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CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

IMMUNE GLOBULIN HYPERSENSITIVITY REACTIONS:

ROOT CAUSES AND MITIGATION

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P R O C E E D I N G S

(8:31 a.m.)

DR. SCOTT: Thank you. Good morning, everyone. I see our audience continues to grow, but we're going to get started now. We're going to begin with welcoming remarks. And the first person - I'm pleased to introduce Dr. Peter Marks, who is the director of the Center for Biologics Evaluation and Research. And we're very grateful to him for sparing a few moments to speak with you.

DR. MARKS: So, thanks, Dot. And I first of all want to thank everyone who's tuned in this morning. I really appreciate the number of people who are joining us. Thanks also to all of our partners and to our FDA staff who've helped put this together -- really appreciate the work of the Immune Deficiency Foundation, Plasma Protein Therapeutics Association or PPTA, and our staff and Dr. Scott for putting together a really nice workshop agenda.

Really, this is something that is an

important issue to address. This is one of those -- many of the things that our center -- I may not have had up close and personal experience with. But as a hematologist-oncologist who used to give a lot of immunoglobulins for a variety of different conditions, to be honest, on label and off label, I have seen what can happen. And so, understanding hypersensitivity reactions and trying to mitigate against them is really an important thing for this class of products, which has a tremendously broad application in so many different conditions, and is -- you know, it's the mainstay of therapy for several conditions, but also finds its way in use for literally dozens upon dozens of other diseases for which it is a preferred treatment, so -- even if it is off label.

So, really appreciate everyone taking the time. I don't want to take too much more time away from the meeting to say anything else other than thank you so much for joining. Really look forward to great presentations and great dialogue

today. Thank you.

DR. SCOTT: Thanks, Peter. Thanks very much. Next, we'll go on to Jorey Berry, who is the president and CEO of the Immune Deficiency Foundation.

MS. BERRY: Thank you. I'm going to share my screen. I just have a couple of slides. And hopefully, everyone can see that.

Okay. Well, good morning. On the -- on behalf of the Immune Deficiency Foundation -- and most people refer to us as IDF -- I just wanted to share a quick word on who IDF is and why we're so pleased that today's workshop is happening. Mission statements can be words on paper for a lot of organizations. I don't think that you're supposed to say that, but I can tell you they are not words on paper at IDF.

We are laser-focused on improving the diagnosis, treatment, and quality of life of people affected by primary immunodeficiency. And we strive to do that by fostering a community - a community that's empowered by advocacy, education,

and research. IDF serves as the trusted organization for the primary immunodeficiency community. And we not only value our role as a patient advocacy organization, we take it very seriously. There are people -- there are people counting on us.

If you have a primary immune deficiency or PI, it means you're born missing part of your immune system or your immune system doesn't function properly. PI is actually, as many people on this call may know, an umbrella term that represents about 450 rare conditions, but they all have one thing in common -- the body's inability to fight infection. And IDF has been fighting for people with a Primary Immune Deficiency since 1980.

For many people with rare diseases, including PI, plasma-derived therapies are the only option for life-saving and really lifelong treatment. In fact, it takes 130 plasma donations to treat one adult for one year who's affected by PI. The plasma-derived treatment for many with

primary immunodeficiency is immunoglobulin replacement therapy -- so much so that we launched Plasma Hero. It's an initiative designed to educate the general public on the critical need for plasma and to connect potential donors with resources to get started.

Because of our mission, IDF is invested in ensuring the safety of plasma products and of plasma donors, as well as ensuring an adequate supply of plasma, all through a lens of transparency. And as an active member of the American Plasma Users Coalition or APLUS, for decades, IDF has joined other organizations that rely on plasma source therapies to engage with the FDA to ensure the health and safety of individuals who rely on these life-saving medications. So, thank you to everyone for joining the workshop today, and to the FDA for inviting the Immune Deficiency Foundation to participate.

DR. SCOTT: Thank you, Jorey. And we'll now go on to the Plasma Protein Therapeutics Association for remarks. And this will be made by

Dominika Misztela, representing the PPTA.

MS. MISZTELA: Good morning -- good afternoon to the audience. My name is Dominika Misztela, and I'm the head of Global Regulatory Policy for the Plasma Protein Therapeutics Association. It is my honor and pleasure to be here today and say a few words on behalf of PPTA, our members, and the plasma products industry.

So, for those of you who do not know PPTA, we represent the manufacturers of plasma-derived therapies, as well as recombinant analogs. They are collectively referred to as plasma protein therapies. We also represent the collectors of source plasma, which is actually then used to manufacture therapies such as immunoglobulins. And as you have heard from IDF, these therapies are essential, and they are used by a very small patient population worldwide to treat a variety of serious and rare diseases.

And I can only echo IDF -- our mission statement is not only just a mission statement. We remain committed to advocate for access to safe

and effective and affordable therapies to all patients, and we engage in constructive dialogue with regulatory agencies. And we collaborate very closely with patient advocacy organizations. So, first of all, many thanks from my side to the organizers of this workshop, especially Dr. Scott, as well as the steering committee to say a few words here today.

So, first of all, the safety and the efficacy of immunoglobulin therapies is of paramount importance to the plasma industry. The safety and the efficacy of these essential life-saving therapies has been demonstrated and well-documented over several decades now. Over the past years, as you have witnessed throughout COVID-19, there have been concerns with access to these therapies for patients, not only in the U.S., but certainly worldwide. Despite these challenges, the plasma industry has continued to provide safe and effective therapies to patients, and is committed to ensure that patients receive the treatments that they so desperately need.

Today, you will hear from three manufacturers on their efforts over the past years to identify the root causes and provide explanations on what we refer to as hypersensitivity reactions, which occurred with specific lots of immunoglobulin therapies since 2018. These hypersensitivity reactions occurred at a higher rate than usually associated with administration of IG medications. And this actually has led to voluntary withdrawals of immunoglobulin therapy lots out of precaution.

So, the three manufacturers have since then been actually working together to address the root causes of these reactions, ensure the safety of immunoglobulin therapies, and at the same time, making sure that patients continue to receive products. The work has resulted in robust scientific exchanges in the form of data, research, and knowledge. The individual, as well as combined efforts and commitment to identify the root causes of these reactions are reflected in the presentations that you will see here today.

I can assure you that the presentations you will see a little bit later on represent tremendous resources, countless hours of manpower and research, and experimental investigations. For instance, the manufacturers have established new R&D methodologies to identify the affected plasma units and lots, have conducted investigations into entire manufacturing chains and methods, and many, many others. In addition, the manufacturers have been working very closely with the USFDA and other regulators to share their investigations and their research.

With this, on behalf of PPTA, our member companies, and the plasma industry, I would like to assure you that we remain committed to work with all stakeholders, including scientific experts, the USFDA, as well as patients to ensure that patients receive safe and efficacious therapies, and have continuous access to those. And it is my pleasure again to welcome, all of you to what really looks like very interesting and insightful discussions, and the insights that we

will gain here today. Thank you very much, and good morning.

DR. SCOTT: Thank you very much. That was -- we've had some excellent introductions. There's one more introduction to go, and that is mine. So, I think we'll get started with my slides. I'm Dorothy Scott from the Center for Biologics Evaluation Research and the Office of Therapeutic Products. My division is the Division of Plasma Derivatives. This is just a disclaimer that my comments are informal communications and represent my own best judgment, and do not bind or obligate the FDA. Next slide.

So, what I intend to convey here is why we're having this workshop and what we hope to achieve, but I want to give you a little bit of background. So, immune globulin lot- specific hypersensitivity reactions are discovered through information collected by the FDA Adverse Event Reporting System. And these consist of voluntary reports of adverse events for any product to the Center for Biologics from manufacturers, from

patients, and from health care providers. Since these are voluntary spontaneous reports, as you will hear, we probably underestimate the number of these adverse reactions.

Typical immune globulin hypersensitivity reactions commonly involve urticaria or hives, itching, other sorts of rashes, sometimes oropharyngeal edema, and less commonly, more serious things, such as hypotension, nausea, chest pain, shortness of breath. And occasionally, these are described as anaphylactic-like reactions. The treatments for these are diphenhydramine, corticosteroids, H1 blockers, epinephrine -- and the intensity of treatments and the number of treatments are based on the severity of the reaction. Next slide, please.

So, what are the consequences of these reactions? We think first about our patients. And these include physical discomfort and also anxiety, particularly with the more intense reactions. They have to get additional medications to treat the symptoms of their

allergic-like response. They also may be undertreated, because sometimes, these reactions occur during the infusion of immune globulin, and the infusion very often is not completed.

In some cases, they may be admitted to an emergency room or have to go -- or admitted to the hospital. And then they're left with subsequent concerns about IG treatments and if they will be able to continue, and if this is going to happen again. And I wouldn't underestimate that particular anxiety that I'm sure many patients have. With respect to the product, these lot-specific hypersensitivity events result in voluntary withdrawals by the manufacturer of that lot of the product, which includes, obviously, loss of that product, at least those lots, and puts us at risk for an immune globulin shortage if there must be a lot of withdrawals. Next slide, please.

So, this is not new. Lot-specific clusters of hypersensitivity events have recently had a greater impact than previously, I think,

perhaps because -- well, there are many possible factors, but perhaps because we also see increased use of immune globulin over the years, and for many different indications. But we do have a number of historical reports of immune globulin lot-specific clusters of hypersensitivity adverse events in between 2004 and 2018. And you will be hearing about 2018 until the present in this workshop in great detail.

What you can see here is that we have five different types of products. And these encompass five different manufacturing processes over the years, which implies to us that this is not a process-specific problem. It could happen to any manufacturer at any time. Now, in the past, implicated product lots have been voluntarily withdrawn. But investigations, although somewhat thorough, did not reveal a root cause. And I think one of the most exciting aspects of today is that we're going to hear about progress in these investigations to a much greater extent than previously. Next slide, please.

So, the purpose of this workshop is to describe the clinical presentation of hypersensitivity reactions, to review the pathogenesis of hypersensitivity reactions -- we have some excellent experts here to do that in the first half of the workshop -- to present scientific investigations to determine the basis of hypersensitivity reactions in this scenario. And again, we're very grateful to our manufacturers for all they have done and for agreeing to present their results at this workshop. We will discuss the likely causes of these reactions and potential mitigation strategies. And at the end, the grand finale will be to seek opinions of clinical and research experts on risk mitigation and directions for future scientific investigations. Next slide, please.

The workshop is structured in the first part to describe the hypersensitivity events and their effects on patients; in the second part, to have expert presentations on the pathogenesis of

these types of reactions; and the third part after lunch will be manufacturers research seeking root causes of these hypersensitivity reactions. And this will be very exciting. I promise you. And lastly, we'll have the expert panel discussion, including all of the workshop speakers and a couple of extra experts as well. Next slide, please.

So, the questions for the expert panel are, what are the most likely biological causes of these lot-specific immune globulin mediated hypersensitivity reactions? What in-vitro methods are most promising for identification of plasma units, plasma pools, or final products that contain hypersensitivity promoting activity? And what can be done in the future to minimize the number of patients affected by hypersensitivity lots? Next slide, please.

So, I want to remind you, the audience, of whom there are 259 present now, that you are very important to this workshop as well. For sessions 1 through 3, we'll have audience

participation, and we expect your avid attention and participation in these question and answer sessions and these panel sessions. If there's extra time after the expert panel, the chat room will be open again for audience participation. It's simply a matter of time. So, hopefully, we will have that time. And we welcome your comments and questions and your thoughts and attention. Next slide.

There are a number of people to thank. I cannot name all of them without spending a lot of time. But we wish to thank our speakers who've gone to extra efforts to make these presentations and spent a lot of time on them, and our additional experts for the expert panel. Next slide. The workshop steering committee who helped formulate the agenda and gave us ideas and also helped us find the expert speakers, we appreciate that. Next slide. And the FDA workshop support staff. And I can't say enough about their assistance, their handholding, in my case, at least, and their attention to making this workshop

work in the virtual format. Next slide.

So, I want to thank you, again, everybody. And now, we're going to proceed to a description of the patient experience with these hypersensitivity events. And we're very fortunate to have Kara, who is a regular recipient of immune globulin infusions, to tell us what these reactions are like and to remind us that we're doing all of this for the patients. Kara?

KARA: Hi there.

DR. SCOTT: Hi.

KARA: I was asked to share my experience with you, which occurred on September 19, 2022. I'm treated for CVID with IVIG every four weeks. My first IVIG was July 10, 2019. And in September of this past year, for the end of my infusion, I experienced a rash waist-up, including my scalp, shortness of breath, and a cough. So, we stopped the treatment. And I was treated with Allegra, Pepcid, Benadryl, steroids, and they observed me for a couple of hours.

I went home and was pretty much up all

night with shortness of breath, and hard to breathe. I should have gone to the emergency room, but I didn't. But what ensued was a bronchial -- I would say, bronchial event for two weeks after that, which was very bad. But that's what happened. And so, subsequently, my treatment, I get pretreatment of steroids, Zyrtec. We've changed from Gamunex to Gammagard. And I get the slowest possible pace. And so far, that's been going well. So, that's -- that was my experience.

DR. SCOTT: Yes. And I'd like Dr. Cunningham-Rundle say -- make comments. But I did want to ask you if you'd ever experienced this before in your years of infusions with immune globulin.

KARA: No, never.

DR. SCOTT: And I think you may have mentioned that they had the epinephrine out when you --

KARA: Yes.

DR. SCOTT: -- were experiencing this

reaction, and there was obviously a question of whether or not to treat you in that fashion.

KARA: Yes, that was the question. So, they observed me, and maybe I should have, but anyway --

DR. SCOTT: Dr. Cunningham-Rundles, did you have any remarks?

DR. CUNNINGHAM-RUNDLES: Well, Kara is a -- has been a patient here of ours at Mount Sinai before she, during the COVID period, relocated for different reasons to Boston, where she was getting infused during that period. And at the Mount Sinai Center, using basically the same product and the same rate with no premedication, she had not had reactions. So, this was very unlooked for. In the Boston area, she's at a very good medical connection there. And I think it was a complete surprise that she had that.

Kara, you -- what was your subsequent infusions? Just remind the group. You had this one on that date. And then what about subsequent infusions?

KARA: I was pretreated with really all that stuff, the steroids -- well, Tylenol, I've always taken -- Zyrtec. And they added fluids, and we changed the product.

DR. CUNNINGHAM-RUNDLES: Right.

KARA: And it slowed the rate down. So, it's now, like, a five-hour infusion instead of, you know, two and a half.

DR. CUNNINGHAM-RUNDLES: And so, you've had no more reactions, or you've had --

KARA: So far, no. So far, no.

DR. CUNNINGHAM-RUNDLES: So, it was one reaction only to this one occasion?

KARA: Correct.

DR. CUNNINGHAM-RUNDLES: Right. The other thing, which, to me, was very interesting, which was that you had asked if they had reported it. And the answer was no.

KARA: Correct.

DR. CUNNINGHAM-RUNDLES: And so --

KARA: The infusion center said, we don't do that. And the doctor's office said, your

infusion was given at the infusion center, so they would be the ones to report it. But we don't do that. So --

DR. CUNNINGHAM-RUNDLES: I see. So, I found that interesting myself, is that it just points out that there's so little that we know about reactions that occur, to be honest, because everyone thinks it's not their job, I think.

KARA: That was my feeling too.

DR. CUNNINGHAM-RUNDLES: Okay.

KARA: But it was scary. It was very scary.

DR. CUNNINGHAM-RUNDLES: Yeah. It is scary. Are you still scared when you go get it?

KARA: Oh, goodness. Yes.

DR. CUNNINGHAM-RUNDLES: You are still?

KARA: Yeah, but I'll still go. Yes.

DR. CUNNINGHAM-RUNDLES: Oh, my. Okay.

KARA: But I'll never get a home treatment. That's for sure.

DR. CUNNINGHAM-RUNDLES: That's another very important point to bring out, I think, is

that it sort of limits your choices and certainly cuts out one more long blast of time in every month.

KARA: Right.

DR. CUNNINGHAM-RUNDLES: Yeah. Yeah, those are just important points to bring out.

KARA: Yeah, I always want to be in a medical facility.

DR. CUNNINGHAM-RUNDLES: Yeah.

DR. SCOTT: Well, Kara --

DR. CUNNINGHAM-RUNDLES: So, thanks, Kara, for sharing that, because --

KARA: Sure.

DR. CUNNINGHAM-RUNDLES: -- we have to check. It's a reality here.

KARA: Well, it's -- I hope it's helpful.

DR. CUNNINGHAM-RUNDLES: Okay.

KARA: And keep up the good work.

MS. NORTON: This is Margaret Norton. Dot, we have some questions from the audience. Would you like to read them now? They're for

Kara, actually.

DR. CUNNINGHAM-RUNDLES: Good.

MS. NORTON: I can read them if you'd like.

DR. SCOTT: Okay.

MS. NORTON: Yeah. The first one is, "Can the patient describe how the adverse events affected her life besides physically? For example, did it impact work?"

KARA: It impacted work in that I really couldn't work that week very well. And the bronchial response that I had -- I do have asthma, very bad asthma, just so you know. The bronchial response I had really -- I was in bed for the rest of the week. And the following week, I was able to, you know, sort of get out of bed, but I was really limited.

DR. CUNNINGHAM-RUNDLES: Yeah.

MS. NORTON: And there's one more. "Thank you, Kara, for sharing your experience. Have I understood correctly, in order to prevent recurrence of the reaction, you now receive IVIG

at a very slow infusion rate?"

KARA: That's one of the things that they do, but I also receive Solu-Medrol and Pepcid and Zyrtec, which I had not previously done regularly.

DR. CUNNINGHAM-RUNDLES: Yeah.

MS. NORTON: And one more just came in. "Did the patient report adverse reaction to the FDA or manufacturer?"

KARA: The patient did not. No.

MS. NORTON: That's all we have for now. Thank you.

KARA: Well, I'm reporting it now. How's that?

(Laughter)

DR. SCOTT: So -- okay. And -- right. I think the other thing to mention is that Kara already said she switched products as well.

KARA: There was a recommendation to make sure that the lot number was different. And then we sort of furthered that and said, let's just change product.

DR. SCOTT: Right.

KARA: Right.

DR. SCOTT: Well, thank you very, very much, Kara. This is a reality check, and we appreciate you coming to do -- coming to speak with the audience. And I think everybody appreciates it, because --

KARA: No problem. Thank you.

DR. SCOTT: Okay.

KARA: Thank you for having me.

DR. SCOTT: All right.

KARA: Have a good day. Thank you.

DR. SCOTT: You too. Bye-bye. So, we're going to move on to our first presentation. This is by Dr. Meghna Alimchandani. She's going to talk about the clinical presentation of these hypersensitivity reactions that have been reported before or after immune globulin infusions. And we can -- you can start.

DR. ALIMCHANDANI: Sorry. Can you hear me okay?

DR. SCOTT: Yes.

DR. ALIMCHANDANI: Okay, great. Okay. So, good morning, everyone. My name is Meghna Alimchandani. I work in the Division of Pharmacovigilance, which is within the Office of Biostatistics and Pharmacovigilance in CBER. And our role is to conduct post-marketing safety monitoring for all of CBER's regulated products, which includes the immune globulins.

I wanted to thank Ms. Glynn for sharing her personal experience of the allergic reaction that she experienced. And as Kara said, her report was not supported -- was not submitted to the FDA or the manufacturer. I just want to remind people again that anyone can submit MedWatch reports to the FDA. And it is important to include the lot number if you have that information available.

So, the goal of my presentation is to provide an overview of the clinical presentations of immediate hypersensitivity reactions that have been reported to the FDA Adverse Event Reporting System or FAERS during or after immune globulin

infusion. Next slide, please. This is my informal communication disclaimer. Next slide.

Okay. So, this is the outline for my presentation. We will go over some background information for the IG product class. Then I will spend a few minutes providing an overview of the pharmacovigilance for immune globulins. Following this, we will focus on the post-marketing adverse event data from our FAERS database. And again, to keep in mind, there is underreporting always in post-market safety reporting. We will provide some examples of clinical presentations of hypersensitivity reactions that have been reported during or after IG infusion, and we will end with summary and conclusions. Next slide. Okay, next.

Okay. So, IG products are manufactured from very large pools of human plasma. Hypersensitivity reaction is a known risk for this product class. So, very important to keep in mind this is a known risk. It's labeled. And I will show you a sample package insert for the -- to show you examples of the sections of the label

that include information about allergic reactions. Another thing to keep in mind is that some subcutaneous IGs may be associated with increased local infusion reactions compared to intravenous IGs.

And the third bullet on the slide highlights the goal for this workshop, as introduced earlier by Dr. Scott and others. So, from time to time, we have seen temporal clustering of immediate hypersensitivity reactions with specific lots. And we have seen this with different IG products -- both IV and subcutaneous products. This occurs episodically and unpredictably, as Dr. Scott said, leading to voluntary lot withdrawals in some cases. Next slide, please.

Okay. So, the next couple of slides display different brands of immune globulin products manufactured by different sponsors. Information on these products is available on CBER webpages. The point that I wanted to make is that we have many different products in this class that

are approved for different routes of administration, either intravenous or subcutaneous, and that are approved for many different indications as well. Examples of approved indications include primary humoral immunodeficiency, chronic inflammatory demyelinating polyneuropathy or CIDP, idiopathic thrombocytopenic purpura or ITP, multifocal motor neuropathy, dermatomyositis, et cetera. Next slide.

Okay. This is just another continuing the listing of IGs. Next -- yes, thanks. So, this slide is displaying sample sections of the label that include information on hypersensitivity reactions for IGs. They are contraindicated in those who have previous history of allergic reactions to human IG. And notably, information is included under warnings and precautions. And I'm going to read it out to you.

So, "Severe hypersensitivity reactions may occur with IGIV products, including whichever the product is that the label belongs to. In case

of hypersensitivity discontinue the product infusion immediately, institute appropriate treatment, have medications, such as epinephrine, available for immediate treatment of acute hypersensitivity reaction." And you will find some wording similar to this in all of the IG labels.

It is also described in the adverse reaction section of the label, if there are post-marketing reports of hypersensitivity, it will be included under the subsection 6.2, post-marketing experience. And if there were hypersensitivity reactions in clinical trial subjects, then that would be included under subsection 6.1, clinical trials experience. So, again, the point is that this is a known risk with IG products. What is -- the clustering is what is unexpected. Next slide, please.

Okay. So, the next few slides, I'm going to give you an overview of the pharmacovigilance activities to monitor the post-market safety of immunoglobulins. So,

pharmacovigilance is defined by the World Health Organization as the science and activities related to the detection, assessment, understanding, and prevention of adverse drug effects or any other possible drug-related problems.

FDA's pharmacovigilance database for therapeutic products is FAERS -- the FDA Adverse Event Reporting System or FAERS. This is a passive surveillance database. These are spontaneous reports that are reported voluntarily, which is very different from adverse events from a study where you would have solicited reporting of adverse events from active follow-up of patients.

We have two broad sources of reports. We have manufacturer reports, and we have non-manufacturer reports. The non-manufacturer reports are also known as direct reports. They are submitted by the public by patients, healthcare providers. We have numerous reports for allergic reactions, for example, submitted by pharmacists.

The sponsor -- for the manufacturer

reports, the sponsor has to comply with mandatory reporting requirements under our Code of Federal Regulations. The manufacturer submits expedited reports, what are also known as 15-day reports, for serious and unlabeled events, or if FDA has required expedited reporting for a particular adverse event of special interest. So, this is important, because hypersensitivity, as I showed you, is a labeled event. So, even serious events of hypersensitivity would fall under that umbrella of serious and labeled events, and would not necessarily be submitted as expedited reports to the FDA, unless we have made that requirement of the sponsor.

So, the database includes serious and non-serious reports. An adverse event is considered serious if it results in any of the following outcomes -- death, a life threatening event, in-patient hospitalization, prolongation of hospitalization, disability, congenital anomaly, birth defect, or other medically important events. Next slide, please.

Okay. So, it is important to understand the strengths and limitations of this passive surveillance database, because it provides important context for the FAERS data that I will be showing you in subsequent slides. So, firstly keep in mind that FDA accepts adverse event reports regardless of the plausibility of the product causing the event or the clinical seriousness of the event. And there are many strengths and limitations of this database.

One of the major strengths which we have seen time and again with the immunoglobulin products is that we can rapidly detect potential safety issues. There is a dramatic difference in reporting trends when we do have lots that are associated with a spike of hypersensitivity reactions. We can also detect rare AEs on this database. It is open-ended for hypothesis generation. There is great geographic diversity not only within the U.S., but we also have foreign reports that the manufacturer submits. And we have, again, an example. A very good example of

this capability to monitor product lots is the story of the immunoglobulins and how we are able to detect these impacted lots.

Now, there are many limitations of the passive surveillance database. One of the key limitations is that we often have missing or inaccurate data, incomplete data. Again, this is not a report that is being generated from a clinical trial. So, there is often missing data. If we don't have the lot number, then we cannot do a lot analysis. So, very important to provide as much information as possible in these spontaneous adverse event reports.

Another important point is that the reported diagnoses are not verified. We are not -- the anaphylactic reactions or the allergic reactions that you hear me talking about, we have not confirmed them with Brighton case definitions. We are taking the diagnoses as they are coming in these spontaneous adverse event reports.

And as you saw from Kara's story, there is underreporting. Not all adverse events are

reported to the FDA or the manufacturer, and there is reporting bias in different forms. We may have stimulated reporting or may have -- or we have underreporting.

There is no control groups. So, there is absence of control group. Patient exposure data may not be available. And this is especially true for the IG products. The manufacturer is able to provide us with distribution data for the quantity of IG distributed over a period of time. But because the dosage varies so much by indication and the age of the patient and the weight of the patient, it is not possible to really estimate a patient exposure. So, we don't have any denominator.

And this is often a challenge. And I often remind people, we cannot calculate incidence rates from passive surveillance data. There's not incidence rates, but we can look for trends in reporting. And we'll go over that more on the next slide. Next slide, please. Thanks.

So, this next slide discusses some of

the things we look for during our review of FAERS reports of hypersensitivity reactions for IG products. In screening post-market data, we take into consideration the baseline reporting rate across lots over time. And this information is very product-specific, because reporting practices vary. Use of the product, market shares, may vary.

So, it is really product-specific information. The baseline for one product may not be the same as for a different product. We conduct analysis by lot to see if there are spikes in reporting for only certain lots. And I have a sample graph on the next slide to display this.

We look at the seriousness of the event, the source of the reports. There are manufacturer reports that include, as I said before, U.S. and foreign reports. And then there are non-manufacturer reports from the public. We take into consideration whether the reports are coming from a single site or a single reporter versus multiple sites and multiple reporters to see if

there is any bias in -- for -- by reporter.

And we also take into consideration the route of administration. We try to understand if there is some factor that is leading to stimulated reporting. For example, after one lot withdrawal has been initiated, or if there are public postings that have been made, we will likely see a spike in reporting.

Another big factor that I want to mention that impacts reporting is whether the IG lot was distributed by a patient support program. And this often happens. And if it was, then the patient support program may be a source of solicited reporting. And this may lead to a dramatic increase in the number of reports, because, again, this is solicited reporting. There is patient contact by representatives of the patient support program who are asking actively about adverse events and reporting that to the FDA.

Okay. And then the last bullet is the information that we seek from the manufacturer as

we do our evaluation of concerning trends. And these are some of the things that we ask about -- were there any manufacturing deviations, are you able to identify additional lots that contain some IG derived from the same plasma pools as the implicated lot, the dates of manufacture, distribution, and expiration, the number of vials in the lot, which can vary very widely, what is the manufacturer's estimate of how many vials are still in circulation, et cetera.

And then, finally, the sponsors' root cause analysis and considerations for lot withdrawal are discussed with the FDA, and potential thresholds for -- that would trigger signal evaluation or lot withdrawal for future. Okay. Next slide.

Okay. So, this is a graphical -- this is a sample. It's not any real product. This is a sample graphical display, but it shows you the hypersensitivity reactions by lot for a -- over a given time period. So, on the X axis, you have the IG lots. On the Y axis, you have the number

of hypersensitivity reports. And there are two things you can see. Firstly, the baseline reporting rate is very low for this product. Most lots have zero to two hypersensitivity reports. And secondly, when you have a temporal clustering of reports with implicated lots, that's indicated by the red arrows, the spikes in reporting are very dramatic and way higher than the baseline reporting rate. Next slide.

Okay. So, as you saw from the last slide, because there is such a dramatic difference in the reporting trends for implicated lots compared to the lots that are not withdrawn, this slide shows a suggested approach for considerations of thresholds to initiate signal evaluation and investigation, which may lead ultimately to lot withdrawal. So, one could potentially compare the data on the hypersensitivity reports from non-withdrawn lots and compare that to the implicated lots that were withdrawn. And we can look at the total number of hypersensitivity reports -- mean, median,

percentiles, ranges. And using this information, we can arrive at a threshold for the number of hypersensitivity reports that would trigger additional assessments. And again, it is important to point out that this type of data is very product-specific. So, thresholds such as this would vary from product to product. Next slide.

Okay. So, we will spend the next few slides looking at post-marketing data on hypersensitivity reports for IG products. Next slide, please.

Okay. So, Dr. Scott went over the IG lot withdrawals that happened prior to 2018. And then this slide shows some of the more recent IG lots that have happened from 2018 to date. So, one very important point to keep in mind is that not all of these lots were associated with increased reporting of hypersensitivity reactions. Some of the lots were indeed associated with increased reactions and withdrawn by the sponsors.

Additional lots were also withdrawn by

the sponsors as a precautionary measure, or quarantined if initial assessments revealed that they may contain immunoglobulin derived from the same plasma pools as the implicated lots. So, I really want to stress this point that several lot withdrawals in this list were implemented by the sponsors out of an abundance of caution as a precautionary measure. Next slide.

