

FOOD AND DRUG ADMINISTRATION

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CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

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NATIONAL INSTITUTES OF HEALTH

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NATIONAL INSTITUTE OF ALLERGY AND  
INFECTIOUS DISEASES

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WORKSHOP ON ADJUVANTS AND ADJUVANTED  
PREVENTIVE AND THERAPEUTIC VACCINES

FOR INFECTIOUS DISEASE INDICATIONS

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TUESDAY  
DECEMBER 2, 2008

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The workshop convened at 8:40 a.m.  
at the Bethesda North Marriott Hotel &  
Conference Center, 5701 Marinelli Road,  
Rockville, Maryland, Jay Slater, M.D., Deputy  
Director, Center for Biologics Evaluation and  
Research, Moderator, presiding.

Introduction and Welcome

ANTHONY S. FAUCI, M.D., Director, NIAID/NIH  
JESSE L. GOODMAN, M.D., M.P.H., Director,  
CBER/FDA

Session 1: Background

NORMAN BAYLOR, Ph.D., Director, Office of  
Vaccines Research and Review (OVR)/CBER/FDA  
DAN ROTROSEN, M.D., Division of Allergy,  
Immunology, and Transplantation/NIAID/NIH

Session 2: Specific Adjuvants Overview

ELIZABETH SUTKOWSKI, Ph.D., Co-Chair, CBER/FDA  
BALI PULENDRAN, Ph.D., Co-Chair, Emory Vaccine  
Center

FABIO RE, Ph.D., University of Tennessee  
Health Science Center  
DEREK O'HAGAN, Ph.D., Novartis Vaccines and  
Diagnostics, Inc.  
EUGENE MARASKOVSKY, Ph.D., CSL Limited  
BRUCE BEUTLER, M.D., Scripps Research  
Institute

NATHALIE GARCON, Pharm.D., Ph.D.,  
GlaxoSmithKline Biologics  
GEERT VAN den BOSSCHE, D.V.M., Ph.D., Bill and  
Melinda Gates Foundation

Session 3: Preclinical Safety

ETHAN SHEVACH, M.D., NIAID/NIH, Co-Chair

MARION F. GRUBER, Ph.D., Co-Chair,  
OVR/CBER/FDA  
JAN WILLEM VAN der LAAN, Ph.D., National  
Institute for Public Health and the  
Environment, The Netherlands  
CARL ALVING, M.D., Walter Reed Army Institute  
of Research

SARAH GOULD, Ph.D., Sanofi Pasteur  
DEBORAH NOVICKI, Ph.D., Novartis  
HANA GOLDING, Ph.D., Division of Viral  
Products (DVP)/CBER/FDA  
WILLIAM WARREN, Ph.D., VaxDesign Corporation

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Adjourn

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

2 8:42 a.m.

3 DR. SLATER: Good morning and  
4 welcome to the FDA NIH Workshop on Adjuvants  
5 and Adjuvanted Preventive and Therapeutic  
6 Vaccines for Infectious Disease Indications.

7 Welcome to all of you. I'm Jay  
8 Slater, and I have a couple of introductory  
9 sort of housekeeping comments before we get on  
10 to the main part of the program.

11 First of all, you are probably all  
12 aware that there are a lot of you here. This  
13 session was well over-subscribed. We were  
14 originally planning for about 250  
15 participants. We have over 400 people signed  
16 up. So my guess is that as the morning goes  
17 on, it will become more crowded.

18 The purpose of this is to warn you  
19 that tomorrow our setting will be a little bit  
20 more intimate. We were successful at getting  
21 two rooms on the first day when we realized  
22 how heavily subscribed we were going to be.

1 But we were unable to get two rooms for the  
2 second day.

3 So tomorrow we will all be in  
4 basically half the space. Obviously the  
5 tables will not be here. So we'll be a little  
6 bit closer. You will get to know your  
7 neighbors and fellow participants a little bit  
8 better tomorrow. But everybody should be able  
9 to safely fit in.

10 Those of you who parked outside in  
11 the parking lot, please make sure at some  
12 point during the day to go to the registration  
13 desk and get parking vouchers in case you  
14 haven't already. I will talk a little bit  
15 later about your lunch options as well.

16 Speakers, if there are speakers  
17 out here who have not yet given in your talks,  
18 Mr. Sandoval, who is sitting right there at  
19 the AV desk in the corner, will help you with  
20 your talks and get you loaded on here during  
21 the next appropriate break.

22 You will also notice that we have

1 a very full schedule today and tomorrow. It  
2 is very tightly packed. The ability to ask  
3 questions will be at the session chair's  
4 discretion.

5 The one thing I would warn you  
6 about is that with a schedule like this, we  
7 could easily be a half hour ahead of schedule  
8 or a half hour after schedule. So don't treat  
9 this like a train schedule that is going to be  
10 particularly precise. If you are a speaker,  
11 please make sure you are here well in advance  
12 of the time of your presentation.

13 There will be time at the  
14 roundtable discussions tomorrow, I hope, for  
15 more questions. The other thing to keep in  
16 mind is this meeting is being transcribed.  
17 Therefore, if you are going to ask any  
18 questions, please take advantage of the six or  
19 seven microphones that are out in the  
20 audience.

21 So it is my pleasure to introduce  
22 for you the first two speakers of the session

1 at this introduction. We're fortunate to have  
2 both Dr. Fauci and Dr. Goodman introducing our  
3 session at this time. Dr. Anthony Fauci, as  
4 you know, is the Director of the National  
5 Institute of Allergy and Infectious Diseases.  
6 Dr. Jesse Goodman is the Director of the  
7 Center for Biologics Evaluation and Research.

8 These are the two organizations  
9 that cosponsored this meeting. And,  
10 therefore, we're going to ask both of them to  
11 come up and give some introductory remarks.

12 Dr. Fauci?

13 DR. FAUCI: Thank you very much,  
14 Jay. It's a great pleasure to be here with  
15 you all this morning. And I'd like to welcome  
16 you on behalf of NIAID. And you will hear  
17 very shortly from Jesse from CBER also  
18 welcoming you.

19 I'm only going to take just a few  
20 minutes because I want to really get us on to  
21 the main guts of this meeting. But I want to  
22 make some introductory remarks on a few

1 slides, likely telling you things that you are  
2 extraordinarily well familiar with. But I  
3 think it is worth mentioning as we start on  
4 this.

5 If I were to be given a 20 second  
6 time slot, I would just come up here and say  
7 you have my absolute commitment that work on  
8 adjuvants and the importance of adjuvants in  
9 both adjuvanted preventive and therapeutic  
10 vaccines is extraordinarily key and of a high  
11 priority to NIAID.

12 Having said that, I'm only going  
13 to spend just a couple of minutes just going  
14 through some issues, again, that I know you  
15 are familiar with. The impact of vaccines in  
16 the United States and globally is profound.

17 If you look at this slide, many  
18 versions of which get circulated, you see that  
19 of all the things we do in countermeasures, be  
20 they drugs or preventive measures, whenever we  
21 go before the Congress or talk to anyone  
22 nationally or internationally, vaccines are

1 always the issue that is brought up for the  
2 highest cost-benefit ratio for the individual  
3 on public health as shown on this slide with  
4 the baseline cases in the 20th century in the  
5 pre-vaccine annual cases of a variety of  
6 important diseases compared to both the cases  
7 as well as the percent decrease.

8 I don't think there is any  
9 intervention in medicine that is as good as  
10 this, something that people in this room are  
11 very well familiar with. Recently, this is  
12 not just something of ancient decades ago, but  
13 recently vaccines that have been developed  
14 have continued to change the course of lives  
15 of people, again both in this country and in  
16 the developing world.

17 And these are just three examples  
18 of relatively recently developed vaccines and  
19 their potential in children less than five  
20 years old with the pneumococcal conjugate, the  
21 Hib vaccine, and the rotavirus vaccines.

22 NIAID from a research component,

1 and again when you are dealing with vaccines,  
2 there are multifaceted aspects of it.

3 Sometimes this confuses people. There are  
4 broad general surveillance and public health  
5 issues. There are fundamental basic research  
6 issues. There are developmental research and  
7 developmental product issues. And there are  
8 regulatory issues.

9 NIAID is one of an important part  
10 of a very heterogenous and multifaceted  
11 approach towards vaccines. I show you this  
12 website of ours, which essentially describes  
13 the research agendas for vaccine development.

14 And I'm not going to talk about  
15 that. And I'm not going to bring up the  
16 research questions. You'll hear a little bit  
17 more about that from Jesse and how that  
18 impacts on the regulatory area.

19 But for those of you who are not  
20 familiar with this -- and I would hope that  
21 you were -- this is a site that you can get  
22 virtually all of the information for what we

1 at NIAID are doing.

2 Now with regard to vaccinology, it  
3 is an old and very well respected discipline.  
4 But as, again, all of you are aware, science  
5 has proceeded at a very rapid pace,  
6 particularly over the last couple of decades.

7 So now what we are -- really it  
8 behooves us -- it is an imperative for us to  
9 do is to apply the new science as it evolves  
10 to this very old and respected discipline of  
11 vaccinology, utilizing the tools and the  
12 platforms that are discussed or listed on this  
13 slide from the explosion in the arena of  
14 genomics, the fact that we now have structure-  
15 based vaccine designs utilizing  
16 crystallography.

17 We have biotechnology with  
18 nanotechnology, systems biology, high  
19 throughput systems, bioinformatics, different  
20 delivery systems, particularly in the arena of  
21 viral vectors, which have caught on very, very  
22 hot over the last several years, production

1           technology, one example in influenza, the now  
2           rapid progression from cell culture-based --  
3           from egg-based to cell culture-based  
4           manufactory.

5                         And last on this slide is the  
6           issue of adjuvants, which is the subject of  
7           our discussions in the workshop here today.  
8           The NIH vaccine adjuvant programs range from  
9           very fundamental, basic research on innate  
10          immunity, looking for the molecular mechanisms  
11          of the actions of adjuvants, to the discovery  
12          and development of actual new adjuvants, new  
13          products, be they from computational models,  
14          high throughput discovery platforms, new  
15          technologies of optimizing of lead candidates,  
16          as well as the important area that we are very  
17          heavily involved in is the clinical studies of  
18          adjuvants.

19                        The paradigm is changing, again,  
20          things that people in this room are  
21          extraordinarily well familiar with, the old  
22          perspective in classic vaccinology, that

1 antigen may be sufficient to induce a  
2 protective immune response, antigen alone,  
3 that is true in some cases.

4 But it is becoming very clear,  
5 particularly with the new challenges that we  
6 have with certain vaccines -- influenza,  
7 influenza in general, influenza for the  
8 elderly, HIV almost certainly, is that  
9 adjuvants, either endogenous or exogenous,  
10 contribute to the effectiveness of vaccines  
11 and that the addition of adjuvants or other  
12 immunomodulators will be necessary for optimum  
13 response in many settings.

14 So this is something that will  
15 happen. The question is how do we, from the  
16 research and developmental standpoint, make it  
17 happen quickly and safely.

18 Now adjuvants were big black  
19 boxes. Many of you -- most of you -- all of  
20 you, I would say, know decades ago. But with  
21 the delineation of the molecular aspects,  
22 receptors, and ligands, and signal

1 transduction mechanisms associated with the  
2 interplay of the innate and ultimately the  
3 adaptive immune system, I found it curious and  
4 interesting as I was going over the history of  
5 this, if you look at this paper from Medzhitov  
6 and Janeway, in 2000, which was just really  
7 relatively recently, in the New England  
8 Journal of Medicine, delineated what we knew,  
9 at that time, of the Toll-like receptors and  
10 other receptors on cells and the ligands that  
11 are used to trigger the innate immunity, and  
12 look at the studies that have gone on from  
13 2000, which is right about the middle of this  
14 slide, up until the end of 2007, and that of  
15 the number of publications, that if you do a  
16 MEDLINE search on innate immunity, much of  
17 which relates to adjuvant potential, you can  
18 see the extraordinary growth in knowledge such  
19 that a recent paper, this one from Host Cell  
20 and Microbe just this year -- and this is a  
21 simplified version of that, is the important  
22 complexity of the host innate immune receptors

1 and how we might use that in our scientific  
2 delineation of where we may go with adjuvants.

3 The pipeline of new adjuvants is  
4 robust, as you see from this slide. There are  
5 a number of them that are in clinical trials  
6 or on their way, ranging from CpG to the Lipid  
7 A mimics, RNA-based peptide carbohydrate,  
8 small molecule activators of TLR signaling an  
9 early discovery. So have a very, very robust  
10 and fruitful, potentially fruitful -- and I  
11 know will be fruitful discovery chain in the  
12 arena of adjuvants.

13 The goals for the future adjuvants  
14 are familiar to all of you. We need earlier,  
15 more robust and durable immunity with fewer  
16 boosters and less antigens. I'm going to show  
17 you an example of that in just a moment -- one  
18 example of many -- broader coverage and  
19 enhanced cross protection, and adjuvants which  
20 are designed according to the immunization  
21 route, be it subcutaneous, intramuscular,  
22 mucosal, or what have you.

1           The example I was referring to was  
2           the issue that we faced a few years ago -- and  
3           this is The New England Journal paper from  
4           2006 from John Treanor and his colleagues,  
5           when we were putting a full court press on  
6           trying to develop an H5N1 vaccine in the  
7           classical manner. It was successful, but it  
8           wasn't optimal, as all of you know.

9           The dose that was required was  
10          non-practical from a domestic or global  
11          standpoint and a relatively small percentage  
12          of individuals were actually induced to give  
13          an immune response that you would predict by  
14          standard guidelines to be protective.

15          There was another study that came  
16          out, this one from GSK using their proprietary  
17          adjuvant, and, in fact, with the same goal in  
18          mind, were able to use a much lower dose.  
19          Instead of 90 mics times two, it was 3.8 mics  
20          times two.

21          Not only did it encompass a  
22          greater percentage of individuals -- 75

1           percent or more -- but there was also  
2           induction of cross-reactive neutralizing  
3           antibody against a clade of H5N1 that was a  
4           bit drifted from the original immunizing  
5           clade, something extraordinarily important.

6                         Now this is a mental exercise on  
7           this next slide. It's not something that has  
8           been done. But if you just take those data  
9           and put them together with the stockpiled H5N1  
10          vaccine of close to 23 million 90 mic doses,  
11          and if, in fact, the adjuvant data proves to  
12          be practically applicable, you would see that  
13          literally we would have 542 million instead of  
14          22 million doses, which is extraordinarily  
15          important when you are dealing with  
16          stockpiling not only for diseases like  
17          potentially pandemic flu but a variety of  
18          others.

19                        Also important, it extends the  
20          supply, it increases the level of the immune  
21          response, importantly for something like  
22          pandemic flu whose strains drift regularly, as

1 we know, an increase of the breadth of the  
2 immune response with protection against  
3 drifted strain, and the option for a single-  
4 dose priming instead of multiple doses.

5 And finally, having said that just  
6 as a quick introduction, the challenges ahead  
7 of us from a pure disease standpoint are  
8 extraordinary. And these are just some  
9 selected examples of vaccine candidates that  
10 almost certainly would benefit greatly if we  
11 had a wider array of adjuvants, ranging from  
12 HIV through malaria, TB, and the neglected  
13 tropical diseases, I already mentioned  
14 pandemic influenza but also seasonal influenza  
15 in the elderly as well as those vaccines that  
16 require multiple doses such as varicella in  
17 children.

18 So in closing, I want to again  
19 welcome you and to again reinforce the  
20 commitment of NIAID from the research  
21 standpoint to work closely with you, the  
22 people who are involved in the research, in

1 the development, biotech, pharma, FDA, CDC,  
2 and all of our other partners in what I am  
3 sure is going to be one of the most important  
4 endeavors that we undertake.

5 Thank you.

6 (Applause.)

7 DR. GOODMAN: Well, thank you very  
8 much, Tony, and thank you for your support and  
9 vision in this field.

10 I'm very happy to be here. It is  
11 wonderful to see this turnout. I was thinking  
12 that I would like to see this meeting be an  
13 adjuvant for adjuvants. Okay. So we can  
14 really stimulate work in the field and  
15 stimulate progress.

16 This is one of the adjuvants you  
17 don't want to get on the cover. This is, I  
18 think, complete Freund's adjuvant. So it  
19 certainly shows you one extreme.

20 But let me just give also a few  
21 introductory remarks, again to thank  
22 colleagues both at NIAID and CBER and others

1 for sponsoring this and also these people here  
2 who I'm informed are the organizing committee.  
3 I'm sure there are others who have helped  
4 support this, and I thank them as well.

5 One of the interesting things that  
6 I think has happened in adjuvants is that  
7 there are almost two universes, and the  
8 crosstalk recently has started. And that is  
9 going to be very productive. One universe is  
10 the vaccinologists who see a problem in the  
11 immune response and basically take an empiric  
12 approach. Another are the immunologists who  
13 tend to focus on their pathway or molecule.

14 And I think one of the goals we  
15 should have, particularly our colleagues at  
16 NIH but also at CBER is to bring these  
17 disciplines together so we're really applying  
18 science to what we're trying to accomplish  
19 clinically. So that's another great reason  
20 for this co-sponsorship.

21 Tony has mentioned many of these  
22 things. What are the needs, potential needs

1       for and benefits of adjuvants. Well, they can  
2       affect the immune response in many ways and  
3       there are many examples of these different  
4       kinds of effects. And they all have meaning,  
5       particularly when we are dealing with emerging  
6       infectious disease threats, bioterrorism, et  
7       cetera.

8                 One is to enhance the rapidity.  
9       And this could also potentially effect the  
10      number of doses needed and/or the height or  
11      intensity of the immune response. So it could  
12      occur earlier. There could be a higher level  
13      of antigen or cellular immunity.

14                This is very important for many  
15      antigens that are out there of poor  
16      immunogenicity. I often like to think that  
17      we've tackled a lot of the infectious diseases  
18      that, for which the host really makes a good  
19      protective immune response, and this is about  
20      the complex interplay of an antigen that may  
21      be poorly immunogenic and a host that may not  
22      respond well to that pathogen.

1                   So we're left with these pathogens  
2                   which either aren't terribly antigenic or with  
3                   a host response that is defective. This could  
4                   also enhance the breadth of the response. And  
5                   you heard Tony mention this with cross  
6                   protection against clades of pandemic flu. So  
7                   this could protect against pathogen evolution.

8                   The duration of the response is  
9                   also important. We are discovering more and  
10                  more issues where memory or priming is  
11                  surprisingly not as good as we may have  
12                  thought it was and where people are needing to  
13                  be re-immunized at various points in life.  
14                  And there is evidence that certain adjuvants  
15                  can direct more immune resources towards  
16                  memory cells.

17                  And, of course, what has driven a  
18                  lot of this has been the finding that H5N1  
19                  influenza was a very poor antigen and the  
20                  exciting results that some of the novel  
21                  adjuvants may really improve that situation  
22                  and, as Tony showed, perhaps result in solving

1           problems of manufacturing capacity, which, if  
2           we think it is critical in this country, is  
3           even more critical globally, and also in  
4           dealing with the likelihood that when a  
5           pandemic strain emerges, it is not going to be  
6           exactly what we have been studying or  
7           predicted.

8                         Okay. And then as I mentioned,  
9           there seem to be a bunch of pathogens in which  
10          either vaccines don't work very well or the  
11          host doesn't work very well. And I suspect  
12          those are just different sides of the same  
13          coin. And these are some of the things that  
14          were listed by Tony.

15                        But, again, as we look at  
16          opportunities to prevent malaria, TB, or HIV,  
17          or in the whole arena of therapeutic vaccines  
18          where we are dealing with a host failure to  
19          mount an effective immune response against an  
20          invader such as a cancer cell, these adjuvants  
21          could be particularly important.

22                        Now Tony listed some of the things

1 under study, and I won't go through this in  
2 detail. But just to say there are many  
3 approaches to adjuvants that range from the  
4 commonly used mineral salt such as alum to the  
5 more recently used oil-in-water emulsions and  
6 then a number of things in earlier stages of  
7 investigation.

8 Also worth pointing out, that as  
9 we understand more mechanistically and we  
10 understand the deficiencies in the immune  
11 response to certain pathogens, it appears  
12 possible and even beneficial to combine  
13 adjuvants that target different places in that  
14 diagram -- that increasingly complex diagram  
15 that Tony showed.

16 What are some of the overall  
17 selective mechanisms of action? I know you  
18 have some talks from people who have really  
19 delved deeply into these. I think the  
20 important overriding message is they are still  
21 often poorly understood.

22 As a non-immunologist who used to

1 work a lot on infectious diseases in the  
2 laboratory, I always found immunology very  
3 frustrating because I always felt like you  
4 could prove anything or nothing. But perhaps  
5 that is an unduly skeptical view of it. And  
6 as we get to a more molecular level, we'll do  
7 better.

8 But we often find -- the flip side  
9 of this is that evolution is wonderfully  
10 complex. And whether you look at the clotting  
11 system or the immune system, nothing is ever  
12 as simple as one receptor or molecular. And  
13 these are really complex control loops.

14 And so that the idea that an  
15 adjuvants works on just one thing would  
16 probably be a very naive idea. And they often  
17 work at multiple steps.

18 But some of the things that have  
19 been identified or interactions with antigen  
20 uptake through antigen-presenting cells, or  
21 prolongation of that uptake, similarly and  
22 related a traction of mononuclear cells,

1 dendritic cells, even neutrophils to process  
2 and present antigen, direct effects on  
3 cellular membranes, and, of course,  
4 increasingly interactions with these pattern  
5 response recognition molecules, including the  
6 whole family of TLRs.

7           And many of these will result in  
8 downstream and fairly nonspecific  
9 manifestations such as cytokine, chemokine  
10 release, and enhanced body and T cell  
11 responses. Another thing that others have  
12 pointed out that I think is worth considering  
13 is when -- while it is a blunt instrument,  
14 when you do unleash this whole cytokine  
15 response, you also tend to unleash a counter-  
16 regulatory response, which actually may be  
17 protective against some of the negative  
18 effects we worry about for adjuvants.

19           So the point is that all of these  
20 mechanisms can lead to immune and inflammatory  
21 responses. That is part of what is desirable.  
22 But it also leads to the increased reactions

1 and sometimes systemic effects that we see and  
2 worry about.

3 And we also -- very important to  
4 think about the complexity of the needs for  
5 adjuvants and the responses. They may differ  
6 with different antigens. There's no reason to  
7 think that all antigens would behave the same  
8 with one adjuvant or vice versa in different  
9 clinical settings, children versus adults, et  
10 cetera, or priming verses recall.

11 So at FDA, we are asked to make  
12 some difficult judgments ranging from clinical  
13 trial judgments to approval judgments to where  
14 to put resources in terms of trying to  
15 stimulate product development. And it comes  
16 down to, in this area, enhanced immunity  
17 versus inflammation, adverse events, and  
18 potentially autoimmunity.

19 And I like to remind people,  
20 particularly when many people here are maybe  
21 engaged mostly in laboratory investigation,  
22 that these products ultimately interface with

1 humans who are your children, our children,  
2 our country's children, the world's children.

3 And it is very important to  
4 understand that large numbers of people get  
5 vaccine products and we don't walk through a  
6 single day at CBER without recognizing that  
7 confidence in all of immunization, which, as  
8 Tony showed you, has been a remarkable public  
9 health advance, and in our very institutions,  
10 is dependent on whether we get this right. So  
11 we have a very serious scientific and  
12 regulatory responsibility.

13 So what are some of these  
14 potential concerns we want to keep in mind?  
15 As I mentioned, you could get an antigen-  
16 specific or nonspecific increase in potency of  
17 immune and inflammatory stimulation.

18 We typically see, for effective  
19 adjuvants, increased reactogenicity, an FDA  
20 term for feverishness, sore arm at the site,  
21 things we typically see with non-adjuvanted  
22 vaccines but often see more in the presence of

1 an adjuvant.

2 I want to point out it is very  
3 unclear whether these ever correlate with more  
4 severe adverse events. You know occasionally  
5 they do. But we have not found, to date --  
6 but the flip side is it would be difficult to  
7 find, for example, that increased local  
8 reactogenicity or feverishness down the road  
9 increases the commonality of some of the more  
10 severe adverse events that we might be  
11 concerned about such as neurologic events.

12 There just aren't those data. I  
13 think to some degree that may reflect the  
14 weakness of our tools to look at it.

15 Issues have been raised about the  
16 potential role of autoimmunity. There is an  
17 interesting article just recently in JID from  
18 the folks at CDC Penn and other places about  
19 what seemed to be antigen-specific reactions  
20 to flu vaccine that may cross-react with the  
21 GM1 neural ganglioside and could potentially  
22 be related to the rare cases of GBS that have

1           occurred after flu vaccine. And also concerns  
2           have been raised about autoimmunity and immune  
3           disease in general.

4                        One question that has been raised  
5           with children is for some potent agonists, are  
6           there plausible risks to a developing immune  
7           system. And I don't know of evidence that  
8           that would be true but I ask the scientists is  
9           this plausible and are there ways that we need  
10          to look at it. And I think that is one of the  
11          questions at this meeting.

12                      And I'd like to end up by saying  
13          we see some reassuring observations to date.  
14          One is, as I said, even strong pattern  
15          recognition signaling is likely similar to  
16          natural infection. It's not -- you know you  
17          go through life and you get some pretty bad  
18          infections.

19                      And you get a lot systemic  
20          reactions, for anybody who has had one of  
21          these bacterial infections, and on the other  
22          hand, a caveat if people are aware of the

1 recent study with monoclonal antibody that was  
2 an agonist to CD28, which would certainly be  
3 a costimulatory pathway, that unexpectedly and  
4 despite negative studies in primates, this  
5 monoclonal antibody stimulated near lethal  
6 effects through essentially T cell  
7 stimulation. And, again, people who know much  
8 more about this could probably comment on  
9 that.

