      not so far.

11                   Does available data indicate a threshold  
12      "level of unsafety" for anti-A or anti-B doses?  
13      We have no evidence of that right now.

14                   If not, is such information obtainable  
15      and of value? And here, I think, is where we are.

16                   DR. ZIMRING: I suppose you could make  
17      the argument that the relative absence of  
18      hemolysis in 0 patients indicates that there is  
19      some level of safety. Whether we could ever  
20      practically achieve it or not, and whether that  
21      would erase the efficacy, I don't know.

22                   But you could use that as a fairly

1 strong argument, I think, if you buy the whole  
2 system.

3 DR. SCOTT: Well, I think that's, to  
4 some extent, the way things are already  
5 progressing. But that is one of the -- well, as  
6 you say, the main hypothesis, it doesn't account  
7 for all the patients that don't get hemolysis.  
8 But that's very analogous to the thrombosis issue  
9 that we had with intravenous immunoglobulin, where  
10 a lot of people got highly thrombotic lots and  
11 never had a thrombus, because they were not prone  
12 to getting a thrombus.

13 So it's the patient factor which, you  
14 know, technically is not in this session, but  
15 absolutely has to be a part of the picture.

16 DR. ZIMRING: It's a ubiquitous problem,  
17 with widespread analogies. We give anti-HLA  
18 antibodies to people all the time, and a handful  
19 get TRALI.

20 DR. SCOTT: Right.

21 DR. ZIMRING: We give ABO-incompatible  
22 red cells by mistake, and half of the people don't

1       even have a symptoms, (inaudible) like, "I feel  
2       great." So, yeah, there's some noise.

3               DR. SCOTT: All right, the next  
4       question: Is there evidence -- perhaps I should  
5       say "enough evidence -- suggesting that current  
6       specifications for anti-A and anti-B should be  
7       revisited?

8               So, several speakers have already  
9       alluded to the possibility of reconsidering that,  
10      although that we have that we can't be absolutely  
11      sure that that's going to be helpful, it is  
12      something that is potentially doable, at least to  
13      some level.

14              So, what comments do we have on this?

15              DR. GOLDING: Well, can I make a  
16      comment? So, what I'm thinking is that, yes, you  
17      know, we've seen problems, we've seen problems  
18      with certain diseases. Neurological's  
19      overrepresented. It's associated with higher  
20      dose.

21              We don't know, from this discussion  
22      today, what exactly in the antibody preparation is

1       doing the damage, and what are the host factors?

2       So there are a lot of unknowns.

3               And until we know that, it's very  
4       difficult to make any kind of decision based on  
5       your first question.

6               But regarding the second question, could  
7       we say for a subset of patients, quite a large  
8       number of patients, it would be reasonable to have  
9       products with lower titers, and it should be --  
10      that should be pursued.

11              So I think, you know, at least from my  
12      perspective, it's easy to say -- I'm not  
13      manufacturing these products, but it's easy to say  
14      to the manufacturers, please think or consider  
15      making a product that has lower titer, using  
16      donors that have lower titer, or using a group of  
17      donors like anti-A donors or B donors to make a  
18      product, and then the label the product  
19      accordingly with the titer.

20              And that would be an interim measure.

21      It's not based on clear evidence, but it could be  
22      an interim measure to try and reduce the hemolysis

1 in those patients.

2 DR. SCOTT: The other idea that was  
3 brought up was simply to label what we have with  
4 the titers. I don't know how easy that would be  
5 for clinicians. They would need to -- or  
6 pharmacists, or both -- they would need to  
7 understand what that might mean, which means  
8 somebody would have to explain to them what that  
9 might mean, and what it doesn't mean.

10 I think it would be logistically  
11 somewhat difficult. But --

12 MR. SCHIFF: Yes, could I comment on --

13 DR. SCOTT: -- it's the same idea. And  
14 the other thing -- just a second -- if,  
15 particularly low-dose lots are -- or I shouldn't  
16 say "low-dose," low-titer lots for anti-A and  
17 anti-B are manufactured, what is the impact on  
18 all the other lots? I know that plasma is  
19 expensive, and people don't like to waste it.