Okay. So, the next few slides capture slices of FAERS data for a specific time period for different withdrawn lots. And the point of these slides is to give you a flavor of the clinical presentation of immediate hypersensitivity reactions that have been reported for lots that subsequently underwent voluntary lot withdrawal. And I wanted to point out some caveats. This is not a comprehensive analysis. You should think of this as a snapshot of FAERS data for different IGs. And you will see that the outcomes are reported in slightly different ways for different products on these slides, because over time, reviews of adverse events were

classified in slightly different ways.

I also want to take a moment to thank my colleagues in the Office of Biostatistics and Pharmacovigilance, Division of Pharmacovigilance, who provided the data for these slides and who spent hours carefully reviewing and analyzing the very large volumes of adverse event reports and the sponsor submissions that were related to the lot withdrawals. So, I'm presenting, really, on behalf of a large pharmacovigilance team. Thanks.

Okay. So, this first slide goes over the FAERS data for Gamunex-C. So, beginning in 2018, an increased reporting rate of hypersensitivity reactions was identified for specific lots of Gamunex-C. And this slide captures information for the first eight lot withdrawals that occurred from 2018 to '19. There were a total of 23 lots of Gamunex-C that were withdrawn from the market between 2018 and 2021.

Gamunex-C was also presented to the Pediatric Advisory Committee in 2020. And the lot withdrawals were discussed at the public meeting

as well. And another thing to keep in mind is that Gamunex-C may be administered via IV or subcutaneous routes depending on the indication. It is IV only for ITP and CIDP indications, and IV or sub-Q for a primary humoral immunodeficiency.

So, as you can see from this slide, there were 271 reports of hypersensitivity events, and 39 percent or 107 were serious adverse events. There were no deaths due to hypersensitivity reports. We had pediatric as well as adult reports. The most common events were urticaria, pruritus, rash, lip swelling.

The serious adverse events included dyspnea, bronchospasm, laryngeal edema, throat tightness, swelling, lip and/or tongue swelling, airway closure. Onset was -- again, there is strong temporal association. Onset was during infusion or shortly thereafter. Some resolve spontaneously. Others require treatment on-site or in the emergency department with antihistamines or steroids.

Another thing that we noted during

review of these reports was that the pretreatment protocols really varied by institution, and that really impacts the severity of the reaction. So, some patients had been pretreated and others had not. Okay. Next slide.

Okay. So, the next two slides go over the FAERS data for Privigen, which has IV route of administration. So, as you can see from this slide, there were 261 reports of hypersensitivity adverse events, of which, about a little bit more than half, to 59 percent, were serious events. Again, there were no deaths due to an allergic reaction. The most common adverse events were urticaria rash, infusion-related reaction -- and the next slide actually shows you a graph of the top referred terms that we see in the FAERS reports.

Serious adverse events included dyspnea, lip swelling, anaphylactic reaction. We had reports in pediatric patients and adults. Time to onset, again, is during infusion or shortly thereafter. And there were patients who were

hospitalized and required intervention. Next slide. So, this is just showing a graphical display of the common preferred terms that were seen with the withdrawn lots. Next slide.

Okay. So, Hizentra is a subcutaneous IG. From this -- this is a slide of -- summarizing data from two withdrawn lots. There were 73 cases of hypersensitivity adverse events, and this exclude any duplicate reports. About 41 percent were serious events. Again, there were no deaths causally related to an allergic reaction. The most common events were infusion site/injection site erythema, infusion site pruritus, infusion site swelling, injection site pruritus, injection site swelling. So, a lot of local infusion site reactions that we see with a subcutaneous product like Hizentra.

About 20 percent cases reported systemic symptoms, including four cases with airway symptoms, throat tightness, dyspnea, mild shortness of breath, tongue swelling. There were no cases reported of epinephrine administration,

anaphylaxis, or hospitalization from this data set. And the age range, again, included pediatric and adult patients. Next slide.

Okay. So, the next slide, this is data summarized for Octagam 10 percent. There were 39 cases -- and this is an IV administration. There were 39 cases of hypersensitivity adverse events, of which, 64 percent were serious events. There were no deaths due to allergic reactions. Majority of reports categorized as serious involved rash, pruritus -- most were treated with Benadryl, Pepcid, steroids, and recovered on the same day. So, again, there is some judgment in submission of serious reports and what is categorized as serious by the reporter.

One lot involved anaphylaxis and use of epinephrine. The age range was -- the -- it was 27 to 88 years old. So, it reported adults. And again, time to onset shows close temporal association with the infusion. Next slide.

Okay. So, moving on to summary and conclusions. Next slide. Okay. So, what have we

learned from our post- marketing experience with lot analysis for IG products? There are many factors impacting reporting to the FAERS database. And again, it depends on the use of the product, the market shares of different brands, reporting practices vary. There may be stimulated reporting, solicited reporting through patient support programs, and underreporting.

Another thing that we learned is that clinical practice varies. And this varies by institution with respect to premedication prior to IG infusion. And this really has an impact on the severity and the seriousness of the hypersensitivity reaction. From the withdrawn lots, we saw that the majority of hypersensitivity reactions were non- serious, and the most common are rash and pruritus. But it covers the spectrum from rash to anaphylaxis with systemic symptoms that can be life-threatening -- lead to life-threatening events. To death, there are no deaths that were attributed to an allergic reaction. And then clustering was not associated with indication

or route of administration. Next slide.

Okay. So, FDA, you know, and manufacturers work very closely. And you'll hear more about this during the workshop, very -- work very closely on these safety signals. So, voluntary lot withdrawals may be initiated by the manufacturer. Website postings and communications may be done by the sponsors. FDA may post safety communications and potential signals of serious risk. And I'll show you a few examples of the postings that FDA has done.

We may require enhanced pharmacovigilance for a period of time, given that you would not [ordinarily have to] to submit expedited reports, since this is a labeled event. We may say that for reports, after lot withdrawal has occurred for a particular period of time, submit all reports of hypersensitivity, including the non-serious reports, as 15-day reports. And this reduces the lag period in FDA being able to review these types of reports.

FDA may also ask the sponsor to propose

certain criteria for a threshold to initiate signal evaluation and consider lot withdrawals. And this is really based -- this is product-specific data. It would be based on a baseline historical data about baseline reporting rates of hypersensitivity compared to the reporting rates for the withdrawn lots.

And then, finally, the sponsor conducts root cause investigation and provides updates to the FDA. So, very important message is that FDA and the sponsors work collaboratively on signal identification and evaluation, risk communication, and mitigation. Next.

Okay. So, this is an example of a CBER safety communication that we did. This is on the CBER webpage, listing out all the voluntary lot withdrawals of IGIV and IGSC that were associated with increased reports of allergic and hypersensitivity reactions. Next slide.

And then we provide some information on the webpage about reporting to the FDA as well and what to do with -- if you have a withdrawn lot in

your stock. Next slide.

And this is an example of what is known as a 921 posting. This is a posting for a potential signal of serious risk that was identified by FAERS data. And this is a posting that was done in 2019 for Gamunex-C.

And I believe that is my last slide. Thanks so much. Back to you, Dr. Scott. Happy to take any questions.

DR. SCOTT: Thank you very much, Dr. Alimchandani. And let's see if we have any questions.

MS. NORTON: Dot, would you like me to read those?

DR. SCOTT: Yes, please.

MS. NORTON: Okay. So, we have some questions for Meghna, but also one more for the patient. So, maybe I could ask that one at the end. So, we have, "Does the FDA collect data on the ABO blood group of recipient and reported reactions?"

DR. ALIMCHANDANI: No, the -- we don't.

We don't. We -- there is information that you can provide in the narrative when you submit MedWatch forms, but we don't collect that information.

DR. SCOTT: Okay. The next question is, "What is the difference between a withdrawal versus a recall?"

DR. ALIMCHANDANI: That's a really good question. Let me read out to you. Give me one second. Okay. So, I'm going to read out to you. This is from the FDA's -- from the CBER webpage for recalls. So, Recalls are a firm's removal or correction of a marketed product that the FDA considers to be in violation of the laws it administers, and against which the agency would initiate legal action -- for example, seizure. Recalls may be conducted on a firm's own initiative by FDA request or by FDA order under statutory authority.

Market withdrawals are a firm's removal or correction of a distributed product, which involves a minor violation that would not be subject to legal action by the FDA, or which

involves no violation. So, really, for these lot withdrawals, there were no [relevant] manufacturing deviations. And you will hear later the complex process for identifying the root cause. So, the violation -- there was no violation at the level that would initiate a recall. And I don't know, Dr. Scott, if you have other comments on that.

DR. SCOTT: Well, I would say that the firms involved have always been agreeable to a voluntary withdrawal, that they initiate. And that's the way it's been working at least since 2004. We present them with the information, and sometimes, they come to us, and they have received a lot of adverse event reports that we haven't received. And those naturally would flow to us in a longer time frame, but they will bring it to our attention. So, either FDA or the manufacturer discovers these clusters, or both, very often both, and then we discuss the need for voluntary withdrawal, which then takes place.

MS. NORTON: Okay. The next question

is, "Injection site pruritus is not included in SMQ for HSN/anaphylactic reactions. Is this event considered as so?"

DR. ALIMCHANDANI: So, the question was that it's not -- why is it not included in the SMQ? I'm sorry, I missed the --

MS. NORTON: It says it's -- "is not included." And --

DR. ALIMCHANDANI: Okay. So, we are really looking at -- we're not just doing the queries by the SMQs. We're looking at any hypersensitive-related AEs. So, it's really a very broad approach when we're looking at these reports.

MS. NORTON: Okay. The next one is, "Is site of care taken into consideration when monitoring adverse events?"

DR. ALIMCHANDANI: The -- as in whether the infusion happened in the hospital site or whether it happened at home? Yes, if that information is available in the report, we will take it into consideration. And it is -- you

know, it is more scary for the withdrawn lots if it is something that the patient is administering at home.

MS. NORTON: Okay. The next one is, "Does FDA have experience of any similar adverse events patterns associated with parenteral infusion of other large volume products, not IVIG, not plasma-derived?"

DR. ALIMCHANDANI: No. We haven't seen this pattern. It's really been a pattern for the IG products.

MS. NORTON: Looks like we have two more, and then a raised hand. "Does the FDA receive data from EMA for those products that are marketed in the U.S. and in the EU also?"

DR. ALIMCHANDANI: Mm-hmm. No, that's a good question. So, we have received information from sponsors, for sure, that have -- you know, that are selling the product outside U.S. And occasionally, there will be -- the root cause investigation involves product both from the U.S. As well as outside U.S. So, we have that data

from the sponsor, and that comes up when we discuss that with the sponsors. We do have standing meetings with EMA, and we have had meetings before on discussions of these allergic reactions associated with specific lots. So, there is, you know, information being exchanged with the EMA, and the sponsor also provides us with foreign information when it's relevant.

MS. NORTON: How is a cluster of adverse events defined per FDA?

DR. ALIMCHANDANI: Yeah. There is no formal definition for a cluster of adverse events. As you saw from the graph, you know, we're looking at reporting trends, and it becomes very obvious when there is a sudden increase in adverse events for a particular lot. And as Dr. Scott mentioned, you know, sometimes, the sponsor sees it first. If they're not doing expedited reporting, we may not have eyes on those reports until they let us know. And sometimes, we see it first, because we get direct reports from healthcare providers that the sponsor hasn't seen and that have come to the

FDA directly. And then we share that with the sponsor through FOIA requests.

So, it is very -- it becomes very obvious very quickly once we see, you know -- it sort of snowballs. One day, you have 10 reports. And you do a query in a couple of days, and you have 30 reports. And for that particular product, maybe the baseline is zero to two reports. So, that is -- you know, that is how we do our analysis for a cluster, but there is no formal definition that FDA has.

MS. NORTON: Okay. I see Dr. MacGlashan has his hand up.

DR. MACGLASHAN: Yes. Hi. Quick detail in -- did your team who was reviewing the -- these reports differentiate between a reaction that was occurring during administration versus afterward in the context of IV administration versus sub-Q? And I'm just getting at the rapidity of the response and whether the sub-Qs -- the reported reaction occurs after the sub-Q administration maybe because of diffusion time or something like

that versus IVIG, where -- I mean, where it's coming in IV. And -- just a question whether you could see that detail there.

DR. ALIMCHANDANI: Mm-hmm. So, you know, for Hizentra, it is sub-Q only. And there were more -- you know, there were these local infusion reactions. There were just more of these. And many of them were nonserious when you compare that to the IV products.

For Gamunex-C, we tried to do a little bit of what you were saying, Dr. MacGlashan, because Gamunex-C is administered both IV and sub-Q, depending on the indication. We did not find a pattern. And that may be because we often have, you know, missing information in these reports. So, I think, you know, that sort of, you know, prevents us from seeing a pattern. I think it's a good hypothesis --

DR. MACGLASHAN: Okay.

DR. ALIMCHANDANI: -- what you're saying. We just didn't see it. We didn't look for it. And often, as I said, we just don't have

all the data in the FAERS reports.

DR. MACGLASHAN: Okay. Thank you.

MS. NORTON: We have four more questions. Do we have time for those?

DR. SCOTT: Let's just -- well, basically, we have one minute left. So, for questions that haven't been answered, we will also have a list of all the questions in the chat room. And we'll endeavor to answer those if there is time, or after the meeting. So, why don't we do two.

MS. NORTON: Okay. I have a comment from Caroline Voltz from EMA. She says, "At EMA, we have not received reports on hypersensitivity reactions from the companies. Perhaps the question to be asked to the companies if they can explain why this is the case." Perhaps later on?

SPEAKER: Yeah, very loaded question. That's fine. Let's --

DR. SCOTT: Yeah, I think that's for later on. Yes. Mm-hmm.

MS. NORTON: Okay.

DR. SCOTT: It's a good question, but we'll get into that -- we can get into that under the manufacturers' discussions in the afternoon.

MS. NORTON: Okay. And then one more. I guess if we have -- so, there's three more, but I'll read one more. "Did you take a closer look at the cases with pretreated patients, in particular, in comparison to those who did not receive premedication?"

DR. ALIMCHANDANI: Yes, we did. And the reviewer for Gamunex-C, for the initial lot withdrawals, is the one who pointed that out that there were some patients where it would be listed that, you know, the patient was premedicated, and usually, those reactions were less -- were not serious or less severe. And that is, you know, something -- it is the practice of medicine. It just -- institutional practice varies with regards to premedication.

DR. SCOTT: Okay. I'm sorry that we'll have to move on. And we'll have a look at those other questions and try to answer them if there's

time, or after the workshop, to the individual.

So, we need to move on. And I'd like to introduce Dr. Fred Finkelman, a physician and professor of internal medicine emeritus at the University of Cincinnati, as well as an adjunct professor of pediatrics at Cincinnati Children's Hospital Medical Center. But the reason he is here today is because of his vast experience of immunology, particularly where it comes to the types of processes that underlie hypersensitivity and other types of events. So, Fred, take it away.

DR. FINKELMAN: Thank you, Dot, and thanks for organizing this workshop. And I want to mention that recently, I become a consultant for CSL Behring with regard to IVIG problems. Next slide, please.

The goals of this talk really to go over the pathogenic mechanisms for immediate hypersensitivity reactions, and to then consider how IVIG might initiate these mechanisms. Next slide, please.

This is data from one case report. And it really underlines the information that you heard about from Kara. This is one patient who, within less than an hour of the start of an IVIG infusion, developed urticarial rash, difficulty breathing, nausea, vomiting, abdominal cramping, hypotension consistent with anaphylaxis, and flushing. All of these are symptoms associated with immediate hypersensitivity reactions, and are reason for considering immediate hypersensitivity mechanisms as a reason for adverse events that could be associated with a particular lot of IVIG. Next slide, please.

So, then it raises the question of what accounts for the rapidity of these reactions. And probably, the most important reason is the very rapid release by activated cells of vasoactive mediators, including histamine, platelet-activating factor, leukotrienes, serotonin, prostaglandins, but especially, according to research that several people have done in this area, histamine and platelet-activating factor.

This is not all that's released. There are a number of cytokines and chemokines, a number of proteolytic enzymes. But the major effect of all of these, especially the vasoactive mediators, is increased vascular permeability with fluid leak that can cause vascular collapse. And this can be furthered by decreased cardiac contractility and by vasodilation. And in patients, as you heard from Kara today, increased airway smooth muscle contraction, which can be underlined by epithelial -- increased epithelial secretion, producing an asthma-like reaction. And the rashes that you see, such as urticaria, are accounted for by this increased vascular permeability with fluid leak. Next slide, please.

Well, that raises the question, what cells produce these mediators? And at least in experimental systems, the two most important cells are mast cells and macrophages. Mast cells produce histamine, PAF, and everything else that I've mentioned. Basophils are cells that are circulating like mast cells, but have many

similarities to mast cells, and can also release histamine. Macrophages produce many of the same mediators, but very little histamine. And there's arguments about whether neutrophils and platelets are important parts of these reactions. Next slide, please.

Well, what activates these cells? And probably the most important activation comes from crosslinking of Fc receptors, either by antibodies that then bind to antigen to cause crosslinking, or by antibodies to the Fc receptors themselves. And the most important Fc receptors are FcRI, which is the high-affinity receptor for IgE, and several FcRs that you'll hear more about later. In mice, the most important one seems to be FcRIII -- in humans, FcRIIA. The IgE receptor binds IgE with extremely high affinity. The FcRs bind IgE -- IgG with relatively low affinity. And they're competed with by circulating monomeric IgG.

But there are also anaphylaxis reactions that can be caused by complement receptors --

receptors for the anaphylatoxins C3a and C5a. And cytokines, particularly IL-3, can cause an anaphylactic-like reaction with activation of some of these cells. More recently, a G-coupled receptor called MRGPRX2 has been shown to be activated by a number of drugs, particularly, agents that are used during general anesthesia for neuromuscular blockade, and some types of antibiotics -- and can also cause mast cell degranulation and these types of reactions. LPS has been described to cause mast cell degranulation. And detergents by perturbing cell membranes can affect mast cells, basophils, and other cell types, including macrophages. Next slide, please.

It's important to realize that a lot of what we know about anaphylaxis comes from models that are easily studied. You can't do these studies easily in people. So, by default, studies have been done in rodent models in which rapid development of hypomobility and hypothermia are easily observed features. The next slide, if you

can activate the left-hand panel, these are BALB/c mice that are happily wandering around their cage. You can see that they meander fairly quickly.

If you look at the next slide, please, and activate that, now, you're looking at mice 10 minutes after they've received an agent that's causing an anaphylactic reaction. And you can see that they're still meandering, but much more slowly. By 15 minutes, as shown in the next slide, they're hardly moving at all. These mice are very lethargic. They're not dead. You can see that some of them are still moving, but there's a huge difference from what you saw in the first slide. And this is one way that we assess anaphylaxis in mice.

The next slide shows the other main way that we assess anaphylaxis. Mice are small critters. They radiate their heat very quickly. And when they develop anaphylactic shock, they're perfusing critical organs of their body much less than normally. And they're making much less heat than normally. And as a result, very rapidly,

their temperature goes down. So, here, in the blue diamonds and red circles, you're seeing mice that are undergoing anaphylaxis mediated by IgG anti-TNP and injection of TNP bound to a protein, ovalbumin, or by IgE anti-TNP in the red circles.

You can see that the anaphylactic reactions are very similar. You're getting about a 5-degree drop in temperature. The nadir is about 5 -- about 30 minutes. You can see some effect by 5 to 10 minutes. And if the mice don't die, they very rapidly recover. And you can see in the right-hand panel that this temperature drop very closely matches this increase in lethargy with decreased mobility. And again, it's very similar for IgG and IgE-mediated anaphylaxis. Next slide, please.

So, those reactions were caused by an IgE antibody or an IgG antibody binding antigens, stimulating the receptor. But the receptors can also be stimulated by IgG antibodies that are specific for the receptors themselves. And here, you can see the temperature drop that occurs in

mice that are injected with an IgG antibody to the IgE receptor, or an IgG antibody to one of the IgG receptors. Again, very similar kinetics to what you saw before. And as we'll discuss in a few minutes, this is, I think, very pertinent to the types of problems we see with IVIG. Next slide, please.

And you can see that a single injection of the two most important mediators for these reactions, histamine and platelet-activating factor, again, will cause a very similar decrease in temperature drop, which is self-limited, if the animals survive. Again, you can see that the similar kinetics of the temperature drop caused by histamine or by an antigen- antibody reaction indicates that the release of histamine or platelet-activating factor must occur very quickly. And we see this within two minutes of stimulation with IgE antibody and an antigen to that antibody. Next slide, please.

While the kinetics of these responses and the severity of these responses is very

similar between IgE receptor and IgG receptor signaling, the -- there are some considerable differences between these two IgE or IgG-mediated mechanisms. Next slide, please.

But first, just to show some of the similarities in signaling, both of the IgG receptors and the IgE receptor involve a signaling molecule called Fc receptor gamma chain. This is activated when the receptors are crosslinked with activation of a Src family kinase called Lyn that activates Syk, and that then activates a number of other tyrosine kinases and other signaling molecules, with one important one being BTK or Bruton's tyrosine kinase.

This is shown for an IgG receptor. And then the next cartoon on the next slide -- next slide, please -- shows a very similar mechanism for an IgE receptor, although a second signaling chain called FcR beta chain is involved. But again, Lyn, the Src family of kinase, starts the ball rolling with activation of tyrosine kinase Syk, activation of Bruton's tyrosine kinase, and

down the line, many other signaling molecules. Next slide, please.

But while there are similarities, there's one very important difference, and that's the quantity of antigen that's required to stimulate the IgE or the IgG-mediated pathway. So, here, you see the induction of a decrease in body temperature in mice by injection of either an IgE antibody to TNP or an IgG antibody to TNP, followed by different conjugates of the hapten TNP with a protein, ovalbumin.

And if you just look at these black squares on these two panels, you can see with the IgE-mediated mechanism, you'd get about half-maximal drop in temperature, with about 20 nanograms of antigen. Whereas for the IgG mechanism, it takes about two micrograms of antigen or about a hundredfold more antigen for the IgG-mediated mechanism than the IgE-mediated mechanism. Next slide, please.

And this occurs because of the different affinity of IgE or IgG for their receptors and

because of the presence of large amounts of competing soluble monomeric IgG in circulation, whereas there's very little competing circulating IgE. So, for IgE-mediated anaphylaxis, there's pre-association of IgE with the Fc? receptor. The relevant antigen comes along, crosslinks these IgE molecules, and activates the mast cell or other cell type.

For IgG, there isn't pre-association with the receptor. Instead, you have to have the formation of aggregates in circulation between IgG and the antigen. And these aggregates, because they have a higher affinity for the IgG receptors than the competing soluble monomeric IgG, can trigger these cells. Next slide, please.

As a consequence of this, anaphylaxis caused by low levels of antigen can only be mediated by IgE. And examples would be insect sting allergy or food allergy, because there's poor absorption of ingested food into systemic circulation. When the antigen-specific IgG antibody levels are high and antigen levels are

low, the predominant effect of the IgG is blocking IgE-mediated anaphylaxis via antigen sequestration and signaling through an inhibitory receptor.

Whereas when antigen-specific IgG levels are -- antibody levels are high and antigen levels are high, then IgG can mediate anaphylaxis. And when we're dealing with a large amount of an antigen injected, such as some of the therapeutic monoclonal antibodies and possibly IVIG, you may have the second situation that could allow both IgG and IgE-mediated anaphylaxis. Next slide, please.

There are also a number of mechanisms that negatively regulate mast cell degranulation. The most important of these is the inhibitory receptor that I mentioned earlier, FcRIIB. It's also a low-affinity receptor, but there are other receptors on mast cells such as Siglec-8.

There are cytokine receptors for IL-10 and IL-37, and there's desensitization to stimulatory receptors by low and slow receptor ligation and crosslinking that can make these

receptors less responsive. You'll also hear from a later talk about certain antibody glycosylation patterns that can negatively regulate this process. Next slide, please.

Next slide, please. So, let's bring this into IVIG. When might IVIG activate these mechanisms, and what might be increased in pathogenic lots? Well, one mechanism could be that certain lots of IVIG could contain IgE or IgG antibodies to antigens present in recipients. So, if a recipient was exposed to large amounts of cat dander, for example, and IVIG contained IgE antibodies to cat dander, that could be a possible way of triggering an immediate hypersensitivity reaction.

Perhaps more likely, though, is that IVIG might contain antibodies to some of these receptors themselves or to IgG or IgE themselves. And these antibodies could then directly activate the receptors and cause mast cell or macrophage activation. Receptors to the complement-derived anaphylatoxins to MRGPRX2 could also be

crosslinked by specific antibodies that might be in the IVIG. And that could then activate the cells that produce the mediators and cause an anaphylactic response.

IVIG might also contain antibodies to things to which might contain antigens such as IgA, which, in IgA- deficient patients who may have anti-IgA antibodies, could trigger an anaphylactic reaction. And IG -- IVIG might contain detergents or might contain preformed IgG-immune complexes that could directly activate cells such as mast cells, or activate them by activating the complement system. Next slide, please.

But there are reasons to be suspicious of any of these possibilities. Most importantly, a lot of IVIG is made from a great many patients, which means that a single donor's plasma is going to be diluted anywhere from 1 to 1,000 to 1 to 10,000, which means that there would have to be an extremely high titer of whatever it is in that patient's serum that's causing the reaction.

That, to me, makes it more likely that you're going to have an IgG antibody to one of the receptors that could stimulate and activate mast cells or macrophages than to have IgG or IgE itself that is specific for an allergen.

So, an anti-receptor antibody rather than an antibody to an allergen seems to me to be more likely. But there are questions. If an autoantibody is involved, such as an antibody to a receptor, then why isn't the donor sick? Well, maybe the donor could become tolerant of that antibody. Or maybe the antibody that's produced would be to an alloantigen that is --doesn't recognize an antigen in the donor, but recognizes an antigen in the recipient.

If that's the case, then you would think that most of the donors would have to be female, because women are much more likely to be induced to have alloantigen -- alloantibodies by pregnancy. It's unlikely to be an -- unlikely to be IgE, because there's very little IgE in IVIG preparations. If high IgA content is involved,

then the only donors who should develop disease should be IgA-deficient donors. That doesn't seem to be the case.

As far as problems with detergent or aggregates, the FDA provides limits for excipients such as detergents, which should be able to prevent those problems. And finally, only a small percentage of recipients who receive pathogenic IVIG develop disease reactions. So, why is only a small percentage of recipients developing the reactions if there's something in common in a particular batch of IVIG that makes recipients develop the reactions? Next slide, please.

Next slide, please. Well, recipients can differ in their susceptibility to develop an anaphylactic-like reaction. Certain cytokines that could be present in larger amounts in some recipients could activate mast cells and basophils and increase their number. Other cytokines can increase the responsiveness of target cells such as vascular endothelium to vasoactive mediators such as histamine. Some recipients more slowly

degrade vasoactive mediators so they can accumulate more. Some recipients such as people with mastocytosis have very large numbers of mast cells.

Others have mast cells or macrophages in a preactivated state so they'd be more easily activated by something that they get through IVIG. There are some drugs such as ß-adrenergic antagonists, often used for people with cardiovascular diseases. An example is propranolol that may make people more susceptible. Alcohol use and exercise can also make people more susceptible to anaphylactic reactions. Next slide, please.

I want to point out that a lot of the information I gave you comes not from human studies, but from mouse studies. And mice and people are very different. So, for example, using hypothermia as an indication of anaphylaxis works very well for mice, but not for people, who are much bigger and lose their heat much more slowly. It's difficult to see urticaria in mice, even

though this is a major way of seeing anaphylaxis in people.

There are differences in the cellular Fc receptors, in the IgG isotypes, and the mediators produced by activated cells and their effects on target cells between mice and people. Basophils have very few granules and very little histamine in mice, whereas they can have much more in people. So, they're likely to contribute more to anaphylaxis in people than in mice.

But despite these differences, studies in mice have held up remarkably well as models for human anaphylaxis. And lessons that we learn from normal mice or mice that have been partially humanized have really helped us understand immediate hypersensitivity reactions in people. And it would be very helpful to have a mouse model of IVIG-induced immediate hypersensitivity reactions that might be used to examine IVIG lots prior to their approval for clinical use. Next slide, please.

So, my bottom line is it seems likely

that IgG- associated mechanisms are involved in immediate hypersensitivity reactions to IVIG, especially mechanisms in which the IgG is acting as an antibody specific for receptors on cells that release mediators, rather than mechanisms in which the IgG is binding to a particular allergen. Understanding why some lots of IVIG are particularly likely to cause immediate hypersensitivity-like reactions and the factors that make some individuals particularly prone to develop these reactions remain elusive.

Better understanding of IVIG-mediated anaphylaxis, as well as better and more practical ways to identify recipient characteristics that increase the likelihood to have these reactions should provide ways to avoid these reactions.

I realize that I've spoken very quickly, and a little cold has perhaps made it difficult to understand me. Anybody who wants a copy of this presentation, please email me, and I'd be happy to send it. And thank you very much. It's my understanding that we'll go on to the next speaker

and take questions at the end of the three talks.

DR. SCOTT: Thank you very much, Dr. Finkelman. That was an excellent presentation and introduction to the complexities of these -- of hypersensitivity reactions. And it gives us some future directions, potentially, that could be explored. Our next speaker is Dr. Robert Anthony. He's an associate professor at Harvard Medical School. And he's going to speak about the role of antibodies and Fc receptors in immediate hypersensitivity.

DR. ANTHONY: Great. Thank you, Dot. Thanks to Fred for that introduction to our section. And it's really nice to be here. So, thanks for this opportunity. Next slide, please.

So, this is a schematic of chromosome 14 in humans that shows the heavy chains that essentially are used to express the antibodies. And underneath the heavy-chain locus, you can see schematics of the antibodies that are essentially produced as a result of the expression. Next slide.

And since we're talking about type I hypersensitivity reactions as a framework, much like Fred did in his talk, I think it's really useful to put this in the context of the way it's taught in the textbooks, which is IgE- mediated reactions. And so, in this context, there's a TH2 response that results in the production of IgE antibodies. They're secreted by B cells. And then they bind very stably with high affinity to FcRI, which is expressed on the surface of mast cells in the tissue and basophils in the circulation.