10 The other good thing is there is  
11 no evidence to date of major problems with  
12 those compounds being most actively  
13 considered. But we always point out the  
14 absence of evidence is not evidence of  
15 absence. It just tells you a little.

16 There are very few of these  
17 studies with adequate numbers of controls with  
18 long-term follow up or with children.

19 So Norman will probably say more  
20 about this but we are here to assess the  
21 current knowledge base. And I think really to  
22 stimulate a research agenda. And I'd take

1           that one step further -- to stimulate research  
2           collaboration, to be sure we learn from basic  
3           science studies what can help us with patients  
4           and be sure we've applied basic science to  
5           patient studies much more often to learn from  
6           those as well.

7                         We're going to review the clinical  
8           data, some of the clinical data, and I think  
9           an important area that I'll comment once more  
10          briefly on is, you know, the toxicology of  
11          vaccines, not to mention the toxicology of  
12          adjuvants has been a really neglected area.

13                        And, you know, we've tended to  
14          only recently pay attention to this. And  
15          we've had just tools of conventional  
16          toxicology, which largely focus on drug  
17          effects on organs. And, of course, when you  
18          are talking about immunotoxicology, there are  
19          not a lot of good models.

20                        I think there is a huge  
21          opportunity for the scientific community to  
22          develop better nonclinical or non-human models

1 and even human studies that could tell us  
2 about the safety of novel vaccines and  
3 adjuvants.

4 The good side is I really think  
5 this meeting and some of the investment --  
6 and, again, I credit our colleagues in HHS and  
7 industry as well in trying to get better flu  
8 vaccines -- that all these things are going to  
9 bring us to a place where we are going to have  
10 successful development and evaluation of  
11 vaccines for some of these unmet challenges.

12 So just to finalize, a few  
13 overarching scientific questions that occurred  
14 in me, more as an infectious disease person  
15 but also as somebody who sees the beginning of  
16 your innovations, the question has been asked  
17 are there some cases where there is a reason  
18 we don't respond to certain antigens that  
19 actually may protect us. I'm not sure how  
20 important that is but we always need to keep  
21 that in mind.

22 Or is the organism designing how

1           it presents the antigen to simply evade our  
2           immune system? And if so, not only could we  
3           possibly design better adjuvants but can we  
4           better design antigens or present them in more  
5           antigenic manners and have an adjuvant effect  
6           in itself without a chemical adjuvant.

7                           And certainly the use of  
8           particulate presentations may, in fact, be  
9           doing some of that. And alum may do some of  
10          that.

11                          As we understand host protection -  
12          - and I think this is where the basic science  
13          is very important -- can we design adjuvants  
14          that work far more specifically? Or will they  
15          not work? I don't think we know the answer to  
16          that yet.

17                          But, for example, if we are more  
18          distal in a pathway, can we get less  
19          undesirable information but let's say more  
20          turning on of T cells? That would be a  
21          question.

22                          And then I mention can we get

1 better approaches to vaccine toxicology in  
2 general. And I see there are a number of  
3 talks at the end of today but I've seen very  
4 little where genomics are applied where  
5 responses of human cells to certain antigens  
6 or substances are applied to look at can we  
7 recognize profiles that would be associated  
8 with both effectiveness and safety. And I  
9 think there is huge opportunity there to bring  
10 together the basic scientists with clinical  
11 and animal studies.

12 Very important to remember as we  
13 look at models, again, the incredible  
14 complexity and, again, just skimming the  
15 surface of some of this literature, all  
16 different mice with different TOR responses,  
17 which may or may not be relevant to humans, so  
18 the importance of looking at animal studies  
19 with more global knowledge than most of us  
20 have and with some skepticism.

21 This is just one study I recently  
22 found though that looked to be a very

1 specific, more distal use of an adjuvant  
2 approach. And this was a study using a  
3 costimulatory ligand for CD137 as an adjuvant  
4 for cytotoxic T cell responses. And what you  
5 can see on the right, that highest line is the  
6 lysis of influenza-infected target cells when  
7 this is occurring in a background of this  
8 molecule for IBBL being constitutively  
9 expressed.

10 So this is just an example of a  
11 very specific molecular tweak on a very  
12 specific pathway. Now what I don't know is  
13 how many other pathways this then goes and  
14 influences. And an immunologist could  
15 probably teach me a lot about that.

16 So, again, I think you for your  
17 interest. I showed this slide in various  
18 places. But I'm hoping what we end up with is  
19 new, improved antigens and vaccines and  
20 solutions to our public health problems that  
21 are safe and that protect our people.

22 So thank you very much.

1 (Applause.)

2 DR. SLATER: Thank you, Dr.  
3 Goodman. Thank you, Dr. Fauci, for those  
4 introductory remarks.

5 Session 1 is focused on background  
6 to get us pointed in the right direction in  
7 terms of our discussions today. The two  
8 speakers in Session 1 will be first Dr. Norman  
9 Baylor who is the Director of the Office of  
10 Vaccines Research and Review.

11 And following him Dr. Daniel  
12 Totrosen, who is the Director of the Division  
13 of Allergy, Immunology, and Transplantation at  
14 NIAID.

15 Dr. Baylor?

16 DR. BAYLOR: Good morning.

17 What I want to try to do, in the  
18 brief time I'm speaking, is to sort of set the  
19 stage, give you a little background about the  
20 meeting and sort of where we are going. And  
21 also build upon a little bit of what Dr.  
22 Goodman and Dr. Fauci stated earlier.

1                   Just as an introduction, when we  
2                   think about vaccine development, what we want  
3                   to do for an ideal vaccine is we want to  
4                   provide the safest vaccine we can, we want to  
5                   provide a vaccine that has a maximum efficacy,  
6                   and we want a vaccine that requires the least  
7                   amount of antigen and the number of doses,  
8                   preferably one dose.

9                   Now as it has been stated today,  
10                  the interest in vaccine adjuvants and new  
11                  delivery systems has significantly increased  
12                  over the past decade. And a variety of new  
13                  technology and advances in vaccine development  
14                  present significant challenges to the national  
15                  regulatory authorities such as the FDA.  
16                  However, these products may present  
17                  opportunities for advancing public health as  
18                  well as have been presented by the previous  
19                  speakers.

20                  The FDA, as the national  
21                  regulatory authority in the United States, we  
22                  must be in a position to develop new

1 scientific and regulatory criteria to  
2 facilitate the development of these new  
3 vaccines, including vaccines with novel  
4 adjuvants. And we need to evaluate these  
5 vaccines for their safety and effectiveness.

6 As most of you know, adjuvants are  
7 not licensed separately from vaccines which  
8 they have formulated in the United States.  
9 And currently only aluminum-containing  
10 adjuvants are used in U.S.-licensed vaccine.

11 It is the individual vaccine-  
12 adjuvant combination in the United States that  
13 is licensed. And this necessitates a case-by-  
14 case evaluation of these compounds. But when  
15 you start evaluating on a case-by-case basis,  
16 this makes it very difficult in developing  
17 guidelines that would apply in all situations.

18 And so what we are trying to do is  
19 collect as much information as we can,  
20 evaluating the science to try to formulate  
21 guidelines that will apply across many  
22 situations.

1                   The other challenges that we see  
2                   with adjuvants, of course, are the safety  
3                   concerns which, as has been mentioned to some  
4                   extent by Dr. Goodman. And so we must, as we  
5                   do with all vaccines, adjuvanted or not,  
6                   evaluate benefit versus risk.

7                   One of the issues with the  
8                   adjuvants is the lack of universality.  
9                   Adjuvants are currently not considered active  
10                  ingredients in prophylactic vaccines. So we  
11                  license and we evaluate the adjuvanted  
12                  vaccine, not as separate.

13                  And also the immune responses that  
14                  are obtained with one antigen adjuvant  
15                  combination cannot always be -- and most of  
16                  the time cannot be extrapolated to another  
17                  antigen or even the same combination given by  
18                  different routes.

19                  Other challenges with evaluation  
20                  adjuvants is the manufacturing, such as scale-  
21                  up, consistency of manufacturing from lot to  
22                  lot, evaluating potency and stability of the

1 combined product.

2 And also from a clinical  
3 perspective determining the clinical endpoints  
4 for assessing safety and efficacy. These are  
5 challenges that are presented to us as not  
6 only from a development point of view but also  
7 from the regulatory point of view.

8 So the objectives of the workshop  
9 over the next couple of days, we will look at  
10 mechanisms of action of adjuvants, try to  
11 identify the scientific gaps, and also look at  
12 approaches to nonclinical safety evaluation  
13 for adjuvanted vaccines, what criteria for  
14 selecting the appropriate route of  
15 administration, doses, schedule, are there  
16 animal models that can be used in evaluating  
17 these new adjuvants. And also alternate  
18 methods. And, of course, clinical experience  
19 with respect to safety.

20 There will be a couple of  
21 roundtables today. And just some of the  
22 questions that we'll try to get out to really

1       tease out in the roundtables, if you think  
2       about this there is really your nonclinical  
3       and your clinical. Those are your big areas.

4               And so looking at the current  
5       approach to adjuvant toxicology testing is one  
6       of the topics we really want to try to get a  
7       handle on today. And find out what  
8       information do we know.

9               For example, is it sufficient to  
10       test only the highest human dose of the  
11       vaccine-adjuvant combination and adjuvant  
12       alone? Should the dose ranging studies be  
13       conducted on the adjuvant alone? Should other  
14       parameters such as cytokine levels or other  
15       biomarkers be assessed in evaluating these  
16       adjuvants? And are other aspects of current  
17       study designs, such as the route of  
18       administration or the regimen appropriate?

19               These are just some of the  
20       questions that will come up in the nonclinical  
21       discussion roundtable today. And there are a  
22       number of others that will come out. This is

1 sort of -- I hate to use the word free-for-all  
2 but it is a free-for-all because we're trying  
3 to collect as much information as we can and  
4 open up the discussion as we try to evaluate  
5 these adjuvants.

6 Less so, there will be a clinical  
7 issue. The clinical issues involved, this  
8 will not be as in depth but the things that  
9 we'd like to know are what type of clinical  
10 studies are needed to, for instance, detect  
11 age-specific differences in adjuvant responses  
12 going from a pediatric population to an  
13 elderly population? What type of long-term  
14 safety information needs to be provided? As  
15 well as dose ranging data on adjuvants as well  
16 as the antigens that they are stimulating.

17 And what kind of clinical studies  
18 can be designed that will incorporate safety  
19 information from the preclinical data? So can  
20 you build upon the preclinical data as you  
21 move into your human studies? Can you  
22 translate that data as you are looking and

1           trying to design clinical studies going into  
2           humans?

3                           And, of course, there are a number  
4           of other clinical issues that are out there  
5           that we probably will not be able to address  
6           all of those today. This is, again, an  
7           evolving dialogue, trying to really get some  
8           understanding of how we're going to evaluate  
9           these products and also bring these products  
10          to licensure.

11                          So just in summary, the  
12          development and evaluation of novel adjuvants  
13          present unique challenges. I mean that's  
14          obvious. The use of adjuvants in vaccines  
15          also can provide an opportunity to improve  
16          public health.

17                          In many of the examples that Dr.  
18          Fauci showed in his presentation of antigen-  
19          sparing, increasing the amount of vaccine,  
20          access to vaccines -- I mean the adjuvants may  
21          have a huge impact on our ability to improve  
22          public health globally.

1                   And then keeping in mind that  
2                   nonclinical safety assessment as well as the  
3                   clinical safety evaluation of adjuvant  
4                   vaccines are critical and those two will be  
5                   the focus of the panel discussions later on  
6                   today.

7                   And I believe that's it. Thank  
8                   you.

9                   (Applause.)

10                  DR. ROTROSEN: Let me thank you  
11                  again for all the participants today joining  
12                  us.

13                  I'm going to finish up the  
14                  introductory session with a little more  
15                  background on the NIAID perspective and our  
16                  goals in cosponsoring the workshop today.

17                  The background has been covered  
18                  amply by all the previous speakers. But just  
19                  very rapidly, there has been a tremendous  
20                  growth in information on adjuvant activity.  
21                  We know a lot now about distinct classes of  
22                  adjuvants in innate and in receptors that is

1 fairly new information.

2 The complexity of the signaling  
3 pathways is clearly evident. And these  
4 insights provide the potential to further  
5 dissect and more important to direct immune  
6 responses.

7 There is growth, although not all  
8 that great yet, in the numbers and classes of  
9 adjuvanted vaccines entering clinical trials.  
10 And we should learn a lot from these examples.

11 And finally, these developments  
12 offer unprecedented opportunities but they  
13 will require new research and regulatory  
14 approaches.

15 And our goal at NIAID in  
16 cosponsoring this workshop, one of our major  
17 goals is to expand the dialogue that we  
18 already have ongoing with many of you more on  
19 an individual basis to a collective dialogue  
20 on how we can position our research portfolio  
21 to address these issues and facilitate further  
22 vaccine discovery and development.

1           I think it is worth taking just a  
2           couple of moments to kind of review some of  
3           the recent history. And Dr. Fauci and Dr.  
4           Goodman have mentioned the tremendous growth  
5           over the past two decades.

6           It was just about 20 years ago  
7           when Charlie Janeway published this monograph  
8           on the Cold Spring Harbor Symposia where he  
9           was musing about what he had termed the  
10          immunologists dirty little secret, the fact  
11          that in animal models immunologists knew that  
12          purified proteins rarely generated an immune  
13          response. And when one was demonstrated, it  
14          was usually weak.

15          What you needed was the addition  
16          of an adjuvant, and at that time, it was  
17          usually Freund's adjuvant, to generate robust  
18          immune responses. And what Charlie posited  
19          was that immune receptors will be discovered  
20          that would recognize generalized structural  
21          patterns in molecules found on microorganisms  
22          but not in mammalian cells.

1                   And it was about ten years later  
2                   that he and Ruslan Medzhitov demonstrated that  
3                   was actually the case with the discovery of  
4                   the Toll-like receptors in mammalian cells.  
5                   And that triggered the explosion of growth and  
6                   publications in this area that Dr. Fauci  
7                   already mentioned.

8                   So there are a number of new  
9                   insights and emerging opportunities that are  
10                  quite recent over the past six months or so in  
11                  fact. We now know that alum signals via the  
12                  NLRP3 inflammasome. And this insight is  
13                  really a wonderful example of basic research  
14                  answering questions that had been rather murky  
15                  for decades.

16                  And the fact that alum is now  
17                  known to signal through a particular innate  
18                  immune receptor and pathway provides a  
19                  tremendous opportunity for growth in adjuvant  
20                  engineering, the design of specific adjuvant  
21                  combinations that signal through distinct but  
22                  complementary pathways and the like.

1                   We have new technologies published  
2                   only in the last year or so that reveal that  
3                   vaccine responses are far more robust than  
4                   previously appreciated. For example, flu  
5                   vaccine elicits unexpectedly high number of  
6                   flu-specific B cells, roughly about six  
7                   percent of circulating B cells if measured at  
8                   an appropriate time after vaccination.

9                   And similarly, smallpox vaccine  
10                  elicits unexpectedly high number of CD8-  
11                  positive T cells, almost 40 percent of  
12                  circulating T cells. And these kind of tools  
13                  for immune profiling coupled with systems  
14                  biology approaches and transcriptional  
15                  profiling may provide a variety of new  
16                  opportunities for dissecting and directing the  
17                  immune response.

18                 Here's just one example published  
19                 last summer from the group at Novartis looking  
20                 at the transcriptional profiles and cytokine  
21                 activity of mouse muscle cells and the  
22                 inflammatory cells in those muscles triggered

1       either with MF59, CpG, or alum -- and you can  
2       see the Venn diagrams show a surprisingly  
3       distinct set of genes upregulated by each of  
4       these with some degree of overlap.

5               And then on the right Venn diagram  
6       a combination of MF59 and CpG versus MF59  
7       alone or CpG alone. So the tools for immune  
8       profiling and transcriptional profiling are  
9       tremendous.

10              Another study that came out just  
11       this week from the Emory group and Institute  
12       of Systems Biology in Seattle took a slightly  
13       different approach looking at yellow fever  
14       vaccine and the correlates of immunogenicity  
15       after yellow fever vaccination. And I think  
16       Bali Pulendran will probably speak about that  
17       later.

18              So the potential utility of  
19       transcriptional immune profiling is obvious I  
20       think. We have great opportunities to  
21       identify correlates of vaccine safety and  
22       efficacy, to disassociate drivers of

1 protective immunity from toxicity and reactive  
2 genecity.

3 And to adjust and optimize antigen  
4 adjuvant content and formulation to achieve  
5 these goals. And explore and compare  
6 responses across species, in vitro versus in  
7 vivo.

8 And in special populations we have  
9 unique problems in vaccinating the very young  
10 and the very old. And perhaps this type of  
11 transcriptional immune profiling will help us  
12 identify approaches that would be more  
13 effective in these populations.

14 So to sum things up, I want to  
15 reaffirm the commitment that Dr. Fauci voiced  
16 earlier to supporting fundamental research at  
17 the interface between innate and adaptive  
18 immunity, in particular to enhance the  
19 understanding of the biochemistry and the  
20 biophysics and formulation issues and how they  
21 influence adjuvant activity.

22 It is a topic that NIAID has not

1 supported all that substantially. And  
2 industry has supported much more robustly.  
3 But there is an important role for academic  
4 scientists in this area as well.

5 We are committed to enlarging the  
6 pipeline of potential adjuvants and developing  
7 safer and more potent adjuvants. And finally  
8 to supporting a highly-trained cadre of  
9 investigators and providing them with the  
10 tools they need to pursue these cross-  
11 disciplinary approaches.

12 And with that I'll thank you for  
13 your participation today. And we'll begin the  
14 main session.

15 (Applause.)

16 DR. SLATER: Thank you all very  
17 much.

18 We're now going to begin Session  
19 2. I'm going to ask the Session 2 co-chairs  
20 and speakers to come up to the lecterns.  
21 Session 2, which is our specific adjuvants  
22 overview, will be co-chaired by Dr. Elizabeth

1 Sutkowski and Dr. Bali Pulendran.

2 Dr. Pulendran is from the Emory  
3 Vaccine Center. Dr. Sutkowski is from  
4 CBER/FDA. And they will introduce the  
5 session.

6 DR. SUTKOWSKI: Good morning  
7 everyone. And thank you for coming to this  
8 NIH and FDA cosponsored public workshop on  
9 adjuvants and adjuvanted preventive and  
10 therapeutic vaccines for infectious disease  
11 indications.

12 I'd like to thank Drs. Jay Slater  
13 of CBER and Chuck Hackett of NIAID for asking  
14 me to co-chair the specific adjuvants overview  
15 session along with Dr. Bali Pulendran.

16 I'd like to open by quickly  
17 highlighting a just a few of the initiatives  
18 that have been undertaken in the past few  
19 years regarding vaccine adjuvants and  
20 adjuvanted vaccines. The first entry here is  
21 a reminder that exactly six years ago today,  
22 on December 2nd and 3rd of 2002, CBER co-

1 sponsored a two-day workshop together with the  
2 Society of Toxicology on the nonclinical  
3 safety evaluation of vaccines in general in  
4 which a couple of talks were on adjuvants or  
5 adjuvanted vaccines.

6 Then came the WHO guidelines on  
7 nonclinical evaluation of vaccines, which was  
8 published in 2003. And it contained a special  
9 consideration section that focused on  
10 adjuvants. And then in 2005, the EMEA  
11 published a guideline that was dedicated  
12 specifically to vaccines adjuvants which was  
13 quickly followed by a note on immunomodulators  
14 in 2006.

15 And now we have this two-day  
16 workshop on adjuvants alone and adjuvanted  
17 vaccines. So we've come a long way.

18 In the EMEA's guideline and  
19 explanatory note, adjuvants are called  
20 adjuvants if they are included in the  
21 formulation with the antigen but they are  
22 called immunomodulators if they are given

1           separately from the antigen, whether given at  
2           the same time or at a different time.

3                       It should be noted, however, that  
4           although there is the distinction in their  
5           names, the principles of the EMEA guideline on  
6           adjuvants published in `05 apply to both  
7           adjuvants and immunomodulators.

8                       In the next few slides, I'd like  
9           to go over just a couple of definitions and  
10          regulations. Our office, the Office of  
11          Vaccines Research and Review, or OVR,   
12          regulates the preventive and therapeutic  
13          vaccines for infectious disease indications.

14                      This is in contrast to therapeutic  
15          vaccines for other types of indications such  
16          as cancer vaccines. Those vaccines would be  
17          regulated by OCTGT, the Office of Cell,  
18          Tissue, and Gene Therapy within CBER.

19                      And since they are targeted for a  
20          different patient population than most  
21          preventive vaccines are targeted for, they  
22          would likely result in a different risk versus

1 benefit assessment.

2 As far as definitions of adjuvants  
3 go, we, in the Office of Vaccines in CBER,  
4 would define adjuvants as agents added to or  
5 used in conjunction with vaccine antigens to  
6 augment or potentiate and possibly target the  
7 specific immune response to an antigen.

8 It is also important to point out,  
9 as was already mentioned, that in the U.S.  
10 adjuvants alone are not currently licensed as  
11 such but rather each specific antigen plus  
12 adjuvant formulation is licensed as one  
13 adjuvanted vaccine.

14 With respect to vaccine regulatory  
15 requirements, the IND regulations are covered  
16 under Section 312 of the Code of Federal  
17 Regulations, or CFR. And these include the  
18 items that are required to be an  
19 investigational new drug application or IND.

20 For example, the chemistry  
21 manufacturing and control or CMC information  
22 and the pharmaceutical. tox. information,

1           which specifically should include data from in  
2           vivo or in vitro studies on the basis of which  
3           it can be concluded that the product is safe  
4           for use in humans.

5                       The licensure-relevant regulations  
6           are covered in Section 610 of the CFR.  And,  
7           for example, these include the requirements  
8           that were already mentioned by Dr. Baylor for  
9           lot release, potency, general safety,  
10          sterility, purity, and identity, et cetera.

11                      Also in this Section 610 are the  
12          regulations that are specifically relevant to  
13          adjuvants.  And these include those under  
14          Section 610.15 on constituent materials, which  
15          includes ingredients, preservatives, diluents,  
16          and adjuvants and states that like all other  
17          vaccine components, adjuvants shall meet  
18          generally accepted standards of purity and  
19          quality.

20                      This means that for clinical  
21          studies a certificate of analysis for the  
22          adjuvant would need to be provided to the IND

1 and is often also provided to a cross  
2 reference master file for the adjuvant. This  
3 regulation also states that an adjuvant shall  
4 not be introduced into a product unless there  
5 is satisfactory evidence that it does not  
6 effect adversely the safety or potency of the  
7 product.

8 This will be the topic of the  
9 session that will follow this session that is  
10 going to occur soon. This following session  
11 will be later today after lunch. And also, of  
12 course, the clinical session would address  
13 this as well.

14 So as far as the product-relevant  
15 data that is required to be submitted in an  
16 IND, it should include sufficient information  
17 regarding the adjuvant and the adjuvanted  
18 vaccine formulation. This routinely includes  
19 info on the source of the products, how they  
20 are purified, the general QC testing, and  
21 product-specific QC testing conducted, as well  
22 as lot release and stability data, if

1 available.

2 For an adjuvanted vaccine, this  
3 testing would include an assessment of antigen  
4 and adjuvant content in the final formulation  
5 and of the particle size distribution for the  
6 adjuvant, for example, if appropriate, as well  
7 as an assessment of the integrity of the  
8 antigen adjuvant mixture upon storage.

9 It is also helpful when a sponsor  
10 provides functional information on the  
11 adjuvanted vaccine formulation to include the  
12 rationale for including the various components  
13 and the rationale for the particular dose of  
14 adjuvant if such data are available from pilot  
15 studies, for example.

16 Also sponsors are encouraged to  
17 demonstrate that the product causes an immune  
18 response in animals and to demonstrate immune  
19 response enhancement by the adjuvant.

20 So having said that, the goals of  
21 this session are to provide updates on how  
22 several different types of adjuvants are

1 thought to work. We've invited several  
2 speakers to provide information on how  
3 specific adjuvants activate both innate and  
4 adaptive immune systems and to discuss their  
5 lessons learned with respect to how well  
6 animal studies predict human responses and  
7 their experiences regarding formulation  
8 issues.

9 So without further delay, I'd like  
10 to just now invite the co-chair of this  
11 session, Dr. Bali Pulendran, to provide a few  
12 introductory remarks. He is a professor at  
13 Emory in the Emory Vaccine Center. And his  
14 area of expertise is the innate immune system.

15 DR. PULENDRAN: Thank you very  
16 much, Liz.

17 Good morning. I'd like to thank  
18 the organizers for inviting me to participate  
19 in this very interesting and exciting  
20 workshop. And basically I'd like to introduce  
21 the speakers for this Session 2.

22 And as Liz mentioned, the goal of

1 the session is to stimulate discussion about  
2 adjuvants, what is known and what we would  
3 like to know about the biology underpinning  
4 the mechanism of action of adjuvants and also  
5 what we would like to know about the  
6 mechanisms that might mediate the toxicity --  
7 mediated by some of these adjuvants.

8 So just to sort of set the tone  
9 from a historic perspective, if you take stock  
10 of the major vaccines that have been made  
11 since Edward Jenner's smallpox vaccine in 1798  
12 right through to the first recombinant  
13 vaccines to be licensed, say, for example, the  
14 Hepatitis B vaccine, what I find very  
15 interesting about this slide is that despite  
16 the success of many of these vaccines, we  
17 really do not understand the mechanisms by  
18 which they stimulate immune responses, okay.

19 Why? Because most of these  
20 vaccines have been made empirically. So the  
21 notion that these induce strong immune  
22 responses is really driven by empiricism and

1 by what we see.