20 There has been success in various donor  
21 recruitment programs for certain types of donors,  
22 and that would be one potential way to do it. But

1       there is a great deal of use of large doses.

2                   But these are all partially  
3       considerations for tomorrow, as well, so hopefully  
4       a lot of you will be saying.

5                   I'm sorry.

6                   MR. SCHIFF:  Sorry -- I mean, you  
7       partially answered, but I think there are  
8       logistical issues.  If 60 percent of the  
9       gamma-globulin is used for the high-dose  
10      indications, and that's roughly the number, it's  
11      pretty hard to -- you know, if it were a very  
12      small subset it would be easy.  You could make a  
13      boutique line.  But with 60 percent, that means  
14      that all the gamma-globulin, you basically have to  
15      eliminate all the high-titer, all the high-titer  
16      would then go into lots, that certainly would, you  
17      know, be an issue -- so, from the practical  
18      standpoint.

19                   And as far as labeling, if 90-some  
20      percent -- and we'll see that tomorrow -- 90-some  
21      percent are 1:8 or 1:16, labeling isn't going to  
22      be very useful.  I mean, there's only the

1 occasional 1:2 or 1:4.

2 So, in theory it would make sense, but I  
3 think, practically, it would be very difficult.

4 DR. THORPE: The labeling option was  
5 actually considered before a specification was  
6 introduced. But it was decided that clinicians  
7 wouldn't read the label, they'd just whack it in  
8 anyway.

9 DR. BUSSEL: And wouldn't know what to  
10 do with it if they read it, anyway.

11 DR. BRANCH: Plus, if they knew what the  
12 titers were, and there were higher-titer ones,  
13 you'd get them back.

14 At the Canadian Blood Services, we send  
15 out the IV IG, and if some of the doctors did read  
16 the labels, they'd send back all the ones that  
17 were the higher titers and say, "We're not going  
18 to use these. Send us a lower titer one."

19 DR. ZIMRING: But I would imagine, no  
20 matter how high the titer is, they'd still use it  
21 in an O patient -- no?

22 DR. BUSSEL: If you told them.



1 DR. ZIMRING: Yeah, I mean, so -- you  
2 know, experimentally --

3 DR. BRANCH: You'd have to make sure  
4 they knew they could use it in an O patient  
5 without --

6 DR. ZIMRING: In an industry  
7 collaborative, as a matter of generating new  
8 knowledge, as well, would it never be feasible to  
9 make one big batch of IV IG from exclusively AB  
10 donors, and see if there -- and then give it  
11 across the board? And if there was zero hemolysis  
12 there, wouldn't that sort of answer the question  
13 we're asking?

14 DR. SCOTT: I think, in theory, it  
15 could, if those patients were actually monitored.  
16 So it couldn't just be distributed, by and large.  
17 And we actually don't know the rate. The closest  
18 thing we have to a rate of this event is something  
19 on the order of 1-1/2 percent, which we heard in  
20 an earlier talk. And I won't comment on the  
21 amount of significance of that hemolysis.

22 But things like that, in theory, could

1 be done.

2 DR. ZIMRING: And that's the whole --

3 DR. SCOTT: We'd need controls, and --

4 DR. ZIMRING: And even practice-wise,  
5 it's the whole basis of the blood bank, right? I  
6 mean, we match plasma and red cells. And here,  
7 you're combining tens of thousands of donors. But  
8 if you had higher-titer lots that you could  
9 shuttle towards O recipients, and lower-titer lots  
10 that you could shuttle towards non-O recipients --  
11 besides logistical problems and people having, you  
12 know, ethical concerns about a grand conspiracy --  
13 what problems would there be?

14 DR. LUBAN: I think one of the  
15 confounders is that, most often, you're not  
16 getting a blood group and type before you're  
17 administering the produce. So that's a major  
18 logistical element of this.

