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.
Deputy Director CBER/FDA

4 MARY GUSTAFSON
5 Vice President, Global Regulatory Policy
PPTA

6 KEITH HOOTS, M.D.
7 Director, Division of Blood Diseases
Research NHLBI

8 Statement of Problem:

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

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

12

13 Moderator:

14 JAMES BUSSEL, M.D.
15 Cornell University

16 Panelists:

17 MARKUS FUNK, Ph.D.
Paul Ehrlich Institute

18 ELAINE TAYLOR, M.D.
19 Health Canada

20 SCOTT WINIECKI, M.D.
CBER/FDA

21 ROGER BERG
22 PPTA

1 A G E N D A

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

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

5 JACOB PENDERGRAST, M.D.
University of Toronto

6 Session II: Product Risk Factors

7 Moderator:

8 DOROTHY SCOTT, M.D.
9 CBER/FDA

10 Panelists:

11 DONALD BRANCH, Ph.D.
University of Toronto

12 SUSAN THORPE, Ph.D.
13 National Institute for Biological
Standards and Control

14 CAROLINE BELLAC, Ph.D.
15 Swissmedic

16 CATHERINE DE COUPADE
LFB Biomedicaments

17 JAMES ZIMRING, M.D., Ph.D.
18 Puget Sound Blood Center

19 NAOMI LUBAN, M.D.
Georgetown University

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P R O C E E D I N G S

(8:35 a.m.)

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

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

You have speaker biographies and you have questions in your agenda. I think that we will not go through the biographies in any detail now, but please refer to those, and without further ado, we'll get started with our introductions and first we have Dr. Peter Marks, who's the deputy director of CBER and who's taken 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

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

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

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

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 Romiplotsim.
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 hemolize 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 hemolize 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 clinical 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 engraphtments, no
2 other dysplasia, and she was given IVIG at a does
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 patter 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 interleuken 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 interleuken 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 tittering.
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 105. 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 106 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 105. For B, it's 7-1/2, as you saw, for

17 105. For A1B, it's 8-1/2, almost a million. For

18 A2B, it's only 105. 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-material 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 then 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 that 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 confractionation 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 lope 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 acanthoccyte, 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 uncompelling, 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 whit,
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 though 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-regs, 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

so that we can address the questions that we have for this session -- and others.

So, speaker questions that you held back on, I think it would be reasonable to ask some of those. We have about half an hour, should it take so long.

But it's certainly daunting, the complexity of these cases, and the bits of information that we have are very difficult to put together. Nevertheless, we do have some questions here. And if folks think of other, more interesting questions, do feel free to ask them.

Could we have the question set, please? The session questions.

Well, first, I want to find out if anyone in the audience has additional questions for the speakers -- and we have one out there. "Other Product Risk Factors"

SPEAKER: This is more a comment than a question (inaudible) between older generation, newer generation.

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 hemovigilence
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 whit: 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 O 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 god 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)
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CERTIFICATE OF NOTARY PUBLIC

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I, Carleton J. Anderson, III, notary public in and for the District of Columbia, do hereby certify that the forgoing PROCEEDING was duly recorded and thereafter reduced to print under my direction; that the witnesses were sworn to tell the truth under penalty of perjury; that said transcript is a true record of the testimony given by witnesses; that I am neither counsel for, related to, nor employed by any of the parties to the action in which this proceeding was called; and, furthermore, that I am not a relative or employee of any attorney or counsel employed by the parties hereto, nor financially or otherwise interested in the outcome of this action.

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