2 So given recent advances in  
3 immunology and innate immunity that Dr. Fauci  
4 and Dr. Rotrosen and others have spoken about,  
5 the question is to what extent we can  
6 deconstruct some of these empirically-derived  
7 successful vaccines. And to what new insights  
8 can be gain from such deconstruction that  
9 might be useful in designing new and emerging  
10 vaccines, okay.

11 So one of the dilemmas that  
12 vaccinologists have is that if you look at the  
13 timeline and if you go from Jenner's smallpox  
14 vaccine right the way through to the first  
15 recombinant vaccine, even though the vaccine  
16 purity has progressively increased with time,  
17 we also see that there is an increasing  
18 requirement for exogenous adjuvants, okay.

19 Now all of us in this room know  
20 why this is the case in hindsight but this was  
21 not so obviously as recently as ten years ago.  
22 I don't think any one of us could have told

1           ourselves why is it that some of these highly  
2           successful vaccines are successful.

3                         Well, we now know that innate  
4           immunity, the so-called science of adjuvants  
5           has really demystified this area of adjuvant  
6           research, which is a bit like a witch's brew  
7           but now with all the new insights about Toll-  
8           like receptors, C-type lectins, NOD-like  
9           receptors and so on that we're going to hear  
10          much more about from Drs. Bruce Beutler and  
11          Fabio Re and other this morning.

12                        And the idea that cells of the  
13          innate immune system like dendritic cells  
14          macrophages play an absolutely key role in  
15          sensing vaccines and adjuvants and then  
16          translating this information into useful or  
17          productive immune responses.

18                        So these insights are now  
19          beginning to guide the future, development of  
20          new adjuvants and vaccines. So just as a point  
21          of example, a few years ago in my lab we  
22          demonstrated that this highly successful

1 vaccine, the yellow fever vaccine, which is,  
2 in fact, a live virus was working because it  
3 was engaging multiple Toll-like receptors.

4 Toll-like receptor 9, 8, 7, and 2.  
5 And here was a vaccine that had been in use  
6 for the past 70 years or so given to 600  
7 million people globally. And it was engaging  
8 good old Toll-like receptors.

9 So in a sense, one might make the  
10 argument that Toll-like receptor ligands,  
11 indeed a combination of TLR ligands has  
12 already been licensed for use to be given,  
13 okay. So deconstructing some of these  
14 vaccines has been very fruitful.

15 Another example that the innate  
16 system does not work simply through Toll-like  
17 receptors comes from the work of Dr. David  
18 Nemazee and Dr. Bruce Buetler who showed that,  
19 in fact, some of the adjuvants that are used  
20 in animals but also in humans, for example  
21 alum, does not engage TLRs or do not require  
22 TLRs for the induction of antibody responses.

1 And I think we're going to hear more about  
2 this from Bruce and from Fabio Re.

3 Now the other side of the coin has  
4 been something that we've neglected. So  
5 immunogenicity is one thing and we are  
6 beginning to apply innate immunity trying to  
7 figure out how to make immunogenicity better.  
8 But I think we have been relatively negligent  
9 about the other side of the coin, which is  
10 toxicity.

11 So some of the questions that I  
12 think we should focus on are number one, what  
13 are the mechanisms that mediate vaccine  
14 toxicity? Number two, are these mechanisms  
15 similar to those that mediate vaccine  
16 immunogenicity or are they quite distinct.

17 So, for example, last year in  
18 Science there was a paper that showed that  
19 MPLA, which is TRL4 ligand activates mostly  
20 the TRIF pathways signaling. And that this  
21 might account for the reduced toxicity of MPLA  
22 relative to some of the other TRL4 ligands.

1                   Another question is to what extent  
2                   can toxicity measurements in animal models be  
3                   extrapolated to humans? And here, you know,  
4                   what comes to mind is the fact that these  
5                   innate immune receptors showed differential  
6                   expression profiles in mice versus humans.  
7                   And so, for example, TLR9 is expressed only on  
8                   human PDCs whereas it has a much broader  
9                   express profile in humans.

10                   And so how does this impact the  
11                   evaluation of toxicity profiles between these  
12                   two species. This is the key, key area which  
13                   I think Dr. Bob Coffman will address tomorrow  
14                   in his discussion.

15                   And then finally, to what extent  
16                   do formulations and delivery systems impact on  
17                   the toxicity of adjuvants in vaccines. So,  
18                   for example, if you have nano particles or  
19                   ISCOMS that target antigen presenting cells,  
20                   does this mitigate the indiscriminate  
21                   bystander activation of undesirable cells of  
22                   the immune system, okay.

1           So this is an area that is under  
2 active research. And Eugene Maraskovsky and  
3 Derek O'Hagan will address this issue.

4           So with that said, let's move on  
5 with the agenda. Here it is. We have eight  
6 presentation -- actually seven presentations.  
7 Firstly Fabio Rey will talk about the  
8 activation of the inflammasome by adjuvants.

9           This will be followed by two talks  
10 on liposomes, micro particles -- first one by  
11 Derek O'Hagan and the second one by Eugene  
12 Maraskovsky on ISCOMS. And then we'll have a  
13 coffee break and then Bruce Beutler will tell  
14 us about TLRs and how they regulate vaccine  
15 responses.

16           This will then be followed by  
17 Nathalie Garçon who will talk about adjuvant  
18 development from an industry perspective. And  
19 then Dr. Geert van den Bossche from the Gates  
20 Foundation will tell us how to use adjuvants,  
21 the perspective from the Gates Foundation.

22           And then finally I will give a few

1           comments on the possible synergy between TLRs  
2           and CLRs and the applications of systems  
3           biology in predicting the immunogenicity of  
4           vaccines.

5                        So with that, I think we can move  
6           on with the next speaker who is Fabio Re from  
7           the University of Tennessee.

8                        Fabio.

9                        DR. RE: Good morning and I would  
10          like to start by thanking the organizer, in  
11          particular Elizabeth and Bali for the  
12          invitation and the opportunity to show you  
13          some of our results still unpublished  
14          regarding deactivation of the NALP3  
15          inflammasome by different adjuvants.

16                       And as we heard before by Dr.  
17          Rotrosen, 20 years ago Charlie Janeway would  
18          famously declare adjuvant immunology's dirty  
19          little secret. And we heard that from that  
20          time that immunity has really bloomed in great  
21          part thanks to Janeway.

22                       And we have learned how some of

1 the adjuvants work or start to understand how  
2 they work, in particular adjuvant-like  
3 microbial products which clearly stimulate  
4 pathway recognition receptor.

5 We -- the general assumption  
6 should be that these substances that act as  
7 adjuvants may mimic biological activities  
8 which are associated with live pathogens. And  
9 that is clearly the case with microbial  
10 products.

11 We know much less about a whole  
12 variety of other substances that works as  
13 adjuvant, in particular particulate adjuvant,  
14 we know very little about how this molecule  
15 works until recently.

16 So particulate adjuvant comprised  
17 a wide variety of substances, including solid  
18 carrier particle such as polystyrene  
19 microsphere, chitosan. Chitosan, I'll show  
20 a little bit about chitosan. Chitosan is, as  
21 you may probably know, a fragment of the  
22 exoskeleton of crabs, basically a

1 polysaccharide, alum, you are all familiar  
2 with, the immune stimulatory complex, which  
3 are lipid particles and saponin, which is  
4 QuilA, QS21, emulsion particles, such as the  
5 adjuvant MF59.

6 So the proposed mechanism of  
7 action for this class of adjuvants, particular  
8 adjuvants at least the most cited is the so-  
9 called antigen depot theory. So what is  
10 believed is that the antigen, by absorbing to  
11 the particle of adjuvant, would lead to an  
12 increased stability and concentration of the  
13 antigen at the injection site.

14 This would prolong the time of  
15 interaction between the antigen and antigen  
16 presenting cells. This would also enhance the  
17 antigen uptake, being a particle, through  
18 phagocytosis or endocytosis. And finally,  
19 also importantly, would ensure the delivery of  
20 antigen and adjuvant to the same antigen  
21 presenting cells.

22 Now these are clearly -- these

1 different effects are clearly responsible for  
2 the adjuvant property of these different  
3 substances, however, the antigen depot theory  
4 has been challenged by quite a few reports.  
5 What is being shown, for example, is that it  
6 is not really true that the antigen remain and  
7 the antigen concentrate and stability is  
8 increased at the injection site.

9 For example, it has been shown  
10 that the antigen elude pretty quickly from the  
11 adjuvant particle. Also it has been shown  
12 that you can still elicit an immune response  
13 even if you inject antigen and alum separately  
14 if you use enough antigen concentration.

15 So that suggests that other  
16 mechanisms may also be involved in the  
17 mechanism of action of these substances. So  
18 among the particular adjuvant, alum is clearly  
19 the most successful one. And, as we heard  
20 before, the only one that is really approved  
21 by FDA in the United States.

22 So these are alum. These are

1 crystals of aluminum hydroxide and aluminum  
2 phosphate. Alum promote bias responses with  
3 high IgG1 and IgE titers. And it probably the  
4 major limitation of these antigen, of these  
5 adjuvant, and these prevents its use in those  
6 situations where you would rather have  
7 elicitation of a Th1 type of immune response.

8 So what is the mechanism of action  
9 of alum? As I said, the most believed is that  
10 antigen depot theory with the caveat that I  
11 mentioned before. So suggesting that other  
12 mechanics may also account for the activity of  
13 alum.

14 Alum is being tested by many  
15 different laboratories and it clearly does not  
16 activate Toll-like receptor and does not  
17 induce dendritic cells maturation.

18 So among the other activity that  
19 has been ascribed to alum, which may well  
20 account for its adjuvant ability, is the  
21 ability of alum to fix complement. It has  
22 also been illustrated that alum injection

1 result in formation of a granuloma containing  
2 antibody-producing plasma cells. And in some  
3 cases, in some extreme cases, this may  
4 actually result in a sterile abscess. So it  
5 is clearly an inflammatory reaction at the  
6 site of injection caused by alum.

7 Alum has been demonstrated to  
8 induce -- injection of alum induce influx of  
9 neutrophils and interleukin-4 expressing  
10 eosinophils in the spleen and these cells are  
11 then being shown to be able to prime D cells.

12 What is interesting is that it has  
13 been demonstrated that in IL4 not compromised  
14 or otherwise IL4 nonresponsive animals, mice,  
15 alum induced only a Th2 response but also Th1  
16 response, suggesting that IL4 has been already  
17 known down-regulate the Th1 response.

18 And finally something that has  
19 been known for some time is alum induces  
20 necrosis at the injection site. And in the  
21 second part of the talk, we will see a little  
22 bit about necrosis and how these may actually,

1 indeed, be mechanisms of action of alum.

2 So one question that was clearly  
3 important to address and that many labs try to  
4 address is does alum mimic features of  
5 pathogens. And so the way we -- that would  
6 mean does alum activate any pattern  
7 recognition receptor.

8 And so as you all know, innate  
9 immunity relies on the ability of cells to  
10 recognize microbial products and endogenous  
11 danger signals through classes family of  
12 pattern recognition receptors, which can be  
13 soluble, it can be expressed on the cell  
14 surface, can be expressed in the cytoplasm.

15 And these recognition events would  
16 trigger a signaling event which lead to  
17 production of a wide variety of inflammatory  
18 molecules and to the reprogramming of the  
19 antigen-presenting functions of the dendritic  
20 cells and other antigen-presenting cells,  
21 which eventually culminate in the antigen  
22 process and presentation and activation of

1           that immunity.

2                        So among pattern recognition  
3 receptors, the mouse studies -- the first  
4 correct rising details are the Toll-like  
5 receptors, which are expressed on the cell  
6 surface or in the endosoma compartment of  
7 several different cell types.

8                        And they recognize microbial  
9 products or endogenous danger signals and  
10 activate signally pathway, most notably the NF  
11 kappa B, the MAP kinase, and interferon  
12 reconstructor pathways which lead to a  
13 transcriptional response without regulation  
14 of a wide variety of proinflammatory  
15 mediators, including here -- and I put here  
16 also including the cytokine belonging to  
17 interleukin-1 family.

18                       Now more recently, too, a family  
19 of pattern recognition receptors has come to  
20 prominence. One is the RIG-like helicases,  
21 which detect viral genomes in the cytoplasm of  
22 cells in contrast to Toll-like receptor which

1 really scanned the extracellular and endosomal  
2 compartment.

3 And the pathway activated by RIG-  
4 like helicases are largely similar and  
5 overlapping with those of TLR, including NF  
6 kappa B, MAP kinase, and interferon  
7 reconstuctor, again leading to production of  
8 inflammatory mediation.

9 Another family of pathway  
10 recognition receptor is the NOD-like receptor  
11 or also known as nucleotide binding leucine-  
12 rich repeat containing receptor. These are  
13 expressing also in the cytosol of cells and  
14 they recognize, again, microbial product or  
15 danger signal.

16 And in contrast to Toll-like  
17 receptor and RIG-like helicases, the pathway  
18 they activate is not really the MAP kinase and  
19 NF kappa B but rather they, as far as we know  
20 right now, they use as an effector molecule  
21 Caspase-1, lead to activation of caspase-1,  
22 and activation of these proteases is a key

1 step for the secretions of the interleukin-1  
2 family of cytokines. And this would be  
3 interleukin-1 beta, interleukin-18, and  
4 interleukin-33.

5 So this slide illustrates the  
6 architecture of these molecules that are over  
7 more than 20 NLRP member in humans.

8 They are correctly classified  
9 central domain, which is a nucleotide binding  
10 domain, a leucine-rich repeat domain, and an  
11 end terminal domain which is either -- which  
12 can be classified into a pyridine domain so  
13 the nomenclature for this family of molecules,  
14 which was quite confusing, is now called this  
15 NLRP if they contain a pyridine domain and the  
16 end terminals or NLRC if they contain a CARD  
17 domain. This is a caspase activational  
18 recruiting domain such as IPAF.

19 So the most studied NLRP family  
20 members are IPAF and NLRP3. And these are the  
21 ones that I will talk in more detail today.  
22 Also NOD1 and NOD2 have received a lot of

1 attention although it is still not clear how -  
2 - whether they are really part of an  
3 inflammasome.

4 So I didn't introduce the  
5 inflammasome yet so activation of caspase-1  
6 mediated by these NLR molecules, of course in  
7 the context of a multi-protein complex which  
8 has been termed the inflammasome.

9 And these illustrate the  
10 composition of the inflammasome that is better  
11 studied right now is the NLRP3 inflammasome.

12 Again, this is also -- this  
13 molecule is also known as NALP3 or cryopyrin  
14 or CIAS1. There are quite a few names. So  
15 the way we think the inflammasome is activated  
16 is is illustrated here.

17 So it is believed that in the  
18 resting state, this molecule is inactive by  
19 probably an intramolecular interaction between  
20 the leucine-rich domain and that NALP domain  
21 and the nucleotide-binding domain.

22 In reference to recognition of

1 ligand, and there is a big question mark here  
2 -- what are these ligand, and if we have time,  
3 we may go into that in detail, there is  
4 oligomerization of these molecules, which  
5 recruit an adaptor molecule called ASC, which  
6 then bridge and activate and recruit also  
7 caspase-1, which is the effector molecules.

8 This brings to activation of  
9 caspase-1, which then proteolytically cleave  
10 pro-interleukin-1-beta, pro-interleukin-18,  
11 and pro-interleukin-33 to give the mature form  
12 of of interleukin-1-beta, which is now being  
13 secreted.

14 So secretion by a synthesis of  
15 interleukin-1 family then requires at least  
16 two key steps, one is the induction of the  
17 messenger and the pro-immature protein, which  
18 is triggered by classical inflammatory  
19 stimuli, in particular Toll-like receptor, and  
20 then activation of these inflammasomes, which  
21 is regulated by these NLR molecules.

22 This slides summarize what we know

1           so far about these NLR molecules, at least the  
2           one that activates inflammasomes. So NLR1 and  
3           NAP1 activate inflammasome in reference to  
4           anthrax leukotoxin, IPAF, or as it is known  
5           now, NLRC4 mediate inflammasome activation in  
6           reference to a wide variety of intercellular  
7           bacteria or bacteria that possess type 3 or  
8           type 4 secretion systems.

9                           And it has been proposed that the  
10          actual ligand might be the flagellin, which --  
11          a immunometric which is injected through these  
12          types of secretion systems into the cytosol  
13          cells.

14                          And finally now, three cryopyrin  
15          or NLRP3, and I will call these molecules  
16          NLRP3 from now on, which is activated and  
17          inflammasome activation in reference to a wide  
18          variety of product. You have intercellular,  
19          and otherwise bacterias which activate this  
20          pathway.

21                          You have muramyl dipeptide. This  
22          is a breakdown product of the fetal ligand

1           which, by the way, has also been demonstrated  
2           to be an adjuvant. This is actually the  
3           active compound of Freund complete adjuvant  
4           which has been demonstrated to activate these  
5           pathways.

6                           It is also interesting that MDP  
7           has also been shown to activate also NLRP1 as  
8           well as NOD2. So this molecule may act on  
9           different -- target different NLR molecules.

10                          Recently it has been demonstrated  
11           that several particles such as asbestos fiber  
12           or silica particles activate this pathway,  
13           thus explaining the activities of these  
14           compounds -- the proinflammatory activity,  
15           which we've known for a long time about is  
16           compounds.

17                          Extracellular ATP has also been  
18           demonstrated to activate this pathway. This  
19           would represent an endogenous danger signal.  
20           So a molecule that is released -- for example  
21           when cells die by necrosis -- and therefore,  
22           it is believed the immune system learned to

1 recognize these as a danger signal and,  
2 therefore, activate pathway that is protected.

3 And importantly, NALP3  
4 inflammasome has been demonstrated to be  
5 activated by monosodium urate crystals. So  
6 these are the crystals that accumulate --  
7 these are urate crystals which accumulate in  
8 the joints of people with gout disease. And  
9 it has been known for a long time this was a  
10 chronic inflammatory disease and nobody really  
11 knew how this works.

12 So the group in Lysine published  
13 these observations. And that is what really  
14 triggered our interest in the inflammasome and  
15 in the alum. So the question that we asked is  
16 well these are what is important, the urate  
17 has to be a crystal -- has to crystalize.

18 Soluble urate would not activate  
19 this pathway so it isn't -- and, in general,  
20 it is now clear that several different  
21 particles and crystals are able to activate  
22 this pathway. So what we ask ourselves is

1 well, does alum, which is a crystal, activate  
2 this pathway. And that is clearly the case.

3 So what we do here, we stimulate  
4 human PBMCs with different combinations of  
5 alum and LPS and then measure production of  
6 natural IL-1 beta in the culture supernate.  
7 And as you can see here, we are using three  
8 different formulations of alum. This would be  
9 aluminum phosphate, aluminum hydroxide, and  
10 this is the alum inject stuff you buy from  
11 peers which may turn out to be not really  
12 aluminum.

13 So in any case, when you stimulate  
14 cells with this compound alone, you don't have  
15 any production of IL-1 beta. When you  
16 stimulate cells with a clean preparation of  
17 LPS at low concentration, you have an  
18 negligible amount of IL-1 beta release.

19 However when you add LPS together  
20 with the different alum, you have a robust  
21 production of IL-1 beta. So this response is  
22 blocked by an inhibitor of caspase-1, showing

1 specificity for the inflammasome.

2 You can achieve the same effect if  
3 you proliferate the cells with a different  
4 Toll-like receptor agonist. This would be a  
5 synthetic lipopeptide that activates TLR2 and,  
6 again, in the presence of alum you have  
7 production of IL-1 beta.

8 So the activation of the  
9 inflammasome by alum is blocked by  
10 cytochalasin B, suggesting that phagocytosis  
11 of the alum particle is required for  
12 activation of this pathway in contrast, for  
13 example, to the response -- the activation of  
14 the pathway by ATP which is now sensitive to  
15 cytochalasin B and also occurs with a  
16 different kinetics.

17 You may look at the other cytokine  
18 belonging to interleukin-1 family, IL-18, and  
19 you will find again that the cytokine, the  
20 mature form, is produced in cells stimulated  
21 with LPS plus alum.

22 So this slide illustrates from a

1           biochemical point of view the activation of  
2           the inflammasome. So caspase-1 is also  
3           produced in an inactive, immature form, which  
4           is then self cleaved into an active p20 and  
5           p10 sub unit so the presence of the p20  
6           caspase-1 sub unit in culture is a measure of  
7           activation of caspase-1.

8                     And, again, you see that caspase-1  
9           -- the mature caspase-1 is present only in  
10          cells stimulated with LPS plus alum. This is  
11          blocked by the caspase-1 inhibitors. And  
12          interestingly, you see that alum alone,  
13          without any LPS, is also able to activate  
14          caspase-1.

15                    And down here, you have also a  
16          demonstration of the cleavage and maturation  
17          of IL-1 beta. So again when you look in the  
18          cell lysis of cells stimulated with LPS in the  
19          presence of -- or with or without alum, you  
20          will see a similar amount of pro-IL-1 beta.  
21          This is the immature form of the proteins.

22                    But then when you look in the

1 culture supernate and you will find the  
2 mature form only in cells illustrated with  
3 alum and LPS. Again, caspase-1 inhibitor  
4 blocked the processing. And importantly, alum  
5 alone does not induce any IL-1 beta  
6 production.

7 So the big question -- so we  
8 published those results and then the big  
9 question was which NLR molecule mediated a  
10 response inflammasome activation by alum. And  
11 so to make a long story short, it took us a  
12 long time to get the mice.

13 So I pair up with -- I team up  
14 with Jenny Ting who had the mice and send us  
15 some bone marrow of mice deficient in the ASK  
16 molecule. This is the adaptor which links NLR  
17 to caspase-1 and then mice deficient in NALP3,  
18 NALP3 cryopyrin or NLRC4, which is IPAF, and  
19 asked -- we made dendritic cells out of these  
20 cells and stimulate them, measuring IL-1 beta.

21 And as you can see, dendritic  
22 cells derived from wild-type mice or IPAF,

1 NLRC4-deficient mice, are still able to  
2 produce mature IL-1 beta in response to LPS  
3 hydrogel and our cells deficient in ASK and  
4 NALP3 are unable to produce IL-1 beta.

5           These are just the specificity of  
6 controls so these are cells infected with  
7 listeria monocytogenes, which is known to  
8 activate NALP3. And, indeed, you lose the  
9 response in NALP3 and NLRP3 knockout cells  
10 and, in contrast, salmonella, which mediate  
11 activation of the inflammasomes through IPAF  
12 is still working in NALP3 knockout cells.

13           You look at other cytokines, such  
14 as IL-6, which do not depend on the  
15 inflammasome, and you will see that they  
16 express an equal amount between what are now  
17 NALP3 knockout cells.

18           ASK cells consistently have lower  
19 amount of these and other cytokines, which  
20 probably suggests that ASK may be involved in  
21 other pathways other than inflammasome  
22 activation

1                   This slide, again, simply is a  
2 demonstration of activation of the  
3 inflammasome at the biochemical level and we  
4 can skip through this one.

5                   So what we asked is how common is  
6 activation of this pathway by adjuvants which  
7 look at other adjuvants, in particular  
8 chitosan, as I mentioned -- these are the  
9 fragments of the exoskeleton of crabs -- and  
10 QuilA -- this is attracted by tree bark.

11                   And as you can see, you have a  
12 combination of LPS plus chitosan and quillaja  
13 results in production of IL-1 beta. This is  
14 blocked by the inhibitor caspase-1, similarly  
15 for IL-18. And then when you look in the  
16 mice, again you see that this response,  
17 secretion of IL-1 beta or IL-18, in reference  
18 to LPS chitosan or LPS Quil, again it is lost  
19 in NALP3 knockout mice.

20                   So here we have at least three  
21 adjuvants and a fourth one if you consider  
22 IMDp, which are known as -- now have been

1 demonstrated to activate a NALP3 inflammasome.  
2 And what is interesting to note is that this  
3 cytokine, IL-1 beta, IL-18, and IL-33 have all  
4 been associated with the Th2-type of response.

5 IL-1 beta has been demonstrated to  
6 be an adjuvant which suggests that they may  
7 play an important role for -- in the action of  
8 alum, which is also a Th2-type of immune  
9 stimulator.

10 And then finally we asked the big  
11 question -- is alum still an adjuvant in IL-3  
12 knockout mice? So what we did here we  
13 vaccinated mice with a commercial vaccine.  
14 This is a pediatric diphtheria, tetanus toxoid  
15 which is adjuvanted by alum or with a homemade  
16 vaccine which is avomine absorbed to aluminum  
17 hydroxide.

18 And as you can see, then we  
19 measure total IgE or antigen-specific IgG1  
20 production. As you can see, this response is  
21 reduced in the NALP3 knockout mice. However,  
22 it is not completely abrogated, suggesting to

1 us that other mechanics are also responsible.

2 So at the same time, we published  
3 our observation, the group of Richa Shlavel  
4 also reported similar results in this paper.  
5 What they see, in contrast to our result, is  
6 a complete lack of response in the NALP3  
7 knockout mice.

8 More recently, two other groups --  
9 Shaw Group and Gabrielle Nuniz in Michigan  
10 they also reported activation of the the NALP3  
11 inflammasome by alum in contrast to our  
12 result. So the -- so the Shaw Group saw a  
13 difference in the IgE -- different than we saw  
14 but not much in IgG1. More interestingly, the  
15 saw an actual increase in the production of  
16 IgG2c, which is a Th1-associated type of  
17 hemoglobin, suggesting that if you lack NALP3  
18 inflammasome activation and the four you lack,  
19 IL-1 beta, IL-18, IL-33 production, you may  
20 skew the response to Th1.

21 And finally, the group of  
22 Gabrielle Nuniz didn't observe an appreciable

1 difference in vivo for the vaccination in  
2 mice.

3 So that tells you that there are  
4 clearly other mechanisms that are responsible  
5 for the alum adjuvant effect. And that  
6 clearly one measure or point that comes out of  
7 this is that we need really to standardize  
8 experiment and vaccination protocols.