19 DR. THORPE: There are other products  
20 that are blood-group specific (inaudible). So, I  
21 think it would be more realistic to label IV IGs,  
22 the high-titer ones, to Group O patients only. I

1 think that would be more acceptable, rather than  
2 putting on a titer.

3 DR. SCOTT: I think we'd also have to  
4 factor in dose. Because what we have seen is that  
5 there aren't very many hemolysis cases with the  
6 low doses. So, it may not matter as much,  
7 providing that we're within the current  
8 specifications. But for high-dose people, then,  
9 that would be a consideration.

10 I can sort of feel like there's a  
11 groundswell of people from the firms kind of  
12 worrying about this. But --

13 MR. SIMON: Well, I was just --

14 DR. SCOTT: And here's one of them.

15 MR. SIMON: Yes, I just wanted Dr.  
16 Zimring to clarify. Because I think what he's  
17 proposing is not a change in the system, but a  
18 study --

19 DR. SCOTT: Yes.

20 MR. SIMON: -- to look at this. Am I  
21 correct?

22 DR. ZIMRING: I was proposing a study to

1 look at this, but also a change in the system.  
2 The comment was made, if these things are done,  
3 then isn't that going to increase the titer in the  
4 other lots because the AB donors are not part of  
5 them? And so, even with the current donors you  
6 have, a purposeful, unequal distribution of ABO  
7 phenotype would result in lots that you could  
8 preferentially put towards one or another.

9 But I don't know if that's -- it's  
10 probably, it's easy to say --

11 MR. SIMON: But you raised issues about  
12 whether we can simply accept the anti-A, anti-B  
13 hypothesis.

14 DR. ZIMRING: Yes. Well --

15 MR. SIMON: So you wouldn't want to  
16 change the whole system until you test it.

17 DR. ZIMRING: Absolutely -- which would  
18 be the impetus for doing a study, where you had a  
19 large lot made exclusively from AB donors and, you  
20 know, seeing -- the prediction is the hemolysis  
21 would be in A patients, it would be equivalent to  
22 the current hemolysis in O patients. You'll still

1 get background problems, you get to control for  
2 them.

3 And if that hypothesis holds, then  
4 whether it's feasible or not, there's at least a  
5 rational basis for lowering titers as low as you  
6 could possibly get them in the product. If that  
7 hypothesis doesn't hold, then there would seem to  
8 be a problem with our thinking.

9 DR. FUNK: May I just add a comment from  
10 my side? I think that we have an increased  
11 reporting rate of severe hemolytic reactions with  
12 immunoglobulins. And I think there is also a  
13 correlation with high-titer anti-A globulins. And  
14 I disagree, in this point, with Dr. Berger.

15 And I mentioned before that this  
16 immunoglobulin A I presented, that they will  
17 perform a PATH study, and they will look at the  
18 period before the implementation of risk  
19 minimization measures, and after. And one of  
20 these minimization measures is the screening of  
21 the donor, and the other one is the implementation  
22 of the modification of the manufacturing process.

1                   And if they can demonstrate that then  
2       there is a decreased number of reporting rate,  
3       then we have, not evidence, but at least a  
4       correlation, and a hint that this would help to  
5       reduce the reporting rate.

6                   DR. SCOTT: I just want to ask how those  
7       recipients are being followed? Is there a  
8       registry, or is it dependent upon spontaneous  
9       reports?

10                  DR. FUNK: No, the company will use a  
11       database in the United States, and they collect  
12       all the patients who receive their product, and  
13       then they follow up the patient during a long  
14       period after the implementation. And then --  
15       before the implementation and after the  
16       implementation of these risk minimization  
17       measures --

18                  SPEAKER: So, that's our study --

19                  DR. SCOTT: Yes.

20                  SPEAKER: And our intention is to do  
21       exactly what you described. That study, we should  
22       know the results in 2019.

1           I think this is significant enough that  
2   we don't want to wait until 2019 to be making  
3   changes.

4           So, our view is not that labeling is the  
5   solution, but that just lowering isos across the  
6   board on the full product line makes sense.