And then upon a subsequent re-exposure to the allergen, you get crosslinking of the cell-bound IgE on the surface of the mast cells or the basophils. And that leads to a signaling cascade that results in degranulation of these cells, releasing these soluble mediators that are responsible for the symptoms. And really, that's -- you know, as a mouse immunologist, we look at that in terms of vascular leakage, impermeability, and temperature loss, as Fred mentioned in his

talk. Next slide, please.

And so, for IgE, it sits on the mast cell in the form of a trimeric receptor, which is shown in the schematic. The alpha chain binds directly to the high -- to the IgE molecule with very, very high-affinity. So, the association constant is in the range of 109 or 1010. And so, this is a very stable interaction. The off-rate is three days. So, it has a -- IgE has a tissue half-life of three weeks on the surface of mast cells.

So, also associated with this receptor is the common gamma chain, which is critical over the signaling of many Fc receptors. And then it's also the beta chain that helps out with the signaling downstream. And so, next slide, please.

So, upon crosslinking of two IgE bound to the alpha chain of FcRI, that will essentially trigger the signaling cascades downstream inside the mast cells and the basophils. Next slide.

And so, this is similar to what Dr. Finkelman showed, but essentially, upon

crosslinking of the alpha chains by IgE that's bound, both the kinases Lyn and Syk will phosphorylate LAT. And that will essentially trigger a number of downstream cascades, including a MAP kinase pathway that's thought to be responsible for release of the lipid mediators associated with type I hypersensitivity -- the prostaglandins and leukotrienes. There's also essentially the phospholipase C pathway that ultimately leads to the calcium flux that's associated with the degranulation in mast cells that's associated with the release of histamine. Next slide, please.

And so, some of the classic models that we immunologists use include this passive cutaneous anaphylaxis model. And so, in this model, we would take a mouse IgE molecule and inject it intradermally into the ear of a mouse. Next slide. And it's essentially sensitizing locally in the ear. They could also be done in the skin, but the fur needs to be removed to do that. And the next day, the mice are essentially

challenged systemically or intravenously with the allergen that's been emulsified in Evans blue dye. And it's an inert dye.

But essentially, when the productive anaphylactic response occurs, it will trigger vascular leakage and permeability. And because of that, the blue coloration will actually migrate into the site where the anaphylactic response occurs. Next slide. So, you can actually get a blue coloration of the ear. Next slide, please. And so, that's visible, and you can quantify that as a surrogate readout for the magnitude of anaphylaxis. Next slide.

And so, this is just the Hooke Lab cells -- these kits that help elicit these types of responses. And this is a picture from their website, where you can see the right ear was treated with the DMP-specific IgE, where there was a mock injection in the left ear. And then upon challenge with the DMP allergen, in the context with Evans blue dye, within minutes -- you know, so, essentially, 30 to 40 minutes, you get this

very robust coloring of the ear that can be quantified. Next slide.

So, there's another classic model of anaphylaxis, which is a systemic model. So, in this model, mice are sensitized intravenously or systemically with the IgE molecule. And then they're challenged systemically with the allergen. And then measured, essentially, temperature is dropped, just as Dr. Finkelman presented. You can get this robust drop in temperature approximately 20 minutes after the allergen challenge. Next slide. Right. One more, please.

Right. So, this is a classic experiment from Kinet's Group (phonetic) where they compared sensitization of mice with human IgE that either lacked the mouse IgE receptor, which is the first bar, followed by mice that expressed one copy of human IgE high-affinity receptor that had both copies of the mouse IgE receptor, or that had -- were deficient in that receptor. And you can see that when there was either a mouse or human IgE receptor, there was a fairly robust drop in

temperature, about 4 degrees, approximately 20 minutes after challenge with the allergen. Next slide, please.

Right. So, with that framework in mind, I thought we would move on to the IgG antibodies. And there are four different IgG antibodies in humans. They are numbered based on their discovery, but also, it correlates with their amount that they're present in the circulation. So, IgG1, IgG2, IgG3, and IgG4. Next slide, please.

So, this is an IgG molecule. I think everyone here is likely familiar with this structure, but it has a Y-shaped structure -- the top of which is responsible for the specificity of the molecule. Next slide.

So, that's what's referred to as the antigen-binding fragment or the Fab. Essentially, the molecule is two identical heavy chains and two light chains. The Fab essentially is the light chain paired with the heavy chain, and it's the variable regions of the molecule in this portion.

Next slide.

The stalk of the molecule is the crystallizable fragment or the Fc or the constant region and this is the business end of the molecule responsible for conveying the effector functions. Next slide. And there is a single N-linked glycosylation site, asparagine 297, that's conserved across all mammalian IgG antibodies. And it's in a fixed position in the molecule, and you can actually resolve it by crystallography, which is shown here in this molecule. All right. Next slide.

So, the canonical functions of IgG antibodies are triggering inflammation. This is typically thought to be in the form of antibody-dependent cytotoxicity. They can also opsonize various antigens by, you know, enabling the phagocytosis, or they can be responsible for inflammation that's mediated through all complement fixation. And this can occur, certainly, at the end stage of some autoimmune diseases, including, you know, lupus nephritis.

Next slide, please.

All right. So, this is a picture of Jeff Ravetch at the corner, who's my postdoctoral mentor. And Jeff spent his career defining how IgG antibodies trigger inflammation. And so, this is historical data generated by Raphael Clynes in Jeff's lab almost 20 years ago. And so, Raphael was using a model of thrombocytopenia driven by a monoclonal antibody specific to the platelet ß-integrin.

And so, he gave this monoclonal antibody to wild- type mice, which is shown in red. And within about two hours, there's a rapid loss of platelets from the circulation of mice, as you can see. Mice in green received an isotype control treatment. And so, they had no loss of platelets. And mice that lacked the C3 protein, that's the complement protein that's common for all three complement cascades, they're shown in blue -- they also underwent a rapid loss of platelets that mirrored what was seen in a wild-type animal.

Next slide.

So, mice that lacked that signaling component of Fc receptors, the common gamma chain, were administered the platelet-specific antibody. And no platelet loss was observed, and that's shown in black here. And so, this result showed that this Fc receptor -- or the signaling component of this Fc receptors was required for this platelet depletion model, and not complement. And so, Jeff has gone on to show how important Fc receptors are for antibody biology. Next slide, please.

And so, this is just a schematic of the Fc receptor family for all of the antibody classes. So, starting on the far left, there's the high-affinity IgE receptor, FcRI. Next to it is the IgA receptor, and then there's the high-affinity IgG receptor, FcRI. Then we have the lower-affinity IgG receptors, FcRIIB, C, and

A Then there's FcRIIIA. IIIB is a G-protein-linked receptor. Then there's these C-type lectin receptors for IgE and IgG that have been described as type II Fc receptors. Next

slide, please.

So, focusing on the IgG receptors, there's -- on the top of this diagram, there's the mouse receptors. And on the bottom, there's the human receptors. But looking at -- so, there's a lot of similarities, but some important distinctions in these two different systems.

So, starting with the orange receptor, which is the high-affinity receptor, FcRI, this is largely thought to be irrelevant in terms of triggering inflammation. And the reason for that is because it binds IgG with a very high affinity. And because of the high serum concentrations of IgG, which is 1 mg per mL on a mouse and 10 mg per mL on a human, it's thought to be saturated by essentially random IgG molecules. So, the likelihood of it being crosslinked by an antigen is incredibly low.

So, generally those of us in the antibody field don't consider IgG1 as triggering antibody effector function. And I guess the exception would be what Dr. Finkelman mentioned in

terms of an antibody that might directly target FcRI. So, the remaining canonical or type I Fc receptors are considered to be low or moderate affinity. So, if we start with the first yellow receptor shown here that's the inhibitory Fc receptor, which is FcRIIB -- and so, it's a single alpha chain molecule that has an ITIM or a immuno tyrosine inhibitory motif in its -- in the chain. So, this induces an inhibitory signal when it's crosslinked on the cells that express it.

In the mouse system, that's the only, essentially, receptor of the II family. Then there's the FcRIII molecule, which is an activating Fc receptor, and uses the gamma chain to signal. There is also another activating Fc receptor, FcRIV, which also uses the common gamma chain to signal. Also shown in this diagram are the neonatal Fc receptor, FcRN, that's responsible for the prolonged serum half-life, and then this C-type lectin receptor, SignR1, that is responsible for conveying the anti-inflammatory signals of IgG.

So, in the human system, again, there's many similarities. So, starting with the low-affinity receptor, there's the inhibitory receptor, FcRIIB -- again, using a ITIM motif signal. There are the activating low-affinity receptors FcRIIA and IIC. So, IIC's risk expression is really limited to natural killer cells. So, we really focus on the biology of FcRIIA, again, which -- it's a single chain, alpha chain signaling molecule with an ITIM motif in the intracellular domain.

There is FcRIIIA, which also is expressed on natural killer cells, and which also uses the gamma chain for signaling. And then there's the GPI-linked receptor, FcRIIIB, which is thought to be expressed on neutrophils. And then, again, there's the FcRN responsible for the serum half, life and then the human ortholog for SignR1, which is DC-SIGN which, again, has been implicated in the anti- inflammatory activity of antibodies. Next slide, please.

All right. So, looking at the signaling

that's caused by immune complexes for these low-affinity activating Fc receptors, which is shown here, and as Dr. Finkelman alluded to, because these are lower-affinity interactions, they require the formation of complexes, which is depicted here in this schematic. And that increases the avidity of the interaction with these low-affinity receptors. And the crosslinking will essentially do some very similar signaling moiety to what we observed in the mast-cell pathway where there's essentially a Syk activation that is required to trigger the downstream events, which, again, include map kinase signaling as well as calcium flux, as shown here in this diagram. Next slide, please.

And so, this activating signal is opposed by this inhibitory signal mediated by the inhibitory receptor, FcRIIB. So, that receptor is expressed in the surface of B cells. And when it's co-ligated in the -- at the same time as the B-cell receptor, that's an inhibitory receptor that shows or tells the B cell to not respond to

the particular antigen that's being complexed and that's shown in panel B.

But in panel C, which is more relevant for this session, just looking at immune complexes engaging the inhibitory receptor on the surface of cells like macrophages, dendritic cells, and neutrophils, where, essentially, you would get an induced apoptosis by BTK in an adjunct-induced pathway following ligation of this receptor. Next slide, please.

So, the four human IgG subclasses are shown here, with, essentially, you know, a cartoon schematic of what they would look like. And they have very similar structures with the sole exception of IgG3, which has this very extended hinge region, which is shown here in the schematic. And the way that I generally think about them is with this blue chart with the plus or minuses, where IgG1 antibodies are very potent in fixing complement, but they also bind, you know, the most -- I guess, the best of all of these antibodies, that both activating and

inhibitory Fc receptors.

IgG2 are, you know, poor binders of complement and activating and inhibitory receptors, so it's thought to be essentially fairly inert. IgG3 binds strongly to complement and activating inhibitory receptors, so it is thought to be fairly inflammatory, similar to IgG1. Then IgG4 is a poor binder of complement and activating receptors, but binds with moderate affinity to the inhibitory receptors. So, it's thought to be either inert or inhibitory in terms of the subclasses. Next slide, please.

And so, the mouse system of antibodies, there are also four different antibodies. But the numbering system, I think, is remarkably confusing, because the effector function doesn't track with the numbering in humans. So, for mouse IgG1, it's essentially -- it's a poor binder of complement and binds with lower affinity to activating Fc receptors, but binds stronger to the inhibitory Fc receptors. So, mouse IgG2a or c, which essentially are the black (inaudible) valve

C parietals of this antibody subclass, binds very strongly to an activating Fc receptor and is a very potent antibody in vivo. Slightly less potent is IgG2b, but it's still a very strongly-activating antibody. And IgG3 in the mouse system does not bind to any Fc receptors. Next slide, please.

Great. So, this -- the importance of Fc receptor binding, and specifically, the affinity, was established by Falk Nimmerjahn, who's pictured here. And so, this is really a classic study that Falk did using the B16 melanoma model. And so, he administered systemically B16 melanoma cells to a mouse, and they'll -- which usually metastasizes throughout the body, and they'll congregate everywhere, including the lung. And then Falk gave a TA99 class switch variant to these animals. And so, TA99 is a monoclonal antibody that recognizes a specific motif on the B16 melanoma tumor.

So, what you can see very clearly is that these antibodies -- which have the same

specificities, but they just differ in the Fc region only -- had different ability to clear the B16 melanoma. So, looking at a mouse IgG1 antibody, you can see it's very much a black lung in panel A. And then when that's quantified in B, you can see that essentially, this dose of the mouse IgG1 had no ability to clear the B16 tumors on the lung.

In contrast, the TA99 with the IgG2a Fc was very potent at clearing these metastasized tumors. And that's true by the -- essentially, the white lung you can see in panel A, and also quantifying it, that it's a complete reduction of tumors. Also potent, but less so, is the mouse IgG2b. You can see it's almost a gray type of lung, and then -- and there is a significant but less reduction of the B16 tumors. And then IgG3 TA99 antibodies did not do any clearance at all. Next slide, please.

And this is from that same paper where it's using the platelet depletion model. So, this is the 6A6 model that Raphael Clynes established.

But again, it's the same pattern where these same specificity of the antibodies, they differ only in the Fc region. And it shows that in terms of platelet depletion, IgG2a and 2b are very potent at triggering platelet depletion, and that's shown in red and yellow, whereas IgG1 is about a 50 percent reduction in platelets, and there's no depletion in platelets shown in the IgG3 in green. So, next slide, please.

And so, Falk, in this paper, established this concept that you can predict the antibody function by essentially establishing the ratio of the affinity of that particular class of an antibody to the activating versus inhibitory Fc receptor. And so, what you can see is for mouse IgG1 antibody, it has a low activating -- low AI ratio. And so, it's essentially not so potent in vivo. The IgG2a has a very high ratio, so it's, you know, close to 70. And that's the most potent subclass. And then IgG2b has a ratio of about sevenfold higher affinity for activating receptors and inhibitory, and it's fairly potent as well.

Next slide.

And so, this is just a list of the affinity constants for both the human and the mouse antibodies and Fc receptors, really, that this concept of the AI ratio was established. Next slide, please. And so, what you can see is that we focus first on human antibodies. The highest affinity of human IgG1 of the low-affinity receptors is this FcRIIA. And then human IgG2 binds with about a tenfold lower affinity to that receptor -- that same receptor, preferentially. Human IgG3 binds with very high affinity up in the 107 range to the, you know, the receptor FcRIIIA. And then human IgG4 has the strongest binding to FcRIIB, the inhibitory receptor -- slightly higher than the other receptors, which is why it's thought to be an inhibitory-type antibody.

And then in the mouse system is essentially what Falk had laid out, where mouse IgG1 binds preferentially to the inhibitory receptor based on its Ka. Mouse IgG2a binds very strongly to FcRIV. And a little bit about --

essentially at tenfold less or so is where the IgG2b binds to that same receptor. And there's no detectable binding of the IgG3 subclass. Next slide, please.

All right. And so, this, again, is the picture of the IgG antibody with its structure. Next slide. Right. And so, one of the other things that might influence the interactions with these receptors is the single glycan. And this is just a schematic of the glycan that's at asparagine 297. It's tremendously heterogeneous. And so, it can vary by the presence of this fucose, the red triangle, the bisecting N-Acetylglucosamine or the galactose or sialic acid -- all of those moieties found outside of the dotted box. Next slide.

And the presence of these -- or absence of these various sugar moieties influences directly the interactions with Fc receptors. So, antibodies that lack fucose actually gain a 50-fold increase in affinity to the FcRIII3A. Next slide. And then antibodies that have sialic

acid have impaired interactions with Fc receptors. So, an additional wrinkle of complexity is the presence of the type of glycan attached on the antibodies. Next slide, please.

So, this is just a MALDI-TOF analysis of IVIG, actually. But it just shows the heterogeneity of glycosylation. So, there's over 30 distinct glycoforms that are present in healthy individuals on the Fc region of the antibodies. Next slide. So, 10 percent of these terminate in sialic acid, and these are represented under the red brackets. Next slide. But the other major forms essentially are these fucosylated, digalactosylated, monogalactosylated, or agalactosylated glycoforms. Next slide, please.

And so, this is just turning back to some of Falk's work looking in the B16 melanoma model in an antibody with and without fucose. And I alluded to earlier how that -- removing fucose from the antibody glycan results in enhanced affinity to an activating Fc receptor. And so, you can see, he did an analogous experiment to

what we walked through earlier, where, essentially, mice that received the B16 melanoma tumor intravenously were treated with the TA99 antibodies of an IgG2B backbone. And the only difference was one of those antibodies had fucose and one didn't. The same dose of the antibodies was administered. And you can see that the afucosylated antibodies have a -- were much more potent in clearing the tumor. And they have an enhanced activating to inhibitory ratio of 20 compared to that of 7 of a fucosylated antibody. Next slide.

So, this is the crystal structure of antibodies with and without fucose, as shown here. And essentially, what happens when fucose is present is there is essentially a contortion of a glycan essentially out of the frame, which is shown here on the top panel in yellow. But when that fucose is removed, you have a very strong hydrogen bond, a glycan- glycan interaction between the antibody and the Fc receptor. And so, that's responsible for this 50-fold gain in

affinity. Next slide, please.

And so, that's actually, you know, popping up in the clinic. And just two examples are that these afucosylated antibodies are associated with severe COVID. And so, essentially, it's thought to exacerbate some of the clinical presentations in COVID. And then also therapeutic antibodies that are -- they're being engineered to lack fucose because they're -- have enhanced potency. Next slide.

Right. So, another confounding factor for antibodies -- or human antibodies is in this idea of Fab-arm exchange, which occurs in IgG4 subclass specifically. So, there are these two cysteine moieties just beneath the hinge region. And they can -- when they're -- when these antibodies are undergoing, essentially, the recycling with the FcRN with a low pH, it's thought that these can undergo, essentially, an intra-arm cysteine pairing that allows for essentially a swapping of the two arms. And so, it's thought that IgG4 actually exists in this

mixture of heterodimers and homodimers, which is depicted in the bottom right of this slide. Next slide.

Finally, I just wanted to bring up this idea that while there are four different subclasses, there are a number of allotypes of the different antibodies as well. So, these are just some of the allotypes that have been identified in the literature. Next slide, please. And so, the so-called constant region has a total of over 30 different glycoforms that are possible to be present. There are 30 different -- either allotypes and/or subclasses, which makes -- there's a possibility of 900 different Fc configurations stored. So, it's much more complex than, I guess, we think typically. Next slide, please.

So, getting into some of the classic models, looking at IgG-mediated anaphylaxis, I think the first thing to point out was this passive reverse Arthus reaction that was described in Jeff Ravetch's lab. And so, this is

essentially mice that are treated with rabbit IgG at a particular site, and then they're administered with an antigen systemically. And you can essentially qualify the -- or quantify the edema and hemorrhage at the site. So, this is very -- this is analogous to the passive cutaneous anaphylaxis reaction that we discussed in the mouse system earlier. Next slide, please.

And so, this is actually pieces of skin tissue that were treated with this passive reverse Arthus reaction. So, you can see that wild-type mice received a fairly productive reaction, which was quantified by essentially the vascular leakage or permeability. There was not the blue dye that was administered here. So, essentially, you can just see the spreading of the blood. Looking at mice that lacked Fc receptors in panel A, you can see there's a marked attenuation of this reaction. Mice that lacked mast cells, which are the W/Wv mice in panel C, also had an attenuated reaction.

These mast-cell-deficient mice that were reconstituted with Fc-receptor-deficient mast

cells in D also had an attenuated reaction. Whereas mice in panel E, that were lacking mast cells, but had been reconstituted with wild- type mast cells that had Fc receptors, had -- were able to recapitulate the productive Arthus reaction as shown in panel A. And so, this experiment showed that it was essentially FcRs on mast cells required for this Arthus reaction in the tissue. Next slide, please.

And this is Dr. Finkelman's classic model in which mice are administered a goat anti-mouse IgG antibody. And this results in production of goat antibody specific IgG1, IgG2a, and IgE. The mice can then be challenged with either a goat antibody or an anti-IgE antibody. And you can see that essentially, you get a similar anaphylactic curve. However, the -- when you challenge with the antigen, which is the goat IgG, this is thought to be an IgG-mediated systemic anaphylaxis. And that's because, essentially, you can block IgE and still get the anaphylaxis. But if you block the FcRs, there's

no anaphylaxis that occurs. And that's in contrast when the anti-IgE antibody is administered. So, essentially, that does require IgE, and blocking FcRs has no effect on the induction of anaphylaxis. Next slide, please. Yeah, next slide. Thank you.

And so, this is a really classic passive model. And I just wanted to add this at the very end, just to further complicate things. But if you look at panel D, this is a TNP- specific monoclonal mouse IgG1 that was administered to a mouse. They also received human IVIG, which is shown in red. And you can see that while the -- there is anaphylaxis that's induced in the mice that received the antibodies, the IVIG was able to attenuate the anaphylaxis, which is shown in panel D. So, certainly, that further complicates thinking about how IgG antibodies trigger anaphylaxis responses. Next slide, please.

And so, getting back into the signaling, this paper identified a key role for the adaptor proteins Fyn and Lyn. And they showed that,

essentially, mice deficient in Fyn had a fairly normal, robust anaphylactic response in A, but the Lyn knockouts did not require -- did not recover nearly as quickly, which is shown in panel B. Next slide, please.

Right. And so, this shows that essentially, the mast cells are the primary mediators of the histamine release, which is what Dr. Finkelman alluded to. Next slide, please. And so, just to sum up with two very quick slides, thinking about Fc receptors in the cellular expression -- next slide -- you can see that looking at mast cells and macrophages or monocytes -- yeah. Next slide, please. Next slide. Yeah. So, the -- oh, I'm sorry. Back one. Yeah. Thank you.

So, looking at FcRIIIA in the mice, that's expressed by mast cells and monocytes and macrophages, but the Fc receptors that are expressed by those cells in humans are predominantly FcRIIA and IIIA. Next slide. And then -- I know I'm short on time. And so,

thinking about FcRIIA and IIB -- next slide -- the -- that is bound most strongly by human IgG1, which is just highlighted here in this chart. Next slide.

And so, with that, I'll just summarize, thanking everyone for their time and attention. Also, just to -- like, it's appealing to think about the similarities and also differences between IgG and IgE systems. And I'd like to thank all of the authors who generated the data and the reviews that were used here, and apologize to anyone that I didn't cite. That was an oversight, my fault. Thanks very much.

DR. SCOTT: Thank you, Dr. Anthony. And hopefully, you have time to stay around for the questions at the end of this session. And it was a very detailed and, to me, exhaustive -- maybe exhausting review of FC receptors, but really excellent. Thank you very much.

Next, we have Dr. Laurent Reber, who is a PhD in immunology research at the Institute Pasteur. And he's going to speak about cellular

mediators of immediate hypersensitivity reactions.

DR. REBER: Thank you. So, I actually moved to Toulouse in the South of France so I'm not anymore at the Institute Pasteur. So, I was asked today to present you more about the cells that could mediate hypersensitivity reactions. And as you will see, there will be lots of overlap with what was already presented by the two first speakers. But I will try to go more into details about three types of specific reactions that could occur. Can you go to the next slide, please.

So, I will focus on the IgE-mediated hypersensitivity reactions and what Fred presented before on MRGPRX2-mediated hypersensitivity reactions, and really looking at the main cells that could mediate that and the main mediators. And then I will discuss more about IgG-mediated hypersensitivity reactions, which I think is really what could occur in IVIG-mediated reactions, and that there are two main pathways that could be activated, as was presented before, the FcR pathways, and which cells are involved in

that, and also the complement pathway. And I will go more into details about experiments that were performed using humanized mice, and especially humanized for these FcRs. Next slide, please.

So, this was already presented. IgE is really well known to mediate hypersensitivity reactions. And I will focus really on anaphylaxis as the main hypersensitivity reaction -- so, a systemic reaction. And this is known for a long time that IgE mediates anaphylaxis, and they mediate it mainly through one receptor, which is FcRI, which is expressed in mice in two main cells -- mast cells and basophils. In humans, it's a little bit more complicated, because there are additional cells such as dendritic cells and some monocytes, macrophages, that could also express a version of this receptor. Next slide, please.

But what is also clear in -- yeah, what is clear is that there are many people that actually develop autoantibodies against this receptor -- so, anti-FcRI autoantibodies, mostly

of the IgG subclass, and also, IgE against self-proteins -- and that these autoantibodies can actually cause disease in humans, which is called chronic spontaneous urticaria. And what these antibodies can do is that they can directly activate. So, if you have antibodies against FcRI, these antibodies can directly activate the receptor, causing the activation of mast cells, and potentially, basophils.

And if you have antibodies against IgE, they can also activate the receptor, because IgE is always bound to the Fcereceptor of the surface of mast cells and basophils. And then if you have IgE against self-antigens, if you encounter these self-antigens, these immune complexes can also directly activate the receptor. So, I think one thing we have to take into consideration, if, in the pool of IVIG, there are autoantibodies against either FcRI or against IgE, these autoantibodies have the potential to activate mast cells and basophils. Next slide, please.

Yeah. And indeed, there is one report

which was published a few years ago using basophils. So, what they did is that they took basophils from the blood of a healthy donor. They primed these basophils using a cytokine, which is called IL-3, to enhance the activation potential of these basophils. And then they incubated these basophils with different IVIG batches. And what they observed is that the basophils got activated.

And when they depleted anti-IgE that were present in these IVIG batches, they couldn't see any more basophil activation. So, what this means is that even though -- of course, the batches of IVIG are a pool of thousands of different donors. But if you have donors that have high levels of these anti-IgE autoantibodies, these antibodies clearly have the potential to activate basophils. And so, if you think about people who could be prone to develop hypersensitivity reactions because they have mutations that could enhance mast cell activation, these people could be likely to develop mast cell activation upon injection of these autoantibodies.

And one thing is that these autoantibodies are very common in the general population. It's about 1 percent to 2 percent of people that have autoantibodies against either the receptor or IgE. And so, it's highly likely that these autoantibodies are present in many batches of IVIG. Next slide.

Then the second main pathway that can trigger mast cell activation is this newly described MRGPRX2 receptor. It's called MRGPRB2 in mice and X2 in humans. And it triggers what we call pseudoallergic reactions. So, it's degranulation of mast cells by many drugs, and especially cationic molecules. Next slide.

And it's clear that -- and this was done with human mast cells. If you knock down the receptor, many of these cationic drugs cannot activate mast cells anymore. So, as Fred mentioned, if there are people developing these anti-MRGPRX2 autoantibodies, and if these autoantibodies are found in IVIG batches, these would be likely to also cause direct activation

and degranulation of mast cells in the recipients. Next slide.

Now, I will present more data on IgG-mediated anaphylaxis, which is also a potential explanation of the hypersensitive reactions in some of these IVIG batches. So, as was presented before, there are different subclasses of IgG in mice and in human. Most of the data that we have on IgG- mediated anaphylaxis comes from mouse studies. So, I will start with studies with mouse IgG, and then I will switch to human IgG that have been studied using humanized mice.

So, there are four main classes of IgG in mouse -- IgG1, IgG2A, IgG2B, and also IgG3. But I will mostly focus on IgG2A and 2B and IgG1. And as you see here -- so, Fred told you that anaphylaxis in mice is quantified by looking at hypothermia. And actually, in mice, the three classes -- so, in the center, it's IgG2A, even though it's not written. So, IgG1, IgG2A, or IgG2B can all induce anaphylaxis in mice. But

IgG2A or B are more potent at inducing anaphylaxis than IgG1. And this likely reflects the fact that the affinity of IgG2A and 2B for the FcR is much higher than that of IgG1. Next slide.

So, as was presented before, there are four IgG receptors in mice -- three that are activating FcR1, 3, and 4, and one that is an inhibitory receptor. And data from many groups have pointed that, actually, there is one main activating receptor mediating anaphylaxis to IgG antibodies in mice, which is FcRIII. So, in white, you have mice that are deficient for FcRIII, and they can no longer develop IgG-mediated anaphylaxis. Next slide.

So, these FcRs are expressed by many cell populations. And if you look at FcRIII, it's actually expressed by mast cells, basophils, also eosinophils, neutrophils, monocyte, macrophages. And so, all these cell populations potentially could drive IgG anaphylaxis. Next slide.

And so, this is actually experiments

from the group of Fred Finkelman. They studied IgG-mediated anaphylaxis and IgE-mediated anaphylaxis. And in white, you have mice that are wild-type, and in black, mice that lack, well, mast cells. So, these WWV mice are actually deficient for mast cells. And what they reported, which was confirmed by many different groups, is that if you have IgE-mediated anaphylaxis, it no longer develops in mice that lack mast cells. So, clearly, mast cells are the main effector cells of IgE-mediated anaphylaxis.

But if you induce IgG anaphylaxis, it can still develop -- at least in most models, it still develops in mice that lack mast cells. So, there must be additional effector cells, different effector cells, that mediate IgG anaphylaxis. And for basophils, I'm not showing data, but the contribution is still controversial. There are some groups that have shown a contribution of basophils for IgG anaphylaxis. And most of the reports have shown little to no contribution, so it's really still controversial. Next slide.

Neutrophils, there were reports about the importance of neutrophils, because they express high levels of FcRs, activating FcRs. And these were studied, published, more than 10 years ago, using depleting antibodies that can deplete

neutrophils in mice. And the white group actually is a group that has been depleted in neutrophils. And as you can see, they were protected -- completely protected in two different models of IgG anaphylaxis. So, these imply that neutrophils must be key players of IgG anaphylaxis. Next slide.

But here, I'm just sharing with you some data that are still unpublished. They are from our lab, but not published yet, in revision. We have confirmed this data that you can block IgG anaphylaxis with depleting antibodies that deplete neutrophils. But we've developed a new model in which you can deplete neutrophils without using these antibodies. And as you can see, this is the second panel and the fourth panel. In red, the

mice are depleted in neutrophils, and they still fully develop anaphylaxis.