9 I'm running kind of slow so I  
10 probably should stop, I guess. So let go to  
11 the conclusion and just to leave you with some  
12 open questions, so one thing that is important  
13 to us, at least to my lab, to understand is  
14 what is the role of the interleukin-1 family  
15 in the alum adjuvant effect. As I mentioned,  
16 all these cytokines are known to be adjuvant  
17 and associated to Th2 type of response, which  
18 is the same, which is activated by alum.

19 And also what is the role of  
20 necrosis and release of endogenous danger  
21 signals indicating an adjuvant effect.

22 I'll just briefly mention -- I

1           didn't have time to show you -- it has been  
2           known that alum activate some form of  
3           necrosis, at least in vivo, and necrosis is  
4           associated with release of these endogenous  
5           danger signals.

6                         And we have evidence that -- I  
7           couldn't show you -- that necrosis activate  
8           inflammasomes and also that it has been known  
9           for some time to be an adjuvant necrosis to be  
10          immunogenic. So I think this is another  
11          important area to explore.

12                        So let me thank Hanfen Li, who  
13          follows most of the studies. And Jenny Ting  
14          that initially provided us with the knockout  
15          bone marrow. And finally Vishva Dixit, which  
16          now has given us -- has provided us the mice.  
17          And NIH for and R01 R21 grant.

18                        So I'll take some questions.

19                        (Applause.)

20                        DR. MALONE: Can you take a  
21          question?

22                        DR. PULENDRAN: Yes.

1 DR. MALONE: Robert Malone  
2 speaking as an empirical vaccinologist. Can  
3 you comment on the role of these pathways in  
4 VLP activity? And can you comment on  
5 potential toxicology associated with  
6 inflammasome activation?

7 DR. RE: Well so -- yes, I'll  
8 start with the last one. So the toxicology  
9 implication, I find it interesting that -- so  
10 there are a few syndromes -- out inflammatory  
11 syndromes which are due to a mutation in  
12 NALP3. And so patient with this disease have  
13 mutation in NALP3 which lead to conservative  
14 activation of NALP3, which results in their  
15 symptoms, which are recurrent fevers and skin  
16 rashes and atralgia.

17 So these mutations result in a  
18 conservative active NALP3 pathway. These  
19 patients don't have -- so far, there isn't any  
20 evidence that they have any other disease like  
21 out inflammatory or, you know, there isn't  
22 much evidence on that so I don't know if that

1           may answer your question.

2                        So you may envision using  
3 substances that activate inflammasomes without  
4 that many side effects. This is just a very  
5 naive -- so I'm not sure I -- what was the  
6 first one?

7                        DR. MALONE: (Speaking from  
8 unmiked location.)

9                        PARTICIPANT: If you could please  
10 use the microphone.

11                       DR. MALONE: Viral-like particles.  
12 So part of your thesis is that alum is  
13 activating -- and other crystalline  
14 formulations are activating your inflammasome  
15 activity. And that's contributing to the  
16 potency of the formulation, right?

17                        So virus-like particles clearly  
18 appear to have enhanced potency relative to  
19 non-particular formulations. And so I'm  
20 wondering whether you can comment on whether  
21 VLPs enhanced potency or apparent enhanced  
22 potency may be a consequence of an activation

1 of inflammasome activity.

2 DR. RE: VLP meaning bacterially  
3 lipoproteins?

4 DR. MALONE: Virus-like particles.  
5 Okay. We're in very different fields, I  
6 guess.

7 DR. RE: Yes.

8 DR. MALONE: Remember I'm an  
9 empiric vaccinologist so we use the  
10 terminology VLP to refer to virus-like  
11 particles.

12 DR. RE: Oh, VLP, okay.

13 Yes, it is, you know, it is  
14 clearly -- you know the consensus is that this  
15 inflammasome, at least the NALP3 inflammasome,  
16 is activated by particle. These are crystals.

17 One of the things that have been -  
18 - not all particles activate these  
19 inflammasomes. For example, some  
20 microsphere, polystyrene microsphere, in our  
21 hands do not really activate inflammasomes.

22 So one of the ideas that has been

1 proposed is that there is this frustrated  
2 microphages. So a microphage that tries to  
3 phagocytose a particle, like crystals or  
4 asbestos fiber, which has not really been  
5 prepared to, in nature during evolution, to  
6 phagocytose.

7 And this may lead -- I didn't have  
8 time to go into detail -- to destabilization -  
9 - may lead to destabilization of the lysosome.  
10 And that may lead to release and leakage of  
11 lysosome proteases in the cytokine. That what  
12 might be what activated the inflammasome.

13 So I don't know if viral --  
14 viralized particle could activate  
15 inflammasomes.

16 DR. PULENDRAN: Thank you, Fabio.

17 We're running behind time so if  
18 you can just take a couple of -- two quick  
19 questions. I think you and then you. Thank  
20 you.

21 DR. REED: As far as the mechanism  
22 of alum in producing the responses you talked

1 about -- this is Steve Reed from Infectious  
2 Disease Research Institute -- you showed that  
3 alum alone was inert in terms of inducing IL-1  
4 for example. But alum plus LPS had enhanced  
5 over LPS alone.

6 How is this relevant to a vaccine?  
7 What happens if you put alum on a real vaccine  
8 target? Did you see anything like that? What  
9 is the conclusion? Alum is actually  
10 responsible for this enhancement rather than  
11 just changing the form of LPS?

12 DR. RE: Well, alum -- so it's --  
13 as I show you, you don't require LPS.  
14 Actually I didn't show you. You can, for  
15 example, pre-activate the cells with TNF.  
16 This will lead to production of IL-1 beta and  
17 alum would activate, again, the inflammasome.  
18 Is that what you were asking?

19 So it's really -- and, again, so  
20 the other things that one might ask is what  
21 happened in vivo when we inject alum but there  
22 is no LPS. So one of the things we are

1 thinking is that, for example, IL-18 is  
2 already present and conceivably able to  
3 express in many cell types.

4 So alum alone could be sufficient.  
5 You don't need in that -- for IL-18 -- and  
6 there is evidence -- and it may be the case  
7 also for IL-33 -- you don't need the classical  
8 proinflammatory priming of cells to build up  
9 the pro-IL-1 beta or IL-18 because many cells  
10 express conservatively pro-IL-18.

11 So in that case, alum may induce  
12 or release just on itself IL-18 and IL-18 has  
13 been demonstrated to then trigger  
14 transcriptional activation -- transcription of  
15 IL-1 and IL-33. So that could be a mechanism  
16 in vivo.

17 DR. REED: Thank you.

18 DR. PULENDRAN: One last question.

19 PARTICIPANT: So given the  
20 restriction of the inflammasome, what is the  
21 role that it plays in the secondary versus  
22 primary responses?

1 DR. RE: You know, I can very  
2 little here.

3 PARTICIPANT: Have you look at  
4 secondary responses before for the  
5 inflammasome?

6 DR. RE: Secondary response  
7 meaning --

8 PARTICIPANT: Memory.

9 DR. RE: Oh, no. Yes, no, sorry.  
10 No, we didn't. Not yet.

11 DR. PULENDRAN: Okay. Thank you,  
12 Fabio.

13 We'd like to move on to the next  
14 speaker, who is Derek O'Hagan from Novartis  
15 who is going to be talking about first  
16 generation adjuvants, the use of liposomes and  
17 microparticles.

18 Derek?

19 DR. O'HAGAN: So, good morning.  
20 And I'd just like to start by thanking the  
21 organizers for the opportunity to be here.

22 So I'm going to talk about first

1 generation vaccine adjuvants and I'll define  
2 better what I mean by first generation in a  
3 moment.

4 I wanted to highlight that I'm  
5 going to be talking predominantly in the  
6 context of vaccines for infectious diseases  
7 against to protect against -- not therapeutic  
8 vaccines. So the risk-benefits evaluation is  
9 somewhat different.

10 I was going to start with a slide  
11 that kind of highlighted why we include  
12 adjuvants in vaccines but I'm already starting  
13 to realize that probably every speaker has  
14 their own version. So this may be somewhat  
15 repetitive.

16 But in essence, you know, we  
17 include adjuvants for practical, pragmatic  
18 reasons. You know some of them are very  
19 important in relation to pandemic influenza,  
20 dose bearing, higher titers, responses more  
21 rapidly. And the breadth of response is  
22 really important.

1                   So adjuvants are here because  
2                   vaccine is increasingly purified, soluble  
3                   recombinant proteins, poly immunogenic, we  
4                   need them.

5                   So this is my attempt, after many  
6                   years looking at it, to find some kind of  
7                   classification that we can understand vaccine  
8                   adjuvants. I've tried it many times in the  
9                   past. They define easy definitions, there are  
10                  no two ways about it.

11                  And in relation to generation one,  
12                  what I'm really talking about are the kinds of  
13                  particulate carriers, dispersions,  
14                  particulates. These have been around for  
15                  quite some time.

16                  Aluminum, we've talked about, but  
17                  clearly the most well established, licensed in  
18                  Europe, licensed in the U.S.

19                  Other approaches came along  
20                  somewhat not long after. Freund first brought  
21                  forward water and oil emulsions. And  
22                  interestingly, it was another famous

1       vaccinologist, Jonas Salk, who really looked  
2       at water and oil emulsions with flu vaccines  
3       in the 1950s and made some pretty key  
4       observations that you enhance the response and  
5       you allow a significant dose reduction.

6                So water and oil emulsions were  
7       very effective but their tolerability profile  
8       was not great and not appropriate for  
9       prophylactic vaccines. So oil-in-water  
10      emulsions were developed. Subsequently I'll  
11      talk a lot about those since they are the most  
12      prominent really for new generation adjuvants.

13               Calcium phosphate was on the  
14      market in Europe for quite some time, then  
15      kind of replaced by aluminum. Liposomes are  
16      licensed in Europe to be used with influenza  
17      vaccines. And tyrosine is used for allergy  
18      vaccines.

19               More recent developments include  
20      microparticles and nanoparticles, which I'll  
21      talk about at the end of the talk if I have  
22      time. So many of these technologies have been

1           around for quite some time.

2                   And generation two adjuvants, in  
3           essence they mostly represent the first  
4           generation with something added. So something  
5           added are the kind of things we've started  
6           talking about today already -- TLR agonists,  
7           NLR agonists. The most advanced is AS04. And  
8           Nathalie Garçon will talk about that, I  
9           believe.

10                   But I just wanted to add, you  
11           know, a couple of dates here. The concept of  
12           generation two adjuvants has been with us for  
13           quite some time.

14                   People were adding TLR agonists in  
15           the '60s and the '70s without understanding  
16           what they were, what they did. We just knew  
17           they were immune potentiators.

18                   So they have been around for quite  
19           some time. But obviously it is only  
20           relatively recently with AS04 have they  
21           started to gain acceptance and approval.

22                   And then there are some newer

1 concepts like ISCOMS and IC31. ISCOMS will be  
2 spoken about later.

3 So here I wanted to try and say  
4 what are we looking for, what are we trying to  
5 achieve with vaccine adjuvants. And kind of  
6 on the right-hand side is what we perceive as  
7 ideal. What do we really want?

8 Certainly we want something that  
9 is safe and not associated with any long-term  
10 effects. But also we need it to be well  
11 tolerated. So short-term reactogenicity is a  
12 key issue if you are going to have a  
13 successful adjuvant.

14 Other important factors -- it  
15 needs to be simple, easy to scale up, the  
16 manufacturing needs to be reproducible, and it  
17 needs to be easily characterized and perhaps  
18 these will be discussed a lot more about  
19 characterization.

20 Ideally, it should be made from  
21 abundant, inexpensive components, things that  
22 are readily available and not hugely

1 expensive. These components should be  
2 biodegradable and biocompatible.

3 Ideally the adjuvants should be  
4 compatible with many different antigens if you  
5 are going to develop the adjuvants, if you can  
6 use it broadly, obviously that is beneficial.  
7 And in relation to generation two, if you can  
8 use it as a platform to deliver other  
9 adjuvants, then that is pretty important, too.

10 So this is an adjuvant I've been  
11 involved with for quite some time. I called  
12 it a successful adjuvant. I mean successful  
13 because it is included in licensed products.  
14 I think generally speaking we have many  
15 adjuvants. We're not short of adjuvants.  
16 We're short of adjuvants that have achieved  
17 success in terms of product licensure.

18 So MF59 is an oil-in-water  
19 emulsion. From a pharmaceutical perspective -  
20 - I'm a formulation scientist -- it is  
21 relatively simple. It is a low content of oil  
22 -- I'll say more about the oil in a moment --

1 squalene. It is biodegradable. It has two  
2 nonionic surfactants, which have been broadly  
3 used in a range of alternative products.

4 It has a low viscosity so it is  
5 easy to inject. It is easy to add to other  
6 components, to add to other antigens. And its  
7 size is important. It is 160 nanometers  
8 prepared by microfluidization.

9 So MF59 is a squalene oil-in-water  
10 adjuvant. Arguably, it is most well  
11 established and there are others coming  
12 behind. You'll probably hear about AS03 from  
13 GSK and also AF03 from Sanofi. So there are  
14 other squalene-based adjuvants coming forward.

15 So the major component of MF59 is  
16 squalene. Chemically it is very simple,  
17 C<sub>30</sub>H<sub>50</sub>. Structurally, it is rather more  
18 complex. It is over here. That is what the  
19 structure looks like.

20 But this is a normal metabolite of  
21 all of us. So it is produced by humans. It  
22 is a precursor to cholesterol and steroid

1 hormones. And you see as simplified  
2 biosynthetic pathway here where you end up  
3 with the steroid hormones and cholesterol.

4 So it is synthesized in the liver  
5 and skin. It is secreted in significant  
6 quantities by sebaceous glands. It is used in  
7 a broad range for other purposes. So it is  
8 biodegradable, biocompatible, a normal  
9 component of all of us. So that is a  
10 fundamentally important characteristic we  
11 believe.

12 So I didn't want to go into too  
13 much of the preclinical data. This adjuvant  
14 has been around since the mid-`90s. And there  
15 is quite a lot of experience accumulated in  
16 the preclinical setting.

17 Certainly in the mouse setting,  
18 significant dose reduction, several  
19 hundredfold. Probably the most important data  
20 we generated a long time ago was that it  
21 restores the immune response of old mice. Old  
22 mice, like old people, respond badly to flu

1 vaccines.

2                   You give them the adjuvants. And  
3 their responses back up to what you see in the  
4 young mice. And just out of interest, we  
5 summarize a lot of this experience about the  
6 mouse model, using the mouse model, the  
7 limitations of the mouse model, but how you  
8 can use them optimally in a publication at the  
9 end of last year.

10                   More recently we've shown improved  
11 heterologous and homologous challenge in  
12 ferrets. And we're looking at the pig model  
13 as a large animal model of flu vaccines.

14                   But to get into a little bit of  
15 data, in Novartis, we are bringing forward a  
16 new generation flu vaccine based on flu cell  
17 culture. So we had the opportunity to ask  
18 again, is MF59 as good as it gets? Or are  
19 there other adjuvants that can be equally  
20 potent or even more potent?

21                   So we did a competitive evaluation  
22 of the ones we had easy access to. So see

1           these are some of the adjuvants you saw  
2           earlier, CpG oligonucleotides, calcium  
3           phosphate, PLG microparticles. This is just  
4           the three strains included in seasonal  
5           influenza vaccine.

6                           And it is pretty striking and  
7           clear that emulsions are very effect adjuvants  
8           for flu vaccines. And this, in essence, this  
9           is rediscovering what was discovered by Salk  
10          in the 1950s but using an adjuvant that is  
11          much better tolerated and, we believe, is very  
12          safe.

13                           Kind of a -- this is an  
14          interesting aside looking in the pandemic  
15          setting but still in the mouse model, this is  
16          actually a collaboration with Kanta Subbarao  
17          with NIH.

18                           And it was asking the same  
19          question. Is MF59 as good as it gets? Or can  
20          we make it better? Can we have a more  
21          effective vaccine. And this is kind of using  
22          MF59 as generation one and adding something to

1           it.

2                       So in this situation, this is  
3           looking at T cell responses to MF59. The one  
4           in the middle, the vaccine alone, and on the  
5           right, MF59 plus CpG oligonucleotides. And  
6           this is the kind of color scheme at the top.  
7           If it bluish, it is a Th1 response. If it is  
8           reddish, yellowish, it is Th2.

9                       So in Balb/c mice, a mouse  
10          predisposed to Th2 responses, MF59 gives a  
11          potent T cell response dominated by Th2  
12          cytokines. If you add CpG, the magnitude of  
13          the response is not increased but the quality  
14          changes significantly. Now it becomes a much  
15          more Th1 response.

16                      And the question is does that make  
17          for a better vaccine or not? And Kanta went  
18          ahead and did some challenge studies. So this  
19          is one of the studies she did. And this is  
20          looking at 50 LD50 challenge dose, a pretty  
21          significant challenge dose.

22                      The observation was PBS or vaccine

1 alone, all the mice died over ten days. MF59  
2 or MF59 CpG all the mice survived. So clearly  
3 one adjuvant helps. In this setting, the  
4 second doesn't.

5 But actually I must say she also  
6 did a challenge dose of 50,000 LD50, a huge  
7 challenge dose. And in that setting, the CpG  
8 combination actually offered improved  
9 protection. So that was interesting.

10 Just to clarify something that is  
11 kind of sometimes misrepresented, MF59 gives  
12 a Th1 response in flu-exposed mice. So this  
13 is the same Balb/c mice.

14 In this situation, you are looking  
15 here at naive mice. The Balb/c mice are  
16 inherently predisposed to a Th2 response. So  
17 the MF59 gives a Th2 response. If you  
18 previously infect the mice, then you use MF59,  
19 it is a completely Th1 response.

20 So in essence, MF59 is more like  
21 T80. Whatever is predisposed in the  
22 situation, the MF59 enhances. Humans are not

1 Th1 or Th2. We are kind of mixed.

2 This is a complicated slide but it  
3 is a very simple message. And I think it is  
4 an important one. This is another study to  
5 look at a range of generation one adjuvants,  
6 different particulate carriers.

7 So we're looking across the  
8 bottom, microparticles, tyrosine, calcium  
9 phosphate, MF59, aluminum, we are testing  
10 these different alternative adjuvants against  
11 a number of traditional vaccines and new  
12 generation.

13 Tetanus toxoid, diphtheria toxoid,  
14 a protein polysaccharide conjugate against  
15 MenC, Hepatitis B surface antigen, and a  
16 recombinant antigen, Neisseria meningitidis  
17 serotype B -- on the left-hand side, ELISA  
18 titer, the right-hand side, a functional titer  
19 if you could do it. And you generate at two-  
20 dose levels.

21 And kind of clear picture emerges.  
22 The MF59, as the architype oil-in-water

1 emulsion, tends to be very potent. And tends  
2 to be the winner amongst all these particulate  
3 carriers. It is very striking for the  
4 recombinant antigens here and here.

5 Probably alum works best with  
6 these traditional bacterial toxoids. And that  
7 is how alum was originally introduced, as an  
8 adjuvant for diphtheria and tetanus.

9 So we've had MF59 for quite some  
10 time. When we first developed it back in the  
11 '90s in Taiwan there was a lot of work done on  
12 its mechanism of action. And we thought we  
13 had a reasonable understanding. It looked  
14 mostly to be a delivery system promoting  
15 antigen uptake, that kind of thing.

16 But more obviously over a decade,  
17 the techniques, the technologies improved  
18 significantly. So relatively recently we've  
19 gone back, applied a bunch of new techniques  
20 and asked the question again. How does it  
21 work?

22 We've looked in human cells and

1 we've look in mouse in vivo because they are  
2 the easy things you can do. And then we've  
3 done the mouse in vitro cells trying to link  
4 the two fine connections between mouse and  
5 human and make sure what you are seeing is  
6 consistent.

7 And this is a slide that was  
8 actually shown already. This is looking at  
9 the gene expression profile in the mouse  
10 muscle. And it was kind of interesting.  
11 Numerically, MF59 is the most active in terms  
12 of activation of transcription. But when you  
13 focus on the immune response genes, it is  
14 surprisingly more active than CpG, for  
15 example.

16 So MF59 activates 891 genes. CpG,  
17 less alum, and then there is some overlap.  
18 And here you see the time profile. And you  
19 see the red for MF59. And the combination  
20 MF59 CpG, in essence some things were down-  
21 regulated, which surprised us a little.

22 But in essence, surprisingly, MF59

1 was the most potent activator and it induced  
2 transcription of chemokines, cytokines. It  
3 was activating innate immunity. It's not just  
4 a delivery system. It is doing a lot more at  
5 the injection site.

6 So to summarize a significant  
7 amount of work on the mechanism of action,  
8 which is still ongoing, in the human work, we  
9 identified three target cells, microphages,  
10 granulocytes, and monocytes. We saw that MF59  
11 rapidly recruits cells into the injection  
12 site.

13 We saw that MF59 induces the  
14 release of chemoattractants and activate  
15 innate immunity. And, you know, relevant and  
16 interesting to some of the other discussions  
17 today, MF59 does not activate any TLR. And as  
18 far as we can see so far, it does not appear  
19 to activate inflammasomes.

20 So it certainly generates a local  
21 immunostimulator environment and the work is  
22 continuing. And we kind of published this

1 work and we tried to put a picture together of  
2 what we think it is doing. And this was  
3 published in JI earlier this year.

4 And this is what we think is going  
5 on in terms of the immune stimulator  
6 environment in the muscle, the release of  
7 chemokines, the recruitment of lots of cells,  
8 the activation of those cells, and then moving  
9 off to the lymph nodes to promote the immune  
10 response.

11 So I'll finish up now with where I  
12 think adjuvants may be going in the future.  
13 Maybe I'm thinking about generation three  
14 here. So we are looking at discovery of new  
15 adjuvants.

16 And because we are a large company  
17 that does drug discovery in addition to  
18 vaccine-related work, we have the capability  
19 to utilize the mechanisms of high throughput  
20 screening drug discovery to look for new  
21 generation adjuvants.

22 And this is a schematic

1 representation of a TLR-based screen to look  
2 for what we are calling small molecule immune  
3 potentiators, abbreviated to SMIPs. So you  
4 look for compounds that activate through TLRs,  
5 activate immune cells. And then you formulate  
6 and deliver these compounds to make more  
7 effective vaccine adjuvants.

8 And so, you know, what are the  
9 advantages of SMIPs? Why are we focusing on  
10 these small molecules? And, you know, these  
11 are some of the advantages.

12 Certainly there are simple  
13 synthetic pathways. We know how to make drugs  
14 very inexpensively. They have well-defined  
15 chemical structure. And there is a lot of  
16 history of manipulating the structure to  
17 modulate the response that you get.

18 Certainly there are 100 years of  
19 successful development so people know how to  
20 develop drugs for a variety of purposes. We  
21 see no reason why we can't develop them for  
22 use in vaccines.

1                   And certainly there is an  
2                   established safety profile. How much of what  
3                   is traditionally done for these compounds is  
4                   relevant to a vaccine setting, obviously we  
5                   need to discuss with the regulators. But we  
6                   believe we know the kind of work that is  
7                   necessary.

8                   Certainly these are easily  
9                   degraded and excreted, biodegradable. And we  
10                  know that the delivery systems are well  
11                  established, the delivery systems to control  
12                  the related release and delivery of these  
13                  drugs.

14                 And one delivery system that we  
15                 are particularly interested in is something --  
16                 it is a biodegradable microparticle. So it is  
17                 a polymer called PLG, which is an abbreviation  
18                 of polylactide-co-glycolide.

19                 It is biodegradable and safe. It  
20                 has already been included in 11 licensed  
21                 products. So the particles degrade and leave  
22                 no tissue residue -- completely biodegradable.

1                   Because of the size of the  
2 particles, antigens stuck on the surface a  
3 targeted to immune cells. And the absorption  
4 of the antigen retains the integrity and the  
5 structural features of the antigen as it does  
6 with aluminum.

7                   But the important feature is that  
8 microparticles can co-deliver an antigen and  
9 an immune potentiator. So microparticles were  
10 developed for the controlled release of small  
11 molecular weight drugs.

12                  So we can utilize that technology,  
13 we believe, to deliver these SMIPs. And the  
14 idea is to encapsulate the SMIPs, to limit  
15 their systemic distribution to improve their  
16 safety profile, keep them at the site, keep  
17 them focused on the immune cells that you want  
18 to activate. Don't allow them to circulate  
19 away from the site.

20                  And this is the basic concept,  
21 again put into a picture, traditional  
22 vaccines, like a whole bacteria, a couple of

1           microns or so in diameter, a lot of immune  
2           potentiators contained inside, antigens  
3           generally on the surface.

4                        So the idea is you make a  
5           synthetic microparticle of this completely  
6           degradable polymer. It is about the same size  
7           for uptake into antigen presenting cells. You  
8           absorb the antigen on the surface.

9                        You put in the small molecule  
10          immune potentiators so they are delivered to  
11          the immune cells that take up the  
12          particulates. And you don't allow them to  
13          distribute any further than the injection  
14          site.

15                      So that's the basic idea. Maybe  
16          this is generation three.

17                      And I don't think I'm going to be  
18          brave enough to actually discuss this but I  
19          think there are certainly many regulatory  
20          challenges in relation to development of new  
21          adjuvants. These will be talked about in  
22          greater detail as we go through the day.

1           I guess I just wanted to highlight  
2           the basic researchers, like myself, need to be  
3           aware of these challenges as we go through our  
4           programs. And we need to design the programs  
5           appropriately to meet the needs.

6           And thanks for your attention. If  
7           there is any time, I'll deal with questions.  
8           Or we can do it tomorrow.

9           (Applause.)