7           DR. GOLDING: You know this uncertainty  
8   about the titer, and how come the titer doesn't  
9   correlate better with hemolysis -- but other  
10   evidence presented that, to me, is fairly  
11   compelling. I think in every case that was  
12   presented, where there was hemolysis and antibody  
13   was eluted off the red cells, it made sense. In  
14   other words, anti-A was eluted off red cells that  
15   were A-positive, and the product had anti-A  
16   titers.

17           So, you could wait for more and more  
18   evidence, but it seems to me that it's very clear  
19   that -- very likely, I wouldn't say "clear" --  
20   very likely. You know, I don't know what  
21   probability -- that what we're talking about is  
22   anti-A and anti-B. And I challenge anybody in the

1 audience to say differently.

2 But the fact that the titer doesn't  
3 correlate well means that we don't understand what  
4 subset of antibodies are in the product are in  
5 involved.

6 DR. SCOTT: Or the patient.

7 DR. GOLDING: But it's very clear that  
8 it's the anti-A and the anti-B.

9 DR. ZIMRING: Fundamentally, I don't  
10 disagree with your conclusion, but I would  
11 challenge one of your lines of reasoning.

12 First of all, it may be a threshold  
13 effect, right? So, if you have to get to a titer  
14 of 1:256 before you drop off, then the  
15 non-correlation at a higher titer is completely  
16 consistent with the hypothesis.

17 But I would posit to you the following:  
18 All batches of IV IG will have anti-A. All A  
19 recipients will express A on their red cells.  
20 Ergo, you would have positive DATs with the  
21 elution of anti-A in any scenario, in any  
22 mechanism, regardless of what's happening.



1           That being the case, I don't see how  
2   that data distinguishes any hypothesis from  
3   another.

4           DR. BRANCH: I'd just like to say that  
5   in our study that we have prospectively going,  
6   that many A patients that receive IV IG do not  
7   have a positive DAT, and those are the ones that  
8   don't hemolyze. So, only the ones that get a  
9   positive DAT, so far, have shown evidence of  
10   hemolysis.

11           So, it looks like at least it correlates  
12   to a DAT- pos, where the antibody to the  
13   corresponding blood group is eluted -- and the  
14   only antibody that's eluted.

15           DR. THORPE: Also, I suspect that some  
16   titers have been underestimated in the past, if  
17   they'd been determined using the indirect  
18   antiglobulin test. We've certainly got data that  
19   would support that.

20           So, I think there might be a broad  
21   correlation, but it's certainly not everything.

22           DR. KLEIN: I think that nobody's going

1 to argue that higher titer is better, so that  
2 lowering titers certainly would be an admirable  
3 thing to do.

4 But, Don, to get to your point, I think  
5 there are thousands of people who receive IV IG  
6 who have positive direct Coombs' tests, from whom  
7 you can elute tons of antibody, they don't  
8 hemolyze.

9 So, you know, I agree with your basis  
10 thesis, but I think there's a lot more to it than  
11 just antibody titer.

12 DR. SCOTT: I think we have a comment in  
13 the room.

14 DR. SHEBL: Yes, just one comment.  
15 Maybe I would like to describe at least what I  
16 understand from this whole story of having titers  
17 playing a role and not playing a role.

18 So, I would like just to say that in a  
19 very simple word, which is, if we have one single  
20 patient, we have specific risk factors associated  
21 with this patient to develop hemolysis. And I  
22 would say roughly 30 to 40 percent constitutes the

1 risk factor of the titer in the lot, and there are  
2 other 60 to 70 percent that would represent a lot  
3 of other factors.

4 So, if we say do titers play a role?

5 Yes, it plays a role, because it adds to the other  
6 factors to reach 100 percent to have hemolysis in  
7 the patient.