So, what these data imply, and it's something that I want to point, is that as Fred mentions, there are clearly many differences between mice and human. And so, what I'm showing you and what we are talking about today are data from mice. But even in these data, there are still many caveats in the approach that we are using. So, the conclusion that we are making cannot be definitive, but they point that, most likely, neutrophils are not mediating anaphylaxis through IgG antibodies. Next slide.

What was mostly shown by the group of Fred Finkelman and what was also confirmed by many other investigators is that monocytes and macrophages are likely to mediate IgG anaphylaxis in mice. This was mainly done using depletion with different drugs, either toxic liposomes or gadolinium in mice, which can deplete macrophages. And using these pretreatments, mice became protected to anaphylaxis. Next slide.

So, these are also data from the Finkelman lab showing that if you mediate IgG anaphylaxis and you treat the mice with antagonists through either the PAF receptor or histamine H1-receptor, you can markedly reduce the severity of anaphylaxis. Next slide.

And so, again, coming from Fred's lab, this is from a review that is no more than 15 years ago, but it's still pretty much what we know about IgE and IgG-mediated anaphylaxis in mice. Clearly, there are two main effector cells that mediate anaphylaxis. These are mast cells and macrophages. Mast cells are likely to mediate -- for sure, they mediate IgE anaphylaxis through the FcRI receptor.

They could also mediate anaphylaxis induced by IgG antibodies, because they also express high levels of this activating FcRIII receptor. But most likely, macrophages are the main effector cells of IgG and anaphylaxis through FcRIII. What these cells do is that they release very fast histamine from mast cells and PAF from

macrophages. But I have to point that mast cells can also release PAF to some extent, and that these two main mediators, as Frank has shown you, on their own, are able to induce all symptoms of anaphylaxis in mice. Next slide.

So, now, this is the situation in mice. But the situation in human might be even more complex, because as was pointed before, human have even more FcRs. Next slide.

So, to study this, there are different groups that have generated mice humanized for this FcRs. So, I'm not entering into the details of all these mice were humanized and which exact receptor they express. But the data I'm showing you are with humanized mice that express most human FcRs in place of the mouse FcRs. So, now, they can respond to human IgG, and they do so through the human FcRs.

And so, this is very important for today's topic, is that this is from the group of Pierre Bruhns in -- at the Pasteur Institute. They use IVIG as a model to induce anaphylaxis.

And what they did is not simply injecting IVIG, but they preheated this IVIG to aggregate the antibody. So, if you heat the antibody, it will aggregate the antibodies. And by doing so, it will mimic immune complexes. And once you have aggregated these antibodies, they are highly potent at engaging FcRs. And so, if you take this heat-aggregated IVIG and you inject that into mice that are humanized for FcRs -- so, these are the black groups -- it induces anaphylaxis -- and actually, a pretty strong anaphylaxis with an hypothermia of around 5 to 8 degrees.

And then, on the right panel, if you block one of the activating human FcRs using a blocking antibody -- so, the FcRII -- it completely abrogates anaphylaxis. So, this is the white square group, meaning, that even though these mice express most of the human FcRs, blocking one receptor, FcRIIA, is enough to block anaphylaxis. So, this implies that, likely, this receptor can trigger a very severe anaphylaxis. Next slide.

So, now, in the same model of heat-aggregated IVIG- mediated anaphylaxis, they depleted macrophages. So, this is the left panel. But they didn't see an involvement of macrophages in this model. They depleted basophils using an antibody which is called Ba103, and they saw very little reduction in anaphylaxis. And here, they depleted neutrophils on the right using anti-Ly6G antibody, and fully blocked anaphylaxis. But as I pointed before, based on our new data, we should take care in the interpretation of this data, because there are problems with the neutrophil-depleting antibodies. Next slide.

And so, in the same model of heat-aggregated IVIG- induced anaphylaxis, they again blocked either the PAF receptor using an antagonist -- or two different antagonists, actually -- or the histamine H1-receptor, also using two different antagonists. And exactly as was shown with the mouse IgG, human IgG present in this IVIG, heat-aggregated, induced anaphylaxis through the release of PAF and of histamine in

humanized mouse. Next slide.

So, these are more recent data that were done with the mouse model that is now fully humanized so that they express all the human FcR in place of all the human receptors -- so, these are the triangles -- and as control in mice that are deficient for all FcRs. So, they have neither human nor mouse FcRs. And so, again, anaphylaxis was induced by injecting heat-aggregated -- sorry, yeah, heat-aggregated IVIG. And as you can see on the top panel, it induces anaphylaxis in the humanized mice, but there is no sign of anaphylaxis in mice that lack FcRs. So, again, it's going through human FcRs.

Again, in red, when you block the Fc?IIA receptor, it fully block anaphylaxis. And so, now, if you look at the red -- the right panels, so this is the platelet counts after inducing anaphylaxis. So, as soon as 10 minutes after injection of this heat-aggregated IVIG, there is a huge drop in the platelet count. So, there is thrombocytopenia that occurs during anaphylaxis.

And this thrombocytopenia is blocked when you block the Fc?IIA receptor.

And now, if you look at the bottom anaphylaxis panel -- so, the C -- here, they depleted platelets using a depleting antibody, and it partially blocked anaphylaxis. So, what this implies is that likely, platelets also can mediate anaphylaxis, and they actually express quite high level of this Fc?IIA activating receptor. Next slide.

And so, I just want to finish by showing you some data that are not done with IVIG, but are done with a therapeutic antibody called omalizumab. It's an antibody that is used in the clinic as an anti-IgE antibody. And there are some reports of side effects, even anaphylaxis, in a minority of patients that are treated with this IgG, anti-IgE antibody. Next slide.

And so, what we've done a couple of years ago is using the same humanized mice -- so, mice that express all human FcRs -- and as control mice that are knockouts for all FcRs. We

looked at both local and systemic adverse reactions to this antibody, omalizumab, when complexed with IgE. So, we preformed immune complexes by incubating human IgE with omalizumab, and we injected these immune complexes either subcutaneously to induce local reactions, or IV to induce systemic reactions.

And so, if you look at the upper panels -- sorry, yeah. What you see, it's a bioluminescent signal that is turned on when neutrophils are activated. And actually, we saw this bioluminescent signal when we injected only the immune complexes, and only in mice that express human FcRs, but not in mice that lack these FcRs. So, what this implies is that local activation of FcRs at the injection site can trigger local inflammation and the activation of neutrophils.

And in the bottom, it's exactly the same, but we injected these immune complexes IV. And again, we observed anaphylaxis in mice that express the human FcRs, but not in mice that lack

these FcRs. And now, if you look at the right bottom panel. In green, you have mice that additionally lack -- so, they express the human FcRs, but they lack the complement C1Q component. And what we observe is that in these mice, anaphylaxis was almost completely abrogated.

So, what this implies is it's not only about activation of FcRs, but IgG immune complexes also activate the complement pathway by binding to this C1Q molecule. And clearly, this C1Q molecule and the cascade of complement activation is also implicated in anaphylaxis. Next slide.

And so, using the same model of immune-complexes- induced anaphylaxis, what we've shown is that you see anaphylaxis in mice that express all of the FcRs, but not in mice that only express the FcRIIA. So, in this model, it's likely that it's a different receptor that actually induce anaphylaxis, different from FcRIIA. And what we found is the blockade of the Fc?III also abrogates anaphylaxis. So, what this

implies is that the story might be even more complex, and that it's not only one receptor, one FcR, but multiple FcRs that can trigger anaphylaxis with human IgG. Next slide.

And so, I will finish with that. So, it's a review that is now two years ago, and I think it's already a little bit outdated, based on some new data we have. But what it shows is that there are many pathways, to summarize, that can induce anaphylaxis with human antibodies. Human IgE can trigger anaphylaxis by activating the main receptor, FcRI, on mast cells, and perhaps on basophils.

Many drugs can trigger anaphylaxis by directly activating mast cells through this newly recognized receptor, MRGPRX2. And then IgG immune complexes can induce anaphylaxis by activating either platelets through FcRIIA or macrophages through IIA or FcRIII. And perhaps, they can also -- they certainly activate neutrophils, and perhaps neutrophils also mediate anaphylaxis. But this is somewhat compromised now, by some of the

new data we have.

And something that is lacking in this drawing is that certainly, these immune complexes also activate complement cascade that also can trigger anaphylaxis. And the two main mediators of anaphylaxis that have been described are PAF, which is released by neutrophils, macrophages, and also, to some extent, by mast cells, and mast cells and basophils release histamine. And all -- and these two mediators on their own can induce all signs of anaphylaxis. And so, I will finish there. And I believe that the three of us can take questions.

DR. SCOTT: Thank you very much. Excellent. And --

MS. ELLER: Okay. Yeah, we have a number of questions. The first one is regarding mechanisms of action. What is the difference between anaphylaxis and anaphylactoid reaction?

DR. FINKELMAN: The terminology is really complicated. And probably, the short answer is, there really isn't any difference. At

one point, anaphylaxis was very tightly defined as being mediated by IgE, FcRI, mast cells, or basophils. And anything else was called an anaphylactoid reaction. But this got so complicated that the powers that the analogy redefined things and just called all of these immediate-type hypersensitivity reactions that occur quickly, that involve mediators, that involve any of these immune cells that my colleagues have spoken about, are called anaphylaxis. If you look in literature, you can still find the older terminology. And that's why it makes it either difficult or interesting, depending on your philosophy.

MS. ELLER: All right, we'll move to the next one. Is there a possibility that an IgG-class molecule can trigger crosslinking between FcRI without involving IgE?

DR. FINKELMAN: Again, yeah, if it's an IgG antibody, as both Robert and Laurent spoke to, that is specific for FcRI -- so, its Fab part binds to FcRI -- then since IgG is bivalent, it

can crosslink FcRI and trigger mast cells or basophils in that way. And that's --

DR. REBER: These autoantibodies are actually very frequent in the general population. It's 1 to 2 percent of people that actually have these autoantibodies. Of course, they are not sick by having these antibodies. And I think if someone developed these antibodies, and on top of that, has a hypersensitive mast cell or has more mast cells than normal, it is likely that then, it can become pathogenic. And so, this might explain also why not all of the patients develop anaphylaxis when they're injected with IVIG -- with these patches of IVIG, is that certainly there is something in the IVIG pool that triggers activation. But then there is something in the recipient --

DR. SCOTT: Mm-hmm.

DR. REBER: -- that makes it more sensitive to develop anaphylaxis.

MS. ELLER: Okay. If no more comments, we'll move to the next question. "Is it known

whether mouse anaphylaxis correlates well to humans? I know animal immunogenicity data generally does not translate due to MHC differences." I know this had been talked about a little bit earlier, but maybe you can expand for this person.

DR. FINKELMAN: My argument would be -- yeah, the mechanism seemed to be very similar. You can do experiments in mice that, as Laurent discussed, have human receptors that are treated with human antibodies, and get very similar disease to what you see in mice with mouse receptors and mouse antibodies. You can do experiments in immunodeficient mice that have been reconstituted with human cells that develop large numbers of human mast cells and that are primed with human IgE that develop very similar disease to what's seen in normal mice, using the normal mouse antibodies and receptors. I think the one thing that we can't do right now is make mice that have human target organs, like a human smooth muscle or human vascular endothelium. So, there

certainly could be differences there.

The types of reactions, the types of antibodies that are involved, the types of cells that seem to be involved seem to be very similar in mouse and human. And there's -- I'm not aware of any major reason to think that the two processes are fundamentally different.

DR. REBER: If you take the example of IgE anaphylaxis, I think there is still one -- one main mystery is that the amount of IgE that we need in mice, it's actually much -- and antigen that we need in mice is much higher than in human to trigger anaphylaxis. In human, you can have -- if you're allergic to peanuts, you can develop very severe anaphylaxis to really traces of peanut. In mice, to model that, we still need much more antigen and probably much more antibodies.

So, probably, we are underestimating maybe the anaphylactic shock. But in terms of effector cells, receptors, et cetera, I think it models very well the -- what we see in human, at

least for IgE.

DR. ANTHONY: Yeah, I think these points are all well taken. And it's also, you know, limited by the access that we have to human samples. I mean, we really are limited to look what's in the blood. It's hard to get into the tissue, which is really where, you know, they're kind of -- the mast cells and the IgE is, right? So, I think, like, you know, to Fred's point, it's -- it really lines up as best as we can. And this is really the systems that we have to work with.

MS. ELLER: Okay. The next question is for Dr. Finkelman. It's from Neil Blumberg. And he says, "Your old Harkness Dorm co-resident from '70, '71 is here." And he asks, "Allergic transfusion reactions might be a potential similar issue. It appears that donors who are atopic may be more likely to cause reactions -- mechanism, largely unknown. Would collecting information about donor-atopic diseases/conditions be a useful way forward, perhaps?"

DR. FINKELMAN: Hi, Neil. And the

answer is absolutely, yes. I think it's been referred to by some of the speakers that people who have asthma, for example, are more likely to get an asthmatic type of reaction to IVIG. People who have mastocytosis or large numbers of mast cells for other reasons or allergic diseases of any sort are more likely to get that type of reaction to IVIG. That information is based on very limited data. If we had more data, we could pin the specifics down, and I think, much more tightly. It'd be great to have more data on the -- both the donors and the recipients.

DR. SCOTT: Yeah.

MS. ELLER: So, he missed part of your presentation, so he's hoping he could get a copy of your slides. But I believe you gave your email address and were willing to share.

DR. FINKELMAN: Yeah.

MS. ELLER: Okay. The next one actually has to do -- she's curious on how exercise makes people more susceptible to anaphylactic reactions. Is it exercise or lack thereof, and can you

elaborate on how, why, and when exercise immediately following exposure to -- due to increased blood flow?

DR. FINKELMAN: Yeah. I don't think this has really been well studied. It's a very interesting question, and it's probably an important question. The hypothesis has been raised that, yes, exercise -- increasing blood flow is going to cause dissemination of the causative agent into the systemic circulation or to the site that's important more quickly. But that's really intelligent speculation rather than something that's been demonstrated by data. It's just a pragmatic observation that people who have recently exercised are more likely to get anaphylaxis after a number of different stimuli, including food-induced anaphylaxis.

MS. ELLER: Okay. Next question is also for Dr. Finkelman. You said only a rather small number of patients develop hypersensitivity reactions after administration of specific lots of IVIG. Does this refer to the number of reports

received by FDA or the manufacturers compared to the number of vials used from these lots, or how do you know that other patients have tolerated product from these lots?

DR. FINKELMAN: I'm probably not the best person to answer that. Maybe we should --

MS. ELLER: Maybe Meghna could, if she's still on?

DR. ALIMCHANDANI: Sorry, could you say that again?

MS. ELLER: They're asking -- they said, "Only a small number of patients develop hypersensitivity reactions after administration of specific lots of IVIG. Does this refer to the number of reports received by FDA or the manufacturers compared to the number of vials used from these lots, or how do you know that other patients have tolerated product from these lots?

DR. ALIMCHANDANI: Yeah. So, this is all based on reporting. So, it's not actual -- again, as I was saying, it's not actual incidence of hypersensitivity reactions. And we heard from

Ms. Kara earlier that, you know, her incidence of hypersensitivity was not actually reported. So, there, you know, may certainly be allergic reactions in patients that do not get reported to the manufacturer or FDA. I hope that helps to answer.

DR. FINKELMAN: But I think these lots go to a large number of people --

DR. ALIMCHANDANI: Yeah.

DR. FINKELMAN: -- in general, so that if -- yeah, if 90 percent of recipients develop the significant reaction, even with very imperfect reporting, you'd probably know about it.

DR. ALIMCHANDANI: Yes, yes, thousands of vials. And as I said -- I mean, the baseline number of reports that we get are very low. It's, you know, either no reports, or one or two.

DR. FINKELMAN: And even in the mouse studies, if you, say, went down on the stimulus, you would see mice that clinically look perfectly normal. But if you looked at their blood, you would see that there was enzymatic evidence of

mast cell degranulation or macrophage stimulation. And that may well be what's happening in people who are less susceptible to anaphylaxis, who are getting one of these -- what I call pathogenic lots. They may have a reaction, but it may never reach the level of being clinically apparent.

MS. ELLER: Okay. The next question is, can lot-to- lot variation of antibody glycosylation give rise to hypersensitivity reactions in patients receiving IVIG?

DR. ANTHONY: So, my understanding is that IVIG is from tens of thousands of donors. And so, because it's so many different donors, I think that there's going to be some very -- I think, globally, the glycosylation pattern is not going to be that different, particularly when people are fairly healthy.

I would be more interested in thinking about what the allotypes that are present to see if that somehow is contributing, you know, because there's definitely these regional differences from where the donors are collected from. But there

certainly is glycosylation batch variability for some of the monoclonal antibodies. That's been reported.

DR. FINKELMAN: Robert, if you look at the single individual level, do you ever find people who have, you know, very unusual glycosylation patterns that would be more likely to cause a reaction?

DR. ANTHONY: So, generally, to my knowledge, not really. That's because glycosylation is such a common pathway for all different cells and systems, right? So, if there is some deficiency in it, usually, there is, you know, very serious symptoms that manifest that make, you know, the immune kind of, you know, sequelae almost, like, irrelevant, right?

So, generally, you know -- yeah, if somebody does have a glycosylation deficiency, there is other very serious complications that are associated with it. But, you know, there are certainly, you know, changes to glycosylation that are associated with, you know, ongoing

inflammation, For example. It can either be an infection or a vaccination, et cetera. You do see differences in glycosylation, for sure. But, you know, I'm just guessing, you know, if it's really just tens of thousands of people, that would really be diluted out. But it's just a guess.

MS. ELLER: If there's no other comments, I'll move to the next question. What is the difference between a hypersensitivity reaction and infusion-related reaction? Does anyone --

DR. ALIMCHANDANI: Should I --

MS. ELLER: Yeah, I think, Meghna, you may want to pick that one.

DR. ALIMCHANDANI: Yes, yes. No, that's a good question. So, infusion-related reaction, I think it's just more a broader term, maybe. I think hypersensitivity is a much more specific term. And that brings up another -- you know, another aspect of the post-marketing reports. They are coded by preferred terms. So, a lot of this is, you know -- there are similar, you know, preferred terms describing the same thing. And

one report has multiple, you know, preferred terms. So, it's used interchangeably in the same report, maybe coded as infusion site reaction, hypersensitivity reaction, pruritus, you know, et cetera. So, just something else to keep in mind. But it's all, you know, describing the same clinical, you know, event. Thanks.

MS. ELLER: Next question is, what markers can we look for in the hosts that react to IVIG with hypersensitivity?

DR. FINKELMAN: When you find one, patent it.

(Laughter) Yeah, I would think that a host with a large number of mast cells, that a host with a strong history of allergy or asthma of any sort. But I don't think there are any specific genetic markers known that would predict susceptibility.

DR. REBER: The only thing -- looking at baseline tryptase level might be important, because this will really reflect if patients are

-- a mast cell pool that is higher than normal, or a mast pool activity at baseline that is higher than normal, these people might be more susceptible to develop hypersensitivity reactions. This is what is observed in mastocytosis, for example.

DR. FINKELMAN: I guess it's been known for a long time that IgA-deficient patients are more likely to have anti- IgA antibodies, which could cause an anaphylactic reaction if they receive IVIG that has IgA in it.

MS. ELLER: All right. If there's no further comments, I'll move to the next. If a patient has anaphylaxis due to mechanisms outside of IgE, would you still expect worsening with subsequent exposure? Should patients not be given the product again, or can you premedicate?

DR. REBER: Well, based on what we heard, I guess not giving the same product batch, for sure. But probably -- I mean, it's a matter of batch. So, if that patients didn't have anaphylaxis for years with different IVIG batch,

it's certainly not giving the same batch. But then changing batch should be okay.

DR. FINKELMAN: It's a really hard question to answer, because -- yeah, in a single individual who develops a reaction, we don't know what's mediating that reaction. And in a single lot of IVIG that causes an increased number of reactions, we don't know why it's causing those reactions. So, as Laurent suggested, we should, I think, err on the side of cautiousness and infuse with a different lot. And if I were the one who was receiving it, I would want to be premedicated.

MS. ELLER: And I believe that's what Kara talked to -- the patient talked to us earlier, that she changed products as well as more premedication. Next question, which mouse models would help screen IVIG to avoid hypersensitivity? Isn't that the gold standard we would like? Any comments?

DR. REBER: Certainly, these humanized FcR mice, I think, would be a way to go.

MS. ELLER: Yeah.

DR. FINKELMAN: Uh-huh.

DR. REBER: I mean, these are the best models that I can think about.

MS. ELLER: Okay.

DR. REBER: But that being said, we -- I mean, we've used these mice with different IVIG batches. And without heat aggregation, we never saw any sign of anaphylaxis in these mice. So, it might still be very hard to model.

DR. FINKELMAN: Laurent, did you use IVIG batches that were known to have an increased frequency of reactions?

DR. REBER: No, no.

DR. SCOTT: That might be worth looking at.

DR. REBER: Yep.

MS. ELLER: All right. Next question looks like it's for FDA. Is any testing specific for anaphylaxis required by regulators before clinical studies or commercial lot release for IVIG or other protein products?

DR. SCOTT: I think we would be happy if

there were such a test. There's no specific requirement, nor have we really been able to come up with one that's rational and science-based and predictive to help us out in that way.

DR. REBER: So, now, there are several mast cell lines that respond pretty well to IVIG --

DR. SCOTT: Mm-hmm.

DR. REBER: -- and that also express this MRGPRX2 receptor. So, maybe one potential thing in the future would be to screen these lots using a mast cell line, at least to avoid any of this mast cell activation. But again, this probably won't catch all potential pathogenic batches, but --

MS. ELLER: All right. Next question. Do patients who experience adverse events to a certain lot of IGIV and subsequently continue therapy with pretreatment regimen eventually be weaned off of pretreatment? In other words, does the body eventually calm down?

DR. SCOTT: So, I'll start answering it,

and Dr. Alimchandani can add to that. We've seen -- or we've had reports of some cases of repeat administration of the implicated lot, and those people at least getting just one re-administration of the implicated lot typically have a reaction again. And then that lot is not given to the patient a third time. In some cases, the brand of IVIG is also changed. So, there may not be any data available to tell us whether there's a desensitization process that can occur after more than one dose.

DR. ALIMCHANDANI: Yeah, we -- as Dr. Scott mentioned, we've had these reports of rechallenge. You know, not very many, but we have reports, and they did, again -- you know, there was no desensitization. They did, again, experience hypersensitivity reaction. So, we don't really have any more information on whether, you know, the body calms down, as the comment as said.

MS. ELLER: Okay. Next question. Has there been studies comparing plasma collected from the U.S. versus Europe, and if AE triggers in IGIV pools are tied to demographics, lifestyle, diet, et cetera, that we can learn from?

DR. ALIMCHANDANI: I think those are all good comments and thoughts. I'm not aware of any studies, but perhaps there will be in the future.

DR. SCOTT: I think what's important to know is that we export a lot of -- or a lot of plasma units are exported to make European products. Not exclusively, by any means, and there have been efforts in Europe always to be self-sufficient in plasma production. Nevertheless, based on logistics, for example, it may be that the European firms have simply not received any implicated plasmas -- or retrospectively, at least, implicated plasmas. But we don't know that for sure.

DR. FINKELMAN: Dot, what's known about the diseases that would exclude somebody from being a plasma donor?

DR. SCOTT: Well, there's -- there are a number of them, but they do not include systemic mastocytosis, for example, or atopic individuals. They -- people are asked about allergies, but that would be common allergies like to medications and so forth. We have looked at a lot of these reports. There's no particular allergy -- for example, to penicillin -- that stands out. I mean, some people have a lot of allergies, but there's no one thing that stands out. That's about the best I can do.

DR. FINKELMAN: Is there a question about chronic spontaneous urticaria?

DR. SCOTT: No, there is not. But what we really need to know more about is our donors -- as you were saying -- and to see if anything would match this. So, we'll be hearing a lot about donors in the second part. And hopefully, that might clarify some things. But donors themselves are sometimes difficult to get a detailed history from. And that would not be -- a very detailed history like that so far is not routinely taken.

DR. REBER: I mean, perhaps screening for anti-IgE and FcRI autoantibodies could be an easy screen to perform on the donors' IgG, or at least on the different pools, on the different batches.

DR. ANTHONY: This is just a comment I thought, and I'm hoping that, you know, Fred and Laurent can comment on this. But, you know, this really does seem to be a pretty unique case. Like, we're all vaccinated against flu and COVID, and would be exposed subsequently to those pathogens, which would cause immune -- you know, IgG production and immune complexes that do not often trigger anaphylactic responses. So, you know, one my fellow panelists think that's so unique to this IVIG infusion, that that's different from, you know, how IgG antibodies typically work.

DR. SCOTT: I think you've asked one of the major questions. And I think what would help us would be to have samples from, if I should call them this, implicated donors, donors in common

with the various multiple lots of hypersensitivity-inducing IgG, and see what they have in common both from a history standpoint, but also from a biological standpoint with respect to these kinds of reactions. But again, I think that there will be a little bit of clarity that we get in the second half of the workshop about ways in which this has been done, and some of the limitations as well.

DR. FINKELMAN: No, it would be -- if it's coming from a single donor, or it's -- that donor must have something incredibly potent that can withstand being diluted 1 to 1,000 or one 1 to 10,000. And if it -- for example, if it were an antibody to FcRI, it would have to be a high-titer, high- affinity antibody.

MS. ELLER: Well, this is a reason we're having the workshop. We hope to come up with some directions for research towards being able to take pragmatic actions to try to prevent, or at least -- even if not totally prevent, to more quickly be able to identify whether or not there's a

particular donor or set of donors that are involved.

DR. FINKELMAN: Yeah. But the humanized mice that Laurent mentioned, even if it's not practical to use them for screening, they're a wonderful resource to -- for scientific investigation of trying to figure out why a particular lot of IVIG might be frequently pathogenic.

DR. SCOTT: Absolutely, absolutely. It would be nice if he had some allergenic immune globulin to test, wouldn't it?

DR. REBER: And what is known about the possibility that there is some aggregates that occurs in the hospital before injection of this IVIG -- because that's the way we trigger anaphylaxis in non-humanized mice, by aggregating this IgG. And it's very potent at engaging FcRs. So, if there is a little bit of aggregates in these specific lots -- that can occur after the batch has been actually opened in the hospital -- this could also generate adverse reactions.

DR. SCOTT: That's potentially possible. But there are some other tests that are done at lot release that help to screen out aggregates. Certainly visible aggregates, vials containing visible aggregates, or lots with a high percentage of visible aggregates, are excluded from being distributed. We -- also, it is routine to perform HPLC, which will give you some information about aggregation.

But also, we've discussed amongst ourselves a possibility, not only for this reason, but for other reasons as well, of looking more carefully at subvisible particulates and aggregates that you won't detect on HPLC, in part, because of the filters at the top of the column that are -- to prevent the column fouling. And we think that perhaps it -- that would be useful.

We do have some unpublished information looking at hypersensitivity lots versus non-implicated

lots. We've looked at dynamic light scattering in a few cases, and that has not shown

us a clear difference. But there are other methods that are perhaps more sensitive and more informative, like FlowCam, where we might also look. I don't know if Ms. Eller wants to say anything additional about this, since she is the one who works on some of -- some aspects of particulates and aggregates.

I should also mention that we have another test, the anticomplementary activity functional assay, which is done for all of our lots of all of our products. And we haven't found any anomalies there. And that has -- that's sort of an indirect measure of whether you have activating immune complexes.

MS. ELLER: So, we've done dynamic light scattering on material that we've received, and it doesn't differ from what we see in our historical lots that we've tested. It has a similar pattern. It has a similar polydispersity index. So, nothing in there indicates that it has a different level of aggregation, but DLS is not very sensitive. It's, you know, comparing small

changes. So, there could be a small increase in subvisible particles, but you would never really detect it by DLS. We haven't done the FlowCam yet, but that is in the future.

DR. SCOTT: Another question?

MS. ELLER: Okay. Can you please expand on the area that platelet cells also have FC receptor sites that also causes thrombocytopenia?

DR. REBER: So, platelets express FcRIIA, which is the receptor that we found is the main trigger of human IgG anaphylaxis in our humanized mice. They actually express quite a high level of this FcRIIA. It is specific for human platelets in, you know, humanized mice because most platelets do not express these FcRs. So, this is something that only occurs in the humanized mice, and perhaps in humans.

And what platelets do is that they can express and release serotonin, which also, on its own, if you inject serotonin in a mouse, will induce the same signs of -- sign of anaphylaxis than histamine or PAF. So, potentially, the

release of serotonin by platelets could trigger anaphylaxis. But again, this is far from being really demonstrated. It's just a few evidence that we have in mice. The thrombocytopenia that we observed during anaphylaxis in mice probably reflects this huge activation of platelets, and probably, they aggregate somewhere in the blood vessels or -- so, it really reflects activation of the cells. And this clearly is mediated by FcRIIA in our model.

MS. ELLER: Dot, we're at 11:38.

DR. SCOTT: I was just looking for the time. All right. Okay. How many more questions do you have?

MS. ELLER: More than 10.

DR. SCOTT: Oh, my. All right. Just pick two more.

MS. ELLER: Oh, okay. What is the role of infusion rate in hypersensitivity reactions?

DR. FINKELMAN: The higher the infusion rate, the more likely the reaction will occur. And in some cases, if people are developing a very

mild reaction, like a bit of a headache, for example, you can slow down the infusion rate and avoid having a more severe reaction. I think, in the patient we heard from, Kara, she mentioned that after she had a bad reaction, they halved the infusion rate for her, which certainly will be protective.

MS. ELLER: What sorts of temperature and duration of heating is required to create aggregation? A 37 C routine blood warmer would presumably not be adequate for this aggregate formation, correct?

DR. REBER: No, no, it's quite a dramatic heat that we -- like, I don't remember. It was, like, 65 degrees.

DR. SCOTT: 60, yeah.

DR. REBER: So, it's not something that will occur in your batch for sure.