10          DR. PULENDRAN: Thank you, Derek.

11          We have time for a couple of  
12          questions.

13          DR. SEAN SULLIVAN: Sean Sullivan,  
14          Vical.

15          Derek, I had a question about your  
16          expression profile studies. You said  
17          something interesting in that if you look at -  
18          - if you have mice that are infected and you  
19          give them MF59 versus if you give them MF59  
20          with the antigen alone, in your PNAS paper you  
21          were characterizing expression profiling.  
22          There's really no antigen present.

1                   And I was wondering -- and I know  
2                   you have, you know, there are a variety of  
3                   antigens you can look at but do you see a  
4                   change in the response when you look at  
5                   expression profiling in the presence of an  
6                   antigen? And also if the animal has been  
7                   exposed to a pathogen?

8                   DR. O'HAGAN: Yes, it is a very  
9                   good question.

10                  You know, of course the gene  
11                  expression profiling work, in looking at  
12                  adjuvants, is kind of novel and new. So we  
13                  start off with the most simple situation where  
14                  you have only the adjuvant.

15                  When you put the antigen, it  
16                  becomes more complicated. And it depends on  
17                  the antigen. So it is a more cloudy picture.  
18                  And I would expect that if you have a pre-  
19                  exposed infected animal, it would be much more  
20                  complicated still.

21                  We may get to that level of  
22                  evaluation. We've started with the relatively

1 simple studies.

2 DR. SEAN SULLIVAN: Could you also  
3 comment in your random screening on what you  
4 use to screen for, like what are the cell  
5 types and what kind of markers you look for,  
6 especially relevant to what you had for a  
7 comparison between human and mouse?

8 DR. O'HAGAN: Yes, I mean we look  
9 at human cells and we look at a variety of  
10 human cells. It is not a single target. And  
11 we look at TLR transfectants. And we look at  
12 native cells. So there is a variety of cell  
13 types we look at for confirmation of the hits  
14 with any one screen.

15 DR. SEAN SULLIVAN: Thanks.

16 DR. PULENDRAN: Okay.

17 So thank you very much, Derek.

18 The next speaker is Eugene  
19 Maraskovsky from CSL in Melbourne, Australia,  
20 who is going to be talking about ISCOMS.  
21 Eugene?

22 DR. MARASKOVSKY: Thank you. I'd

1           like to thank the organizers for inviting me  
2           to present at the workshop.

3                           And today I wanted to give  
4           everyone an overview of saponins and ISCOMS  
5           and, in particular, ISCOMATRIX adjuvant. So  
6           basically I'll introduce what saponins are.  
7           I'll summarize what saponin-based adjuvants  
8           there are out there and what are currently in  
9           clinical development. And in particular then  
10          focus on our understanding of our particular  
11          saponin-based adjuvant, that is ISCOMATRIX  
12          adjuvant.

13                           Now saponins are actually high  
14          molecular weight glycosides that are  
15          consisting of sugar moieties linked to a  
16          triterpene. Now there is a distinction that  
17          I need to make between ISCOMS and ISCOMATRIX  
18          to basically clarify that they are not  
19          interchangeable terms.

20                           ISCOMS are actually a complex of  
21          saponin, cholesterol, and phospholipid where  
22          the antigen has been purposely incorporated

1           into the cage-like structure during the  
2           formulation. So the full components are  
3           formulated together. And the antigen is  
4           associated.

5                        ISCOMATRIX adjuvant is actually  
6           the cage-like structure made out of the  
7           saponin, cholesterol, and phospholipid. And  
8           you can make an ISCOMATRIX by then adding the  
9           antigen to that cage-like structure. So that  
10          is quite a different componentry.

11                      Now the structure of quillaja  
12          saponin is essentially this triterpenoid  
13          moiety component here with a fatty acid and  
14          there's also three areas of carbohydrate or  
15          sugar moieties attached to that.

16                      It's actually derived from the  
17          quillaja saponaria tree, which is an  
18          indigenous tree to Chili and Peru. And crude  
19          quillaja has actually been used in many  
20          industrial processes from agriculture,  
21          cosmetics to the foaming agents in our beers  
22          and soft drinks, so we actually ingest

1 saponins during our lifetime, too also  
2 extraction purposes in mining. And clearly  
3 what I'll focus on is the vaccine use of  
4 saponins.

5 The other important point to point  
6 out is that saponins have actually been used  
7 in the context of vaccines for over 80 years.  
8 So -- and they've actually been going through  
9 an evolutionary process of further defining  
10 what is immunogenic in the saponin and what is  
11 actually the reactogenic component.

12 But I think it is important to  
13 note that we have quite a long history of  
14 experience of the use of saponins in the  
15 vaccine adjuvant setting.

16 And it has been in the more recent  
17 terms where we have made some revolutionary  
18 steps in minimizing the reactogenic potential  
19 within the saponin fractions and focusing on  
20 what is really the immunogenic potential of  
21 the saponins and how to actually formulate  
22 these in a safe and robust way.

1                   Now there are several types of  
2                   companies and commercial versions of saponins  
3                   that are being used. They vary from the QS21,  
4                   which is a highly defined saponin, to what we  
5                   use in the ISCOPREP, which is actually saponin  
6                   that's formulated with the lipids,  
7                   cholesterol, and phospholipid. And various  
8                   other sort of formulations.

9                   Now the issues with saponin are  
10                  that the naked saponin or saponin alone  
11                  actually has quite haemolytic activity,  
12                  particularly at the injection site, which  
13                  results in reactogenicity.

14                  And also it is quite susceptible  
15                  to alkaline breakdown. And one of the  
16                  solutions for actually overcoming some of  
17                  these issues was to complex it with  
18                  cholesterol and other lipids. And also to  
19                  optimize the fractions that are selected,  
20                  particularly to move towards fractions that  
21                  maintain the immunogenicity and minimize the  
22                  reactogenicity.

1                   ISCOMS, which is the four  
2           components associated together -- that is the  
3           antigen with the saponin and the lipids --  
4           also have issues in terms of they are quite  
5           complicated to produce and manufacture in sort  
6           of a robust process.

7                   And so the solution that CSL has  
8           used is to actually devise the ISCOMATRIX  
9           adjuvant, which is the cage-like structure  
10          that you can then formulate with your antigen  
11          independently, and inject into patients.

12                   Now in terms of the saponin-based  
13          adjuvants that are in advanced clinical  
14          development, these not only include the CSL  
15          ISCOMATRIX adjuvant but also other saponin-  
16          based adjuvants such as the AS series that are  
17          being developed by GSK as well as the more  
18          naked QS21.

19                   The one thing to point out here is  
20          what we understand of saponins from our  
21          studies is they don't actually act through the  
22          TLR or Toll-like receptors at all. And in

1 order to activate that pathway, you'll need to  
2 actually add some of these TLR agonists. But  
3 ISCOMATRIX adjuvant is quite a potent adjuvant  
4 but doesn't actually act through the TLR  
5 pathway.

6 What we do know, though, is that  
7 we get quite a balanced immune response that  
8 is generated in mice and monkeys and also in  
9 humans in measuring antigen-specific vaccine  
10 responses. We can actually detect both Th1  
11 and Th2 cytokines in mice and humans.

12 We definitely see a broad and  
13 Th1/Th2-type profile when it comes to antibody  
14 isotypes where we've looked and also we see  
15 quite a robust responses, both CD4 and CD8  
16 responses against multiple epitopes to the  
17 protein antigen that is used in the vaccine,  
18 which gives this quite an advantage in terms  
19 of what type of immune response you want to  
20 actually gear towards.

21 So just to summarize quillaja  
22 saponins, in particular, they have a long

1 history as immunomodulators in vaccines.  
2 Purified fractions are required for human use.

3 Although in vet vaccines you can  
4 away with the more crude fractions, you need  
5 to complex them with lipids such as  
6 cholesterol and phospholipid. And ISCOMATRIX  
7 adjuvant has no addition immunomodulators that  
8 are added to it in the way that we are using  
9 it in the clinic.

10 And sort of the program of  
11 activity that CSL is pursuing is really  
12 setting where we have vaccines that are not  
13 actually showing sufficient immune conversion  
14 in patients, whether they are hyperresponsive,  
15 such as settings in the elderly, chronic  
16 infectious disease in cancer where patients  
17 may be immunosuppressed, and our need to try  
18 and focus on the therapeutic vaccine setting  
19 has basically made us want to understand what  
20 the mechanisms by which vaccines induce CDI T  
21 cell responses are at.

22 And most of our work is really

1 focused on the CTL side of the equation  
2 although we have quite a good understanding of  
3 antibody responses as well with this adjuvant.

4 In terms of mechanisms of action  
5 in vivo, what we do understand is that the  
6 ISCOMATRIX adjuvant is really a sort of dual-  
7 focused adjuvant. It has both antigen  
8 delivery and immunomodulatory capacities. And  
9 it seems to integrate these two very nicely.

10 It's not a depot adjuvant in the  
11 sense of depot antigen at the injection site  
12 but we believe that -- and I'll show you today  
13 -- prolonged antigen exposure in vivo at the  
14 antigen presenting cell level is where we are  
15 getting some of this benefits of the adjuvant  
16 in terms of its delivery capacity as well as  
17 the cytokines that are responsible for the  
18 immunomodulatory effects. And that's  
19 summarized here.

20 ISCOMATRIX targets and activates  
21 APCs in vivo -- and I'll show you that data --  
22 enhances the mechanism of cross-presentation -

1       - this is by which antigen, exogenous antigen  
2 gets into the antigen presentation pathway  
3 into dendritic cells in a noncanonical way.

4               It escapes into the cytocell,  
5 which allows it to be processed in the Class  
6 I MHC pathway, which is critical for the  
7 generation of cytotoxic T lymphocytes.

8               Most other exogenous antigens  
9 would normally be processed in Class II MHC,  
10 which is great for CD4 T cell responses and  
11 antibody responses. ISCOMATRIX has this  
12 unique ability to also target the Class I  
13 pathway.

14              We get prolonged presentation in  
15 the drained lymph node and, as I mentioned  
16 earlier, the immunomodulatory potential  
17 relates to recruitment and activation of  
18 innate immune cells as well as cytokine and  
19 chemokine induction.

20              And I'll show you the data for  
21 that right now. If we inject mice with  
22 ISCOMATRIX alone and have a look in either the

1           draining node or the spleen, we find that most  
2           of the activity of T cell generation after the  
3           prime is essentially in the lymph node.

4                       And when we actually look at what  
5           is happening in the lymph node, what we find  
6           is that very shortly after injection, we get  
7           a large influx of dendritic cells into the  
8           draining lymphocyte. This is transient so  
9           it's rapid and transient in the setting of RDC  
10          influx.

11                      What we also find, which is quite  
12          unexpected, is that we get a very rapid  
13          presentation of peptide on Class I MHC  
14          molecules on the surface of those dendritic  
15          cells actually within about four hours after  
16          injection.

17                      And what we are finding is that  
18          ISCOMATRIX actually is directly trafficking to  
19          the draining node as opposed to remaining at  
20          the injection site, loading dendritic cells in  
21          the node. They are initiating the  
22          presentation process very rapidly and

1 generating these T cells.

2 The other thing to point out is  
3 that we get about a hundredfold more  
4 presentation going on in the draining node  
5 than if we used antigen alone. And the other  
6 advantage is that we have this prolonged  
7 presentation over a period of three days,  
8 which is continuously stimulating the T cell  
9 immune response.

10 So we asked the question what  
11 dendritic cells are the ones that are actually  
12 either recruited into the node and are the  
13 resident dendritic cells in the node also  
14 responsible for the T cell expansion that is  
15 going on?

16 And the first thing we found was  
17 that ISCOMATRIX activates these dendritic  
18 cells in the node. It activates both the CD8  
19 positive lymph node resident dendritic cells.  
20 It also activates the plasmacytoid dendritic  
21 cells that are in the blood.

22 It causes transient but rapid

1 induction of cytokines that can be detected in  
2 the lymph -- and this is using sheep  
3 cannulation studies -- and this is quite  
4 important to point out, they are quite rapid  
5 and high production of cytokines but they are  
6 transient and reversible.

7 And these also result in a rapid  
8 and transient recruitment of many types of  
9 innate immunoeffectors into the node. Within  
10 24 to 48 hours, we have NK cells, neutrophils,  
11 macrophages, NK T cells directly trafficking  
12 into the node that is downstream of the  
13 injection site.

14 If we look at the contralateral  
15 node, there is no influx of those cells in the  
16 contralateral non-injected node.

17 When we actually harvested the  
18 dendritic cells out of those draining nodes  
19 and asked can they present peptide from the  
20 vaccine that was being carried with the  
21 ISCOMATRIX adjuvant, what we find is that in  
22 the early time points, predominantly the CD8

1 resident dendritic cells are doing all the  
2 presentation of the vaccine, which is evidence  
3 that the vaccine is getting to the node  
4 directly.

5 We have a second wave of  
6 presentation that occurs at about 24 hours  
7 onward. And these are actually the migratory  
8 dendritic cells from the injection site  
9 finally getting to the node and starting to  
10 present themselves.

11 And we find that this is probably  
12 responsible for the prolonged presentation  
13 that we are seeing within the node following  
14 ISCOMATRIX vaccine injection. At the later  
15 time points, interestingly, most of the cross-  
16 presentation is occurring by the migratory  
17 dendritic cells.

18 In terms of the TLR pathway and  
19 with ISCOMATRIX as a TLR agonist, we've done  
20 various types of experiments. We've looked at  
21 NF-kappa B activation as one of the surrogate  
22 readouts for TLR downstream effects and find

1           that ISCOMATRIX adjuvant does not activate the  
2           TLR pathway at the level of NF-kappa B.

3                         We've also looked at knockout T  
4           cells and knockout APCs and even knockout mice  
5           from the various TLRs, and again we find no  
6           evidence for a TLR mechanism. We have found,  
7           however, that there is a MyD88-dependent  
8           pathway that ISCOMATRIX adjuvant employs.

9                         And what I can say is that MyD88  
10          is not only a TLR downstream signaling moiety  
11          but also shared between the IL-18 and IL-1 and  
12          IL-33 pathway.

13                        And we found in particular that  
14          IL-18 signaling is important for the way that  
15          ISCOMATRIX activates the immune response in  
16          that IL-18 receptor knockout mice, RL-18  
17          knockout mice are showing defective T cell  
18          responses following ISCOMATRIX vaccination.

19                        So ISCOMATRIX adjuvant targets and  
20          conditions multiple dendritic cell populations  
21          in vivo, enables DCs to cross-present to CDI  
22          T cells, does not activate TLRs, but does

1           require IL-18 for induction or CTL responses  
2           via MyD88, and conditions the draining lymph  
3           node environment for both Th1 and Th2  
4           responses.

5                       In terms of how ISCOMATRIX gets  
6           into the dendritic cells, we've looked at this  
7           by confocal microscopy. If you look at tagged  
8           antigen alone, fed to human dendritic cells,  
9           what you find is that within the first ten  
10          minutes, most of the antigen is actually  
11          within endosomal compartments that we actually  
12          can define using various late and early  
13          endosomal markers.

14                      If you look at ISCOMATRIX, within  
15          ten minutes a lot of the antigen is actually  
16          in the cytosol. So we have cytosolic escape  
17          or translocation into the cytosol of the  
18          antigen, which is a prerequisite for getting  
19          into the Class I pathway for stimulation and  
20          presentation to CD8 T cells. And we think  
21          this is quite an important mechanism by which  
22          we get this robust CTL response in vivo.

1                   The other aspect is that dendritic  
2                   cells are particularly sensitive to  
3                   translocating antigens into the cytosol. If  
4                   we look at monocytes and macrophages --  
5                   monocytes being the precursor of this type of  
6                   dendritic cell. They actually can't  
7                   translocate very efficiently and ISCOMATRIX  
8                   vaccine into the cytosol as compared to the  
9                   monoDCs.

10                   And macrophages, similarly seem to  
11                   capture the antigen to these endosomal  
12                   compartments and very little is translocated  
13                   into the cytosol.

14                   So there is something very  
15                   particular about dendritic cells and their  
16                   ability to translocate.

17                   Now the final sort of points that  
18                   I want to make is in terms of pulse chase  
19                   experiments in human DCs, if we look at  
20                   pulsing human dendritic cells with peptide,  
21                   washing them, putting them back in culture,  
22                   and then sampling those cells periodically to

1 see how much peptide is on the surface that a  
2 T cell can see and make interferon gamma, we  
3 find that peptide pulsed dendritic cells  
4 rapidly lose peptide over time so that by 48  
5 hours, those dendritic cells have hardly any  
6 peptide recognized on the cell surface as it  
7 has been replaced by other competing peptides.

8 If you pulse and chase dendritic  
9 cells with protein, it is very poorly cross-  
10 presented onto Class I MHC so very little is  
11 detected on the surface of those dendritic  
12 cells.

13 If you use an ISCOMATRIX-  
14 formulated antigen, you find that this is  
15 rapidly translocated and expressed on the  
16 surface of Class I peptides as detected by T  
17 cells and you have this very prolonged  
18 presentation over a 72-hour period.

19 And it is at this time point where  
20 you see the big differential and the advantage  
21 of an ISCOMATRIX adjuvant. And this is where  
22 I'm talking about the intercellular depot of

1 the saponin-based adjuvant as compared to it  
2 being an injection site depot adjuvant. This  
3 is very similar to the sort of findings we  
4 found in the mouse studies, too, by the way.

5 Now the other things that we found  
6 out about ISCOMATRIX is that it not only  
7 translocates antigen into the cytosol but it  
8 can actually generate epitopes in a proteasome  
9 independent fashion as opposed to using the  
10 more canonical proteasome-dependent mechanism.

11 And these rules seem to also vary  
12 depending on that antigen and the epitope  
13 within the same antigen. So there is a very  
14 complex array of rules which we are analyzing  
15 at the moment in terms of how isotopes are  
16 expressed on MHC Class I.

17 But the bottom line is that what  
18 we get is a very broad capacity for epitope  
19 generation that dendritic cells can express as  
20 a result of an ISCOMATRIX-formulated vaccine.

21 So the final summary really is  
22 ISCOMATRIX targets and conditions multiple

1 dendritic cell populations in vivo. It  
2 actually enables multiple dendritic cells to  
3 cross-present. And this actually results in  
4 prolonged Class I presentation, which can  
5 either be proteasome dependent or independent,  
6 and generates tumor-relevant T cell effectors  
7 of broad specificity in humans.

8 The sorts of things we're  
9 currently doing now is extending some of these  
10 mechanisms of action questions. We're also  
11 looking at cytokine profiling that can be  
12 detected in vivo, either by ISCOMATRIX alone  
13 or ISCOMATRIX vaccines, and looking at some of  
14 the more sort of systems biology approach and  
15 network biology to understand what is  
16 happening in the draining lymph node because  
17 that's really the side where most of the  
18 vaccine ends up in our system.

19 So the four take-home points, I  
20 think, for today's talk is that ISCOMATRIX  
21 adjuvant is an immunomodulator and an antigen  
22 delivery vehicle. And these are both

1 integrated sort of properties of this  
2 adjuvant. They recruit and activate immune  
3 cells not using the TLR pathway.

4 They accelerate and provide  
5 prolonged presentation. And that these  
6 integrated mechanisms result in the production  
7 of broad specificity, both antibody and T cell  
8 responses both in mice and man.

9 And I'll leave it at that. Thank  
10 you.

11 (Applause.)

12 DR. SUTKOWSKI: Since we're  
13 running overtime, perhaps we can -- unless  
14 there are any burning questions for  
15 clarification, leave the question -- Jan  
16 Willem? Maybe just one question. Then maybe  
17 we'll have to shorten our break.

18 DR. van der LAAN: Just a very  
19 short, very short one.

20 Your colleague from Isconova last  
21 year presented the idea that you can give your  
22 ISCOMATRIX in your left arm and your antigen

1 in your right arm. So then it is more an  
2 immunomodulator. What is it? Is it an  
3 immunomodulator or an adjuvant?

4 DR. MARASKOVSKY: We actually  
5 haven't done those experiments ourselves so I  
6 can't really comment on their data. But what  
7 I can say is that from the experiments that  
8 we've actually been looking at, we have both  
9 an immunomodulatory and an antigen delivery  
10 component there.

11 We don't see the immunomodulatory  
12 effects in the contralateral nodes in terms of  
13 recruitment of cells at least with our  
14 adjuvant system. So most of the activity we  
15 tend to find, at least in the priming phase,  
16 is all happening in the draining node.

17 Upon boosting the vaccine, you do  
18 see now activity going on in the spleen. So  
19 a lot of the boosting of the immune response  
20 will result in antibody and T cell responses  
21 detectable in the spleen. So you do end up  
22 with a systemic effect after boost.

1                   But I can't really comment on  
2                   their data in terms of, you know, delivering  
3                   antigen in one side and the adjuvant in  
4                   another.

5                   DR. SUTKOWSKI: Thank you.

6                   Dr. Slater, do you think we need  
7                   to shorten the break or -- no? Okay. Okay.

8                   If the speakers for the next --

9                   (Applause.)

10                  DR. SUTKOWSKI: Thank you  
11                  everybody.

12                  (Whereupon, the foregoing matter  
13                  went off the record at 11:15 a.m.  
14                  and went back on the record at  
15                  11:35 a.m.)

16                  DR. SUTKOWSKI: Okay. So now  
17                  we're ready to finish up this session on the  
18                  various specific adjuvant overviews. And the  
19                  first speaker is Dr. Bruce Beutler. He is  
20                  coming to us from Scripps Research Institute.  
21                  And he will talk about his many years of  
22                  experience with Toll-like receptors.

1 DR. BEUTLER: Well, thank you very  
2 much. It is a great pleasure to be here. And  
3 it has been a very interesting meeting for me  
4 so far.

5 I was given a very long title  
6 sometime during the lead up to this meeting.  
7 So I parrot it here. But what I have to say  
8 will be relatively simple. And I'll  
9 concentrate on the biochemical mechanisms of  
10 TLR adjuvanticity.

11 But while I was sitting listening  
12 to the first talks, I had a number of thoughts  
13 of my own. And so the first slide is based on  
14 those.

15 It is a big question in immunology  
16 just what the switch is that activates an  
17 adaptive immune response. And as was pointed  
18 out, we've known about adjuvants for close to  
19 a hundred years beginning with alum, then  
20 there was Freund's complete adjuvant and  
21 Freund's incomplete. And there were many  
22 serious attempts to understand just what the

1 relevant molecules were that would ignite an  
2 adaptive response over all that time.

3 By 1989 when adjuvants were  
4 effectively renamed the immunologists' dirty  
5 little secret, this, in itself, didn't really  
6 advance our understanding of how they work.

7 If you think about it for a  
8 moment, just substituting a synonym like that  
9 or making a catchy phrase, it's nice and it  
10 helped to focus attention on the field but in  
11 itself, it wasn't really an advance nor was  
12 the use of the term pathogen associated  
13 molecular patterns to lump molecules like LPS  
14 double-stranded RNA and also DNA that were  
15 already very well known to have endogenous  
16 adjuvant effects.

17 On the other hand, finding  
18 discreet receptors for these molecules was an  
19 important advance. It did enhance our  
20 understanding and it continues to do so. And  
21 understanding the signaling pathways that lead  
22 to adjuvant effects is also important.

1           But at this point, what I think  
2 all of you know, is that there is a lot of  
3 redundancy in this field. There are many ways  
4 to activate an adaptive response. And maybe  
5 that is the central message that I have to  
6 give you.

7           The TLRs are extremely important  
8 in this regard. And their discovery was part  
9 of the broader question of how innate immune  
10 sensing operates. How we know when we have an  
11 infection.

12           And the story of TLRs, from my own  
13 perspective, began with the story of  
14 lipopolysaccharide, which again was more than  
15 a hundred years in the making. LPS was  
16 identified as something that was inherently  
17 toxic about gram negative bacteria.

18           And by the early 1980s, it was  
19 clear that it worked by interacting with  
20 macrophages. And in some of my own early  
21 work, I found that it would induce the  
22 production of cytokines that had LPS mimetic

1 effects, TNF being the key one among these but  
2 certainly not the only one.

3 Victor Jongeneel and his  
4 colleagues showed by 1990 that the TNF  
5 response was entirely dependent on NF-kappa B.  
6 And if you mutated more than two of the NF-  
7 kappa B binding motifs in the TNF promoter,  
8 you didn't get TNF production.

9 TNF and other cytokines, of  
10 course, work in a very complicated way. They  
11 interact with receptors present on many cells  
12 throughout the body.

13 And where this meeting is  
14 concerned, the most important point to make is  
15 that since 1955, since the work of Condie and  
16 Good, it was known that LPS was endowed with  
17 adjuvant activity. If co-administered with a  
18 protein antigen, it would greatly augment the  
19 antibody response that could be measured.

20 If we went forward a few decades  
21 from then, we would say it was not the  
22 macrophage but the dendritic cell that was of

1 key importance there. And people pointed to  
2 the up-regulation of costimulatory antigens  
3 and also the Class I and Class II MHC antigens  
4 themselves as being key events in driving an  
5 adaptive immune response.

6 But for LPS, the mystery remained.  
7 What is the LPS receptor? That is where it  
8 all must start.

9 We had good information from the  
10 1960s that there must be just one LPS  
11 receptor, one solitary pathway for LPS  
12 responses because it had been shown that there  
13 were mice of the C3H/HeJ strain, for example,  
14 also C57 black/10ScCr where a single mutation  
15 that had been mapped to chromosome 4 could be  
16 ablate all responses to LPS. And they said  
17 that probably there was an LPS receptor and  
18 only one such receptor.

19 Where adjuvant effects went, it  
20 was shown by Skidmore and Weigle in 1975 that  
21 these animals derive no adjuvant response from  
22 LPS. So the adjuvant effect, like all effects

1 of LPS, was mediated by this receptor.