8 And once I talk about that, I would say  
9 there are a lot of other things, product-related,  
10 which might be other irregular antibodies, it may  
11 be subclasses of specific titers as part of this  
12 30 to 40 percent. And also a lot depends also on  
13 the patient risk factors constituting, as an  
14 example, the disease -- we have today a very good  
15 presentation regarding the association of  
16 inflammatory factors in developing hemolysis, and  
17 others. So the underlying disease, the  
18 concomitant medications, and others.

19 So, the underlying disease, the  
20 concomitant medications and all of that, all  
21 together.

22 And the way is -- yes, they play a role,

1        titers play a role. But it doesn't play this role  
2        in all patients.

3                    And the evidence of that, that there are  
4        a lot of titers with very high titer that did not  
5        induce hemolysis. And, vice versa, with low  
6        titers, we have seen hemolysis.

7                    And this goes back to this equation,  
8        which is "patient-specific plus product."

9                    DR. ZIMRING: The question has been  
10       asked. The role of titer has been assessed.  
11       There are empirical, natural phenomena that have  
12       been observed, that have been described to us  
13       today, that within the range of the titer that has  
14       been examined, within the confidence of the assay  
15       that has been used, there is no correlation. And  
16       so why 30 percent? Where do you -- I'm not --  
17       where is this confidence of yours coming from?

18                   DR. SHEBL: Now, I'm just saying a rough  
19       idea of what I see that, at least from all the  
20       data we have presented today, where we -- there  
21       are titers with -- sorry, lots with high titers  
22       that never showed any events, and vice versa.

1                   So, it's multifactorial. We have to  
2    know that it's multifactorial. It depends on --  
3    what is clear from the data that everyone has  
4    presented today, and previously, is the high dose  
5    -- of course, this is one factor that could be  
6    added to that.

7                   But the titer itself is not a triggering  
8    factor for hemolysis, but it's part of a  
9    multifactorial process to induce hemolysis.

10                  DR. SCOTT: I think it's pretty clearly  
11    -- could I have the mic, please? It's pretty  
12    clearly controversial, the extent to which titer  
13    may matter. But that's okay. One of our  
14    approaches in the past has been that if there's  
15    something we can do to try to make a product  
16    safer, we're generally in favor of doing that,  
17    even if we don't have all the evidence on which to  
18    do that. In other words, we prefer to be  
19    proactive where it's possible. And I think  
20    everybody does. If we can do something about this  
21    adverse event, which clearly is problematic, and  
22    may occur in more people than we know.

1 I just want to go on to the next set of  
2 questions. And I'm sorry we don't have a wine bar  
3 after this, because I think it would be very  
4 useful. These are the last two questions. And  
5 the first is -- and we will be out of here, I  
6 think, in the next six minutes. I don't think we  
7 have to be out of here until at least 5:30.

8 Is there a rationale for revisiting  
9 anti-A and anti-B test methodologies?

10 So, what we've heard is that there are  
11 downsides to the existing method, but it has shown  
12 reasonable reproducibility, and may be okay -- may  
13 be okay -- well, is the best we have for the  
14 purpose of testing products for lot release.

15 We did bring up new assays. We don't  
16 have a lot of presentations about that. It  
17 probably could take half a day if we did, and  
18 would be very interesting.

19 But I just want to hear what the  
20 rationale might be. And I think that Dr. Thorpe  
21 and Dr. de Coupade had opinions about that, and  
22 they both recognize the positive and negative

1 sides of both types of technology -- flow  
2 cytometry and direct agglutination.

3 But I wondered if people in the audience  
4 who do these tests, as well as the two of you,  
5 have additional comments?

6 MR. ROMBERG: Maybe from one of the  
7 manufacturers. This is Val Romberg.

8 You know, the agglutination methodology  
9 has a lot of noise. But I'd like to hear -- I  
10 would love a better assay. And I think the FACS  
11 assay looks very attractive. It's not an  
12 agglutination assay.

13 And then I think it was Dr. Branch who  
14 suggested, well just make an ELISA, which -- it  
15 was even easier, and be more straightforward.