MS. ELLER: One last one. Considering that the U.S. Exports a good volume of plasma for production of IGIV for ex- U.S. countries, but that we don't see similar clusters of AEs, could

there be other factors masking this information -- differences in donor screening, AE reporting criteria, standards of health care, childhood vaccinations, et cetera?

DR. REBER: Yes, certainly all of that. I mean, one thing for sure is that because you don't observe that in the majority of patients, it is patient-dependent. So, it is -- it can depend on genetic environmental factors. I guess that's what we're discussing today, is we don't know what are these factors, but they are donor and recipient-specific.

MS. ELLER: One last one. Do you observe any differences in the rate of hypersensitivity reactions between the patients treated with IG for various medical conditions?

DR. FINKELMAN: Yeah, I think the FDA can probably give the better answers to that. But the answer is yes, that there are some conditions -- well, it's certainly -- there are some conditions that really don't classify as immediate hypersensitivity reactions, like platelet

problems, in which IVIG is more likely to cause exacerbation of a problem. And the preexisting presence of asthma is -- makes it more likely that IVIG is going to cause an asthma-like reaction.

DR. SCOTT: Meghna, do you want to add anything?

DR. ALIMCHANDANI: Yeah, I didn't have anything to add. Thanks, Dr. Finkelman.

MS. ELLER: Okay, Dot. I think we --

DR. SCOTT: Okay. I think it's time to let everybody go to lunch. And what is the time we reconvene, Nancy? That's okay, I've got it -- at 12:15. So, we'll see everybody at 12:15. And for those of you who have not had your questions answered, we'll be showing a -- an email address. Actually, I think it's on my slide, and I will post it as well, where you can -- where we will answer your question to the best of our ability, but it won't be right away. So, I'll post that during the intermission. And everybody, have a good rest or snack and so forth. And we'll see you back soon. Thank you.

(Recess)

DR. SCOTT: Hello, good afternoon. I hope everybody had a nice break. We're going to move on, but first I want to thank our speakers from the first half of the workshop for their excellent presentations, very informative, and I think we'll find them helpful as we move into this next phase of the presentations. It looks as if a moderate number of people have returned, and I think for the sake of time we should go ahead and proceed.

We first have, these are the manufacturer investigations of the immunoglobulin-mediated hypersensitivity reactions. And first we have Dr. Nathan Roth, Vice President of Global CMC, Global Pathogen Safety and Plasma Product Development, as it's written here, from CSL Behring. So he's representing the work done by CSL Behring on this particular issue. So thank you, Nathan.

MS. REVELL: Could I just interject really quickly, if you're not speaking, can you

make sure you put your microphones on mute, please.

DR. SCOTT: Thank you. So are you ready to begin?

DR. ROTH: I'm ready, thank you.

DR. SCOTT: All right.

DR. ROTH: Thank you, Dr. Scott. Good afternoon. I would like to thank the FDA for organizing this public workshop today. My name is Nathan Roth and I am Vice President of Plasma Product Development at CSL Behring. I have the pleasure of opening up the manufacturers' session to help set the stage for our discussion. Next slide.

I am going to start off by providing a general overview on behalf of the manufacturers here with some general information surrounding the manufacturing processes and quality controls for immunoglobulin products. And then I will transition into sharing CSL's experience with regard to these lot specific hypersensitive events. Following the presentations from all

three companies, we will open up for questions and answers. Next slide, please.

To start we want to communicate our shared goals to ensure patient safety and preserve immunoglobulin product availability. This is important because immunoglobulin products are used to treat -- next slide, please. To treat a variety of conditions, including for patients who have immunodeficiencies and those who live with other autoimmune diseases. They are also a precious resource as they are derived from donated human plasma. I think the slide has not moved forward yet. Next slide, please. Great.

And while hypersensitivity events are listed as common reactions for all immunoglobulin products, we're here today due to an unusual increase in lot specific sporadic groupings of hypersensitivity reactions that resulted in voluntary market withdrawals of IG products. As an industry we are committed to working collaboratively to identify the root cause of these observations, share scientific knowledge,

and find effective and efficient solutions applicable across all manufacturers to minimize future occurrences, maintain patient access and ensure patient safety. Next slide, please.

To grant us all in the scope of use for these products FDA recently published a paper in the Journal of Transfusion which described immunoglobulin usage trends. Between the 10-year period from 2009 to 2019 there were more than 300,000 patients treated with immunoglobulin products, both chronic and acute conditions, with infusions occurring either at infusion centers for i.v. administrations or at home with subcutaneous injections. Next slide, please.

Let's now move into a high-level overview about how these immunoglobulin protein products are manufactured and the controls that are in place to ensure their quality. Next slide, please.

The immunoglobulin manufacturing process starts with donor screening and collection of plasma from qualified donors. To become a

qualified donor applicant donors must pass two separate medical screenings. The collected plasma then undergoes testing for a variety of biomarkers and other attributes. Plasma donations that meet the quality standards are shipped to manufacturing sites for pooling. Plasma pools generally consist of several thousand donations. And then the pooled plasma undergoes cold ethanol fractionation to produce intermediates that are enriched in immunoglobulins. These intermediates are stored frozen and shipped to sites for further manufacturing.

The final stage of manufacturing is the immunoglobulin purification process, during which the immunoglobulin enriched intermediates are resuspended and undergo further purification and viral inactivation and removal steps to produce the final product that is primarily immunoglobulin G with trace amounts of IgA and IgM. The final product undergoes final quality control testing, quality release, and distribution for patient use. Next slide, please.

The manufacturing of immunoglobulin products is done under FDA approved licenses at FDA inspected facilities per strict regulations. There are extensive quality measures in place across the manufacturing stages to ensure the reliable production of safe and efficacious products. These include screening of donors and plasma, testing of raw materials, intermediates, and final product to registered specifications, in-process controls, quality review of batch records, quality release of final product, and post-marketing surveillance.

This comprehensive quality approach ensures the reliable production and supply of safe and effective immunoglobulin therapies for our patients both within the United States and globally. Next slide, please.

So with that as a general background for immunoglobulin products let me now move more specifically into CSL's recent experience with these rare lot specific immunoglobulin hypersensitivity reactions that resulted in a

voluntary withdrawal of product from the U.S. marketplace in 2021 and early 2022. Next slide, please.

So for the next 30 minutes or so I will be discussing CSL's experience, the root cause investigations that were conducted, along with the current working hypothesis from these investigations. I'll finish off with our learnings and next steps. Next slide, please.

To provide some historical context, CSL has over 15 years of experience manufacturing our current generation of immunoglobulin products with over 3,000 lots of final product, resulting in an estimated 1.4 million patient years of exposure. However, in 2021, specific lots of CSL's immunoglobulin products were associated with an increased number of hypersensitivity reactions. Not only was this the first time in our 15 years of manufacturing experience but it was actually the first time that we had any specific lot of our current generation immunoglobulin products associated with an increased number of

hypersensitivity reactions.

As a result, CSL took precautionary action on 16 lots by voluntarily withdrawing eight lots from the market which had shown an observed increase of hypersensitivity reactions. In addition, CSL preemptively withdrew three additional lots from the market that had not yet produced an observed signal and withheld an additional five specific lots from release to the market.

These preemptive measures were taken out of an abundance of caution based on our initial investigations of preliminary analysis, which will be discussed later in this presentation. Next slide, please.

This slide graphically demonstrates the observed phenomena with these specific lots. Shown here you can see a four-year depiction of hypersensitivity cases reported by lot with our subcutaneous immunoglobulin shown on top and the intravenous immunoglobulin shown on the bottom. Each lot is represented by a blue bar, or where no

HSR cases were reported, the absence of a blue bar.

On average the number of cases of hypersensitivity reactions reported per lot was less than one. To provide perspective, approximately 1,000 to 2,000 patients are treated per lot, depending on the size of the lot and the doses given to patients. The impacted lots, represented in the red bars, are the lots which were voluntarily withdrawn that showed a markedly higher number of hypersensitivity cases versus other typical lots. Next slide, please.

On this slide we describe the most commonly reported adverse events from the identified lots across both products, which importantly are consistent with the known and labeled hypersensitivity reactions that can occur with use of these products. Also consistent with labeling, most events were transient and reported as resolved or recovered without complications with or without treatment.

Most of the reactions were reported as

hypersensitivity skin reactions. In a few IVIG cases shortness of breath or throat or face swelling was reported. For the SCIG product, most of the adverse events were localized around the site of injection, whereas with the IVIG there was also more general or diffuse adverse events.

The type and severity of these events were consistent with the product label for IVIG and SCIG. Nevertheless, as just discussed, it was the total number of hypersensitivity events in the specific impacted lots that was highly unusual. Next slide.

This slide provides further details on pharmacovigilance reporting. Reporters noted that many patients have been using the same intravenous or subcutaneous product for years with no issue, and only reacted to the impacted lots. Interestingly, we did receive reports on an IG rechallenge for some patients who experienced hypersensitivity reactions with the impacted lots. There were greater than 30 cases of repeated receipt of the same IG lot and an additional

greater than 10 cases of administration of a different impacted IG lot, all leading to a positive rechallenge and the patient experiencing repeat hypersensitivity reactions. Importantly, all of the patients were then further exposed to non-impacted lots did not have hypersensitivity reactions. Next slide, please.

Now I'd like to provide you an overview of our root cause investigations for these specific lots. Over the last year -- next slide, please. Over the last year CSL has conducted a very rigorous and comprehensive root cause investigation.

Our current understanding from our investigations is that the most probable root cause is a single donor who produces what is currently hypothesized to be a unique component of their IgG repertoire. Now that's through the FCeR1 pathways, although other pathways may also be involved.

Importantly, our investigations have ruled out root causes related to excipients, known

product characteristics, and other process-specific causes. Next slide, please.

Our investigations took a very structured approach and were grouped into three main buckets; Manufacturing investigation, and extended characterization of the final products of the affected lots, and a hypersensitivity hypothesis investigation. These investigations were initiated and run in parallel with one another. Next slide.

In the manufacturing investigation we conducted an extensive overview which demonstrated that the affected lots were not associated with specific common [lots of] raw materials, fractionation processes, manufacturing facilities, or manufacturing sites. The manufacturing and quality records also showed no difference in attributes versus normal lots of immunoglobulin and met all quality control testing and release requirements. Next slide.

In the extended characterization analysis we tested the effected lots, including

vials returned from the market, for a wide variety of attributes and trace impurities. And again found no difference versus reference lots. Next slide, please.

Finally, in the hypothesis-driven investigation we asked the question, what in these specific lots but not in routine lots, could trigger mast cell activation and degranulation resulting in the observed hypersensitivity reactions? Although there were more potential causes that were investigated than depicted here, the four main leads that were evaluated are shown on this slide. Next slide, please.

The first three potential causes, Polysorbate 80 degradation products, protein aggregates, and anti-IG auto antibodies, were thoroughly tested and showed no differences compared to normal reference lots. This left the potential contribution of something in plasma from a donor or donors common to the affected lots that caused hypersensitivity. This commonality hypothesis was encouraged in part by separate

scientific discussions we had with the FDA and with Grifols. We began our common donor analysis very early in our investigation once four lots had been associated with these elevated reactions. Next slide, please.

Our common donor analysis was done by identifying all donors who had contributed to the affected lots and then assigning a score to each and every donation that the donor had given over the past two years. A donation was scored as positive if it was associated with an affected lot and was scored as negative if it was in an unaffected lot. In addition, a temporal factor was included in the donation scoring to ensure that the lot that a donation went into was present in the marketplace long enough to actually have sufficient usage to show a clinical symptom.

This was an enormous digital-data exercise. At four lots there were over 125,000 donors represented within the lots, having donated a total of over six and a half million donations. Of these 125,000 donors, there were 674 common

donors. And upon evaluating the scoring, no actionable conclusions could be reached.

We continued the common donor analysis with each subsequent withdrawn lot. And once we hit eight IG lots associated with the increased hypersensitivity reactions we still had 100 common donors. However, at this point our common donor screen analysis, combined with some temporal data, showed that one donor stood out from all the others. And this is digitally depicted on the following slide. Next slide, please.

On this slide we show the percentage of the donor's donations that were associated with the sffected HSR lots in red, and the percentage of the donor's donations that were associated with unaffected lots in gray. Each bar on the graph depicts one of the top 15 scoring donors out of the 100 common donors. Strikingly, you can see that only one donor, the donor that we labeled Donor Zed, had donations that were 100 percent associated with effected lots. All other 99 donors had many many more donations that appeared

in unaffected lots than in the affected lots.

So it was with this data, under an abundance of caution, the CSL took additional voluntary market action and withdrew an additional three lots from the market as well as withheld an additional five other immunoglobulin lots for distribution because they contained Donor Zed plasma. Donor Z was also permanently deferred from donating plasma for further manufacturing.

I want to point out that as a Canadian American I am bilingual when it come to the letter Z and therefore will be using Z and Zed interchangeably throughout the remainder of the presentation. Next slide, please.

This slide summarizes the percentage of plasma that Donor Zed contributed to each of the IG lots that he appeared in, in relation to the total volume of plasma used within each lot. As you can see, the percentage of Z was very low in each lot, and varied from a high of.0063 percent to a low of.0001 percent. Of the IG lots that were associated with increased numbers of

hypersensitivity reactions, as little as.0016 percent of Donor Zed was sufficient to produce the clinical signal, and this is highlighted in yellow. It is important to note that the 11 lots that had market distribution were all solely distributed in the U.S. Next slide.

So what do we know about Donor Zed? Well frankly we don't know a whole lot. He resides in the northeast of the United States, he's an above 60-year-old male, and gave 17 donations in two clusters of donations in 2020 and 2021. These donations were distributed amongst 16 immunoglobulin lots shown on the previous slide. Of course Donor Z met all donor screen requirements, and otherwise we know nothing else about his medical history to date. I want to reemphasize that CSL has permanently deferred this donor from donating. Next slide.

One of our initial hypotheses was that Donor Zed had an idiopathic monoclonal gammopathy, and

was overproducing an atypical antibody

of high quantities that was causing this effect. So we evaluated his plasma by electrophoresis. Donor Zed's plasma is represented on the left showing his electrophoretic profile with the immunoglobulins represented by the gamma band shown in orange. Comparing Donor Zed's control plasmas, shown in the middle two panels, the electrophoretic profile looks very typical. Whereas for the monoclonal

gammopathy, you would have expected a spike in the orange gamma region, as is shown in the far right panel. Donor Zed does not have a monoclonal gammopathy. Next slide.

We also evaluated some other basic plasma characteristics of Donor Zed. Donor Zed's measured values are shown as black dots, whereas the reference range is shown in pink bars. Tests for Donor Zed that fell outside of the reference range are highlighted in yellow.

You can see that his albumin ratio is slightly lower than the normal range, whereas his beta globulins are slightly higher. All alpha

globulin levels were normal. When you look at his free kappa and lambda light chains, shown in the lower panel, they are well above the normal ranges, but overall he has a normal kappa to lambda ratio. At this point in time these are observations, and we don't know specifically what they mean or if and how they would contribute to the observed hypersensitivity events. Next slide.

After identifying Donor Z as standing out from the other common donors and taking precautionary market action it was now absolutely critical that we biochemically link Donor Z as the root cause by demonstrating the ability of Donor Zed's plasma to induce hypersensitivity reactions in vitro. Next slide.

As we heard in the morning session, immediate hypersensitivity reactions involve the activation of immune cells like mast cells or basophils, the degranulation results in the clinical effects associated with these hypersensitivity reactions. So to biochemically investigate this we asked three questions. Can

Donor Z's plasma or Z containing IG product induce mast cell deactivation and granulation? If so, if we do see activation, what is causing it? Is it the IgG, IgE, or some other trace plasma protein? And what is the relevant molecular pathway of activation? Next slide, please.

Our first studies looked at the ability of Donor Zed's plasma and affected immunoglobulin lots which we call in this presentation Z-IG to induce basophil activation. Next slide, please. Advance. Thank you.

In these initial studies we chose to study basic degranulation using the cell surface marker CD63 as basophils circulate in the blood and they are far easier to isolate and study than mast cells, yet both share common receptors and pathways. These assays utilized basophils from basophil donors. However, since the sensitivity of the basophil can differ from donor to donor, we made sure to look at a variety of basophil donors in these studies.

The two bar graphs on the left show the

basophil activation results caused from donors that, shown in the red box, versus a typical control plasma. The large bar shown on the right of each graph is the spike positive control that shows the assay is working. The control plasma samples showed no activation, whereas Donor Zed's plasma showed significant activation at various dilutions of plasma used in the assay.

Although not shown here, Donor Zed's plasma, but not control plasma, produces consistent activation albeit to varying degrees across the different basophil donors. So these data confirm that Donor Zed's plasma can activate basophils, where control plasmas cannot. In an attempt to further biochemically link Donor Zed to the observed hypersensitivity reactions we looked at the ability of Z-IG affected lots to induce basophil activation. The results are shown in the far right.

Again the bands on the right of each graph show the spike positive control showing that the assay is functional. Surprisingly, we didn't

see any activation in lots of IG that contained Donor Zed's plasma. Our initial explanation for these results was that the assay sensitivity was not good enough due to the very high dilution of Donor Zed within the final product. Therefore, to investigate the activation potential of Z-IG lots further, we modified the assay to increase its sensitivity. Next slide, please.

To increase the sensitivity of the assay, instead of monitoring the CD63 degranulation marker we monitored an activation marker known to be a bit more sensitive called CD203c. In this more sensitive assay we analyzed final container lots of either effected Z-IG or non-affected IG controls.

This slide shows the test results conducted with two different basophil donors. On the left-hand side we have a more sensitive basophil donor, and on the right-hand side a less sensitive one. For each basophil donor we show two Z-IG lots, depicted in red shading, that contain Donor Zed plasma at different proportions.

The highest percentage on the left and the lower percentage in the middle.

The last slot on the right of each trio of graphs is a control lot IG. Here again, the highest bar on the right of each graph is the spike control to show the assay is working. For the more sensitive basophil donors, and this example represented as Donor 1, we started to have a low but reproduceable activation signal in some Z-IG lots. Also notable was that the activation signals were highest in the Z-IG lots with the highest proportion of Donor Zed's plasma and decreased as the proportion of Donor Zed in this Z-IG product decreased.

We also again noticed a difference in sensitivity and detection associated with the basophil donor. For the less sensitive basophil donors, here depicted as Donor 2, the signals were weaker and more difficult to detect, both for Z-IG and also for the positive control spike into the Z-IG control IG lots.

Although the signals we observe are very

low, we believe there's a positive activation that occurs and is best observed when using more sensitive basophil donors and Z-IG lots containing the highest proportion of Donor Zed's plasma.

Although not shown on this slide, we also observed consistent low levels of basophil activation for Zed plasma pools, a process intermediate, which contained about a 1:5,000 dilution, or.02 percent of Donor Zed's plasma. So now we have confirmed basophil activation by Zed plasma, Zed plasma pools, and Z-IG final purified product. Next slide.

To further advance our investigation we used the more sensitive assay and basophil donor and tested the eight Z-IG affected lots containing various proportions of Donor Zed's plasma for their ability to induce basophil activation. The plot on this slide shows the percentage of activated basophils on the y-axis versus the percentage of Donor Zed's plasma present in the Z-IG lots on the x-axis. As you can see there's a linear positive correlation. Although the signals

are low and most are below what's considered to be the positive threshold, we were encouraged by these results and believed we were on the right track. Therefore we decided to explore this correlation using a different assay. Next slide.

Thanks to scientific collaboration with Grifols, we were able to use the histamine release assay that they had established within their internal labs. This test still relies on basophils but measures the histamine released upon basophil activation degranulation.

In this graph we show the percent total histamine released on the y-axis as a function of the percent of Donor Zed in the eight IG lots. With this more sensitive assay we observed stronger signal in Donor Zed containing IG product lots and a strong and positive correlation between the percentage of Donor Zed's plasma present in the Z-IG lots and the percentage of total histamine released. Next slide, please.

Lastly, we wanted to confirm that Donor Zed was the only common donor who elicited such a

positive linear dosage response to these assays. Therefore similar to the previous slides, we plotted the histamine release assay results for each of the Z-IG lots against the percent of each of the other common donors in the Z-IG lots. The graph on the left shows the linear correlation of Donor Zed shown on the last slide. On the right are results for the 20 other common donors across the lot that had had market exposure. Of the common donors, only donors that showed a very high R-squared value, a direct linear positive correlation of percent of donor's plasma in the affected lots, no linear correlation of the histamine release results with the donor's percent plasma was observed for the other common donors, as shown by the scatter of points in the low R-squared values.

We also conducted a similar evaluation with the CD203 basophil activation test results. And again, Donor Zed was the only donor that showed this positive correlation. Next slide.

So in conclusion, the biochemical

investigation that we performed supports the hypothesis that Donor Zed's plasma is the source of the hypersensitive activity. We've shown that Donor Zed's plasma, Zed plasma pools and Z-IG final purified product activates basophils whereas the corresponding control samples do not. We identified Zed as the single common donor, his plasma is present in the affected lots, and exclusively elicits a positive linear donor response in the basophil activation and the histamine release tests. Although these assays are investigational, time consuming, and the signals were relatively low, the totality of the biochemical results support the single donor hypothesis. Next slide.

So we address the first of our three questions of our investigations. Can Donor Z's plasma or Zed containing IG product in use in mast cell or basophil activation granulation. The answer is yes.

So we set up to answer the second question. What in Donor Zed's plasma is causing

this activation. Is it the IgG, IgE, or some other trace plasma protein. Next slide.

To answer this question we performed an elegant fractionation study. We used affinity columns to prepare IgG enriched as well as IgG depleted, and IgE depleted Zed plasma fractions. We tested these fractions with the basophil activation tests, and the results are shown in the graphs on the right-hand side. The activation results for Zed plasma are shown in yellow. And as expected, we observed pretty strong activation signals at different plasma dilutions.

The second fraction, shown in dark gray, is the IgG enriched fraction. Notably the basophil activation ability of the samples has been preserved. This indicates that samples that have IgG but where all other proteins were removed, still show activity.

The third fraction, in red, is the IgE depleted fraction. Here the activity is also still present. So IgE is not the source of activation in Zed's plasma.

The results for the fourth fraction are interesting. What we see in the light gray box is that when we deplete the immunoglobulin G from the donor's plasma, we eliminate the ability to active the basophils. So this provides strong evidence that the IgG fraction of Donor Zed's plasma is responsible for causing basophil activation. Specific removal of the IgG results in a loss of ability of the plasma to active basophils. Next slide.

So these results answer our second question. And this immunoglobulin G fraction of Donor Zed's plasma that is responsible for activating the cells. Perhaps not too surprising then that it would end up in the purified immunoglobulin products.

Finally, we sought to answer the third question regarding biochemical pathway involved. Next slide, please.

We identified four pathways that were more probable for mast cell activation as described in this morning's session. The first is

the MRGPRX2 pathway. This is a G protein coupled receptor, and this receptor's often responsive to small molecules. The second is the Fcgamma receptor pathway. This is a receptor for IgG antibodies and is specific for the Fc portion of these antibodies that we heard about this morning. The third pathway is the C5a receptor complement dependent pathway. And the fourth pathway is the high affinity IgE receptor pathway. We use different assay systems and specific inhibitors to investigate these pathways.

I want to point out that our investigations on these pathways are still currently ongoing and additional work is needed to solidify our datasets, understandings, and conclusions. Next slide, please.

So our initial data suggests that the first three pathways are not activated by Donor Zed's plasma. However, in our innovation studies we did get indications that the high affinity IgE receptor pathway was involved. Next slide,

please.

In the FcRI inhibitor studies we used a different assay system based on humanized Hoxb8 mast cells. We first conducted a study comparing Donor Zed's plasma samples with control plasma in the absence of any inhibitors. With this mast cell assay we observed a low but reproducible activation signal for Donor Zed's plasma that was higher than for the control plasmas. Next slide, please.

So we pursued investigation of inhibition of the FcRI pathway using two different approaches. In the first study we performed a competition assay with increasing concentrations of soluble FcRI alpha chain. As shown in the bar graphs, we observed decreased activation as the soluble alpha chain concentration increased. We consider this to be partial inhibition. We know the assay system is working properly as we were able to achieve full inhibition with an anti-FcRI receptor antibody control at the same concentrations as the free

alpha chain inhibitor.

The fact that we do see partial inhibition here suggests that Donor Z may be at least partially activating through this pathway. Next slide.

In the second approach we investigated an internal signal component to this pathway called BTK. By using a specific inhibitor Ibrutinib. And what we observed here is a strong inhibition. The combined results of these two studies led us to conclude that Donor Zed acts at least partially through this FcRI pathway, although this story may be more complex than that. Next slide, please.

So the preliminary answer to the final question is that activation is at least occurring through the high affinity IgE FcRI pathway. However, it is important to note that other pathways could also be activated that have yet to be identified and more work in different assay systems needs to be done to further elucidate the underlying biochemical mechanisms. Next slide,

please.

So I'll finish up my talk by describing our learnings and next steps. Next slide, please.

Through our lot-specific hypersensitivity investigations -- next slide, please -- we demonstrated that the root cause is independent of process and not related to process impurities. The product was normal by characterization, and ultimately we identified the most probable root cause, a single donor, Donor Zed, whose plasma donations were 100 percent associated with lots of IG that produced the elevated hypersensitivity reactions. As a result, Donor Zed has been permanently deferred.

We demonstrated that Donor Zed plasma, Zed plasma pools, and Z-IG final product all elicit basophil activation and activation is due to a unique immunoglobulin G specificity of Donor Zed that acts at least partially through the FcRI pathway. Next slide, please.

There are a lot of challenges in the work we're doing. Although we made great progress

over the last year, our reagent Donor Zed plasma is really quite limited overall for conducting investigations. We have to be very considerate in choosing what additional investigative avenues to pursue. It is important to recognize that this was the first time in our 15 years of manufacturing experience with our current generation of IG products that we had experienced an increased number of hypersensitivity reactions with specific lots. Over this time there have been millions of donors that have contributed plasma to these products and now we've identified a rare single donor with unique components of his IgG repertoire that appears to be the root cause of these set of circumstance.

Due to this extreme rarity, it may be difficult to generalize the applicability of CSL's findings from Donor Zed to the root causes of other lot specific events experienced by other manufacturers. All the assays that we used today are highly investigational. We are the at the very low end of sensitivity and assays lack

robustness as illustrated by data where we showed you that the ability to produce the signal is heavily dependent upon the basophil donor. As such, these types of investigational assays fail to meet the rigorous quality standards, specificity, sensitivity, and robustness required of GMP assays to ensure reliable testing of products.

In order to identify and develop an appropriate assay system that is fit for purpose for GMP use we obviously need greater understanding of the commonalities and generaliz-ability of the root causes for similar events experienced by other manufacturers.

This FDA workshop serves as an important first step for knowledge sharing and addressing some of these challenges. Next slide, please.

So CSL will continue our investigations to confirm the activation pathway or pathways as well as investigate the irregularities in donor Z light chains and potential gammopathy. CSL has dedicated breakthrough innovation teams that are

willing to partner with others who may have technologies that may be useful in providing solutions. Next slide, please.

As we move forward, the patient interest remains at the core of CSL's commitment. We will continue to partner with stakeholders, including FDA, industry members, academics, and technology innovators to further our investigations and understandings with the underlying goal of ensuring patient safety and preserving IG product availability. Next slide, please.

So finally I want to acknowledge the key people in collaborations behind this data and presentation. First of all I want to acknowledge my amazing Bioanalytic Team at CSL, specifically Tino and Lorenzo, who have lived and breathed Donor Zed investigation over the past year. And the fabulous data-driven detective work by Peter Vogel, along with our safety physicians Amgad and Adrian, to find a needle in a haystack. Our Grifols and Octapharma colleagues for their very open scientific discussions and sharing their

experiences of these rare events.

An extra special thanks to the Grifols Bioanalytic Team for their collaborations on the histamine release tests on donor plasma in their labs. Our network of partners, shown here from the Mayo Clinic, Charite Thomas and the University of Burnham. And finally a big thank you to Dr. Dorothy Scott and her team from FDA for making themselves available on multiple occasions for very open scientific discussions and suggestions that have helped propel our investigations forward.

Thank you for your attention in listening to CSL's experience.

DR. SCOTT: Thank you, Nathan. It's a wonderful narrative of how this all progressed and exciting findings that you have. But for the sake of time I think we need to move on, we're a little bit behind, to the Grifols Biotherapeutics presentation, which will be split into two parts. The first with Clark Zervos, the Vice President of Quality, and the second by Peter Vandeberg, Dr.

Peter Vandeberg, the Director of the R&D Program. So we thank you very much for presenting and look forward to your talk.

DR. ZERVOS: Thank you, Dr. Scott. And I would like to thank the FDA and the PPTA for the opportunity to talk about Grifols' experience with Gamunex and hypersensitivity.

I'm going to begin by presenting a high-level overview of our investigation and findings. And after that Pete Vandeberg will go into a detailed discussion of the analytical methods and results from the investigation. Next slide, please.

Again, I'm going to talk specifically about Gamunex and hypersensitivity events. Gamunex is indicated for the conditions you have seen here, which have been discussed previously. Next slide.

So Gamunex was first launched in 2003, it has a well-established safety profile. However, beginning in 2018 we observed an elevated rate of hypersensitivity adverse events for

specific lots of Gamunex. The types of adverse events are listed here, they've been discussed earlier in the workshop, so I won't go into detail here. And as a precautionary measure Grifols initiated a voluntary market withdrawal for numerous lots of Gamunex, the total number was 23 over the course of the investigation. And then subsequently as we've learned, some products from other manufacturers were withdrawn for similar events, and we began a collaboration to investigate this. Next slide.

So this graph goes from 2015 to the very end of 2022. My intent here is to show first in the left portion of the graph the baseline before we started seeing this issue with certain lots. And you can see that the majority of lots have either zero or maybe one or two adverse events. There are a few exceptions in there, but a very low baseline until we enter 2018 and we saw the first lots with an elevated number of events. So these were very definitive lots, and you can see in between the lots with the high number of events

there were lots with again very near baseline.