2 We positionally cloned this  
3 receptor over a period of about five years and  
4 discovered that it was a mutation in Toll-like  
5 receptor 4, which to that time had been  
6 described as something similar to the  
7 Drosophila receptor Toll, something that was  
8 known to activate NF-kappa B but it had no  
9 known ligand nor did any of the other Toll-  
10 like receptors.

11 And so the picture that emerged  
12 was one in which Toll-like receptor 4 was the  
13 membrane-spanning component of the LPS  
14 receptor. It was assisted in recognizing LPS  
15 by CD14 and later, as it turned out, by a  
16 small molecule called MD2, which we now know  
17 really directly engaged the lipid A moiety of  
18 LPS.

19 Also very exciting at the time was  
20 the fact that this was one member of a family  
21 of paralogues that we now know have 13  
22 representatives at least in mammals, 12 in the

1 mouse and ten in humans.

2 And as we suggested, each of them  
3 has a specificity for different molecules of  
4 microbial origin. And this is quite a  
5 minimalistic view of how they work.

6 In my lab over a period of years,  
7 we've taken a genetic approach to deciphering  
8 how Toll-like receptors signal. And by  
9 screening about 30,000 mice with randomly  
10 induced mutations, we began to put together a  
11 fairly comprehensive picture of the  
12 biochemistry of TLR signaling.

13 We know first of all that the LPS  
14 receptor, TLR4, is predominantly on the  
15 surface of cells and signals there. We now  
16 that it activates two pathways by interacting  
17 with a pair of adapters called MyD88 Mal on  
18 the one hand or Trif and TRAM on the other  
19 hand. And where the MyD88 signaling pathway  
20 goes, it activates NF-kappa B and drives the  
21 production of hundreds of cytokines.

22 The key thing to remember about

1 the Trif TRAM pathway is that this is the only  
2 way the LPS receptor is able to drive the  
3 production of Type 1 interferons. And it does  
4 so by interacting with a kinase called TBK1,  
5 then with IRF-3, a transcription factor that  
6 activates interferon beta and one thereby gets  
7 interferon production.

8 By combining different mutations  
9 that we created, we are able to ablate parts  
10 of the pathway piecemeal. And we know that by  
11 deleting two of the adaptor proteins, MyD88  
12 and Trif, we arrive at a situation where the  
13 Toll-like receptors can't signal at all  
14 anymore.

15 And under those circumstances,  
16 mice are severely immunocompromised. It is  
17 quite rare that they survive to weaning age,  
18 although they sometimes do. And with great  
19 effort, one can maintain a stock of double-  
20 deficient mutants.

21 But the important thing to note,  
22 which I will return to, is they retain very

1 robust adaptive immune responses to all  
2 adjuvants except those that worked directly  
3 through the Toll-like receptors, purified  
4 ligands like LPS or CpG DNA or poly IC and the  
5 like.

6 The adjuvant effect of LPS we know  
7 now is mediated chiefly through Trif. And we  
8 know that applies also to double-stranded RNA  
9 or poly IC. Remember the adjuvant effect of  
10 LPS has been known since 1955 and in 1975, it  
11 was shown to depend upon the LPS locus in that  
12 it was absent in C3H/HeJ mice. So we've known  
13 for a long time it must depend on TLR4.

14 And we decided to look at adjuvant  
15 effects by monitoring the up-regulation of  
16 costimulatory proteins, including CD80, CD86,  
17 and CD40 on antigen-presenting cells in  
18 response to LPS. And we used our mutant mice  
19 to see whether the MyD88-dependent pathway or  
20 the Trif-dependent pathway was of key  
21 importance.

22 We looked both at LPS and at

1 double-stranded RNA, or really poly IC, in  
2 order to make our judgments about this. And  
3 you can see quite clearly just from the top  
4 panel here that in the wild-type, you get up-  
5 regulation of all three of these molecules on  
6 bone marrow-derived macrophages, for example,  
7 or on dendritic cells I think are shown here  
8 if you use LPS.

9           If you take a Trif mutant, one  
10 with a point mutation that we called Lps2, you  
11 have no up-regulation. If you take a MyD88  
12 knockout, then you have fairly robust up-  
13 regulation.

14           The situation is more complicated  
15 for double-stranded RNA. There neither  
16 mutation will independently ablate the up-  
17 regulatory process. And we know today, this  
18 is because there are redundant pathways for  
19 sensing poly IC, especially MDA5, a  
20 cytoplasmic sensor of the RIG-I-like helicase  
21 family, will do the job.

22           So not only a mutation in Trif but

1       also a mutation in TLR3 fails to completely  
2       abrogate the up-regulation of costimulatory  
3       molecules, again because there is this  
4       redundant pathway embodied by MDA5.

5                Because the endpoint of the Trif  
6       pathway, at least in large part, is the  
7       production of Type 1 interferons, at least  
8       that is the unique endpoint, we wondered  
9       whether the interferons were what was really  
10      causing the up-regulation. And prior to this  
11      point, it had been assumed and written quite  
12      widely that this was an NF-kappa B-dependent  
13      response.

14               But it turned out not to be. It  
15      turned out to be an interferon-dependent  
16      response. And it was specifically IRF3  
17      dependent if you are talking about the TLR  
18      signaling pathways.

19               In Panel A, you can see that if we  
20      take wild-type mice and we stimulate with LPS,  
21      we get up-regulation of costimulatory  
22      molecules. If we use the Trif mutant mice, we

1 don't get such up-regulation. And up-  
2 regulation isn't restored by any of a panel of  
3 cytokines that we applied, including IL-18,  
4 IL-15, IL-12, IL-1 TNF. But it is restored if  
5 we co-administer either Type 1 or, to some  
6 extent, Type 2 interferon.

7 If you look down then at Panel C,  
8 you can see that the Type 1 interferons, by  
9 themselves, do a pretty good job of inducing  
10 the up-regulation of costimulatory molecules.  
11 And finally in Panel D, we antagonize the Type  
12 1 interferons with antibodies and we get a  
13 significant decrement of the response.

14 But antagonism with antibodies  
15 isn't always 100 percent effective. So, of  
16 course, we looked at interferon Type 1  
17 receptor knockout mice.

18 And just to be quick, if you look  
19 at the bottom panel of the slide here, you see  
20 that in mice that are IfnR mutants, you get no  
21 up-regulation of CD80 or 86 or CD40 in  
22 response to either LPS or double-stranded RNA.

1 This is an absolute requirement for the  
2 costimulatory response.

3 Now in the meanwhile, as you may  
4 remember, some years ago in early years after  
5 the turn of the century, there was a lot of  
6 excitement about TLRs being the pathway for  
7 activation of adaptive immunity. And you will  
8 recall there were a lot of papers published  
9 saying that TLRs were required for an adaptive  
10 immune response.

11 It was written that Toll-like  
12 receptors control activation of adaptive  
13 immune responses by APCs, that they play an  
14 essential role in the induction of innate and  
15 adaptive immune response, that they are  
16 responsible for the induction of DC  
17 maturation, which is responsible and necessary  
18 for the initiation of adaptive immune  
19 responses. And also the generation of T-  
20 dependent antigen-specific antibody responses  
21 requires activation of TLRs in B cells.

22 These statements led many to

1       assume that this was a concomitant of adaptive  
2       immune activation, that this was it, this was  
3       the pathway. And we had reason to be  
4       skeptical. And we, ourselves, began to look  
5       at it very closely.

6                   By we, I should mention that this  
7       was the work of my lab together with David  
8       Nemazee, who is the senior author on these  
9       studies. We noted first that by no means were  
10      MyD88/Trif double-deficient mice  
11      agammaglobulinemic. They could clearly make  
12      adaptive responses to some antigens if we  
13      simply looked at their serum immunoglobulin  
14      levels.

15                   But what we did notice was that  
16      there was skewing so that in the double mutant  
17      mice, for example, there was exaggerated  
18      representation of IgE and there was diminished  
19      representation of IgG3. And so we thought  
20      perhaps there was a problem in these mice with  
21      class switching in the ambient microbial  
22      environment.

1                   We then began to immunize the mice  
2                   and look directly at their immune responses.  
3                   And in one instance we used alum as the  
4                   adjuvant. Alum was used just to test the  
5                   thesis that anything would require TLRs to  
6                   activate an adaptive response.

7                   And as you can see, it certainly  
8                   did not. We got an IgM response, an IgG1,  
9                   IgG2c, IgG2b, IgG3, IgE response. And where  
10                  there were significant differences, it was the  
11                  double deficient mice that actually were  
12                  hyperresponsive.

13                  Of course we could be criticized  
14                  and we could face the objection that of course  
15                  alum doesn't require TLRs to generate an  
16                  adjuvant effect because it is not microbially  
17                  derived. But to our surprise and, I think, to  
18                  the surprise of many, complete Freund's  
19                  adjuvant also doesn't require TLRs.

20                  It works perfectly well in mice  
21                  that can't signal the TLRs. And so you see  
22                  again you've got good responses of all the

1 different subtypes. And you find that where  
2 is a significant difference between the  
3 knockout, the double knockout, and the wild-  
4 type, it is usually in favor of the double  
5 knockout, which is hyperresponsive.

6 So neither alum nor CFA depend  
7 upon TLR signaling for adjuvant effect. We  
8 asked since that time what about a real  
9 microbe? What really is TLR dependent when it  
10 comes to adaptive immune responses?

11 We began to test this question, to  
12 look at the question using mouse  
13 cytomegalovirus, which provokes a very  
14 strongly Th1-biased IgG2c antibody response.  
15 It is known to trigger signaling via TLR3 and  
16 actually it should read TLR9, not 7, but no  
17 other TLRs.

18 We know that signaling by TLRs 3  
19 and 9 but no other TLRs is essential to  
20 survival during the first week following  
21 infection with ten to the fifth PFU of the  
22 virus. So it is part of the innate response.

1                   And on the other hand, if lower  
2 doses of the virus are given, the mouse can  
3 mount an adaptive response quickly enough to  
4 survive infection. And even in mice that lack  
5 those signaling pathways, we can evaluate the  
6 adaptive response overall.

7                   We looked then at mice that were  
8 wild-type or that lacked TLR9 of the CpG1  
9 mutant induced by ENU or have a mutation  
10 called 3D which abrogates signaling via TLRs  
11 3, 7, and 9 or mice that lacked all TLR  
12 signaling.

13                   Now as you can see, you've got  
14 perfectly adequate adaptive immune responses  
15 looking out to 90 days. We wanted to repeat  
16 this experiment, of course, and so we did.

17                   And we did it again all over with  
18 fresh mice. This time we looked at just  
19 MyD88/Trif double deficient mice or wild-type.  
20 And again you get adequate responses. Notice  
21 that the wild-type responds much better than  
22 the -- rather the wild-type responds not as

1 well as the double deficient mouse.

2 Our interpretation of this is that  
3 probably the double deficient mouse gets a  
4 higher burden of virus and so it makes a more  
5 robust antibody response in time.

6 We then looked at the question of  
7 memory B cells were compromised in any way if  
8 there was a lack of TLR signaling because  
9 this, too, had been claimed. And so we took  
10 B cells from either wild-type or double  
11 deficient animals and here I simply show you  
12 that these animals, which had been inoculated  
13 with the virus themselves mounted a very good  
14 response in terms of IgG production against  
15 MCMV. Those were the donors of B cells.

16 And we transplanted the B cells  
17 into a T deficient environment. And then we  
18 challenged the mice with irradiated virus to  
19 produce an anamnestic response.

20 If the mice were naive and had  
21 never been immunized, if we used that kind of  
22 a donor, we got no response. If we used

1 immunized cells -- cells from an immunized  
2 donor but didn't boost them, we got no  
3 response.

4           If we took wild-type B cells from  
5 an immunized mouse and boosted, we got a  
6 robust antibody response. And if we took B  
7 cells from double deficient mice and boosted  
8 them, we got a robust response although  
9 perhaps a little bit less than what was  
10 observed in the wild-type. In no way could we  
11 say that the B cell response was really  
12 dependent on TLR signaling.

13           Of course we repeated this  
14 experiment as well. And I just show you the  
15 repetition. Exactly the same thing was done  
16 except in this case I'm not showing you the  
17 controls where we didn't immunize or where we  
18 used naive cells. Again, you see that the  
19 wild-type B cells respond to immunization and  
20 so do the double knockouts.

21           So our conclusions are that TLR  
22 signaling certainly does augment an adaptive

1 response as could be deduced from experiments  
2 that were performed more than 50 years ago.

3 But TLR signaling is not required  
4 at any level, whether it is antigen  
5 presentation or helper T cell function or B  
6 cell activation for an adaptive immune  
7 response to classical adjuvants nor to MCMV  
8 nor to any microbe as far as we know at this  
9 time.

10 TLR signaling does influence class  
11 switching in the ambient microbial  
12 environment. And there is a modest decrease  
13 in B cell memory responses to an authentic  
14 viral pathogen if primary immunization is  
15 performed in mice that lack both MyD88 and  
16 Trif. But TRL signaling is not required for  
17 B cell memory per se.

18 So we might guess that there are  
19 redundant pathways for adjuvant effects. And  
20 the question is how can we look for these  
21 pathways? We've always favored a genetic  
22 approach, particularly when we don't

1 understand a system very well.

2 And we've begun to look at this  
3 question using recombinant Semliki Forest  
4 virus. And we used this system as an  
5 immunization protocol because it was shown by  
6 Gunilla Karlsson Hedestam and Asa Hidmark, her  
7 graduate student, to be a type of adaptive  
8 response that is completely TLR independent.

9 So we wanted to begin with a  
10 system where we knew there wouldn't be  
11 interferons from TLRs and try to understand  
12 exactly how the adjuvant effect might work.

13 We know that in this system if you  
14 take an antigen and you immunize it in  
15 recombinant Semliki Forest virus vector, then  
16 there is a strong response to immunization  
17 with boost. We can use vectors that have  
18 variable expression of the encoded antigen and  
19 one can then run a genetic screen in both  
20 directions. One can test for both high and  
21 low responders to two different antigens at  
22 the same time in the same mouse.

1                   And as was shown already by Asa  
2                   Hidmark paradoxically in view of what I told  
3                   you about the interferon dependency of TLR  
4                   adjuvant effects, a weak antigen gives a much  
5                   stronger immune response in the absence of  
6                   Type 1 interferon signaling.

7                   Now here is the beginnings of our  
8                   screen. We've gone through six or 700 mice by  
9                   this time. And this is work that is funded by  
10                  the Gates Foundation.

11                  But you see we run it in both  
12                  directions. We can take a weak antigen, which  
13                  is OVA, or a strongly expressed antigen, which  
14                  is Beta-gal and on the one hand we look for  
15                  mutants where there are exceptions and you  
16                  have an exaggerated response to the OVA -- and  
17                  here we have three candidates which we are  
18                  evaluating now -- or we can look for mutants  
19                  like perhaps this one where you have a  
20                  diminished response to the strong immunogen.

21                  And in this way we hope to ferret out non-  
22                  redundant components of these signaling

1 pathways.

2 To show you the effect of an  
3 interferon mutation -- I don't know how well  
4 you can read from there -- but this is the  
5 primary response to immunization with the OVA  
6 vector, the weak antigen, weakly expressed  
7 antigen.

8 This is the boost response. And  
9 in this column you see the effect when we  
10 immunized mice that are deficient in the Type  
11 1 interferon receptor. There we do get a  
12 response. We don't get a response in wild-  
13 type mice. And in this case, we are looking  
14 at black6 mice given varying doses of the  
15 viral vector. This is a secondary response  
16 looking strictly at antigen-specific IgG.

17 So I want just to conclude at that  
18 point. This is my group as it stands now. I  
19 didn't tell really what most of them do. But  
20 for the most part, we do take a forward  
21 genetic approach. We make no judgment about  
22 how adjuvants really might work.

1                   But our goal is to dissect them by  
2                   making random mutations that impair their  
3                   function or augment it.

4                   And I want also to thank Kasper  
5                   Hoebe who is now at the University of  
6                   Cincinnati, Gunilla Karlsson Hedestam at  
7                   Karolinska Institutet and David Nemazee at  
8                   Scripps whose work I mentioned during the  
9                   course of my talk.

10                  I'll take any questions you might  
11                  have. Thanks.

12                  (Applause.)

13                  DR. SUTKOWSKI: Thank you for very  
14                  provocative data there.

15                  We have time for a couple of  
16                  questions since the speaker stayed on time.

17                  DR. PULENDRAN: Bruce, thank you  
18                  that really elegant presentation.

19                  Maybe I could ask you a question.  
20                  All the data for the immune responses you  
21                  showed us concerned humeral responses. Have  
22                  you looked at T cell responses in response to

1 various viruses and TLR knockouts?

2 And I ask this because some of our  
3 own work shows that the yellow fever vaccine  
4 responds very poorly in terms of T cell  
5 responses in MyD88 knockout mice suggesting  
6 that at least for that vaccine that you do  
7 need MyD88 signaling to get a T cell response.

8 DR. BEUTLER: It's a particular  
9 interest of Kasper Hoebe to look at responses  
10 of CD8 cells. And that's where we've done the  
11 most work.

12 We find that a CD8 response is  
13 elictable, let's say, by TLR signaling. But  
14 not very strongly. And what drives a CD8  
15 response much more is the induction of  
16 programmed cell death by several different  
17 means.

18 One can cause death my NK cell  
19 killing, by UV or gamma irradiation, by fas  
20 ligation, all of these things will drive a  
21 strong response to any antigen that is carried  
22 by the cell that is undergoing death.

1                   We know that a CD8 response is  
2                   also strongly elicited by recombinant Semliki  
3                   Forest virus. To what extent that's death  
4                   dependent we don't really know as yet.

5                   Oh, I'll mention those are totally  
6                   TLR independent, by the way, the death  
7                   pathways.

8                   DR. SUTKOWSKI: Okay. So now our  
9                   next speaker is Dr. Nathalie Garçon. Nathalie  
10                  was Vice President and head of research and  
11                  R&D in North America in GSK. And since  
12                  September of this year, she's heading now the  
13                  Adjuvant Center for Vaccines at Overseas  
14                  Adjuvant Activity from Research to Life Cycle  
15                  Management.

16                  DR. GARÇON: Good morning. Thanks  
17                  for the introduction, Elizabeth.

18                  So we're going to switch gears a  
19                  little bit here. The presentation and the  
20                  topic, as I understood it also, was to bring  
21                  lessons learned that we have learned in the  
22                  development of adjuvants. And in the 20

1 minutes that I have to present, I will try to  
2 put you through some of the experience we have  
3 had and the lesson we took from that.

4 So I think we have discussed that  
5 several times this morning but again I will  
6 tell you where need new adjuvants or adjuvant  
7 systems.

8 One of the lessons is that you  
9 need to know your component and your adjuvant  
10 system;

11 That they need to be designed to  
12 elicit a tailored immunity that you are  
13 looking for;

14 That the formulations need to  
15 consider the physical/chemical properties of  
16 your component;

17 That the formulation can impact on  
18 the immunogenicity of the vaccine even if you  
19 use the same immunomodulator;

20 And also that one name for one  
21 molecule can refer to different molecules when  
22 you look in the literature.

1                   And I will conclude.

2                   So as it was already presented  
3                   several times, clearly infectious diseases  
4                   require new strategy for the development of  
5                   efficacious vaccine. And that can be linked  
6                   both to the target population but also the  
7                   pathogen you are targeting.

8                   And if we consider the targeted  
9                   population, clearly there is a need to induce  
10                  a long-term persistence of the protection.  
11                  There is a need for vaccines that are adapted  
12                  to fully-responsive population and that can be  
13                  elderly in particular. And there is, in some  
14                  cases, clearly a need for antigen sparing.

15                  That can be linked also to the  
16                  target pathogen. There are complex pathogens  
17                  that can evade or subvert the immune defenses.  
18                  There are pathogens that require complex  
19                  multi-stage immune response. There are  
20                  antigens that are potentially weak and we had  
21                  this morning a presentation actually where  
22                  clearly by going from live vaccine to purified

1 recombinant, we did lose part of the ability  
2 of the antigen to induce an immune response.

3 There are pathogens that exist  
4 with multiple strains, serotype, or genotype.  
5 And there is a need for that to induce a  
6 cross-protection. And there are pathogens  
7 that may not give us time to develop a vaccine  
8 and pandemic flu is a clear example.

9 So there are clearly needs for new  
10 approaches in adjuvant and adjuvant system is  
11 one of them. So what is the GSK approach for  
12 adjuvant system? Basically that was touched  
13 upon already also this morning is that  
14 classical vaccines are made of antigen and  
15 what you could refer to as classical adjuvant,  
16 which are aluminum salt, emulsion, and  
17 liposomes.

18 And adjuvant systems basically are  
19 based on the combination of one of those  
20 and/or an immunomodulator, which can be MPL or  
21 QS21, CpG and alpha-tocopherol.

22 I won't talk about CpG today and I

1 will talk about the three others. And clearly  
2 the goal of doing that is to tailor the immune  
3 response to achieve an enhanced protection.

4 What is obvious also is that if  
5 you don't need an adjuvant system, you don't  
6 use an adjuvant system. So a new component  
7 adjuvant system -- so MPL is registered so  
8 MPL, as defined by Corixa and produced by  
9 Corixa, which is now part of GSK, is a pure  
10 TLR4 agonist.

11 It is derived from the  
12 lipopolysaccharide from Salmonella minnesota.  
13 This is a detoxified form. And MPL can return  
14 the adjuvant activity with a much reduced  
15 toxicity.

16 So what does it do? So as I was  
17 telling you, it is clearly a TLR4 agonist.  
18 MPL acts on monocytes, mDC, but not the plasma  
19 situate CD8 T CELL. And this is per the TLR4  
20 expression on cells.

21 What is important is that one of  
22 the adjuvant systems we are using is called

1 AS04. It is a combination of aluminum and  
2 MPL. If you look at the ability of AS04 to  
3 activate dendritic cells -- and this is a box  
4 I just added -- there is no difference in the  
5 production of TNF-alpha whether the MPL is  
6 absorbed or not on aluminum.

7 So that formulation maintains the  
8 ability of MPL to activate DCs. And that  
9 translates in vivo in mice when we use the HPV  
10 VLP antigen also to an increase in the  
11 antibody production for both VLPs as compared  
12 to the aluminum dioxide alone.

13 So QS21 enhances CTL induction.  
14 There was a presentation earlier on ISCOMS and  
15 ISCOMATRIX. QS21 is a purified fraction from  
16 Quillaja saponaria so you have a certain  
17 number of fractions in Quillaja. QS21 is one  
18 of them. And it is part of the triterpene  
19 saponin family.

20 So what it does, QS21 enhances the  
21 CTL induction and as observed in animal  
22 models. And here you see an example with OVA

1           where whether you use PBS over OVA MPL, there  
2           is no really detectible CD8 response. Only do  
3           you see such a response when you use QS21.

4                        So you can go one step further and  
5           depending on the type of immune response you  
6           are looking for, you can combine different  
7           immunomodulators. And this is a case here of  
8           a combination of MPL and QS21. And by  
9           combining them, they act synergistically on  
10          the innate and adaptive immune responses.

11                      So you combine both of those  
12          molecules and what you see is that it does  
13          impact on the innate immune responses is the  
14          lower left box. And looking at interferon  
15          gamma production by APCs, the production you  
16          induce with the combination of MPL and QS is  
17          more than MPL and QS separately.

18                      And this has an impact also on the  
19          immunity as you see that looking at the  
20          antibody response which is induced. There is  
21          a clear increase in that antibody response  
22          when you combine both MPL and QS versus each

1 of them separately.

2 What is important also to note is  
3 -- and that's what I've circled -- those  
4 molecules have different physical/chemical  
5 properties. And this is important because not  
6 only do you need to ensure that you induce the  
7 type of immune response you are looking for  
8 but you also need to ensure that your  
9 formulation can be done through a process that  
10 could be done at large scale and used for  
11 final product.

12 So MPL is a hydrophobic molecule  
13 and tends to aggregate for clumps so you need  
14 to have a process that allows you to have  
15 particulate that are serofilterable and QS21  
16 is a nonspecific molecule with clearly defined  
17 properties.

18 So alpha-tocopherol directly  
19 impacts the immune response in the elderly.  
20 So this is also a hydrophobic molecule.

21 And it has been recently published  
22 that tocopherol helped to reverse the excess

1           acidity effect in T cell response. And we  
2           have also seen that when in oil-in-water  
3           emulsion, alpha-tocopherol results in the  
4           increased of production of cytokines. And  
5           this translated to an increase of antibody  
6           response.

7                        Tocopherol has been used in a lot  
8           of vaccines, veterinary vaccines since many  
9           years, in particular in poultry. So it's not  
10          such a new immunomodulator.

11                      So adjuvant systems are designed  
12          to elicit immune response. We use, as an  
13          example, the RTS,S malaria candidate antigen.  
14          RTS,S is a particulate antigen which is based  
15          on mixed particles that are made of S antigen  
16          from hepatitis B and a part of the  
17          circumsporozoite surface protein. And this is  
18          referred to as RTS,S.

19                      If you look at the left panel,  
20          which is the first experiment we did in  
21          monkeys and we looked at antibody response in  
22          immunity, we tested three different adjuvants

1 and adjuvant systems. The LMNPL, which is  
2 known as AS04, the MPL QS21, which is known as  
3 the AS01 family, and the emulsion MPL QS,  
4 which is known as the S02 family.

5 What came as a surprise to us was  
6 the results we had with MPL QS because from  
7 the mouse data, we didn't expect to have such  
8 a low response and in particular when  
9 comparing to LMNPL.

10 Actually it turned out that in  
11 that formulation, QS21 was not stable. We  
12 were at a pH that was inducing degradation of  
13 QS21. And hence we didn't have any adjuvant  
14 effect.

15 The oil-in-water emulsion that we  
16 used in that first experiment turned out to be  
17 unstable after six months. So though we had  
18 great results pre-clinically, clearly there  
19 was an issue of production of the formulation.