16 I'd like to hear from the regulators on  
17 their view of this. You know, regulators tend to  
18 like cellular assays.

19 They think it's more based in biology.

20 DR. SCOTT: Yes, we do like bioassays.  
21 We tend to prefer them over binding assays,  
22 because they're indicators of function.

1                   I'll let Jay speak to that. I saw you  
2       raise your hand.

3                   MR. EPSTEIN: Yes, well, certainly  
4       assays that are more reproducible are desirable,  
5       prima facie.

6                   I'm concerned about the antigenic  
7       substrate, though, because we don't know what  
8       we're measuring if we haven't characterized the  
9       red cell or the red-cell antigen. And the one  
10      thing we know is that there's a lot of variability  
11      about these red cell membranes. And if you were  
12      to move to an ELISA, and you selected certain  
13      synthetic antigens, well, which ones do you pick?

14                  And so I think that, given that gap in  
15      understanding, it would be good to have very  
16      robust systems that display, you know, lots of red  
17      cell antigenic variance.

18                  And so I like the idea that, you know,  
19      the intermediary position is this pooled antigen  
20      or antigenic substrate.

21                  So, with that caveat, I think that an  
22      analytic method that has higher precision is



1       desirable, but it still begs the question of not  
2       knowing the relevance of the thing we're  
3       measuring.

4               Because I think, when you boil it all  
5       down, there's some specificity in the setting of  
6       hemolysis of a patient. There was something in  
7       the immunoglobulin that affected something in the  
8       patient's red cell. And I don't think we really,  
9       really know what bound what, and what, you know,  
10      biological response was caused.

11             So, I think if we acknowledge the  
12      uncertainty of what we're measuring, and I think  
13      if we tried to use a broadly reactive assay, then  
14      I would vastly prefer an assay that has higher  
15      precision, because at least you can reproduce that  
16      measurement.

17             MS. DE COUPADE: Maybe I can also add a  
18      comment. I mean, the good thing with the flow  
19      cytometry is that you have your antigen in the  
20      right conformation, whereas in the ELISA, you will  
21      just do an artificial kind of setting. So that's  
22      probably one of the best things you can have with

1 the flow cytometry, because you have the whole  
2 physiology of your antigens integrated inside the  
3 membrane.

4           Maybe -- I mean, I remember the comments  
5 of one of you said that we may try also cells from  
6 patient. That could be a good thing to do, and  
7 make the correlation, and see whether or not there  
8 are other proteins that may interact or  
9 participate in the hemolysis stuff. Even if --  
10 with the flow cytometry, you're only looking at  
11 the binding, not at the hemolysis (inaudible).

12           DR. SCHLEIS: Well, and I think we now  
13 have two papers that basically -- your paper and  
14 the Kahwaji article -- where it does show some  
15 specificity that seems to explain some of the  
16 clinical data we're seeing. And, if anything, it  
17 sort of at least drives us to the area that we  
18 need to research further -- okay? This is showing  
19 a bigger difference than the direct or indirect  
20 methods.

21           And so, if this is the case, and it does  
22 sort of push us toward anti-A being the culprit,

1       you know, at least these are two assays where we  
2       can build upon and maybe address that question.  
3       Because if we nail it down that at least we have a  
4       general feeling it's anti-A, you know, then we can  
5       move to the next step of, well, what do we do from  
6       here?

7                 DR. SCOTT: I think we'll go ahead and  
8       -- thank you very much.

9                 I'll progress to the last question. And  
10       we've talked about this a lot, and we've listed  
11       some of the possibilities.

12                So, is there evidence for  
13       product-related risk factors other than anti-A and  
14       anti-B? And we've even heard that we can't  
15       necessarily be completely sure that anti-A and  
16       anti-B are exactly the culprits. Many of us feel  
17       convinced that they often are, based on various  
18       types of data.

19                But the question is, what clinical or  
20       lab research efforts might clarify of other  
21       factors?