So during this period between 2018 and 2021 we withdrew 23 batches from the market. And then for the reasons I'll talk about later, since about March of 2021 after we had taken some actions based on our findings, we've really returned to baseline here.

And to give you an overall perspective, there are about 3,700 lots of Gamunex shown on the x-axis in this graph. And 23 were withdrawn, that's less than 1 percent, so just to get some perspective of the number of batches. Next slide, please.

So just a quick summary of our manufacturing and quality investigation. So from the very beginning we looked at detail at the lots withdrawn, looking at all the raw materials, the production process, in-process testing, final container testing. So we're not only looking at, you know, did the batches, all the steps meet specifications, meet the specified ranges, we were also carefully comparing to our comparator set of

batches just to see if there were any shifts or trends that could give us a clue as to what was happening with the specific batches. But we weren't able to identify anything as part of that review, and there were no significant manufacturing deviations for the withdrawn lots. Next slide.

So a quick look, we performed quality control testing on all the finished product, so we went back to look at all of that data. All of the withdrawn lots met the product specifications. And in addition we looked within those specifications again against comparator lots to see if there were any slight shifts or trends that might give us an indication, but we were unable to find that.

We also periodically do expanded characterization testing for final container lots so we have a large reference set of data. And we did that testing on the withdrawn lots and again were unable to see any difference between these lots and our comparator lots. Next slide, please.

So beyond QC testing we performed a wide range of testing as you can see here, both in vivo and in vitro testing. This was done on the final product. And again, we were comparing back to comparator lots that were tested in parallel with the withdrawn lots. Again, unable to see any difference in signal that would point us in a certain direction. And Pete will discuss a little more in detail some of these methods later in his presentation. Next slide, please.

Common donor analysis. So during the investigation looking at common donors, we did determine that lots that were withdrawn contained some portion of plasma from a single donor center. So this was something we obviously looked at right away. And as a precautionary measure we put plasma from that donor center on hold and restricted its use from manufacturing. And since we looked at a common donor center, or one donor center, we wanted to make sure it wasn't some center-level issue that might be a cause for these reactions. So we looked at everything, all the

operations of that center, the commodities used, their SOPs, but nothing came out of that that indicated it was a center-level issue. Next slide.

So common donor analysis, we performed this after we withdrew more than one batch and then it had to evolve after every additional batch was withdrawn. And in this case you're looking at thousands of donors that go into the manufacture of each Gamunex lot and comparing that donor population to the ones for subsequent lots. And the challenge with that analysis is if you have for example three lots withdrawn, there could be thousands of common donors ranging from, you know, hundreds to thousands. So that complicates the analysis.

However we eventually were able to identify 18 donors in common to all the withdrawn lots. And those 18 donors did all donate at that same plasma center that I mentioned earlier. These donors at that point were deferred from further plasma donation. And then we obtained

plasma samples from each of these 18 donors and performed analytical testing. Next slide.

And we found one of the 18 common donors produced the positive response in the basophil activation test and the histamine release assay. Pete is going to go into much more detail on this in just a bit. Plasma from the other 17 donors produced negative results by the basophil activation test and histamine release.

And even though that indicated, you know, we had one donor, really our first clue in all of this, we didn't feel there was sufficient evidence to unequivocally assign a cause to that donor so we excluded all of these 18 common donors from further manufacturing. Next slide.

So since that time we have resumed use of plasma from that donor center that we have put on hold. And that was of course excluding the 18 common donors. So this was about 50,000 liters of plasma that was accumulated. And we took that and manufactured several Gamunex lots during 2022 and then those lots were released on a very

controlled, staggered basis and monitored closely by our pharmacovigilance team. And no reports of hypersensitivity have been received for any of these lots. Next slide.

All right. And at this point I'm going to hand it off to Pete Vandeberg.

MR. VANDEBERG: Great. So thank you. Thank you, Clark. And I'd like to talk about our in vitro methods now that we used for this investigation. First to let you know that what I'm going to talk about is just a really small flavor of what we've done. We did quite a bit of compositional analysis. We looked at aggregates, we looked at some different functional methods as well, to look for aggregates and did not see anything different from normal in batches that caused the hypersensitivity. Next slide, please.

So first off, a little discussion about the 18 common donors. We looked at the information available on these 18 donors, and each had an extensive donation history. There were no remarkable medications, nothing remarkable in

their medical history. We had given a supplemental questionnaire to the donors at this donor center, so we had some additional information beyond the normal medical reports and the donation information from them.

Some of them reported common allergies but nothing remarkable. You know, nothing like tick bites or likewise occupational, there was nothing that stood out. They also had normal serum protein electrophoretic profiles. We did some diagnostic testing on the plasma of these 18 donors and several of them did have elevated IgE, which is quite common. We tested for anti-alpha-gal, an emerging allergen, and there were no signs of that in these 18 donors. We also looked at anti- FcRI and anti-IgE antibodies, and nothing [was

different in these donors. So there was nothing in these donor histories that stood out as remarkable. Next slide, please.

So we wanted to look for in vitro methods that would predict the hypersensitivity.

And since the reactions in the patients, the hypersensitive reactions in the patients were suggestive

of mast cell reactions.. There are many different receptors on mast cells which can activate them. So we did think about going to some of the commercially available mast cell lines, but we wanted to stay as close to human cells as possible.

And so initially we did explore the use of a skin- based system where we obtained skin from elective surgeries and set up a system to look at it that way. Unfortunately we did not see any consistent histamine response with samples or controls. So we discarded this system and moved to what we thought was the next best thing, which was basophils sourced from human blood. And so most of the rest of my talk will focus on our work with basophils. Next slide, please.

So we had two basic assays looking at basophil activation. The first is the histamine release assay where we used a commercial ELISA kit

to measure the release of histamine. Both of these assays, the histamine release and basophil activation test, essentially you take whole fresh blood, you add your sample with any kind of buffer dilutions that are required, you incubate and then you measure the response. The histamine release, again it's histamine with the basophil activation test. We used a commercially available flow cytometry kit to measure the expression of CD63 or CD203c. Nathan from CSL mentioned that CD203c seems to be a little bit more sensitive. I think it's also a little bit more prone to background activation.

It's important running these assays that you have the proper controls, and so having a buffer control where you see a low background response. And then the two positive controls, you would use either anti- FcRI alpha or anti-IgE to detect the deactivation of the FcRI on our one pathway, and we would use a peptide FMOP which activates to the G protein coupled receptor pathways.

We set up these assays and we tested our Gamunex product that had shown signs of hypersensitivity. We also tested plasma pools that were used to manufacture those products. We retained samples from the plasma pools. And we did not see a response with any of the hypersensitivity batches. Next slide, please.

So before I talk more about some of our other results I'd just like to talk about the challenges of these methods. These are research methods that we used. And the first bullet there is really the specificity. In our history we've had hundreds of thousands of plasma donors, we've made thousands of batches in our particular collective history at CSL, and these events are very rare. We're talking about what looks like a very small set of donors. In two instances here we were able to detect a response in the histamine release assay on CSL's product but not on our product that caused the hypersensitivity batches.

So really the specificity here is unknown and there's not a good way to test it

because of the rarity of the events. It also could be a sensitivity issue, and perhaps our assays are just not sensitive enough to detect the differences in the product that causes the hypersensitivity.

There's also logistical issues of performing these assays. You need to collect fresh blood for the assays, there is variability in that blood, the assays are fairly labor intensive, the data analysis is cumbersome. And there's also a lack of any kind of official reference standard for calibration. So in short, these are challenging assays, the predictive value is uncertain, and they're not appropriate for use beyond research applications. Next slide, please.

So I'd like to next jump into some of the data from testing the plasma of the 18 donors. And the bar graph there shows results using five different reagent basophils, so that's blood from five different healthy people, and the mix of the histamine release or basophil activation test. Way on the left side of the graph you see the

basophil response. You want that to be low, you don't want your basophils to self- activate. Next over is a set of bars for the positive controls, the anti- FcRI alpha or anti-IgE, which has been normalized. All the results here have been normalized to 100 percent of that, and then [here is]the fMLP peptide positive control.

And so for all of our 18 donors we saw a response in these assays in only a single one and that was Donor 18. And it was consistently, Donor 18 showed consistent activation of basophils and none of the other donors did. So we chose to characterize the Donor 18 plasma response a little bit further. Next slide, please.

So one of the first things we did is look at a dilution series of plasma from Donor 18. And what this shows is the results of dilutions. We did dilutions to 1:2,000 but really we can only see the response out to 1:100. Now for us to be able to detect this donor response in plasma, an individual donor's typical diluted on the order of 1:1,000 to 1:10,000 in the final product. We

could not detect beyond 1:100. This is very different than CSL, who could detect at least down to 1:1,000 in their donor. And it could be a matter of specificity. It could also be a matter of just the activation in Donor 18 was not as strong as

that from their Donor Zed or Donor Z, sorry. Next slide, please.

We were very fortunate to be able to get retain samples from the bleeds of Donor 18. So the plasma donations Donor 18 made, we were able to get some of the samples from those back covering a span of about three and a half years. And looking at the basophil activation response over that time period you see several interesting things. First of all back in 2017 plasma collected from that donor did not cause basophil activation. Then in April of 2018 you see a jump. And this does coincide with plasma that went into the first batches where we saw hypersensitivity results in patients.

But then the interesting thing is that

the activation of basophils by that donor over time is not consistent, it does go up and down. I did look at this for some seasonal relationships and there was no apparent time of year, seasonality to that response. But nonetheless interesting. Next slide, please.

If you replot the data, this graph here shows the basophil activation response replotted on the x-axis. And on the y-axis is the adverse events of the batches that that donor went into normalized by the quantity of plasma from that donor in the batch. And you see something very interesting. You see essentially a flatline until you reach 70 percent CD203c positive. And, you know, this looks like a threshold relationship where when you reach a certain reactivity in Donor 18, you see hypersensitivity in the batches produced by that donor. Next slide, please.

We also performed a fractionalization experiment on the Donor 18 plasma. We used IgG affinity resin specific for IgG so it should not be retaining any IgA, D, E, or M, and if you look

closely at the reduced SDS-PAGE on the left you'll be able to see the plasma lines versus the IgG depleted versus the IgG. You can see the heavy chain and the light chain. At any rate if you look over on the right at the bar charts, you can see the last three sets of bars that the basophil activation we see in the plasma is essentially identical in the IgG fraction and in IgG-depleted fractions there is just very little response. there. So there it looks like IgG is the agent of interest that's causing the basophil activation. Next slide, please.

We wanted to probe a little bit more about the nature of the reactions, so we purified basophils using a commercial kit to remove all the other cell types, so this system would have been just a purified basophil system. And what we see from that on the bar chart at the very far right, that Donor 18, whether it's whole blood or whether it's the purified basophils, activates essentially the same. This implies that it's a direct activation of basophils and not acting through

some other cell types. Next slide, please.

And we wanted to look to see if we could get some clues of the mechanism of that reaction within basophils. And so we looked at the addition of a PI3K-delta inhibitor. And that inhibits downstream the basophil activation initiated with the cross linking of FcRI alpha units, and the signal cascade downstream can be blocked. And what you see in the bar chart on the very right side of that, you see a dose response inhibition of that response. You do not see the same inhibition with the FMLP positive control, which activates an alternate G protein couple receptors. But you also see the inhibition by the anti-FcRI positive control. So this suggests that Donor 18 is activating through FcRI pathway. Next slide, please.

This slide summarizes all of the reagent basophils we looked at with Donor 18 plasma. We looked at a total of 28 different reagent basophils, so that's basophils in whole blood from healthy donors. And I'll start at the bottom

because this is kind of interesting. So some donors you get fresh blood from and they just have a low basophil count, you just can't use them in the assay. Next up you see some that have enough basophils, but they don't respond to FMLP or anti-FcRI positive controls. So they're just not activatable. Next up you see that there were two reagent basophils that only responded to FMLP, they did not respond to the anti-FcRI positive controls. And interestingly enough, those two donors, those two reagents, also did not respond to Donor 18 plasma. However, the 18 fresh bloods we used, 18 different donors that were activatable by both FMLP and the anti-FcRI also showed activation with Donor 18 plasma.

Now this is suggestive that Donor 18 activation, or it's consistent with Donor 18 activation through the FcRI pathway. But it also suggests that the activation isn't through some specific allergen but it's a very broad mechanism. Next slide, please.

So we were interested in trying to find

out what the specific, what a specific auto IgG autoantibody would be that would cause such an activation. So we did testing by the HuProt microarray with technology that was developed at Johns Hopkins and available at a lab called CDI. We submitted samples of plasma from these 18 donors. Initially we were looking at things that had a high signal, we were looking for a high response and something that was connected to mast cells. And looking at all the 18 donors we didn't see anything that had a large signal.

So next I looked at some of the specific mast cells and looked down at some of the lower signals, some of the specific mast cell receptors. And I see something very interesting. We see with one donor, and it happens to be Donor 18, there was a signal showing for FcRI beta. Next slide, please.

So some of our earlier speakers have talked about the FcRI complex. The beta unit is involved in the signaling transduction that occurs. It is a membrane protein, only small

parts of it are exposed. And I do want to comment that the kind of data you get from a microarray screen is just really a screening test, and for us to show that this is really the target it would be nice to get some confirmatory response. We did try some competition experiments and we were able to get some commercial antibody of this against this receptor to see if we could initiate the same response, the same activation basophils. I would say at this point our results have been inconclusive. But this is certainly an area that I think deserves a little bit more work, that it's an opportunity for us to do some more work on. Next slide, please.

So in conclusion, we've done a thorough investigation, looked at lots of different things like manufacturing and characterizing materials and really traced these hypersensitivity events back to 18 common donors. Plasma from one of those donors, Donor 18, showed activation of basophils when tested in a human basophil system. The time course of that basophil reactivity does

match the timeframe of hypersensitivity adverse events. We've isolated that plasma reactivity to the IgG fraction. It occurs in basophils, it's blocked with the PI3KA inhibitor. And then finally by the HuProt autoantigen microarray we have identified some binding to the FcRI beta.

Unfortunately, our in vitro assays did not show any kind of elevated response in the Gamunex hypersensitive product batches or pools, and thus we have excluded all 18 donors from further use in manufacturing. And since we did that, the hypersensitivity adverse events returned back to baseline. Next slide, please.

I would like to acknowledge the efforts of all the different Grifols teams that went into working on this. Our Biomat plasma collection people, pharmacovigilance quality team, and regulatory affairs. Especially I'd like to acknowledge the bioanalyticals team who did all of a lot of characterization testing, but especially the histamine release and the basophil activation tests. And the collaboration of Dr. Scott and

her team at CBER.

I'd also like to acknowledge the advice we got from a few outside experts, Steve Dreskin from University of Colorado Medical School and Don MacGlashan from Johns Hopkins. And I'd like to thank you for your attention.

DR. SCOTT: Thank you very much Dr. Vandeberg, that was quite informative and an interesting contrast in some ways to the CSL investigations. Or rather I should say a different approach and somewhat different kinds of donors maybe for all we know. Anyway, for the sake of time I think we need to move on to the Octapharma investigation. And thank you both for those very excellent talks that you just gave.

So for Octapharma we have three speakers. I would ask you to maybe speak a little faster if you can so we can get partially back to schedule because I don't want to have to take time out of the break. But I think in fairness we probably will do that so that you have adequate time to give your presentations.

So we have Juergen Roemisch, Ph.D., Senior Vice President of R&D Plasma; Balazs, and I apologize if I'm not saying that correctly, Toth, Dr. Toth, MD, Ph.D., head of Corporate Drug Safety for Octapharma, and Josef Weinberger, also Ph.D., Corporate Quality and Compliance Officer. So thank the three of you in advance, and let's get started.

DR. ROEMISCH: We are on?

DR. SCOTT: Yes.

DR. ROEMISCH: Okay. Thank you very much, Dr. Scott. So good afternoon, good evening. My name is Juergen Roemisch, I'm heading the Pre-Clinical R&D Plasma for Octapharma. And thank you very much for the opportunity to present at this workshop for FDA. The next slide, please.

So we split this talk in three parts. The first one will be Octapharma elevated rate of hypersensitivity cases that will be done by my colleague Balazs Toth. And the donation center's single donations and risk mitigation by Josef Weinberger,and biochemical and root causes

analysis by myself. So please follow us. And the next slide, please.

DR. TOTH: Thank you, Juergen. Dr. Ross, colleagues, I would like to give you a short review of Octapharma's part, what we have observed beginning of last year 2022 in connection with certain Octagam batches.

As you have heard before, we could establish quite early on that the observed events were all hypersensitivity related. And hypersensitivity as an entity is labeled for Octagam, both in the company core safety information and the U.S. prescribing information as well.

The reactions which we observed in connection with these batches were mostly non-serious, skin-related hypersensitivity type events. There was only one product affected, Octagam 10 percent. We tried to also establish some kind of geographical spread of the events. These cases were received mostly from the U.S. and certain parts from Canada and certain European

countries as well. Next slide, please.

We identified, and actually eventually we had to withdraw four batches, Octagam batches, with this elevated number of hypersensitivity cases. All these happened within a short period of time, end of January, middle, end of February, 2022. We identified three batches and withdrew three batches from the U.S. market and one batch from Norway, the Norwegian market. The numbers you can see in the table present the situation at the time of the regional decision that were made by Octapharma. As you can see that we had a number of cases, between 10 and 4 in connection with individual batches. All the cases which we received in connection with these batches were hypersensitivity type batches.

We also identified two additional batches on the European market with a similar picture. However, those batches were already distributed and in the later stage of their life cycle, say it this way. And in agreement with the local responsible authorities these batches were

not withdrawn from the market. However the batches were involved in the further investigations. Next slide, please.

We also wanted to be sure whether there are other reactions, hypersensitivity reactions, were observed in certain groups of patient subjects. However we could not identify any particular characteristics of the patient group, nothing we got in demographics, geographic locations, treatment indications, dosages or any other respects. As with the cases we have seen with CSL and Grifols' presentation, (inaudible). Our batch documentation review could not identify any particular batches where the production would have affected the quality of the observed batches. And we also could not identify any changes such as new manufacturing raw materials or

chemicals that were correlated with the hypersensitivy batches. Thank you, and Josef will take it over.

DR. WEINBERGER: Thank you, Balazs. Next slide, please. Can we have the next slide,

please. Thank you. No, one back. Yeah.

As already mentioned by Balazs, within one month we had these four lots with clusters of hypersensitivity reactions, identified in the US (3) and Norway (1). Part of these batches were delivered to Canada but not distributed there. So on one hand the production investigation, we think the batches are as normal as batches could be. In parallel we immediately started the investigation on the plasma sources.

We could very fast clarify that all the plasma was U.S. Source Plasma. And we also found immediately that there is no over-representation of any one donation center. So it was nicely spread over a lot of centers. And also we found quite fast 133 donors in common with the four withdrawn batches. And there's always a different number of donations of such donors in a batch between one and 10. Twenty-four donors of the 133 US donors contributed to the two

hypersensitivity batches in Europe, In parallel the first step was to

defer all those 133 donors from manufacturing medicinal products and removed all the available donations from those 133 donors from our

[plasma] stock. So that was the first investigation.

And the next step we tried to find more information based on donors, as already mentioned, related to including possible tick bites (resulting in

anti-gal antibodies. We also verified the gender, the ethnicity, the age, allergies, medications, and

underlying diseases of these donors. And also this comes in a little more

detailed on the next slide, how many donations contributed to hypersensitivity batches and how many of these donors did not contribute to such batches but were used in standard Octagam batches. And we found in no case any striking

result. We could not see that all those donors have certain

allergies or whatever else. Next slide, please.

So as said, we immediately deferred and we decided based on initial information from CSL at that time, that we would also perform a ranking of those donors based on their contribution to hypersensitivity batches, contribution to withdrawn batches. And based on that ranking to start additional biochemical investigations of those donors. You see below in the right lower corner the ranking of the highest risk considered donors. Only the very first one contributed more than 50 percent of his donations to relevant batches. The next one was already at lower than 50 percent, and then we were almost at 80 percent in non-hypersensitivity batches. So it is just a few donors who were indicated to be responsible for that. Unfortunately we could not get from all our donors retention samples when new donations. We tried to call them to invite them for new

donations but unfortunately in many cases we failed. Next slide, please.

And in parallel we had of course discussions with on one hand the FDA in the United States, on the other hand with the German Paul Ehrlich Institute, which is more or less the CBER of Germany. And from a later discussion we got the hint tick bites may lead to anti-gal antibody development which can cause hypersensitivity types of

events. And with that I would like to hand over

to Juergen for the R&D part.

DR. ROEMISCH: Thank you, Josef, yes. The next slide, please.

So as you have heard extensively in the talks before and also in the first session, there are many factors that can activate cells or could be capable of sensitizing cells. We picked here a number of cytokines or anaphylatoxins like C3a and so on for relative activity to compare the hypersensitivity associated batches with control

batches which with no report at all.

To make it very short here and for the sake of time, I won't go into the detail here. You don't see any difference between the associated batches and the control batches. They were either not detectable or on a comparable level. So the next slide, please.

Also the one assay that shows complement activation. You see it asserts a pair or a couple of columns from the left-hand side, you see [exposure of

whole blood] to heat aggregated immunoglobulins which have been referred to in the talks report and talks before. This is our our positive control, they were heated for two hours at 60 degrees, and they nicely activating complement here. We show the activated

complement protein C4a because it gives more reliable results and after 30 minutes of incubation.

[see slide 10] So the left-hand side are controls,

sodium chloride or maltose controls, and the right-hand side in the green rectangle are control

Octagam batches. But no reports in the red rectangle of the associated batches, and tested with 10 different healthy donors. And you see already that there was a kind of a variability from donor to donor which comes not as a surprise but the overall there is not really a trend between the affected batches and the control batches. But in this relatively sensitive assay we have done a number of times could not refer also to an aggregated IgG or so as a reason for it [sic]. Next slide, please.

Referring to the anti-Alpha-Gal IgG levels, we compared again the four associated batches with the green control batches with ELISA, and you see there is no significant difference in turns of these levels, and they are not higher in the control batches so we could also exclude this possibility. Next slide, please.

So then I have to acknowledge here the

cooperation also with CSL and Grifols and the exchange of knowledge and learning in investigating the root causes that encouraged us also to look into the basophil activation and to get them tested at Octapharma in the mast cell activation assay.

So we used the Octagam 10 percent batches, control batches, as a control, the maltose, the excipient solution and also selected plasma samples from the group of deferred plasma and as a control also our Octaplas LG as a reference for a number of food plasma and SG treatment test done. Next slide, please.

So without going into details here, just a summary of what we found. So referring to the activating anti-FcRI receptor we found that all IgG

concentrates investigated showed weak anti-FcRI receptor 1A activity exclusively at the highest tested concentration. Already the next dilution showed levels below limit of quantification. So we don't know whether this

holds really true, but as we had earlier heard, that a normal population has around, I don't know, 1 or 2 percent of these kind of antibodies circulating, so it does not come as a surprise but they would just be then above the threshold of measurement, but at really low level.

And none of the tested plasma samples showed such kind of an activity. While we have to say, as was said before, that a number or a couple of samples were not available at this time of testing, which may represent risks to patients of that and it could be that a couple of them were also had come into the pool for production.

The other assay activating anti-IgE antibodies revealed nothing at all. So we didn't see any batches that showed any anti-IgE activity. One of the selected plasma samples of those we tested showed a detectible anti-IgE activity, which was low with only 4 percent of the mast cells activated, still it was above the limit of quantification. But the conclusion here was that the dilution of such sample in such a huge pool of

donations comprising IVIG would not be of physiological

relevance if you refer just for this samples and the group of others we cannot state at the moment. Next slide, please.

Just a brief short look not only at this current investigations but, I mean now and then this occurs, we all know. And we have experienced now also for Octagam investigations for I would say 15 years at all in summary of them and having tested them. And we have done a lot of tests when these kind of events occurred. Many of you have seen, I don't have to repeat the first one, but we also did testing in fresh whole blood samples exposed to these kind of positive controls and here we have those associated with hypersensitivity reactions, always in comparison with control batches without those reports.

And we tested a lot of parameters in them. Namely the expression of these things compounds like interleukins, like we used PCR to follow the FC receptor expression of that facts

analysis enzyme. And we never ever found a correlation between the hypersensitivity reaction and any reactions we have seen in these more complex blood systems.

Of course we also have to, and has been mentioned before, it is difficult, more difficult because we have to use fresh blood from healthy donors. So these cells have to be brought into a condition where sensitivity is increased. So we did priming of cells like with fMLP or with very low levels of anaphylatoxin. And just to get them primed but below the threshold of real activation, just to make them more sensitive. But we didn't find any positive reactions that would have helped us.

And it is always a question here, and as I will come to in a minute, what is the correct priming of a cell for hypersensitivity. I mean that has to be discussed also seriously if we talk about cell models and talk about animal models. And also we considered a microarray based systems where we look for specific antibodies

specificities and titers. If we knew or suspected them in our donors or donated in a distinct region, like food specialties or plants and so on, but we never found a really good correlation to that. Next slide, please.

Last not least, I come to animal models. I mean I have not mentioned them and we have not performed them because we did them many years and we also used here associated batches and have never found a positive result in these animal models. So you see, as mentioned before on the talks and the scientific talks, we used an intracutaneous mode; and a lymph node assay and to look for sensitization. In the bronchospastic animal model we never saw a positive reaction versus control.

So in conclusion, unfortunately none of these assays revealed results that help us to explain these episodes and these hypersensitive associated reactions and therefore for ethical reasons, also animal healthcare, we did not proceed in doing these experiments because we

never found a correlation with them. And the next slide, please.

So finally we did not really find the biochemical reason. but of course also the hint and the kind of positive reactions if we looked into the basophil activation assays - at least in one plasma sample which could be the reason. In the end I have to stress again, and it has been discussed excellently before, I mean we have only the key but we don't have the lock. I mean what we see is that we have batches that have mediated some hypersensitivity reactions, but a lock, meaning the patients, we don't know. So any predisposition we can just assume. And if you don't have such [patient] samples it will be hard to understand what the real reason is. And is there only a general reason? And are there more substances or predispositions that have to be there, or even a group of these? So we make a conclusion, but it has to be discussed seriously how to set up such an assay which would help us to learn more about the association with

hypersensitivity. And always taking into account individuals. The next slide, please.

So in summary, and on behalf of the three companies having presented here, and as a group of manufacturers we want to reiterate our shared goals to ensure patient safety and preserve the immunoglobulin product availability. Again, this is important because immunoglobulin products are used to treat a variety of conditions, including for patients who have immunodeficiency and

those who live with autoimmune diseases.

And as reviewed, hypersensitivity events are listed as common reactions for all immunoglobulin products. And today you have seen information from each manufacturer related to the recently identified events, which are rare, sporadic, and lot specific. We are all committed to work collaboratively to continue investigations in order to identify the root cause of these observations, share scientific knowledge, and find effective and efficient solutions applicable

across all manufacturers to minimize future occurrences, maintain patients' access, and ensure patient safety.

Thank you very much for your attention.

MS. NORTON: I believe I can start asking some questions now, Dot?

DR. SCOTT: Yes, thank you. And thank you to Octapharma for your presentation. You didn't have that many lots to work with and you were able to isolate still, you know, relatively low number of donors to be concerned about. So that was very good.

Okay, Margaret.

MS. NORTON: Okay. I'll start with some more common questions that I see a lot of. First one is are we going to get copies of the presentations or is there an email, can we provide an email for them to be sent or --

DR. SCOTT: Well after the workshop we're going to confirm with our presenters whether or not they would like to share their slides. And in my experience all or nearly all presenters are

willing to do that. And then we consolidate the slides and then we post those slides, or sometimes the Plasma Protein Therapeutic Association posts the slides. But at any rate we can let the audience and the presenters know when that will be online, and where.

MS. WARREN-HENDERSON: Dot.

DR. SCOTT: Yes.

MS. WARREN-HENDERSON: The recording of the workshop itself will include the slide presentations, and that will be available on our website shortly after the event. But the recording includes the slide presentations.

DR. SCOTT: Excellent.

MS. NORTON: The other common question is for Grifols and CSL. Could it be possible that Donor 18 is the same as Donor Z?

DR. ROTH: Maybe I'll take that question. So what we do know is that these donors have very different geographical centers that they donated to. And I think the biochemical markers between these two donors are very different. They

have a very different biochemical signature to them, so we don't believe that they are the same donor, no.

MS. NORTON: Okay.

DR. VANDEBERG: And we agree I think, right?

MS. NORTON: Okay, thank you.

DR. ROTH: Yes.

MS. NORTON: Also were the donors added to the PPTA Donor Deferral Registry?

DR. ROTH: I don't know whether, who wants to answer that question. You know, I can say that the donor, the National Donor Deferral Registry has very specific criteria that has to be met in order to add the donor's name to it. And unfortunately these criteria don't meet them. So we are actively working on a solution with other manufacturers in PPTA on how this donor information can be shared in the future.

MS. NORTON: This is also related, how can we ensure Patient Z does not affect other products, or the other patient as well? Would it

be possible that he went to donate to another company?

DR. ROTH: What I can do is I can provide some reassurance at this point in time that the donation center that Donor Zed donated into is fairly geographically isolated from other donor centers at this time. It doesn't mean that Donor Zed hasn't moved, but that's reassurance at this time.

MS. NORTON: Okay. Thank you.

MR. ZERVOS: From Grifols, this is Clark. We have indication that the donor, at least the Donor 18 in our case, had discontinued donating altogether. That's not a complete assurance and, you know, we still feel there has to be some kind of, maybe something similar to the NDDR if not that, to ensure this moving forward. Thanks.

DR. SCOTT: I just wanted to add one thing to the previous question. I presume that you have access to the donor birthdates and the donor ages and other epidemiological information

that really confirms these two donors, the Grifols donor and the CSL donor, are different people; is that correct?

DR. ROTH: So we do have that information obviously. I'm just not actually sure we've actually at this point in time shared that information.

DR. SCOTT: Okay. Can I share it with you?