20 So we went back and reworked the  
21 emulsion and we tested different types of  
22 emulsion that actually were all based on the

1 same principle of density and particulate  
2 size. So we defined the particulate size that  
3 we were looking for and a density. But they  
4 were varying in their composition.

5 And what you can see here is that  
6 if you look at oil-in-water 2 and oil-in-water  
7 3, both of those are emulsion, oil-in-water  
8 emulsion, however they don't give the same  
9 type of immune response in the monkeys,  
10 whether it is antibodies to DTH.

11 When you start an immunomodulator  
12 to those systems, you see that you impact both  
13 on the DTH and the antibody response. And the  
14 highest impact is seen when you combine the  
15 three together.

16 So here what you see that not all  
17 oil-in-water emulsion are equal, that you can  
18 have different types of immune response, and  
19 that when you do add an immunomodulator,  
20 depending on the one you add had how you add  
21 it, you do induce a different type of immune  
22 profile.

1                   This is also true when you look at  
2 challenge models so that is the ferret  
3 challenge model for flu where we used ferrets  
4 that were first infected with the virus and  
5 then vaccinated once with trivalent flu  
6 vaccine. And they were challenged 49 days  
7 after the immunization.

8                   And what you follow here is  
9 temperature. So the ferret has the ability to  
10 give you the set time of clinical symptom that  
11 you have in humans in a raise in temperature.  
12 It is a marker if you don't have any raise of  
13 temperature of the efficacy of your vaccine.

14                   And what we saw is that using two  
15 different split trivalent activated vaccine,  
16 whether you used one type of oil-in-water  
17 emulsion or none, there was no difference.  
18 There was no protection that was seen.

19                   However, if you use those two  
20 split trivalent activated vaccine, we saw that  
21 by adding the oil-in-water emulsion that did  
22 contain alpha-tocopherol, we saw a complete

1 protection in those ferrets.

2 That was also correlated with a  
3 decrease in the virus shedding and in the  
4 ferret we can't look at cell-mediated immunity  
5 but there was a clear difference in the  
6 antibody level when comparing both the group  
7 that protected and the one that didn't.

8 So, again, not all emulsions are  
9 equal. So when you do the formulation of your  
10 adjuvant system or your adjuvant, you need to  
11 consider the physical/chemical properties of  
12 the component.

13 As it was pointed out earlier for  
14 ISCOMS, QS21 has the ability to degrade in an  
15 alkaline pH. And by doing so, QS21 becomes  
16 what has been described as DS1 or it is known  
17 also as QS21h.

18 And what you see in the right  
19 panel is that indeed QS21 in water remains  
20 stable even at 16 hours at 37 degrees. This  
21 is the lower line. However when you put QS21  
22 at a pH 9.0, QS21 transforms in 16 hours in 94

1           percent of QS21h. So you lose almost all of  
2           your immunomodulator.

3                           And this does have an impact on  
4           the immunomodulator property of your molecule  
5           and that has been published around 2000 when  
6           it was seen that QS21 was capable of inducing  
7           CD8 immune response whereas the degraded  
8           QS21h, which is the black square at the  
9           bottom, didn't have this ability anymore.

10                          And actually you do see the same  
11           impact on the humoral immune response as well.  
12           So losing the the acylated chain that is on  
13           the QS21 abrogates its activity.

14                          One other property of QS21 is that  
15           it is an amphiphilic molecule. And again it  
16           was pointed out earlier that QS21 has lytic  
17           activity. This is what you can see here if  
18           you take red blood cells in water, this is the  
19           first group. There you see your red blood  
20           cells.

21                          When you put them in PBS it  
22           settled. And when you saw that in QS21, you

1           have amylase that appears in your sample. And  
2           that amylase is proportional to the amount of  
3           QS21 you introduce.

4                       Doing it in vitro on red blood  
5           cells actually is a marker of what happens  
6           when you inject QS21 intramuscularly. Lysis  
7           of cells is not restricted to red blood cells.  
8           It is a phenomenon you can see also in muscles  
9           and can lead to necrosis at the injection  
10          site.

11                      So how can a formulation help you?  
12          Well, you can reformulate QS21 in such a way  
13          that putting it at pH 9.0 for 16 hours at 38  
14          degrees, you do not have any degradation any  
15          more. This is the lower line of the table.

16                      Your QS21 remains as QS21 and does  
17          not perish into QS21h. And also you can  
18          formulate your QS21 in such a way that the  
19          necrosis that you can see in the picture here  
20          at the bottom, which is induced in the muscle  
21          of a rabbit when you inject 50 microgram of  
22          QS21, disappears when you reformulate QS21

1           that abrogates the lytic activity.

2                       So formulation can impact the  
3 immunogenicity of the vaccine. Again, this is  
4 using the malaria antigen as an example. And  
5 here the two adjuvant systems that were used  
6 both contained the same immunomodulator and  
7 pure QS21. What is different is the  
8 formulation which is used.

9                       And what you can see on the left  
10 side is that in mice, looking at antibody --  
11 this is the upper panel -- they do have the  
12 same type of antibody profile induction  
13 profile. However, when you look at CD4 T cell  
14 response, you can clearly see that -- the AS01  
15 is the next to the last if that can help you -  
16 - you clearly see there is a difference in the  
17 induction of CD4 double positive CD4 T cells  
18 to an interferon-gamma.

19                      When you look at what happens in  
20 humans when you compare both of those  
21 formulations, you do see that there is a  
22 difference -- in that case a significant

1 difference in the antibody titers that were  
2 induced with AS01 versus AS02. And you do  
3 also see a trend for a difference in the CD4  
4 T cell positive induction. And here clearly  
5 we saw the same ranking from mice to humans,  
6 going through monkeys.

7 So one then can refer to different  
8 molecules. So different LPS of different  
9 agonist activities, gram-negative LPS, rTLR4  
10 agonist, gram-positive bacteria, rTLR2. And  
11 also depending on which MPL you are looking at  
12 -- and here I'll take the MPL what is called  
13 the MPLR and is sometimes referred in the  
14 literature as a GMP form of MPL versus mpl8,  
15 which is referred in the literature as non-GMP  
16 material.

17 If you look at the MS profile of  
18 those molecules, you clearly see they are very  
19 similar but they are different and, in  
20 particular, both MPLR produced from Salmonella  
21 minnesota.

22 However, mpl8 does show a peak at

1 the profile which it doesn't exist in MPLR.  
2 And this is most likely due to the difference  
3 in process where the first one only has an  
4 acidic hydrolases in the production process  
5 whereas the MPLR includes both acidic and  
6 basic hydrolases. And this is important  
7 because actually each do present different  
8 cytokine activation patterns on human monocyte  
9 cells.

10 And actually what we have seen is  
11 MPLR is a poor inducer of Trif pathway on  
12 human monocytes, which is different from what  
13 was discussed earlier that showed that MPL was  
14 a Trif-inducer. So one has to be careful and  
15 specific on what he's using when he is testing  
16 molecules.

17 So in conclusion, adjuvants and  
18 adjuvant systems, clearly the knowledge of the  
19 molecular action guides the vaccine  
20 development on the what and the how. The  
21 formulation can impact the physical/chemical  
22 property of the adjuvant or the adjuvant

1 system.

2 And it is possible through  
3 formulation to reduce or abrogate the core  
4 reactogenicity. And clearly formulation of  
5 semi-immunomodulator can lead to increased  
6 immunogenicity.

7 And finally, adjuvant systems are  
8 designed to elicit immunogenicity. And not  
9 all adjuvant/adjuvant systems induce the same  
10 immune response and they need to be selected  
11 and justified appropriately.

12 And I can't name all the people  
13 that have been involved that work since we  
14 have ten years. But we certainly thank all of  
15 them whether they are within GSK Bio or  
16 external collaborators.

17 Thank you.

18 (Applause.)

19 DR. SUTKOWSKI: Does anybody have  
20 any questions for Dr. Garçon or lessons  
21 learned?

22 (No response.)

1 DR. SUTKOWSKI: Okay. Thank you.

2 Okay, our next speaker, Dr. Geert  
3 Van den Bossche comes to us from the Bill and  
4 Melinda Gates Foundation where he is the  
5 Senior Program Assistant for Global Health  
6 Discovery. And he will be talking to us about  
7 additional lessons learned.

8 DR. VAN DEN BOSSCHE: Hello  
9 everybody.

10 I thank the organizers for  
11 inviting me and I congratulate them on this  
12 initiative.

13 It's just amazing if I look at  
14 this audience, such an interest and attention  
15 paid finally to adjuvants. I would say wow.  
16 I mean this really seems the field is moving.

17 And we are really happy about it.  
18 So I will come back to the mission of the  
19 Gates Foundation later on in this talk.

20 So obviously since I joined the  
21 Gates Foundation, I consider myself as a  
22 knockout scientist. And my hands-on gene got

1 severely deleted. I hope a couple of other  
2 genes felt up-regulated but it probably  
3 changed my phenotype. So whatever.

4 So the agenda -- I skipped a  
5 number of slides for the introduction. I was  
6 just -- you know since we saw that biophysical  
7 aspects are going to be maybe on the priority  
8 list of adjuvants and better understanding  
9 their interaction, the interaction of  
10 adjuvants with membranes and so on, I'm just  
11 going to limit this to one single slide.

12 And then move straight on to  
13 adjuvant safety. What are the challenges?  
14 What are the issues? And what can we do about  
15 this just to end up with a number of practical  
16 recommendation?

17 So obviously I'm not going to show  
18 you any hard data. What I want to do is just  
19 to share with you some insights that are based  
20 on my background in adjuvants. And you will  
21 see the statements that I'm going to make are  
22 backed up by a number of references from

1 literature that I appended at the end of the  
2 presentation.

3 So this is really the one slide  
4 that I always start out with where you see --  
5 I call it the discrepancy we are currently  
6 observing between the world of the two Ps --  
7 the publications and the products.

8 On the one hand side, we have  
9 adjuvant discovery where I think we have been  
10 doing a fabulous job over the last ten,  
11 fifteen years. There has been tremendous  
12 progress in, you know, for example, innate  
13 immune biology, discovery of new adjuvants,  
14 discovery of new receptors.

15 We have established discovery  
16 tools to better analyze immune signaling  
17 cascades, transcription, activation of  
18 transcription factors, and also to analyze the  
19 expression of inflammatory cytokines.

20 And frankly this has lead to a  
21 huge amount of information and we don't always  
22 know what to do with all this information.

1           It's -- one is getting the impression  
2           sometimes -- getting a little bit lost in the  
3           whole thing. But it is very obvious that this  
4           has been very, very useful and contributed to  
5           a better understanding of how adjuvants work  
6           and of innate immunity in general.

7                        So when it then comes to  
8           adjuvanted vaccines, I would say to vaccine  
9           development, well, the approach has been quite  
10          different. It has been largely characterized  
11          by empiricism so far.

12                       And we have had some difficulties  
13          to translate this discovery into really  
14          product development, adjuvant development.  
15          And at the end of there, adjuvanted vaccines.  
16          That is what we are looking for.

17                       So this is basically due to the  
18          fact that, of course, we have -- and we  
19          acknowledge this, of course -- that we have to  
20          formulate these compounds. And we have to put  
21          them into delivery vehicles.

22                       We have to process these

1 compounds. And by doing so, by formulating  
2 them, we sometimes change the physical  
3 properties as we just heard. And then we  
4 sometimes encapsulate this stuff and we absorb  
5 it on particles or we present this at  
6 multimeric particles and whatever.

7 So in the meantime, we maybe have  
8 forgotten that we are generating, by doing  
9 this, a number of physical interactions that  
10 not only we don't always understand but that  
11 we don't usually characterize enough. And  
12 that we do not always control.

13 And this may have lead to a number  
14 of issues. I think that the lack of  
15 rationale, sound rationale to why -- how do we  
16 formulate these things and also a lack of a  
17 more multidisciplinary approach to the  
18 understanding of what is the relationship  
19 between the physical properties and the  
20 biological behavior has led to a number of  
21 issues like, for example, reproducibility.

22 How much aid the stability -- and

1 I'm not talking about only chemical stability,  
2 also physical stability? And one of the  
3 challenging things -- and this is a question  
4 I would like to address in this presentation -  
5 - to what extent could these be responsible,  
6 for example, for the lack of the association  
7 between adjuvant potency and toxicity? I  
8 think this is one of the key targets of use of  
9 adjuvants.

10 So what are the challenges to  
11 adjuvant safety? Well, we have already seen  
12 this before. An adjuvant shall not be  
13 introduced into a product unless there is  
14 satisfactory evidence that it does not  
15 adversely effect the safety or the potency of  
16 the product.

17 We all know, of course, that  
18 vaccines are going to induce some side  
19 effects. We have some local side effects. We  
20 have some, you know, systemic effects often  
21 due to some cytokines circulating around.

22 But what we really want to avoid

1 is that the use of adjuvants would enhance  
2 local reactogenicity or even worse, would also  
3 enhance systemic reactions.

4 So what we want to avoid is severe  
5 reactogenicity. And we are especially, I  
6 think, scared of a kind of generalized,  
7 unspecific stimulation of innate immune cells  
8 breaking tolerance, for example, things that  
9 may lead to immune pathology.

10 So how can we avoid this? Well  
11 just first of all a couple of statements that  
12 I -- citations from literature. And we may  
13 have a number of questions around these  
14 statements.

15 But, you know, at least I think  
16 they clearly illustrate that, indeed, vaccine  
17 safety and tolerability are critical  
18 regulatory issues. And probably one of the  
19 greatest barriers to the approval of new  
20 adjuvants. And the fact is we have only a few  
21 adjuvants that are approved right now -- only  
22 alum in the U.S. and a couple of others in

1 Europe.

2 So do we have to live with this?

3 No pain, no gain. So this was, you know, the  
4 kind of spirit I tried to raise my kids in  
5 saying, you know, if you want to achieve  
6 something, it is first going to hurt you. But  
7 they are saying, you know, this is obsolete.

8 I'm kind of old fashioned and they  
9 may be right because if it hurts too much,  
10 people don't want to have vaccines anymore.  
11 And that's not good either. It would be bad  
12 for the perception and the acceptability of  
13 the vaccines.

14 So we know where this dogma is  
15 coming from. And it seems like in the past we  
16 thought we have to make a kind of trade off.  
17 If it is for a very important disease, you  
18 know, it can hurt a little bit more.

19 So the question really is can we  
20 disassociate this? Do we need to continue to  
21 live with this dogma? And what can we do  
22 about this?

1                   So first of all, very simple, I  
2 would say let's have a look in the causes of  
3 adjuvant-related safety issues. On the left-  
4 hand side, we see local reactogenicity.

5                   And what are the reasons for local  
6 reactogenicity or the cause? Well, it is  
7 either going to be some local -- I don't know  
8 whether this works -- it doesn't seem to work  
9 -- some local irritation, some local tissue  
10 insult that could be caused by a number of  
11 compounds. And that is going to generate some  
12 local inflammatory reaction.

13                   Or it could be by a local  
14 activation of the -- I don't know, it would be  
15 useful if it -- I don't know, I can't see it -  
16 - well, at the bottom on the left-hand side,  
17 it can also be provoked, of course, by the  
18 local activation of the innate immune system -  
19 - so to say the danger signals.

20                   And if these influences become  
21 spread, become disseminated into -- over the  
22 body, then we get some systemic toxicity

1           which, of course -- oh, sorry -- systemic  
2           toxicity which could be, for example, some of  
3           these compounds that pose some unspecific  
4           inflammatory reaction start gaining the  
5           systemic circulation and disseminate in  
6           different organs, we could get possibly some  
7           organ toxicity.

8                        You know, this is not something  
9           not something that we are often observing. We  
10          know this material can, to some extent, be  
11          degraded, excreted by urine, et cetera.

12                       So I think what we are more  
13          concerned about is when these danger signals  
14          start spreading to the systemic circulation  
15          and then, you know, send the immune system in  
16          a kind overdrive where we then get this  
17          generalized stimulation of innate immune  
18          cells. And that is definitely something we  
19          want to avoid.

20                       So how do we achieve a potent  
21          adjuvant effect while reducing its likelihood  
22          of causing local reactogenicity? Well, it is

1           pretty simple in fact. First of all, we'll  
2           use adjuvants with low or reduced intrinsic  
3           toxicity -- and I'm going to come back to this  
4           point and give you some more explanation.

5                       And then secondly, and maybe even  
6           more importantly, restrict -- we have heard  
7           this already before today -- restrict the  
8           delivery to the site where you expect them to  
9           exercise their effect and certainly not into  
10          the systemic circulation.

11                      So the message, obviously, is  
12          increase really the retention of the adjuvant  
13          at the injection site and avoid release from  
14          the injection site. This will also allow you  
15          to lower the dose, of course, of the adjuvant.  
16          And on top of this it is favorable for safety.

17                      So first of all, yes, we'll use  
18          adjuvant with low and reduced intrinsic  
19          toxicity. I'm not going to explain all this  
20          in detail but, you know, we know that  
21          adjuvants can, indeed, be detoxified  
22          genetically or chemically. And I've listed a

1 couple of examples there.

2 Some of the adjuvants, especially  
3 detergents, for example, can be physically  
4 quenched so that they are less toxic. And  
5 there are even natural mechanisms of  
6 detoxification which, for example, enzymatic  
7 degradation, this is a way, for example, some  
8 of these polyelectrolytes or polyionic  
9 adjuvants work.

10 They can cross-link structures on  
11 the surface of membranes and, therefore,  
12 induce signaling. So when they get degraded,  
13 this signaling will finally be weakened and  
14 stopped.

15 So the other way to reduce  
16 toxicity is to restrict adjuvant delivery to  
17 tissue- resident dendritic cells at the  
18 injection site, basically, I guess, dealing  
19 with parental vaccination.

20 I think we all agree that  
21 dendritic cells are the cells that we want to  
22 target. These are the guys that are going to

1 traffic to the lymph nodes, that are going to  
2 present the antigen, that are going to be  
3 responsible for immune signaling, et cetera.

4 So if we think about adjuvants,  
5 for example, that enhance antigen  
6 presentation, we often call them antigen  
7 carriers, and we see that first of all --  
8 well, if you look at this part of the cartoon,  
9 I think the message I want to convey there is  
10 that it is very important and we know that  
11 particulate formulations, for example, are  
12 particularly well suited for delivery and  
13 targeting to dendritic cells.

14 And so in order for dendritic  
15 cells to make optimal use of the antigen and  
16 to prime CD4 T cells, for example, and to  
17 induce the differentiation of CD8 and CLs, et  
18 cetera, well, to make optimal use of the  
19 antigen, the antigen should ideally be  
20 presented in MHC Class II or MHC Class I  
21 presentation.

22 And I'm not going to go into the

1 detail of the immunology here but the way the  
2 antigens are presented and the way they are  
3 processed by the dendritic cells very much  
4 depend on the mechanisms of internalization of  
5 the antigen, of the mechanisms of entry.

6 And there is -- so, as I said, I  
7 appended a number of literature -- references  
8 from literature on this -- it has now be  
9 fairly clearly proven, I would say, that, for  
10 example, lipid clathrin-mediated endocytosis  
11 is very favorable to the presentation of the  
12 antigen into MHC Class I presentation whereas  
13 receptor-mediated uptake by the dendritic  
14 cells through phagocytosis, for example, would  
15 rather favor the presentation of MHC Class II.

16 Now what is interesting is that  
17 these mechanisms of internalization very much  
18 depend upon the physical properties of the  
19 antigen. And so you see some sizes that I  
20 have, I would say, copies from what is cited  
21 in the literature, but it is, of course, much  
22 more complex than that.

1                   It is not just about the size. It  
2                   is also about surface charge, about potential,  
3                   et cetera. Globally speaking, it is about the  
4                   surface properties of these particles.

5                   We should not forget that  
6                   particles, in order to get internalized, they  
7                   are going to interact with the lipid bilayers  
8                   and the interfacial properties between the  
9                   particles and the surface membrane is going to  
10                  be very important.

11                  These are some of the things we  
12                  didn't pay enough attention to, I guess, in  
13                  the past. We may want to not forget that, for  
14                  example, all these things like antigen  
15                  presentation, endocytosis, phagocytosis, like  
16                  signaling, for example, also the key junction  
17                  between the APC and the T cell, which is the  
18                  key link between innate and adaptive  
19                  immunities, all are about signaling membranes.

20                  So if we manage to present small -  
21                  - the antigen using these antigen carriers,  
22                  for example, as small particles in multimeric

1 colloids, it is going to favor these  
2 mechanisms of internalization, particularly  
3 colloids are very appropriate to induce lipid  
4 raft-mediated endocytosis whereas small  
5 particles, monodispersed particles are  
6 favorable to be taken up through phagocytosis.

7           So this is going to ensure maximal  
8 antigen presentation and very efficient  
9 antigen presentation. If you don't do this,  
10 for example, and we leave the antigens just  
11 like free monomeric compounds, then we will  
12 find out that they will simply diffuse in the  
13 systemic circulation. There is no antigen  
14 uptake whatsoever.

15           And we may think, well, it is not  
16 efficient so we will increase the dose. But  
17 then, in some cases, we may even end up with  
18 organ toxicity, which is, for example, if you  
19 are using cationic peptides or things like  
20 that, those things may be toxic if they start  
21 to circulate, broadly circulating.

22           So on the other hand, if we now

1 use carriers that really transform these  
2 particles into large, large aggregates, for  
3 example, micro sized particles and big  
4 droplets, for example, then we are probably --  
5 because this is not the ideal size -- this is  
6 not the ideal conformational shape and  
7 confirmation for the antigens to be taken up  
8 by the dendritic cells -- we are probably  
9 rather causing local inflammation than any  
10 kind of beneficial antigen presentation.

11 And to gain, because this is not  
12 very efficient, we may want to increase the  
13 dose and even make the situation worse.

14 So the very same effect applies to  
15 immune potentiators. So the adjuvants, the  
16 real adjuvants that have the immune signaling  
17 effect -- and, we know, of course, their  
18 target cells are also the dendritic cells --  
19 again small particles and multimeric colloids  
20 have been shown to really enhance -- and I'm  
21 dealing here especially with TLR receptors to  
22 enhance TLR signaling.

1                   We know, for example, that  
2           transmembrane TLR signaling is associated with  
3           phenomena of lipid membrane dynamics, of lipid  
4           rafts, for example. This has been nicely  
5           documented by many scientists.

6                   So well these multimeric colloids,  
7           for example, are going to favor lipid raft-  
8           mediated endocytosis. This is also, by the  
9           way, the mechanism by which we make the  
10          signaling transient because really these  
11          receptors get then endocytosed into the cell.  
12          And that makes the signaling transient.

13                   So we also know that -- and this  
14          is really based on some empirical findings --  
15          that if we want to make agonists for TLR C7,  
16          8, 9, that interact with endosomal receptors,  
17          if we want to make them more active, we need  
18          to formulate them.

19                   And people have found out that  
20          turning those guys into particles, for  
21          example, that then get taken up by endosomes  
22          and phagosomes is going to make their effect

1 much more sufficient.

2 So it seems that also for the  
3 immune potentiator itself, it is going to be  
4 favorable to present them as small particles  
5 or multimeric colloids. It is going to allow  
6 you to reduce the dose and to improve on the  
7 ratio between biological activity and  
8 toxicity.

9 So, again, if you don't do that  
10 and you end up with small molecules like  
11 SMIPs, and there is nothing to say against  
12 these molecules, the only thing is, you know,  
13 you need really to formulate them. We know  
14 this.

15 If you leave them alone, they are  
16 going to diffuse in systemic circulation.  
17 There is no local adjuvant uptake. And this  
18 may result into poor biological activity. And  
19 then what do we do?

20 Well, we increase the dose and it  
21 is even worse. It might -- that's where the  
22 questions come up about immune pathology or we

1 go into to send the whole immune system in  
2 overdrive because these things start to  
3 circulate.

4 And, again, the same if we  
5 formulate adjuvants in a way that we end up  
6 with large particles. And there are some  
7 examples, for example, of lipid A aggregates,  
8 and I will show you some of those from  
9 literature, then again these particles will  
10 not be taken up efficiently by the dendritic  
11 cells, will not end up into efficient  
12 interaction with transmembrane or endosomal  
13 TLR receptors. And finally may be causing  
14 more local inflammation than anything else.

15 So ideally adjuvants should come  
16 in particulates and/or colloid suspensions.  
17 So that also means that inappropriate adjuvant  
18 formulation or control thereof may lead to  
19 diminished adjuvant potency. But also it is  
20 a major -- it could be a major cause of  
21 adjuvant reactogenicity, toxicity, and lack of  
22 consistency.

1                   And we know -- I'm not citing  
2           here, I'm not mentioning the companies -- but  
3           all these are different techniques that  
4           companies are now using to make their  
5           adjuvants more particulate, to give them more  
6           complexity, to give them a more confirmational  
7           structure.

8                   And it is all about association  
9           with particles, with delivery vehicles,  
10          absorbing on particles, integrating,  
11          encapsulating into particles and so on.

12                   And I just wanted to show you one  
13          example on the physical importance, for  
14          example of a lamellar versus an inverted  
15          micellar lipid A, which -- for example, this  
16          adjuvant comes naturally in two different  
17          shapes. You see the lipid particles here and  
18          over here. And you should see the lamellar  
19          form here.

20                   Well, this is called a lipid A  
21          polymorphism. And there is a kind of  
22          equilibrium between both. Well, people have

1 found out about 20 years ago -- and the tests  
2 they were using at that time were not as  
3 sophisticated as the immunological panels of  
4 tests that we have right now -- but they  
5 really found out that, for example, if you  
6 present lipid A -- and as I said, it is an  
7 equilibrium between the lamilar form, which is  
8 here on top of this slide, and the inverted  
9 micellar form.

10 And you can shift this  
11 equilibrium. It is depending on the  
12 environmental conditions. I'm not going to go  
13 into the detail but anyway they clearly found  
14 out that this is the biological form which is  
15 active, which is causing signaling, which is  
16 having the biological activity.