22                So, we've already talked about

1 immunoglobulin subclasses. We have touched on the  
2 subject of looking at recipients' Fc-receptor  
3 polymorphisms, of actually looking at anti-A,B  
4 antibodies in the products which may be even more  
5 hemolytic.

6 We haven't really touched on looking at  
7 whether people are secretors or non-secretors.  
8 And not being a blood banker or a hematologist,  
9 I'm not sure that will make a difference. But,  
10 certainly in the literature, it's hypothesized to  
11 make a difference. And that's something we really  
12 don't see much about when we look at case series.

13 So, I'm going to stop myself here and  
14 ask what I've missed.

15 DR. BRANCH: I would just like to just  
16 make a comment on the secretor/non-secretor  
17 status. It's preliminary, but we've looked at  
18 quite a few individuals, more than what was  
19 presented today, and there's no correlation yet  
20 with secretor status, done by Lewis typing.

21 We only did one patient's saliva typing,  
22 but by Lewis typing, if you believe that predicts

1       secretor, which it should, there's no correlation  
2       to secretor status.

3               But I would like to point out that what  
4       we -- you know, we're talking about anti-A and  
5       anti-B as red cell- related, but they're a  
6       histo-blood group. It's a histo- blood group  
7       antigen, which means that all of our tissues have  
8       A-antigen on them. So some of this could be  
9       related to how much A-antigen do we have on our  
10      tissues, how much is being absorbed out by the  
11      tissues -- not by secretor A substance, because  
12      that's different than what we endogenously produce  
13      on our tissues.

14             So that could be a factor. And I'm not  
15      sure how you would look at that, but that might be  
16      a factor to explain it.

17             DR. ZIMRING: So, in asking for  
18      laboratory research efforts, if the momentum ever  
19      were gathered to generate experimental lots of IV  
20      IG from exclusively AB donors, and there were  
21      hemolytic events -- or if you had a transfusion  
22      network large enough that you could capture the O

1 recipients who had hemolytic events -- then those  
2 would we platforms in which you've eliminated the  
3 anti-A, anti-B variables, and you could look for  
4 underlying causes that may be contributory. I  
5 don't know if either of those are feasible.

6 DR. GOLDING: It seems to me that the  
7 red cell membrane is important here. And I think  
8 that some research should be done pursuing what  
9 exactly is happening on the red cell membrane.

10 So, obviously, we have as a possible --  
11 and I think it's very likely, that the target is  
12 the A-antigen or the B-antigen, which is a  
13 carbohydrate. But that could easily be associated  
14 with other red cell membrane proteins, lipids,  
15 whatever.

16 And, you know, that kind of research, I  
17 don't hear it being talked about. And I think  
18 that would be worthwhile pursuing, because it  
19 could be -- part of the host factor, is the  
20 contribution of the red cell membrane, which could  
21 be different, and could be particularly liable to  
22 (inaudible) hemolysis, if we understood, we had

1 samples from patients that had hemolysis, and  
2 looked at their red cell membranes, and  
3 precipitated out that part of the membrane that  
4 binds to the antibody, and analyzed it.

5 I mean, the techniques for looking at  
6 that are available. And I think, at least at that  
7 level, that should be done.

8 DR. FUNK: Well, I would add another  
9 risk factor which was not discussed very much  
10 today.

11 When we analyzed this data, we have seen  
12 that a lot of patients, more than 50 percent,  
13 received immunoglobulin in off-label use. And  
14 maybe I'm a little bit old-fashioned on this  
15 point, but for me it means that there are no  
16 clinical studies which have shown us the risk and  
17 the benefit of the off-label use.

18 And for me, it would be also very  
19 important, as a person from the competent  
20 authority, to see really relevant clinical studies  
21 which show us the benefit, and also the risk, and  
22 also the doses we need in this indication. For

1 me, it's not very evident to understand why such  
2 high-dose therapies are started in these  
3 indications.