DR. ROTH: We would like that, yes.

DR. SCOTT: I believe they are different donors.

DR. VANDEBERG: I believe we shared enough, Nathan, that we know it's different donors.

DR. ROTH: On this cross testing they have very different biochemical signatures to them.

DR. NORTON: Okay. The next questions will be mainly for CSL. So actually this also part of my question as well. Your purification method for IgG, my question would be, would it

include the free light chains as well, would those co-purify?

DR. ROTH: So we've discussed this internally, it's highly unlikely light chains will end up in final product. We haven't actually tested that specifically yet, but the manufacturing process is such that it would be unlikely.

MS. NORTON: And the other question is, would your depletion of IgE allow IgG complexes?

DR. ROTH: Yeah. So again, very likely that the column captures specifically of IgG or of IgE would unlikely remove the IG, would very likely not capture the IgG complexes, yes.

MS. NORTON: Okay. The next question is, did Donor Z have a history of COVID-19? If so, had Donor Z donated COVID-19 convalescent plasma and any info on that infusion?

DR. ROTH: As I had mentioned in the presentation that we don't know a lot about Donor Z's history, medical history. His first donations occurred sometime in June of 2020. But we don't

know anything about his COVID history at that time.

MS. NORTON: Okay. Thank you. The next question is, Dr. Roth, congratulations to you and the CSF Grifols team on this intricate study. Would you expect lower incidents of adverse events in IVIG manufactured from recovered plasma which has a lower proportion of repeat donation in each batch than product from Source Plasma which can contain multiple donations from the same donor in a single IVIG lot?

DR. ROTH: So I think the question's really asking is the, would you expect the incidents from source donations be tested larger donations than recovered donations. Would you expect more hypersensitivity events from that. I think the answer's quite complex to answer from that because not necessarily due to the pooling and then the splitting of intermediates that induct into a final product. It's not really just dependent upon whether or not it's recovered plasma or source plasma. And a donor may not be

represented multiple times in a lot.

So what I can say is donors that in some of the lots that caused hypersensitivity, these elevated hypersensitivity signals, he donated or was represented by about a half of a donation, which is equivalent to what a recovered donor would have given. So maybe not.

MS. NORTON: Okay. Thank you. The next one is, thank you very much, Nathan, for this interesting talk. Please, how long did the investigation take?

DR. ROTH: So we have two aspects of the investigation here. So one was the investigation which was the data driven investigation where we went from four lots to eight lots. And at that point in time that only took about two and a half weeks to do that, going from four lots to eight lots and identifying that individual donor as a root cause.

And then the biochemical investigation has been going on for just a little bit more than one year. So about one year's worth of

biochemical investigation to link Donor Zed specifically to the manufacturing, to the plasma pool entry as well as the final container lots.

MS. NORTON: Okay. Thank you. The next question is to the FDA. The question of HSR, could this be just due to a better pharmacovigilance reporting post-COVID?

DR. ALIMCHANDANI: Hi, this is Meghna. So, you know, I think one thing to keep in mind is that we've seen these spikes and hypersensitive reactions pre-COVID as well. As you saw from the data for the Grifols lots, there were eight lots withdrawn that had happened in 2018 and '19 that is all, you know, pre-COVID. So I am not sure that, you know, COVID has given due increased pharmacovigilance and therefore we have seen lot withdrawals. I don't think that's the case.

MS. NORTON: Okay. Thank you. The next questions I believe are for Grifols. So the first one is, the other 17 donors confirmed also contribute or not known to the hypersensitivity? I guess this is it confirmed whether they

contributed to hypersensitivity.

DR. VANDEBERG: So all 18 donors were common in the hypersensitivity batches, but they had no other markers for, the other 17 did not have any markers for hypersensitivity.

DR. SCOTT: This is Dot. I just have a question about the assays. Have you or Grifols considered pre- activating your human basophils with IL-3 or something else to find out whether or not that increases your sensitivity? That might also be done if there were mast cell lines perhaps?

DR. VANDEBERG: Yeah, we have tried some things along those lines. The problem with that is you also amplify your background, right.

DR. SCOTT: Yeah.

DR. VANDEBERG: And make them more difficult to work with. And so we have not seen any kind of consistent responses.

DR. SCOTT: Okay.

MR. MacGLASHAN: This is Don MacGlashan, I can also support Peter's comment. The problem

is you're testing with plasma, sometimes very high concentration of plasma or serum. And our experience is that when you use enhancing agents you make things really noisy. And in fact if you do an IL-3 overnight with basophils, every plasma we've ever tested, doesn't matter what its intrinsic ability to induce release, they all cause release. So for whatever reason IL-3 would be off the table.

The one that I have suggested to others is Deuterium oxide, which is a really very good way of enhancing any kind of response from the basophil. Like IL-3, it has, there are issues that come with that, which is greater responsivity. However, I don't know, I've never tested it in the context of plasma. We just know that plasma itself, even in fairly reasonable dilutions, will cause an overnight IL-3 treated basophil to respond, and we don't know the reasons for that. We do know that it's not IG needed. We know if you put in inhibitors of the IG or IgE signaling pathway that does not block that

response. So whatever it is, it's just something else that's driving the cell response. So that gets to be obviously an issue. So.

DR. SCOTT: Okay. Thanks. That's entirely plausible. We've seen that in other situations.

MS. NORTON: Okay. The next question is, Dr. Vandeberg, congratulations also to your team. Do your data suggest that Donor 18 experienced some type of event around 2018 which changed the composition of their donation? If so, would further investigation of the donor's history be informative to root cause analysis and/or advise donor deferral rules?

DR. VANDEBERG: Yeah, looking at the time course data it really does look like that donor had some event that then caused the appearance of an autoantibody. And then maybe was re-exposed to something or re-boosted over time. We reached out to try to reach this donor for more information but have been unable to get in contact with him.

MS. NORTON: Thank you. The next question is, do IVIG provide passive immunity against COVID-19, especially when the plasma donor receives the COVID vaccine? Was there a correlation identified with the hypersensitivity reaction?

DR. ROTH: So I can answer this question maybe more generally. You know we've continued to manufacture the IVIG products through the course of the pandemic as well as creating a hyperimmune. So early on in our investigation one of the very first things we asked was well was this COVID related. And we did do a COVID-related investigation. At the time I couldn't talk about it today. But we found absolutely no correlation at all for these lots versus regular lots versus our COVID hyperimmune lots. So we think it's highly unlikely.

DR. VANDEBERG: And I'll just comment from Grifols that, you know, our events with our Donor 18 started, pre- dated COVID, and I believe most of it also pre-dated the vaccine. So there

does not seem to be a relationship there.

DR. SCOTT: Yeah.

DR. VANDEBERG: As for passive immunity, I think, you know, the good thing for our immune deficient patients is you do get antibodies from people who are convalesced and have been vaccinated. That's part of the general population and that's one of the benefits of our IVIG products.

MS. NORTON: Thank you. Have cells from patients having an adverse reaction been used in any assays?

DR. ROTH: So this actually raises a really good question. So far all of our efforts have been related to looking at Donor Zed and what was his unique contribution. But we also recognize that it's really only a subset of patients who are actually reacting to that. So as we move forward in our investigation this is one of the avenues that CSL is quite interested in exploring and trying to understand that aspect. What is it about the patients that cause only a

subset of patients to react to these specific lots.

As such we're looking at potential clinical trial moving forward, but we're in the early phase of this evaluation. If we do something it needs to be meaningful, have some sort of question result that we would be able to answer on it, like if we have some hypothesis that we're starting to put together. And of course we'll need to work with the FDA to initiate this if that's the next step we proceed to.

MS. NORTON: Thank you. To all the presenters, great work, thank you. What did you tell the donors as the reason for deferral?

DR. ROTH: Well again I can tell you from our experience we didn't actually have to tell the donor anything because early on, once we identified him, we had him come back in a couple of times for donations, which were not slated for use in manufacturing. That was in order to biochemically link him to what we had observed, and he hasn't returned since. So now that we have

him in our system as permanently deferred, we haven't had that conversation with him at this point in time.

DR. WEINBERGER: Maybe I can also add some comment on that. As said in our presentation we have deferred those donors for manufacture of medicine and products.

DR. NORTON: Thank you. To Dr. Roth, the donor contributing to HSR was greater than sixty years old, at which age did he start to donate?

DR. ROTH: At around that same range - he only donated within the period of one year, so he was a relatively new donor to our system. He entered in, donated those 17 or 18 donations, I can't remember what it was, over that short amount of timeframe, and then has since departed. So, 60 years.

MS. NORTON: This is one is, I apologize, I joined your call late, so if this was covered already please ignore. Have the plasma fractionators traced this issue to certain

collection devices or certain lots of collection device disposal kits? So I guess no.

MR. ZERVOS: So in the case of Grifols, no, we did look that as part of identifying initially the one donor center that happened that all these donors were at. But no, there was no connection to any of the consumables used in collection of the plasma. So not for us anyway.

DR. WEINBERGER: I can only add for the Octapharma site we had more or less the same experience as said right now. But several years ago we investigated all those disposables and could also not find any relation.

MS. NORTON: Thank you. Do you have information about allergenic blood donations performed by either of the identified at-risk donors?

MR. ZERVOS: I can. So for this current investigation we've been discussing we do not. However, if you recall I think on Dr. Scott's introductory slides there was some, a product called IVIGnex which is distributed exclusively in

Canada, and this was probably 2014. In that case there was a donor plasma suspected to be the cause of that issue on a much smaller scale than what we're talking about now.

But one way that was identified was that this was recovered plasma and so the other blood components that came off of those units by that donor, such as platelets and fresh frozen plasma, did cause rather significant allergic type events in those recipients. So there was a definite correlation in that investigation.

MS. NORTON: Thank you. The next question is, should donors be tested for gammopathy and/or autoantibodies?

DR. VANDEBERG: So I'll take a start at that. And just to say that all donors get an electrophoretic profile of their serum to show that it has normal profile. A gammopathy would be identified there. As for autoantibodies, that's a very broad area. Autoantibodies are ubiquitous, everyone has autoantibodies in them and there might be some specific autoantibodies identified

that may be relevant to this, but just a in general, you know, we all have autoantibodies so it's not a general class that could be tested for.

MS. NORTON: Okay. The next question, I believe it was answered, but again, has Donor Z was lost for further investigation?

DR. ROTH: Donor Z has been lost from further donations. He has not come in for additional donations. We are exploring, I think as Grifols mentioned, we are also looking at potentially putting together a donor questionnaire and going out and seeing if we can find Donor Z at this time. It may be a difficult or challenge to do but we would like to see whether we could find him, explain to him the situation, and then see whether he'd be willing to answer questions about his medical history that could help further our investigation.

MS. NORTON: Okay. The next question is, are there any high throughput testing options that may be implemented by the companies that could possibly indicate individual plasma donor

samples that may promote hypersensitivity reactions if patients were to receive an IVIG batch manufactured using their donation prior to pooling the plasma donations? Would there be any high throughput testing?

DR. ROTH: So I think that's part of the goal of the workshop ultimately. Currently there's nothing that we're aware of. I mean if there was, and then the other big question is, you know, what is it that we would be testing for? We still need more understanding of the commonalities between the Donor 18 and a Donor Zed where we could make sure that whatever tests that we developed would be meaningful or suitable as a GMP assay, and as Dorothy, Dr. Scott has mentioned earlier, that it would be predictive as well. So we're not there at this point in time.

MS. NORTON: Thank you. What steps is Octapharma taking to ensure a root cause is identified? What types of investigations are still in progress?

DR. WEINBERGER: We are still trying to

contact the missing donors of the blood pool of 133. But this is a voluntary effort to convince those donors to provide plasma from them. And then it would go, based on this ranking, into further testing on basophil cells.

MS. NORTON: Thank you.

DR. ROEMISCH: That's the only hint we have at the moment. I mean as I summarized, I mean there is a hint that one donor would have elevated levels but the dilution would be so high that the only donor would not really be the reason for what we have seen. Maybe there are others who contribute as well, and the sum of all of this would make it more probable that the reason there is also this anti-FcRI receptor. But this is still ongoing, and we don't know, it's just speculative at this point.

MS. NORTON: Thank you. Does Grade 1 infusion related reaction, chills, mild fever, mild headache, etcetera, require rate reduction right away or would you wait until symptoms progress to Grade 2 as defined by CTCAE?

DR. SCOTT: That sounds like a clinical question obviously, and up to individual physicians. I mean if it were me I would slow it down. But I'm not a major treater with immunoglobulins. I've done it during my residency and fellowship, but I don't have the experience that some of the audience and some on the panel have with infusing immunoglobulins. So I mean we should really ask Dr. Finkelman and Dr. Cunningham-Rundles, among others, to see what they do.

DR. FINKELMAN: Well since I'm a rheumatologist I'm going to punt to Dr. Cunningham-Rundles and Dr. MacGlashan.

DR. MacGLASHAN: I cannot speak to that since I also do not administer IVIG or those products. I do have someone in my group that is an adult immunodeficiency expert and uses it as a tool for lots of his patients, but he's not here so I can't ask him.

DR. SCOTT: Is Dr. Cunningham-Rundles on the line?

DR. CUNNINGHAM-RUNDLES: Here, I just had to unmute. So to repeat that question, what do you do clinically when you have a reaction basically?

MS. NORTON: Yes. So does Grade 1 infusion rate related reactions require rate reduction right away or would you wait until symptoms progress to Grade 2?

DR. CUNNINGHAM-RUNDLES: The answer's very obvious, you're going to slow down the moment anything happens, otherwise you have a very nervous patient on your hands.

DR. SCOTT: A nervous physician maybe too.

DR. CUNNINGHAM-RUNDLES: Well you've got both ongoing and you don't want it to get any worse because if you can stop it then why would you wait around.

MS. NORTON: Thank you. Next question is, are donors who participate in clinical trials allowed to donate plasma. Example, initial clinical stages. If yes, are there any

restrictions such as type of exposure of specific class of investigational drug?

DR. WEINBERGER: Maybe I can only answer I'm not aware that this is a reason to defer a potential donor as long as he or she is healthy and complies with all the requirements.

MS. NORTON: There are a couple more questions actually about donor screening. Thank you all three companies for sharing your valuable investigation results. Have you used any of your learning from your investigation to revise your donor screening process?

DR. ROTH: So at this time we haven't revised anything in our donor screening process because there's nothing yet we know about Donor Zed which makes him stand out from our regular donors. So at this point there's no pragmatic or practical way to add additional screening measures.

MR. ZERVOS: Yeah, it's the same for Grifols. Certainly we did, as part of the investigation, a lot of additional donor

questionnaires at that one center and then a closer look, we don't have anything tangible to add to screening questions or donor screening at this point.

DR. SCOTT: I'm not sure we know enough to know what questions or question to ask.

MS. NORTON: Another related question is, do you do drug testing screening for donors before donations?

DR. SCOTT: Drug tests?

MS. NORTON: Yes, drug tests or screening. Or I guess drug screening.

DR. ROTH: So the questionnaire would specifically ask about drug usage for our donors.

DR. SCOTT: I think we're going to have to stop there and that puts us back at time for our short break. And thank you, these are extremely informative and fascinating presentations from all three of you. And it makes one feel that moving forward you are moving forward at a steady pace. And I think beyond that it shows what can be done when manufacturers weigh

in and investigate problems, it can be very fruitful. And I know it's a lot of work, but it is also I'm sure going to be helpful in the long run, and it already is, you know. At least there are ways to exclude suspect donors after collecting a relatively small number of lots for any given cluster. And so that's useful I think for everybody to see.

And then of course the investigations are something we will discuss at our next discussion period after the break. Get down to brass tacks and see what we can think of pragmatically and brainstorm a bit on where to go next.

So thank you very much, and I'll see you all in half an hour.

(Recess)

DR. SCOTT: I think we just about have a quorum. One moment, I'm getting a little feedback. All right, we're assembled here with our speakers and some extra experts as well. We have three questions to discuss. There may be

other important questions, which I suggest people can propose either in the chatroom or if you are a speaker, pose them while you have a chance to speak. So, this is where we're going to talk about some of the main challenges that we have and where to go in the future.

So, first of all, the first question is, what are the likely biological mechanisms or mechanism for lot-specific clusters of immediate hypersensitivity reactions caused by specific lots of immune globulin? And I think, you know, we've heard a lot this afternoon about the very plausible etiology based on the research data that we have so far and that's been reported at this workshop so far. So, I would like to know people's opinions of that and where to go next. So, I'll call on Fred. Dr. Finkelman.

DR. FINKELMAN: Thank you, Scott, Dot. Thank you. Thank you, Dr. Scott. So, these presentations by the three companies were really very revealing. And I think they all go towards the idea that there's an IgG antibody against a

determinant that most likely is involved with FcRI signaling. So, either on basophils, or mast cells, or both.

The surprising thing is that it doesn't seem to be an antibody to FcRI alpha chain, which is the chain that's most exposed and which is the chain that binds IgE. And the data from Grifols suggests the possibility that maybe it's the beta chain of the FcRI that's involved. I understand that it's going out on a limb to say that. But it's an interesting possibility at least in part because the beta chain is the most polymorphic of the chains of the high affinity IgE receptor FcRI.

So, if it were the beta chain, that would have some very interesting implications. First, because it's polymorphic and as far as we know, neither Donor 18 nor Donor Z has had allergic symptoms that would be associated with an antibody to their own FcRI. Maybe they were somehow alloimmunized like a blood transfusion, for example, and made an antibody against this.

And it only recognizes the beta chains of the right polymorphic variant sufficiently --

DR. SCOTT: Yeah.

DR. FINKELMAN: -- to cause a clinical reaction at the dilution that's present in IVIG. If that was the case, then it would really be useful, as I think one of the questioners already suggested, to be able to test their serum against basophils from the recipients that developed the severe reactions to see if there's a -- if you dilute the serum more and still get some evidence of basophil activation or degranulation in those cases.

I know also that there's not necessarily reason to believe that there's only one mechanism involved.

DR. SCOTT: Yeah.

DR. FINKELMAN: It could well be that you could have antibodies against several different determinants that could be involved in activation of mast cells, or basophils, or macrophages. Or even through some other mechanism

that we can't readily think about. But this seems to be the strongest lead that we have. And I can't thank the speakers from the three companies enough for the wonderful and difficult investigations that they've done that point us in this direction.

DR. SCOTT: Thank you, Fred. I think that the alpha chain might also be a potential target. We found a paper from 2005 that suggests there's an epitope on the alpha chain at least that is capable of having this kind of activity. And Ms. Eller might be able to find that reference pretty quickly. And if she does, she can post it.

DR. FINKELMAN: Well, we've done a long of work with injecting mice with antibodies against the alpha chain --

DR. SCOTT: Mm-hmm.

DR. FINKELMAN: -- and it's brilliant at inducing anaphylaxis. But my understanding was that both Peter and Nathan had mentioned that the titers against the alpha chain didn't seem to be particularly strong or there was something else

that kind of deemphasized the alpha chain as the --

mechanism. --

DR. MACGLASHAN: Can I -- this is Don. Fred, I actually took that data a little differently. You know, they used a soluble alpha to inhibit and got some inhibition. But it's not the full alpha. In other words, the piece that goes into the insertion necessarily. I don't know what the construct looked like. But there is a, you know, a soluble alpha may not be the best way to inhibit. It might be better just to pull it out with an alpha column or something.

But I think there still is that short section and there was 50 percent inhibition. So, I think it's -- I think it may be that it's a little of both for the two different donors. It is really intriguing.

And I have to say I agree with Fred, this is -- when I first stepped into this arena a few years ago with Grifols, it was a complete black box.

DR. MACGLASHAN: And my impression today based on the data is you've got a pretty good warning flag about the possibility of an antibody of really high titer, high affinity antiFcRI regardless of which subunit it may be. The data just looks pretty strong.

And what also really strikes me is just how unusual it is. You know, it's not that frequent and it goes away with time in all cases. So, that, you know, to have that happen is, having studied a lot of auto -- the presence of auto alpha and IgE in CSU [chronic spontaneous urticaria]

patients, you know, the titer of this is much, much better than we've ever seen. But there are some really good auto alpha subunit antibodies in chronic urticaria. Fred has asked a really good question earlier whether it would be worth asking any given donor whether they have ever been diagnosed with chronic urticaria.

One of the things that really pops out when you study a timeline of a given CSU patient's

serum for autoantibody functional activity, is it's up and down and up and down. And it's just really unclear what stabilizes its presence or makes it go away. And it can happen in months.

So, it's not totally inconsistent with something like this. It would be worth asking. My guess if they had really significant chronic spontaneous urticaria, they might not be popping in to give a donation. I think Fred raises a really good question to ask them just for screening purposes.

The other thing that struck me is besides the fact that it's still quite remarkable that with the extent of dilution that you get with all the plasmas being pooled that it could still work is the question that sort of arose during the Octagam discussion, which is that there's variable inclusion of a given donor in a particular lot of the IVIG. And it just sort of struck me at the moment that it was said that it almost leads to a potential way out if these things are relative unicorns, don't happen very often, is that you

never include a given donor in a lot more than one donation so that you can't get much, much higher titers of whatever it is it got.

I have no clue how practical that suggestion is. But it's certainly in the data from CSL, you know, you got more or less activity as a function of how much of that donor's plasma represented the total immunoglobulin. So, it certainly suggests that as a practical -- maybe not so practical -- but as a thing that could be done almost without doing anything else is just as a policy kind of thing.

But anyway, I'm really struck by the advancement. Real kudos to all the teams for chasing from a black box state just five or six years ago to something that to me was fairly striking. So, yeah.

DR. FINKELMAN: That's great, Don. As in vivoist, I have to mention that there are mice with human FcRI alpha chain as Laurent mentioned. And so, you can make mice extremely susceptible to anaphylaxis by treating them with a drug and a

cytokine.

DR. MACGLASHAN: Right.

DR. FINKELMAN: So, it would be really interesting to see whether a high dilution of the suspect plasma would induce anaphylaxis in those mice. Laurent, are there any mice that have the human beta chain?

DR. REBER: Not that I know. No. What I would suggest also is because all of the in vitro assays that you use, the basophils and the Hoxb8 mast cells, they all have a very high level of the inhibitory FcRIIB. And this will lower the sensitivity of your assay a lot because those are all IgG?Z? (phonetic). So, I would suggest that you use FcRII blockers. So, antibodies against --

DR. REBER: -- try using antibodies against FcRII before you test the serum because that might really increase the sensitivity of the degranulation.

DR. MACGLASHAN: Yeah, that's a good idea. I know it works for some of the monoclonals

that if you -- that because of the nature of that particular interaction, it is the case that you've got sort of a captured species on the cell membrane and any inhibitory receptor's going to grab that very readily. And so, certain monoclonals that we use for stimulating in vitro are in better -- you get a better response by including a blocker FcRIIB. So, I think it's a great idea.

DR. REBER: And in human mast cells in the skin there is no IIB expression. So, they will respond much more --

DR. MACGLASHAN: Yeah.

DR. REBER: -- than what we see with basophils, for example.

DR. SCOTT: Mm-hmm. Charlotte, did you have a comment?

DR. CUNNINGHAM-RUNDLES: Well, I was here wondering how much we're sure it's got anything to do with the gamma globulin, the IgG molecule and Fc receptor, and whether pepsin or papain had been used. That would be -- or

anything like protein A just to say is it really an IgG molecule that's the issue. And is it binding to Fc receptors?

I mean, I put a comment in because we're talking about immediate reactions which are the most common. But we have patients who have reactions in two days, extreme migraine, and other things where they have to go to the emergency room. And we haven't gotten into delayed reactions whatsoever. I think we're not collecting data on those to any extent either partly because the patient has gone home. But they are rather common as well.

DR. SCOTT: That could probably be the subject of another workshop.

DR. CUNNINGHAM-RUNDLES: I think so. I think it's much more elusive though. I mean, this one at least --

DR. SCOTT: Right.

DR. CUNNINGHAM-RUNDLES: -- you have a handle on it.

DR. SCOTT: Mm-hmm.

DR. CUNNINGHAM-RUNDLES: Yeah.

DR. SCOTT: Because of the work that's been done recently.

DR. CUNNINGHAM-RUNDLES: We have really much more ability to get the data and to see what these amazing lots, you know, are capable of doing and narrowing it down into who's contributed. You know, we do have a lot more data to look at this way. And I think it's really pretty stunning, actually, the work that's been done.

DR. SCOTT: Additional comments from our panelists on this work?

DR. ROTH: Maybe I'll just add in another piece of data that, you know, we weren't able to present all our data today due to time. But we also did try the same human protein micro array that Grifols had done. And unlike Grifols, we did not get the same hits against the beta chain.

DR. SCOTT: Okay.

DR. ROTH: So, just know that we have that comparator.

DR. MACGLASHAN: I guess the question is did you get hits on the alpha?

DR. ROTH: We didn't.

DR. MACGLASHAN: Okay.

DR. SCOTT: Okay. All right. Okay.

DR. MACGLASHAN: But I do have to ask, you know, the preparation of the alpha subunit for a chip or for a soluble inhibition assay is maybe quite important because not all the constructs have enough of the external peptides to represent the whole thing, so.

DR. VANDEBERG: And I'll just comment. You know, having the hit on the beta chain, you know, that's something I'd like to go in and see some confirmation, some specificity experiments that confirm that. It's a strong lead certainly but at this point, I think it's a lead.

But just getting back to the alpha chain, we know that there are lots of people that have antibodies to the alpha chain. And in our testing, we looked at it in the product and do see a low-level response. So, could it be specific

parts of the alpha chains, specific epitopes on the alpha chain and we're just missing it because we're looking at the whole thing?

DR. ROTH: Yeah, Pete, it's the same, you know, with us. We know that binding to alpha chain is not the same as eliciting activation, so.

DR. MACGLASHAN: Yeah, I mean, it's worth noting that many years ago, a number of monoclonal antibodies were made to the IgE alpha [chain]

and, you know, they're not all activating. And I think Ruben Zarganian (phonetic) may have done a lot of this where, you know, it just, it's critical what the stereochemistry is. It depends on where the epitope is and whether you can actually create a crosslink. And then there's the strength of that crosslink. And there are just a lot of other variables that go into whether in a monoclonal state or a polyclonal state, you can actually properly crosslink the receptor. Let's put it that way.

So, it doesn't serve to focus too heavily on whether or not it binds. The best assays are kind of the ones that have -- are starting to be suggested. Fred's idea or Laurent's idea of maybe using basophils with a blocker. But when we did our recent chronic spontaneous urticaria study, we already knew that there was what Peter was saying. There are people that have apparent ability to bind to alpha or E for that matter and yet they don't seem functional.

Admittedly, we have sensitivity issues as well when we do those assays. But it is the case that we decided we would look for only those that clearly could be easily demonstrated to have functionality. And, you know, they're quite heterogeneous so, the functionality goes anywhere from you need practically need serum to induce a response, to I think probably the best we ever saw was something like one to maybe 250 or something, our best chronic urticaria patient.

So, a lot of heterogeneity there so, it

wouldn't surprise me that there is a certain amount of heterogeneity. Then you do have the donor side response as well. And that's a whole other story as to the heterogeneity that exists. So, even if it was as simple as an anti-alpha antibody, the heterogeneity in both mast cells and basophils to the number of crosslinks needed to initiate a decent response is quite large as well. And so, you may not even have to invoke too many other recipient biologies to still considerable heterogeneity in response to a pretty high titer of a very high affinity antibody in some particular lot.

Certainly, all the other factors that go into whether a person responds and which organs respond is a whole huge issue. So, but you don't have to go too far to at least find a fair amount of heterogeneity at the cells, the two cells themselves that bear this receptor. So, anyway, yeah, it's a -- there's a lot of possibility here still without digging too deeply, so.

DR. SCOTT: That may lead into our next

question, as a matter of fact. What are possible in vitro methods that could be used or should be tried that would identify plasma units or product lots that could cause excessive numbers of immediate hypersensitivity reactions? So, now we're talking about in vitro methods and what would you wish to do, and I hope it doesn't happen, but I think it's going to happen again because we have these almost every year from one product or another. What would you start out with next time and what do you wish you had known this time? But first, let's just talk about in vitro methods that could be used or should be tried to identify plasma units or product lots with this problem, or plasma pools.

So, I think, obviously, we've talked about mast cells. We don't have any data right now on mast cell lines and whether those can be manipulated to be particularly sensitive or more sensitive than they are. But perhaps development or testing of the various mast cell lines that exist might be useful to have a more, well, easier

test that still is sensitive.

DR. MACGLASHAN: Well, this is Don. I would weigh in on two. I think there is some room to workout the basophil sensitivity issue. Just grabbing donors at random is not necessarily the best. Whether you could develop the basophil assay into standardized -- an assay that was sufficiently standardized that would satisfy the regulatory agencies for, you know, a consistent test, that still would have to be worked out.

But even just to know whether or not on a research level you could crank up the sensitivity of that. Because we know there are ways. The plasma story makes things a little more complex. But I like the notion of, you know, blocking the FcRII and I like -- we are in the process of exploring how radically we can change sensitivity with deuterium oxide, which is a potential approach.

And then I like Fred's idea. I mean, Fred, you've been able to kill mice really effectively. You put the right drugs in with a

small, with a very small amount of stimulus. So, it may be a pretty sensitive way for lots, which is a purified product and probably relatively easily administered for a reaction. So, I like that idea too.

So, I think you could tweak that into something to know its boundaries and how well it could work. And I think you could tweak the basophil assay perhaps into at least knowing whether it ultimately would be useful at all. So, that's my thoughts on it, so.

DR. FINKELMAN: Yeah, I'm thinking along the same directions as Don. It might be nice to have a screening test that's simpler than that than either of those to start out with and then go further with the more definitive tests that are the more definitive functional tests.

When you think of a screening test, you're thinking -- I think of an ELISA. And just seeing does the lot or does a particular plasma sample have a high titer of antibody to a particular chain of FcRI or FcRII, or any of the

other suspect receptors. I don't know and I'd be interested in hearing from Peter and Nathan what they think about how sensitive you should want to make that assay. Would you look with a low dilution or a high dilution of plasma or a lot?