4 DR. SCOTT: Well, you've probably --

5 MR. ROMBERG: Well, maybe --

6 DR. SCOTT: Yes, go ahead.

7 MR. ROMBERG: -- just to respond. You  
8 know, the industry has been doing, looking at  
9 alternate indications pretty heavily, right? And  
10 I take my hat off to Baxter, who did the  
11 Alzheimer's trial -- right? Grifols and CIDP were  
12 doing other indications.

13 You're never going to get all of those  
14 indications covered, all right? We can work for  
15 years and years and years, and we won't get half  
16 of them. So, as a competent authority, you're  
17 going to have to deal with off-label use.

18 Oh -- Val Romberg, from CSL Behring.

19 DR. SCOTT: Well, as you know, we don't  
20 regulate how a product is used, precisely.

21 Off-label use is not really within our authority.

22 But if you look at the review by Jordan



1 Orange about -- and many others -- about uses of  
2 IV IG, they certainly did set out what available  
3 literature was present to support or not support  
4 these various off-label uses. It's extensive and  
5 informative.

6 What we can say about off-label uses is,  
7 in many cases, there's literature to support it,  
8 but we, ourselves, don't have data. So, that  
9 doesn't mean it doesn't work, it just means we  
10 don't have data.

11 But it's not in our authority. And I'm  
12 curious, is it within the authority of the EMA, or  
13 of independent nations in Europe? Price-wise it  
14 might be, I suppose.

15 DR. FUNK: But our opinion is that we do  
16 not have to regulate off-label use, though this is  
17 not our business.

18 We have to regulate the label use. And  
19 therefore, for us, there is no need to find  
20 criteria to regulate the off-label use.

21 DR. SCOTT: It goes way beyond  
22 immunoglobulins. And I'm very glad we don't have

1 to do that.

2 So, I'm going to find out if anybody has  
3 some additional words on the last question, or  
4 additional words in general. Or if everybody  
5 would like to go somewhere to get warm and get  
6 ready for tomorrow's exciting sequel.

7 DR. SCHLEIS: Well, you know, one thing  
8 -- and when I look back on today, and then -- I  
9 don't want to keep everyone here.

10 So, we've sort of looked at a lot of  
11 things that we don't know, but I think there is  
12 one thing we do know, and hemolysis occurs at high  
13 doses. All the literature, all the cases, these  
14 are patients getting high doses. So that's one  
15 thing we know for certain.

16 And maybe that's one thing that we can  
17 look at, in terms of are there ways to mitigate  
18 this and, you know, whether it's infusion rate,  
19 how it's administered, administered more slowly,  
20 those sorts of things. Certainly that could be  
21 done.

22 And, you know, without trying to figure

1 out the mechanism, you know, it could be a way to  
2 make these products safer for patients.

3 DR. SCOTT: That's a very reasonable  
4 last word. So, I want to thank our speakers, our  
5 steering committee, and the audience for some very  
6 helpful comments and input, and great discussion.

7 And we'll see you tomorrow. (Applause.)

8

9 (Whereupon, at 5:28 p.m., the  
10 PROCEEDINGS were adjourned)

11 \* \* \* \* \*

12

13

14

15

16

17

18

19

20

21

22

## 1 CERTIFICATE OF NOTARY PUBLIC

## 2 DISTRICT OF COLUMBIA

3 I, Carleton J. Anderson, III, notary  
4 public in and for the District of Columbia, do  
5 hereby certify that the forgoing PROCEEDING was  
6 duly recorded and thereafter reduced to print under  
7 my direction; that the witnesses were sworn to tell  
8 the truth under penalty of perjury; that said  
9 transcript is a true record of the testimony given  
10 by witnesses; that I am neither counsel for,  
11 related to, nor employed by any of the parties to  
12 the action in which this proceeding was called;  
13 and, furthermore, that I am not a relative or  
14 employee of any attorney or counsel employed by the  
15 parties hereto, nor financially or otherwise  
16 interested in the outcome of this action.

17  
18  
19 (Signature and Seal on File)

20 -----

21 Notary Public, in and for the District of Columbia

22 My Commission Expires: March 31, 2017