DR. ROTH: So, let's talk about the different stages, areas where one could test, right. So, there's the ability to test on a plasma donation level or small pools of plasma donations. This is what we do right now for viral marker testing, you know, pools of about 100. You can test at the plasma pool level, which is about a dilution of 1:5,000 to 1:10,000. Or you can test at the final product, which can go from 1:10,000 up to, you know, 1:50,000 or even more.

As you move out towards final product, you have -- your assay has to be much more sensitive, right? And we saw here that CSL has been the only manufacturer that's been able to biochemically link the final product to the hypersensitivity index. The dilution's just so high, right, on this. I think the same is true at

the plasma pool level. Even at a 1:5,000, 1:10,000 dilution, I think and I'd like the other manufacturers to confirm that, I think we're the only ones that with our donor we're able to get a signal on the plasma pool that was positive.

So, that really puts you back and even if you're testing at those levels, you're losing a lot of other perfectly good donations in that pool, which you wouldn't want to put at risk, right? What we want to try to do is identify that specific donor's donations. So, the big preference would be to test at the donation level and one could do that, you know, either individual donations or up to, you know, pools of 100 or something like this so that you had some level of sensitivity.

But there we're looking at millions of donations so, that test has to be highly specific, highly sensitive, and open to automation. So, it's quite a challenge, I mean, we know what the user requirements would be for that test. And, you know, you can see now we're going from primary

cell lines essentially to what could we develop or what is out there or who is innovating in this space that we could get something that we would be able to then make into a test that would be -- fit all the requirements for GMP testing. And that would be suitable to identify these donors.

And then the last thing is, you know, is our donor Z, does he have something common you could test for versus Donor 18? And we're not quite there yet even in that understanding. What exactly is the molecular entity you would be actually screening for or just looking for a hypersensitivity or a test that indicates that you can get an activation out of it, so.

And I ask Pete and Juergen if you guys can just kick in for a moment on the pools and the final product.

DR. VANDEBERG: I think what you said on the, you know, the dilutions is pretty accurate. Just to, you know, talk a little bit more about that. You know, any such test has to be very reliable whether you're testing pools, or product,

or back to donations, or pools of individual donors. It's got to be very reliable. You've got to understand for sure that it is testing what we think it's testing.

And we're not there yet. I think we've got some good ideas. Going back to the donor level or I think even pool level, if we can identify a specific epitope on FcRI alpha or beta to look at, then perhaps we can come up with an ELISA that works. But, oh, it's going to be so hard to know that that is really addressing our problem.

DR. ROEMISCH: Yeah, I can comment as well. I mean, of course, a screening assay has to be on a kind of an ELISA or it has to be done on a PCR assay. It has to be a high throughput assay. And until you are there to have high throughput assays, you have to go through functional assays and in vitro -- and cell cultures or animal models, and so on, so that you are not at the end of the learning curve.

And as I showed, I mean, for our

results, is that there is a hint that this could be the same reason as presented by Nathan and Pete. But it is not as clear at all whether this is really the one. And I would like to say that here chasing one wolf outside, maybe there are 20. And so --

DR. SCOTT: Yeah.

DR. ROEMISCH: -- yeah, that's -- we don't know. I mean, maybe as I said, maybe it's a combination of two. Or there is a cofactor needed to that.

So, I think before we start setting up screening assays -- this is my opinion -- on one distinct epitope, there has to be more information about that. And it is also about, like Nathan said, it is not about just R&D assays. I mean, these assays have to be validated and they have to be set up properly. And the failure rate has to be extremely low. So, there's quite a way to go until we have such an assay available for that. But, of course, the results are encouraging and more investigations are really needed for that.

DR. REBER: If I can add on the assay. To me, it's clear that the basophil cell lines would

know would be a way to go because there will always be too much variability between donors. The way to go is the cell line for sure.

And maybe also the readouts that you are using because you are looking at degranulation, but if I remember the first -- the paper that I added in my presentation, the first one that shows actually that anti-IgE purified from an IVIG preparation could induce activation, they actually focused on cytokine release. I think it was IL-8 release, which is much more sensitive because sometimes you don't trigger degranulation but you still trigger mediator release. So, maybe that's also a way maybe to look for increased sensitivity. And maybe not chase for degranulation but chase for something that is really is a more sensitive activation of the receptor.

And if it's cytokine release, then we

will see per the analyzer you can really screen then. The pure cell line analyzer to screen, it becomes easier to standardize.

DR. SCOTT: Any other particular comments on this question? It's very good, very thoughtful. Then we have our third question. How can we minimize the number of patients affected by immediate hypersensitivity-inducing product lots? So, if it happens again, is there a way that we could have a shorter period of not knowing who or what set of donors might be implicated? Is there a speedier way to protect our patients than what's already been done?

But I think the broader question is how to minimize the affected number of patients. And some of that has to do with pharmacovigilance reporting. And I think, you know, pharmacovigilance can't be very strong -- as strong as we would like with our adverse event reporting system when we're looking for this type of event. Is Dr. Alimchandani still here?

DR. ALIMCHANDANI: Hi, Dot. Yeah, I

have to drop off soon though but I'm here for five more minutes.

DR. SCOTT: Okay. Five more minutes --

DR. ALIMCHANDANI: Thanks.

DR. SCOTT: -- is good.

DR. ALIMCHANDANI: Sorry.

DR. SCOTT: What ways can you think of to improve our reaction time to these lot-specific hypersensitivity case clusters?

DR. ALIMCHANDANI: Yeah, so, I think one of the things as you know that we've been working with the sponsors is to come up with some kind of threshold that would lead them at least to start an investigation and also to notify FDA. And the reason for this is because hypersensitivity is labeled and a majority of these are nonserious events. The companies don't have to submit them to us as 15-day reports. When we do the enhanced pharmacovigilance, we usually request it for a period of

time. We don't keep doing it for the lifetime, you know, of the product.

So, the only way I think that we can avoid that lag time is if there is an internal, you know, even when they are doing their internal evaluations, you know, there are many factors that go into a voluntary lot withdrawal for sure. Even if it's not at that step, if they could notify the FDA or and they would have to recalibrate the threshold as more data becomes available because, you know, the number of reports, the postmarking experience is always changing and evolving. And I don't know if the sponsors have any comments or any thoughts about that.

DR. TOTH: Yeah, we from Octapharma, we already use, as you might have known, such a formula to inform FDA on the alternatives regarding such lots. And as you mentioned, we try to always go back and rethink the formula, refine it a little bit, and so far as you could see, we informed FDA with quite low numbers. So, at an early stage so we were quite transparent and we would like to keep this way.

DR. ALIMCHANDANI: Yes, thank you.

DR. TOTH: Thank you, sure.

DR. ALIMCHANDANI: Yes.

DR. SCOTT: Another thing we've noticed is that even after a product withdrawal, and in spite of the fact that usually the distributors are tasked with notifying the end users, the way the system is set up either this doesn't happen or it happens, but the end users miss it or don't pay attention. And we see patients treated, you know, I've seen them treated with a lot that's been withdrawn up to several weeks after the withdrawal. I see that as a problem.

And I don't think that we expect to solve that kind of problem here. But I do wonder if Dr. Cunningham-Rundles also has some ideas about it, in general, how we could improve our reaction time.

DR. CUNNINGHAM-RUNDLES: Oh, gosh, I wish we could. I think that it's not entirely easy about the recording or the reporting, which is what you've already said. And so, if there is one in Boston, how is anyone in New York going to

know about it? And I find that very daunting to even wonder how that could happen.

So, first thing is the reporting seems to me. If everyone had an easy to find website to say this particular lot was one that was a problem, but it does not exist.

DR. ALIMCHANDANI: I was going to try to build on that. So, we on the communication aspect, Dr. Cunningham, so, you know, we've seen that it kind of varies from sponsor to sponsor how they share their information about lot withdrawals. I know for Grifols, they had all of the lot withdrawal letters actually very easily available for Gamunex on their website. But we couldn't always, you know, find those letters sort of publicly posted on the sponsor's website.

So, last year, we did a CBER safety communication, which had a whole listing of, you know, all the lot numbers, the expiration dates. And I think that was, you know, one way to consolidate the information and, you know, make it available publicly. But I would encourage, you

know, sponsors, as well to, you know, post the letters publicly or sort of have more communication.

DR. CUNNINGHAM-RUNDLES: I think that's too late, honestly. Because by the time a letter goes out, those lots have already been used.

DR. ALIMCHANDANI: Well, I think, you know, what we've seen is sometimes there are still, you know, many vials that are not expired and that are in circulation and the company is not able to, you know, get back all of those vials. So, this was, you know, our hope was that this was a way to encourage awareness that this is a withdrawn lot to not use it, you know, send it back or, you know, dispose of it, what have you.

DR. CUNNINGHAM-RUNDLES: I think with infusion centers such as ours though whenever anything like that would happen, the lot is already gone. And it's, you know, we get -- any letter I've ever seen was always usually something a month later. So, that's very good but a month later is too long.

DR. ALIMCHANDANI: Yeah, I think that goes back to having an earlier threshold and sort of picking it up even when there are few cases but you think there is an issue to take action as expeditiously as possible I think.

DR. CUNNINGHAM-RUNDLES: Yeah, I mean, I've looked at lots before when patients have told me that they had a reaction so I've looked them up. And it's, first of all, it's not easy to find. And I don't necessarily guarantee that our place has every time done it because the patient begins to itch when they go home. Are they going to call back the center and tell the center I had a problem this time? Not necessarily.

You know, if it was bad as it was for the case of, you know, Kara that we talked to this morning, you know the center didn't think --

DR. ALIMCHANDANI: Yeah.

DR. CUNNINGHAM-RUNDLES: -- that it was their job.

DR. ALIMCHANDANI: They didn't report, yeah. Mm- hmm.

DR. CUNNINGHAM-RUNDLES: No, they said it wasn't their job.

DR. ALIMCHANDANI: Mm-hmm.

DR. CUNNINGHAM-RUNDLES: She's being taken care of by Mass General. I have to tell you that.

DR. ALIMCHANDANI: Wow.

DR. CUNNINGHAM-RUNDLES: She's at a Mass General Infusion Center, you know, in another site, not at Mass General, but one they oversee.

DR. ALIMCHANDANI: Yeah, I don't have a solution for underreporting that we see and passive surveillance. You know, the MedWatch numbers are in the package inserts. It's on the FDA's website. And when we do post, I mean, again that's sort of after the fact, but when we do post information about the lot withdrawals, we also include information there about reporting to the FDA or the sponsor.

DR. SCOTT: Yes, just interestingly I would say the majority of our reports are from pharmacists. Would you agree or?

DR. ALIMCHANDANI: Yeah, yeah.

DR. SCOTT: Which is good. They seem to have a better handle on what to do and maybe it's part of their education in pharmacy school they do report at least I would say the majority of the data.

DR. ALIMCHANDANI: The majority, you are absolutely right.

DR. SCOTT: Yeah, mm-hmm, mm-hmm.

DR. ALIMCHANDANI: Yeah.

DR. SCOTT: Okay. Now, I want to ask the group, do you have any other questions you think we should discuss today? Or big questions?

DR. FINKELMAN: I have a question I'd like to ask Dr. Cunningham-Rundles. It was mentioned that pretreatment, prophylactic pretreatment with anti-H1 blocker and H2 blocker, perhaps with Solu-Medrol has a protective effect and decreases incidents of severe reactions. Do you use this type of pretreatment? Do you think it's justified or is the treatment worse than the disease?

DR. CUNNINGHAM-RUNDLES: Well, you can certainly use it. If the patient really needs the gamma globulin, you're going to end up doing that anyway. And you might do it for the first infusions. But our practice here is to give the first two or so infusions with half the dose that we intend to use in the long haul because that cuts down reactions hugely. So, that's the first thing is you never give the patient a loading dose, not ever, not under any circumstances in a hypogammaglobulinemic patient.

And then over time, they become accustomed to it. As Kara was saying, many, many days go by and many, many infusions go by. Four or five years go by, no reactions at all. And then all of a sudden you get one of these things. She's probably never going to go off the premed because she'll be too nervous to do it and so will the infusion center. But the great majority of our patients, and we have about 150 who come here for infusions, and another 100 or so at home, you know, do not get premeds. And it's not necessary

for most patients.

The other point, which we never went into at all is we're talking about this being something that's due to the infused product. But we published in JCI last year that in fact what you have is DNA in the blood of the patients from bacterial organisms from the GI tract. And IVIG does bind to that material. So, our original work a long, long time ago I admit, was looking at immune complexes in blood of patients that are getting gamma globulin. And most patients do develop immune complexes in their blood after getting gamma globulin. And one of the contents is in fact this bacterial DNA.

And so, in a way you have a situation of almost antigenemia. It's not a positive blood culture you understand. It's the ribosomal DNA that's coming from the GI tract through mucosal, you know, barrier defect. Most of our patients don't have any IgA so, that's part of their kind of leaky gut I would say. And it's a real thing and since IVIG binds to that, you'd expect a

certain amount of immune complexes to always appear. So, it doesn't have to be against an FC receptor of a particular point, it would just land there anyway because it is an immune complex, basically. And that's how it's going to ever be eliminated. And we didn't get into that particular side of things here.

DR. SCOTT: It's interesting. I would assume that to be less the case in people who were immunocompetent but receiving immune globulin for autoimmune diseases.

DR. CUNNINGHAM-RUNDLES: Well, it's a little harder to compare there though because for the autoimmune diseases, the doses tend to be quite a bit higher.

DR. SCOTT: Yes. Yes.

DR. CUNNINGHAM-RUNDLES: So, that's just a little bit different.

DR. SCOTT: It is.

DR. CUNNINGHAM-RUNDLES: Yeah. Anyway, to answer what Fred was saying, we normally don't use premeds on patients who have been treated over

a long period of time. It's generally not necessary. And in fact, I discourage it because you have a patient who, you know, is perhaps a little bit sedated and going home, starting their car on, you know, getting, you know, home on the highway and being sedated is just not a good idea. In fact, there have been lawsuits about that. And so, you know, you don't really want to sedate.

And in fact, the patient that talked about it this morning, she's pretty immune deficient. Do I love the idea of her getting Solu-Medrol once a month? Not particularly.

DR. FINKELMAN: Have there been any studies that look at the differential protective effect of the H1 or H2 blockers versus Solu-Medrol?

DR. CUNNINGHAM-RUNDLES: That has never been looked at in any methodical way is my bet. I mean, here at most infusion centers, I think they pull out both and just call it a day, you know. Just here's your cocktail.

DR. FINKELMAN: You know, I'm biased by

our mouse studies, but --

DR. CUNNINGHAM-RUNDLES: Yeah.

DR. FINKELMAN: -- the anti-H1 blocker has a huge protective effect.

DR. CUNNINGHAM-RUNDLES: Yeah.

DR. FINKELMAN: Whereas, even high doses of steroids have very little effect.

DR. CUNNINGHAM-RUNDLES: Yeah, I think it's --

DR. FINKELMAN: And H2 blocking also doesn't do very much.

DR. CUNNINGHAM-RUNDLES: You know, some kind of real study on that would actually be really interesting because you know as, you know, when you kind of just use your cocktail, you don't really know what was the good part of it, you know? You don't get your data that way.

DR. SCOTT: There are some questions from the audience here. I think we can address them. Margaret or Nancy are you still here?

MS. NORTON: Yes, I'm still here.

DR. SCOTT: Okay. I think --

MS. NORTON: Let's see, I want to see where it starts.

DR. SCOTT: -- Dr. Rossi has a question. I think there are just two or three and I'm trying to get Dr. Golding to be unmuted.

MS. NORTON: Okay. I'll start with Dr. Rossi. It seems that it would be very difficult to further investigate Patient C and Patient 18 as they are not in contact anymore. Okay, That's a comment. Sorry, follows previous question. Do you think Nathan, or Dr. Zervos, that if another cluster of hypersensitivity events occur, you would be able to retain the suspicious donor for deeper investigation to searching for a market that could be identified in donors?

DR. ROTH: Well, that's a great question. Look, we have learned a ton through this through our investigations. And I think absolutely, the next time if this ever unfortunately occurred to CSL, I think we have learned that and we would we definitely have a set of questions already in mind to be able to ask

such a question about their donor history, or their medical history, and so on. And I'm hoping that other manufacturers, you know, will learn from this and also be better prepared in the future if this were to happen.

I just want to also reiterate though that we are still formulating an approach that we hope to be able to reach out and see whether we can find patient 18 or Donor Zed again and see whether we can find out more information. I think it would be very useful to see if we can contact him and see whether he'd be willing to share medical history that could help advance our understanding.

MR. ZERVOS: Yeah, this is Clark. Yeah, on our end with these 18 donors, we were fortunately able, we had units collected that were already on quarantine. So, we were able to access plasma for testing. There's also retention samples of previous donations that are kept.

We did early on, even with larger sets of donors, looking at we began, you know,

interviewing them. Some medical professionals from our Biomat plasma collections were interviewing donors, you know, asking additional questions about any medications they were taking, any allergic-type history that they had. So, yeah, as our investigation progressed, it became, you know, something we learned was the ability -- needing the ability to be able to engage the donors, have samples available, and information. Thanks.

MS. NORTON: Thank you. This is not a question or suggestion for Dr. Scott's last question. The FDA should sponsor/provide a session on AEs reporting and withdrawals at the IGNS[sic] and NHIA[sic] meetings. Also, withdrawals

notices should be posted in IG Living[sic] and the IDF Facebook page. And their last question is what about the Patient Notification System? I'm not sure what that's pertaining to.

DR. SCOTT: Well, actually, we do ask

firms to notify the Patient Notification System. And since I signed up for patient notifications, I can tell you that I have a lot of those notifications from the various withdrawals over the past several years. So, it works for me. I'm not sure how well it works for patients. But I think to get down to that level is a very good idea and it ought to be useful even if not all of the patients, if you will, notice it.

The problem might be that not so many of them necessarily sign up. But I think Jorey Berry was on the phone a short time ago, or on the panel. She might be able to comment about the Patient Notification System. I guess she had to leave.

MS. BERRY: I am here. I was just unmuting for a moment. I apologize. We would be open to looking at sharing something on the IDF website. I mean, we have our database of patients but that's not going to be everyone. And where, you know, there are things that we can do on our website as well. But that's only for the people

that go to -- that would be aware of that. So, that is something that we would be open to exploring but it is also something that has its own limitations.

DR. GOLDING: I was unmuted. Can I go ahead, Dot?

DR. SCOTT: Yes, you can. This goes back to earlier questions about the alpha and beta chain of FcRI. So, go ahead.

DR. GOLDING: Thank you. So, well, first of all, I just wanted to say that, you know, this was an amazing workshop and Dot and her team should be congratulated. But I also want to thank the speakers of the morning session and the afternoon session were all done at a very high level. So, I think it was a wonderful workshop.

But I was intrigued by Dr. Finkelman talking about polymorphisms of the alpha and beta chains. So, I have some related questions that maybe he or somebody else could answer. And that is -- well, first of all in CSU [chronic spontaneous

urticaria), is there a correlation between the polymorphisms in either the alpha or the beta chains that are associated with that condition? And related to that, would it be helpful to get a cell line from one of those patients and would that, you know, to try and use as a cell line to screen these products?

And the other part of this is the donors. So, I understand that it's very difficult -- sorry, not the donors, the recipients -- I under it's very difficult to get a hold of the recipients. We've had multiple clusters and I didn't -- maybe I missed it -- but I didn't hear any information about the recipients and any characteristics that make them more susceptible. It's only a small percentage of the recipients that receive a particular batch that have these reactions and could it be because they have unique polymorphism that makes them more susceptible. So, I'll stop there. Dr. Finkelman, can you help with answers?

DR. FINKELMAN: Well, those are all the

questions I would love to see investigated. And as you pointed out, it's not so easy if it's difficult to bring back somebody and to -- I guess you have to have something set up for research in advance so that you can get a blood sample and preserve blood and certainly making a cell line would be very useful if there's somebody who has the resources to do it and preserve it.

DR. GOLDING: But is there a correlation between the polymorphisms in CSU? Has anybody studied that?

DR. FINKELMAN: I haven't seen that. I'm not an expert at this and beyond doing a couple of quick Google searches. And for people with CSU, of course, it's an autoantibody not an alloantibody. And when you're talking about polymorphisms, you're perhaps thinking about somebody without disease that has an antibody to an allotypic variant that they don't have but that might react with a recipient who then develops a reaction.

DR. GOLDING: So, do you know the mice

that were humanized mice that people mentioned, do you know what kind of Fce receptors they have in terms of being more --

DR. FINKELMAN: Yeah.

DR. GOLDING: -- sensitive?

DR. FINKELMAN: In general -- and Laurent can correct me if I'm wrong -- they just made with the human alpha chain, the one that my lab has used is really a knockout for the mouse FcRI alpha chain. And then has a transgene for the human FcRI alpha chain. That then pairs with the mouse beta and gamma chains.

DR. GOLDING: Mm-hmm.

DR. REBER: Absolutely, we have the same one and there are also some knock-ins that is just the alpha chain that is knocked in. And this I wanted to point out because I think it's for Patient Z that he used the HoxB8 cell line and this is derived from the human Fce(alpha) transgenic murine mast cell. So, it has to be the alpha chain. It's really a true exception because they don't have a humanized beta

chain.

DR. GOLDING: Mm-hmm. Okay. Well, thank you again for a really educational workshop and a lot of great work. Thank you very much.

DR. SCOTT: I think we have a few more questions and then I will gather myself together and try to give a brief summary. But I do believe to have a really good summary, I would go over all of this again. It's been a rich and deep scientific workshop I think. And very useful to us and I hope to everybody. So, we can go on with these few questions and comments.

MS. ELLER: Okay. Then the next question is in regards to better communication of product withdrawals and hypersensitivity reaction reporting, could a database or app possibly be developed to bring together patients, hospitals, infusions centers, distributors, manufacturers, and regulatory agencies that would have a higher visibility or quicker mode of communication, including patients reporting hypersensitivity reactions?

DR. SCOTT: I think Dr. Alimchandani may have left. That is a tall order but in terms of needing to modernize and improve communications, I think the tools exist and this sort of seems like an ideal world, but it could be done. I'm sure there are plenty of potential complications like possibly HIPAA, and certainly getting a lot of different organizations to work together. But it would definitely make things better potentially for patients and maybe speed our reaction time and the reaction time for the firms as well.

MS. ELLER: Okay. Thank you. The next question is for the retention samples mentioned earlier, how long are these samples kept? And I think this was for Grifols.

MR. ZERVOS: This is Clark. We're going to mute here. I'll take that. So, you know, I don't think any of our colleagues from Biomat can manage our plasma operations on the phone. But I know we were able to go back several years to get the retention samples from some of these common donors we were looking at. So, I -- all right.

And I'm getting something internally from a colleague in Los Angeles. So, we keep them essentially forever. So, thank you for that.

MS. ELLER: All right, thank you. I think the last question was answered but the person wasn't -- didn't get the response. This was about does grade 1 infusion rate related reaction require rate reduction right away? Or would you wait until symptoms progress? And I believe Dr. Cunningham-Rundles says that they would reduce the rate right away and they wouldn't wait. Do you want to expand on that or anything else?

DR. CUNNINGHAM-RUNDLES: Not really. I mean, the other thing is you might hydrate. You might give an extra bit of fluid because, you know, being dehydrated is probably a little bit of a negative here.

MS. ELLER: Okay, thank you. There is let's see, is there any information regarding the recipient's exhibited reaction? Did AE happen after first infusion or are they primed with Z or

Patient 18 plasma first and responded to second or subsequent infusion?

DR. ROTH: So, for those patients receiving product that contained Z-plasma and if they're -- which we refer to as Z-IG, they responded on the first infusion.

MS. ELLER: Somebody did ask, I assume that albumen and other products were also made from the plasma donors who may have caused the hypersensitivity reaction. But have any effects on products other than IG products been identified?

DR. ROTH: This is a really good question. Sorry, Clark.

MR. ZERVOS: Go ahead.

DR. ROTH: Thank you. So, it's a really good question. Yes, you know, there were other products made, particularly albumin was made off of the same pools where Donor Z had contributed to. And none of the albumin lots that were associated with this donor exhibited any sorts of elevated safety signals, hypersensitivity events,

or otherwise.

MR. ZERVOS: Yeah, thanks, Nathan. Yeah, it was the same for Grifols. We did look at that early on any product sharing the same plasma pool. But there was no signal on any of the other products including albumin.

MS. ELLER: This is sort of related. Has the learning and research output from these studies been shared with blood collection organizations? And related to this, has this type of observation been detected in whole blood transfusions?

MR. ZERVOS: So, I can answer. All right. So, I can take part of the question. From a previous investigation in Canada, probably 10 years ago, we did see this in --

DR. SCOTT: Yeah.

MR. ZERVOS: -- transfused components. And that actually led us to narrowing down a donor. His recovered plasma was used for fractionation. And I'm sorry, could you repeat the first part of the question?

Oh, I think it was sharing information with blood collection companies. We haven't had any direct communication with Red Cross, for example, or other blood collection organizations.

DR. SCOTT: I think we have to stop here since I only have five minutes to make some kind of summary on the spot here. So, to summarize, I agree with everyone who has said it's a splendid workshop and that's because of our speakers, and our very smooth IT crew, and the facilitators, and the extra panelists. I thank all of you and if I forgot anybody, that's my fault. And there are many important people who had a contribution to the workshop.

So, I'm just going to briefly summarize some of the topics that we've discussed. I think we all recognize that the -- what is seen for these patients that have the lot- specific immune globulin hypersensitivity, that this is very consistent with a typical type of hypersensitivity. And I would point out that maybe it's differentiated from an infusion related

event or an event simply by being infused in that the hypersensitivity reactions almost always includes

rash, or hives, or some skin manifestation in the patients with this adverse event.

We talked about or heard about how hypersensitivity reactions come to be, what can trigger them, what the components of that reaction are both in terms of antibodies and Fc receptors,and

other receptors, but also in terms of effector cells that cause the symptoms that patients have. As Dr. Golding said, at a very high level.

We also learned about development of mouse models and importantly the kinds of testing that can be done and is done in these mouse models to evaluate hypersensitivity and to perform research on hypersensitivity reactions. These include the Fce receptor alpha chain transgenic mouse, which sounds like a wonderful tool for

investigations. Dr. Anthony told us about the variations in antibodies, allotypes, IgG4 light chain switching, and so forth. All of these things we learned about including to some extent receptor variations and other receptors that can mediate hypersensitivity events. I mean, not IgG or IgE receptors, but other receptors on the surface of basophils and/or mast cells.

We then heard about the data that supports a single donor hypothesis for these clusters of hypersensitivity events. And I think that probably is an eyeopener for many people who had not been aware of these investigations before and really quite a breakthrough in trying to figure out what is going on and what to do.

We recognize there are a lot of challenges in continuing along the path that we are on in terms of figuring out in the first place how to speed our reaction time to these hypersensitivity clusters to try to assure that as few patients as possible are exposed to such a lot. And it obviously takes time even to

deconvolute a lot to the extent that you know at least a relatively small group of donors that should be at least temporarily deferred until deeper investigation can be done.

We have heard about the challenge that donors such as Donor Z or Donor 18 are difficult to track down after these events have happened. And probably one would start doing this sooner rather than later but it takes some time to get to that small group or single donor level. We also lack information about the recipients. We recognize the need for more sensitive tests when we're looking at plasma pools and final products.

We also talked about the different ways in which a test could be done and what might be needed if there were a release test or a screening test versus what might be done for an investigation. And the characteristics of a screening test if we knew exactly what to screen for and could eliminate the noise, still has a lot of hurdles with respect to being high throughput, not too difficult to do, and suitable for use on a

large number, potentially, of samples.

And I would say that at this moment, I personally don't think that we are ready to do that. I think we still need some more scientific information to find out how to develop a feasible test. Either for investigations or even for testing of donors.

So, in the discussion, we heard about the promising results focused on IgE receptor alpha and/or beta as a potential epitope targets for the type of antibodies that appear to be in Donor 18 or have the characteristics of the antibodies in Donor 18 and Donor Z. But we know that there are other possibilities and other receptors that still need to be fully explored, and other hypotheses even. But right now, I think we're all excited about the results that we heard and are hoping that these pan out to become very useful and applicable, as well as interesting.

We've also talked about how to improve tests, including the basophil activation test,

using an antibody to block FcRIIb, and

to further activate or make the cells more sensitive in the basophil activation assay and other assays in development. This

and other methods might be tried to improve the sensitivity of such assays

. We also talked about some of the

difficulties in reaching all the donors and getting all the adverse event reports, which will not inherently -- it's inherent in the system that currently we and firms receive spontaneous voluntary reports about adverse events. And we know that these do not represent the sum-total of

occurrences. We've talked about premedication for

immune globulin recipients and where that may be or may not be needed. And many other things. But it is 4:18 so, I am going to stop but I want to ask the group is there anything major that I have missed in this extremely brief summary of this extremely detailed and fascinating information that we've received?

Okay. I think I have my answer then. I

want to thank our speakers, panelists, and everyone else who has participated in the workshop. There will obviously be follow- up with respect to the questions. And I would propose that we meet together if needed, or wished, by the panelists and with the panelists and the FDA basically as needed for ad hoc conversations or perhaps consider writing a summary of the workshop or publishing articles related to the workshop in an issue of a journal. So, we can talk about that later. I just thank everybody and say have a great remainder of your day. And enjoy the sunlight at least if you're on the U.S. East Coast. And goodnight to the people who are in Europe and elsewhere. And so, farewell. Thanks, again. And hope we'll all talk together soon. Bye bye.

(Whereupon, at 4:20 p.m., the PROCEEDINGS were adjourned.)

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CERTIFICATE OF NOTARY PUBLIC

COMMONWEALTH OF VIRGINIA

I, Kendra L. Hammer, notary public in and for the Commonwealth of Virginia, 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.

Kendra L Hammer

Notary Public, in and for the Commonwealth of Virginia

My Commission Expires: September 30, 2025

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