17 If you manage, by the way you  
18 treat this in the formulation, to shift the  
19 equilibrium to the inverted micellar form, you  
20 will end up with higher biological activity  
21 whereas the opposite is true if this fraction  
22 is going to more important. So I think this

1 clearly illustrates the importance of physical  
2 constraints on the biological activity. And  
3 safety is, of course, part of biological  
4 activity.

5 So just to give a couple of  
6 practical maybe implications of this, and to  
7 wrap up very clearly safety is the major  
8 concern of regulatory authorities, and we have  
9 seen some of these regulations summarized  
10 already. I just kind of wanted to focus on  
11 the three last bullets, which are about  
12 characterization, stability, and critical  
13 process parameters.

14 I would like to insist that we  
15 think of these bullets as not only being  
16 applicable to chemical and biological  
17 characterization, stability and critical  
18 parameters to be in control of, but also the  
19 physical -- the physical. So the  
20 characterizations need to include physical  
21 aspects. The stability needs to look after  
22 physical stability. And critical parameters

1           need to be also applicable for physical  
2           parameters.

3                       So in order to try to convince you  
4           even more of this, well, this is mayonnaise,  
5           right, this is the mayonnaise made by my wife.  
6           It's the perfect dressing. It smells good.  
7           It tastes good. It is just perfect.

8                       When I'm doing the same thing, I'm  
9           using exactly the same ingredients, the same  
10          vinegar, the same mustard, I'm using eggs from  
11          the same hen. But obviously not knowing  
12          exactly what are the critical parameters, I  
13          don't do the mixer right. And you can see it  
14          is just a mess.

15                      And the biological activity seems  
16          to be different, right? So -- and the only  
17          thing which is different is the physics of the  
18          whole thing.

19                      So what is important, I guess, is  
20          that we control adjuvant delivery and ensure  
21          consistency. Well to do this, we usually tend  
22          to prefer using small-sized colloid

1       particulate suspensions. I would say it is  
2       better, probably, to stay away from soluble  
3       molecules, monomeric detergents, things like  
4       this, and certainly large, irreversible  
5       aggregates that do not dissolve, it's probably  
6       not going to contribute to biological activity  
7       either.

8               Characterize your adjuvant's dirty  
9       little secret versus well characterized  
10       product. I think to some extent the dirty  
11       little secret comes from all kinds of physical  
12       interactions that we don't well characterize.  
13       And we do have the tools right now. I cannot  
14       go into the detail of this but we do have the  
15       tools today to well characterize interaction  
16       and to well characterize also biophysical  
17       features of adjuvant formulations.

18               I just wanted to mention that also  
19       delivery can be an important aspect for  
20       safety. It is because the environment is  
21       different, depending on the route of  
22       administration, you may change also physical

1 constraints, physical parameters, and, hence,  
2 biological activity.

3 If you think, for example, of  
4 intradermal delivery, well it's going to be  
5 usually pretty safe. I mean we have already  
6 kind of topical administrations so you are  
7 likely to avoid systemic effects.

8 And also with the way we do this  
9 intradermal administration, for example, and  
10 also due to the physiology of the skin, it is  
11 less likely that you are going to induce local  
12 reactor.

13 There are some disadvantages, of  
14 course. Less local reactor means that you  
15 cannot rely on inflammation as a kind of  
16 initiator of adjuvanticity, which you can, for  
17 example, in a muscle. If you induce some  
18 local inflammation already, well, we know that  
19 inflammation can trigger adjuvanticity.

20 So other routes of delivery -- I'm  
21 not going, for the sake of time, to go into  
22 the detail but, for example, intrapulmonary,

1 I think we all agree that this one is pretty  
2 likely to favor systemic distribution.

3 Now is that what you want to do  
4 with adjuvants? I don't think so. So this  
5 one would be pretty tricky, you know, talking  
6 about adjuvanted vaccines of course.

7 As always, use common sense. I  
8 don't think it is very useful if you have a  
9 very complex adjuvant mixture, which is partly  
10 characterized, and then you envisage to  
11 administer this in a prophylactic context to,  
12 for example, young children. I think this  
13 makes sense.

14 Also avoid the delivery, as I was  
15 just saying, of adjuvants through  
16 administration routes that enhances systemic  
17 uptake. I would advise against this. And I  
18 think this is something we should be very  
19 cautious about.

20 So keep it simple is also very  
21 important. Avoid cocktails. Avoid chemical  
22 association between adjuvant and antigen.

1           Avoid interactions between antigen and  
2           adjuvant.

3                       I know this is quite revolutionary  
4           but it is true -- and I'm not think especially  
5           about regulatory constraints there -- but it  
6           is true that if you generate all these  
7           interactions knowing that physical  
8           interactions and the outcome thereof may have  
9           an impact on the biological activity, it will  
10          be important to characterize this.

11                     It will be important to control  
12          these things. And the less interactions you  
13          are generating, the easier you are going to  
14          make your job.

15                     So avoid adjuvant that are  
16          potentially immunogenic. I think this is a  
17          no-brainer. And keep it TLR dependent. Well,  
18          I think TLRs or the TLR agonists, we have a  
19          lot of them already, and we have some tools to  
20          characterize them, we have these knockout  
21          systems. We can over-express the genes. We  
22          have kind of reporter gene systems that we can

1 use.

2                   They are fairly well  
3 characterized. And I think one of the  
4 advantages, as well, is that for the TLR  
5 agonists, we have kind of integrated action  
6 with the immune system. It's not only about  
7 stimulating innate immunity. They do have  
8 impact on adaptive immunity and even on the  
9 regulatory networks.

10                   So optimize formulation and  
11 delivery as to be able to reduce the dose is  
12 obviously key. So keep it simple, which  
13 doesn't mean that we need to say with alum for  
14 the rest of our lives, right.

15                   I'm not going, you know, to open  
16 this box of Pandora, but I think we all agree  
17 that, you know, we feel talking about diseases  
18 that require cellular-mediated immunity and  
19 things like that, alum will not be sufficient.

20                   So just two words -- preclinical  
21 safety assessment. Preclinical safety  
22 assessment is obviously important because it

1           can give us some warning signs on safety  
2           profiles of adjuvants.

3                           And I just wanted to highlight  
4           that -- and we have seen this already in the  
5           other sessions this morning -- that it is not  
6           about the effect in isolated human cells.  
7           Also the peripheral cells and the tissue can  
8           significantly contribute to induction of  
9           innate immunity.

10                          And, therefore, it is interesting  
11           that today we have kind of systems that  
12           integrate several different immune-competent  
13           cells and also the inflammatory compounds.  
14           And those systems may be interesting to use  
15           for assessing and better understanding some  
16           mechanisms of innate immunity and adjuvants in  
17           general.

18                          With regard to the animal model, I  
19           mean we could discuss for hours and hours.  
20           I'm just thinking that if you want to study  
21           really the delivery and the distribution of  
22           adjuvants and adjuvanted vaccines, well the

1 mice may not be the ideal model.

2 And I'm just thinking of this  
3 like, you know, squeezing an elephant in a  
4 Mini Cooper. And then you would ask the  
5 elephant to only sit in the driver's seat, for  
6 example, right.

7 So if you look at the mice and the  
8 volumes we are giving to the mice in relation  
9 to what we are doing in human clinical trials,  
10 it is very likely that because the routes of  
11 administration -- intranasal, for example, in  
12 the mice versus humans, it is not comparable.

13 Putting a large volume in the mice  
14 or a small animal, it may have an impact on  
15 the distribution, on the retention of the  
16 adjuvant and, therefore, not be a good model  
17 in terms of studying distribution and local  
18 retention.

19 So large animals are particularly  
20 useful for testing different delivery systems.  
21 I don't want to say that we should, you know,  
22 use only large animals but in terms of

1 distributing the local effect, then the  
2 distribution may be very useful.

3 Should we do pharmacokinetics?

4 Well, regulatory agencies have already been  
5 thinking about this. If there is an  
6 indication that an adjuvant might be  
7 distributed over the body and/or accumulate in  
8 well-defined tissues, pharmacokinetic studies  
9 should be considered.

10 Well this may be a way, you know,  
11 of finding out whether some of these adjuvants  
12 are distributing into the systemic  
13 circulation, something we would like to avoid.

14 It is not usually performed with  
15 vaccines because there is no relationship  
16 between plasmic concentration of antigen and  
17 immunogenicity but there might be some kind of  
18 relationship between systemic side effects and  
19 circulating adjuvants.

20 So conclusions in -- well just in  
21 a nutshell, I think it is important that you  
22 have a kind of good rationale for all the

1 different ingredients that you are using.

2 It is particularly important to  
3 really focus the effect on the key immune  
4 cells. And that can be done by using  
5 formulations -- formulations that are well  
6 conceived, that are well thought of, and that  
7 are also well characterized.

8 And I think this is going to help  
9 us to make products that are -- because these  
10 are the requirements of a product to be  
11 consistent, to be maybe more safe, and to make  
12 optimal use of the antigen and the adjuvant.

13 And it is my personal belief that  
14 this is not going to be possible to get the  
15 guys, you know, science, and technology to get  
16 these people around the table as well. This  
17 is going to be very, very important because it  
18 is going to trigger the upstream mechanisms of  
19 immune signaling.

20 We are mainly focusing always on  
21 downstream signaling. This is going to  
22 condition the interaction of antigen and

1           adjuvant with the target cells.

2                   And obviously there is some more  
3           discussion needed on the animal model.

4                   So adjuvant dose, the less the  
5           better. I mean, you know, also I would say in  
6           terms of the number of adjuvants. Well, the  
7           fewer adjuvants you are using, I think the  
8           more easy -- the easier it is going to be to  
9           develop them into true products that we can  
10          use in adjuvanted vaccines.

11                   And well the more it is targeted,  
12          of course, the less the likelihood that you  
13          are going to run into toxic effects.

14                   So we have apparently a mission  
15          here. There is a call for more interest, for  
16          more investment, for more resources in to  
17          adjuvant development, to make it possible to  
18          move some of these candidates forward into  
19          clinical development.

20                   There is a call also to funding  
21          agencies. Well, we at the Gates Foundation,  
22          we are taking this very, very serious.

1                   We have in our portfolio, for  
2                   example, diseases like HIV, malaria,  
3                   tuberculosis, and we have a firm commitment to  
4                   the development of vaccines. And we cannot  
5                   have this commitment -- make this commitment  
6                   without being also firmly committed to the  
7                   development of adjuvants.

8                   So that is basically what are our  
9                   goals. We want to foster efforts that help us  
10                  to move candidates forward into clinical  
11                  development and to also make them available  
12                  for developing countries.

13                  So with these stats, I thank you.  
14                  And, well, if there are any questions, I will  
15                  be happy to take them. Thank you.

16                  (Applause.)

17                  DR. SUTKOWSKI: Okay. Just one  
18                  quick question.

19                  DR. MALONE: The Foundation has  
20                  been an advocate for alternative vaccine  
21                  delivery technology including jet injection,  
22                  for example. My understanding -- when you

1 think about it, vaccines are really  
2 combination products. We have an  
3 administration device and the formulation.

4 Is there any evidence for any of  
5 these alternative delivery technologies  
6 altering the properties of adjuvant-formulated  
7 vaccines? Does that make sense?

8 DR. VAN DEN BOSSCHE: Yes, well I  
9 think we have been moving forward some of  
10 these efforts quite rapidly. And I think we  
11 are in the process of reviewing and better  
12 understanding what is going on because if you  
13 add on top of this -- so, for example,  
14 alternative routes of delivery, there is an  
15 additional component that you add on top of  
16 this which is, for example, the device, which  
17 is different from the needle.

18 So this is going to add to the  
19 complexity. And as I was just saying, the  
20 route of delivery, it may impact. So we need  
21 to take these things into consideration. We  
22 really need -- we don't have all the answers.

1 We're looking for the answers.

2 But we are sure that if we are  
3 going to better understand what is the impact  
4 of all this, that we are going, you know, to  
5 make better choices and that we are going to  
6 move forward these alternative technologies in  
7 a way that is going to be very useful for what  
8 are our goals in the end, which is to deliver  
9 safe vaccines to developing countries that are  
10 very efficient and that are widely available  
11 at low cost, right.

12 DR. SUTKOWSKI: Thank you.

13 Okay, our last and final speaker,  
14 Dr. Pulendran, he's -- if you listen to all  
15 this advice and you get the physical/chemical  
16 characteristics right and you get back to the  
17 biology here, Dr. Pulendran will be talking to  
18 us about a slightly different title than what  
19 is in the agenda. He'll be talking on his  
20 most recently published work on systems  
21 biology and vaccine development.

22 DR. PULENDRAN: Thank you very

1 much, Liz.

2 I realize that we are overdue for  
3 lunch so I'll be -- I'll try and be brief and  
4 not hold you back too much from lunch.

5 So the focus of my presentation is  
6 some work that we're doing to apply systems  
7 biology to try and understand how vaccines  
8 work and how adjuvants work.

9 And we've heard this -- this  
10 particular slide the message from this slide  
11 should be very obvious to all of us -- I mean  
12 a lot of speakers have spoken about this today  
13 and that is that the quality of the adaptive  
14 immune response is absolutely key in  
15 determining protection against different  
16 microbes or viruses.

17 So, for example, Th1, Th2, Th17,  
18 Treg cells are all very important in  
19 conferring protection against different  
20 microbes or pathogens. And, in fact, Tregs  
21 are also useful in controlling the immune  
22 system altogether.

1                   So from a vaccinologist's  
2                   perspective, a central question is what are  
3                   the adjuvants and what are the mechanisms by  
4                   which you could stimulate these different  
5                   types of immune responses that could be  
6                   optimally effective against different  
7                   microbes?

8                   And, again, this is now obvious to  
9                   all of us that the dendritic cell, the  
10                  antigen-presenting cell is absolutely key in  
11                  this process.

12                 In fact, there are many types of  
13                 dendritic cells. And these seem to be  
14                 programmed differently. They express  
15                 different markers on their surface. They make  
16                 different cytokines. And then they can lodge  
17                 different types of immune responses.

18                 And so the question is how can  
19                 different adjuvants target these specific  
20                 types of dendritic cells to program the immune  
21                 response in a given direction? And then as  
22                 we've heard from Bruce and others, the Toll-

1       like receptors and other pathogen recognition  
2       receptors are key in recognizing vaccines and  
3       adjuvants and then in programming the adaptive  
4       immune response.

5                There are many different TLRs,  
6       some expressed on the surface, some inside the  
7       -- in the intracellular compartments in  
8       dendritic cells. And so the question is how  
9       can we exploit them in vaccine design?

10               So with this very broad kind of  
11       perspective, really the questions that we'd  
12       like to focus on are as follows:

13               Firstly, can we apply this new  
14       knowledge in innate immunity to understand the  
15       mechanism of action of some of our most  
16       empiric, highly successful vaccines? And then  
17       if we can get insights from this kind of  
18       approach, in what way can these insights guide  
19       the development of new vaccines against  
20       emerging infections and pandemics?

21               So this is Sir Edward Jenner more  
22       than 200 years ago doing his most historic

1 experiment in human immunology of actually  
2 vaccinating this child with cox pox pus and  
3 then showing that this kid was then immune to  
4 further infection with smallpox.

5 And then as we've seen before,  
6 since that time, many, many vaccines have been  
7 developed, many highly successful vaccines.  
8 And so as I mentioned earlier, one of the  
9 paradoxes is that we really don't understand  
10 the mechanisms by which they stimulate immune  
11 response.

12 And so some work in our lab has  
13 focused for the last three or four years on  
14 understanding how exactly the yellow fever  
15 vaccine works. Why the yellow fever vaccine?  
16 Well, it just happens to be a very successful  
17 vaccine.

18 It is one of the most effective  
19 vaccines ever made. It is a live virus. One  
20 injection of the vaccine give you a very broad  
21 spectrum of immune responses, Th1, Th2,  
22 cytotoxic T cells, neutralizing antibody. And

1           remarkably, one injection of this vaccine  
2           gives you neutralizing antibody that can last  
3           for up to 30 years, okay.

4                         So here we have a model, a model  
5           vaccine that we've given to 600 million people  
6           globally but we don't really understand how it  
7           works. So the simple question is can we  
8           deconstruct this vaccine immunologically such  
9           that then you could design adjuvants that do  
10          exactly the same thing, or facets of these  
11          kinds of immune responses that this vaccine  
12          does.

13                        So this really summarizes all that  
14          we have been talking about this morning and  
15          that we believe that this black box called the  
16          innate immune system is absolutely key in this  
17          regard.

18                        So what are some of the pathways  
19          and the receptors within this black box that  
20          control these different types of immune  
21          responses?

22                        So as I mentioned to you earlier,

1 we began a few years ago by demonstrating that  
2 this vaccine was triggering multiple Toll-like  
3 receptors and that this seemed to be relevant  
4 because depending on which TLR was engaged,  
5 you seemed to get either a Th1 or a Th2 bias,  
6 suggesting that one reason -- it's not a  
7 reason but one of the manifestations of  
8 activating multiple TLRs is to give you a  
9 balanced Th1/Th2 response.

10 And since then we've gone on and  
11 we've also understood that this vaccine is  
12 engaging or activating this pathway within the  
13 plasmacytoid dendritic cells, the so-called  
14 mammalian target of rapamycin, which seems to  
15 control the production of Type 1 interferons  
16 by plasmacytoid dendritic cells.

17 So this is an example of some new  
18 biological insight that one can get by trying  
19 to deconstruct a really successful vaccine,  
20 okay, that we wouldn't necessarily have done  
21 this experiment if not for the fact that we  
22 were looking at how this yellow fever vaccine

1 is working, okay.

2 But one major focus of our lab is  
3 this. And that is to apply this new science  
4 of systems biology to trying to understand if  
5 we can predict the immunogenicity of vaccines  
6 and, indeed, to predict the toxicity of  
7 vaccines, okay, in a completely unbiased kind  
8 of way.

9 So put simply, the question is are  
10 there innate signatures that are induced by  
11 vaccination in humans with which you can  
12 actually predict the subsequent immune  
13 responses or the toxicity of vaccines or  
14 adjuvants?

15 So a couple of years ago, we began  
16 to address this question with a small clinical  
17 trial in humans who were vaccinated with this  
18 yellow fever vaccine. Blood samples were  
19 removed at these different time points,  
20 including very early time points like Day 1,  
21 Day 3, Day 7. And then later on at Day 120,  
22 180, 160.

1                   And we made various measurements.  
2           We measured the antigen-specific CD8 T cell  
3           responses and the neutralizing antibody  
4           titers. And then we also made measurements of  
5           so-called innate responses in the blood.

6                   And so the question was by looking  
7           here early on, could we predict what is going  
8           on later on, okay? And why would we want to  
9           do this? Well, because when we design new  
10          vaccines of questionable efficacy, we would  
11          like to think that this kind of strategy could  
12          be informative in evaluating the potential  
13          success or efficacy of emerging new vaccines  
14          is the first reason.

15                   Second reason is that the same  
16          kind of approach, we would like to think,  
17          could be useful in predicting potential  
18          toxicities that might be stimulated by  
19          vaccination or adjuvants.

20                   And thirdly, because we think that  
21          this kind of unbiased global analysis would be  
22          useful in providing new biological insights

1 about the mechanism of action of vaccines and  
2 adjuvants, okay.

3 So the approach is shown here,  
4 that we measure cytokines in the blood using  
5 Luminex platform. We look at the activation  
6 of different types of dendritic cells and  
7 monocytes in the blood.

8 And then we do a microarray  
9 analysis of the gene expression profiles in  
10 the blood. And this was done in collaboration  
11 with Alan Aderem at the Institute for Systems  
12 Biology in Seattle.

13 So first when we look at the  
14 immune response -- so this is -- we have a  
15 tetramer that can stain for the yellow fever-  
16 specific CD8 T cells and you can clearly see  
17 that this is the population that comes up two  
18 weeks after vaccination.

19 And you can characterize the  
20 phenotype of those T cells using a number of  
21 different markers. And this was done in Rafi  
22 Ahmed's lab at the Emory Vaccine Center.

1                   And what is apparent is that if  
2                   you gate on those tetramer-positive T cells  
3                   shown here as red dots and you overlay this on  
4                   a profile that shows HLA-DR versus CD8 on  
5                   gated CD3-positive T cells, in fact there is  
6                   a remarkable correlation between the  
7                   coexpression of CD8 HLA-DR and tetramer  
8                   positivity. And this is shown nicely in this  
9                   linear graph here.

10                   And so, as I mentioned, you could  
11                   phenotype these cells and it turns out that  
12                   these tetramer-positive T cells at Day 15 are  
13                   mostly effector cells.

14                   They express high levels of CD27,  
15                   higher levels of CD28. They are dull for  
16                   BCL2. They are dividing because they are Ki67  
17                   positive. And they are CCR5 high and CCR7  
18                   dull, suggesting that they are highly  
19                   activated effector cells.

20                   And so if you look at the  
21                   variation in the magnitude of the CD8 T cell  
22                   responses in these 15 individuals, the first

1 surprise was that, in fact, there is a  
2 striking variation. So, for example, these  
3 vaccinees here seem to have a relatively poor  
4 response compared to these vaccinees, okay.

5 So we were initially worried  
6 because we thought that perhaps this was a  
7 technical flaw in terms of the vaccine not  
8 being administered successfully in the clinic.

9 But, in fact, when we looked at  
10 the neutralizing antibody titers in these  
11 individuals, for example, 1910 and 1920, who  
12 were relatively poor at CD8 T cells responses  
13 do just fine with the neutralizing antibody  
14 titers suggesting that no, these people, in  
15 all likelihood, did receive the vaccine.

16 So for us the question was could  
17 we predict this. For example, we see this  
18 variation in T cell responses or variation in  
19 the antibody responses. By looking early  
20 after vaccination, could be identify  
21 correlates that would predict how strong a T  
22 or B cell responses a particular vaccinee

1           might have.

2                       So, as I mentioned, we did a  
3           comprehensive analysis of cytokines in the  
4           blood. And the short answer is that despite  
5           the up-regulation of these two molecules, IP-  
6           10 and IL-1 alpha, in the majority of the  
7           vaccinees, really there was nothing that  
8           correlated with the magnitude of the T cell  
9           responses or the B cell responses.

10                      And then we also looked at the  
11           activation of these various dendritic cell  
12           monocyte subsets and, again, you know, we see  
13           up-regulation of CD80/86 and so on but nothing  
14           that seemed to segregate people who had a high  
15           CD8 versus a low CD8 or a high antibody versus  
16           a low antibody.

17                      This suggested to us that the so-  
18           called traditional correlates that we might  
19           have been programmed into looking for were not  
20           sufficient for this exercise. That we needed  
21           a much more unbiased kind of approach to  
22           identify potentially new correlates, okay.

1           So the approach we took was a  
2           microarray analysis of total peripheral blood  
3           mononuclear cells using the Affymetrix  
4           GeneChip platform. And the first question was  
5           whether we could identify genes that were  
6           reproducibly up- or down-regulated in the  
7           majority of the vaccinees who got this  
8           vaccine, okay.

9           And in the interest of time, I'm  
10          not going to belabor the statistical approach  
11          but we can talk about that later on if you'd  
12          like to.

13          So the message that came across  
14          quite strongly was that yes, there were a  
15          subset of genes, about 65 genes, that were  
16          reproducibly expressed in the majority of the  
17          people who got the vaccine.

18          And so this is shown here in this  
19          heat map. And if you focus on Trial 1, which  
20          is the first trial with 15 individuals -- and  
21          so here we have the kinetics, Day 0, 1, 3, 7,  
22          and 21. And unfortunately you can't read the

1 names of these genes here but I can point out  
2 some of the important ones.

3 So, for example, you have  
4 oligoadenylate synthetase 1, synthetase 2,  
5 synthetase 3, synthetase L, RIG-I, protein  
6 kinase R, MDA5, TLR7, Lgp2, all the usual  
7 suspects that you might normally associate  
8 with a viral infection. And remember this a  
9 live attenuated virus, okay.

10 So this was reassuring because  
11 this kind of approach had not been done with  
12 vaccines. And so this was -- and going into  
13 this, we didn't have any idea that we would  
14 see anything that any signature could be  
15 detectable in the blood because we are giving  
16 this vaccine subQ. But this was actually  
17 reassuring, that told us that perhaps this  
18 kind of approach does have merit, okay.

19 So the question was to what extent  
20 is this simply an artifact? And can we  
21 validate the signature using other approaches?

22 So the one thing that we did do

1           was to use a realtime PCR approach to  
2           systematically evaluate the expression of all  
3           of those 65 genes that we had seen. And,  
4           again, we see a very nice correlation between  
5           the expression based on the microarray data  
6           and the realtime PCR data.

7                        And the second thing was a couple  
8           of years after the initial trial was over, we  
9           set up a completely independent trial with  
10          funding from Sanofi Pasteur to vaccinate an  
11          independent cohort to vaccinees with this  
12          vaccine, again did the same kind of approach.  
13          And we see a remarkable concordance between  
14          the signature that we had seen with Trial 1  
15          and Trial 2.

16                       And also of interest in the  
17          signature are things like IRF7, STAT1, again  
18          transcription factors that mediate the Type 1  
19          interferon response, okay.

20                       So that was all very well. So you  
21          can actually now some bioinformatics modeling  
22          and you can put in those list of 65 genes.

1           And, you know, what comes out is a signature  
2           which is pretty much a textbook signature of  
3           Type 1 interferon induction in response to  
4           viruses.

5                       And so this was a nice  
6           confirmation that, indeed, this kind of  
7           approach is viable in terms of picking up  
8           genomic signatures in response to vaccination.

9                       Now previously, as I mentioned, we  
10          had shown that this vaccine is triggering  
11          multiple Toll-like receptors but the  
12          expression of RIG-I, MDA5 raised the question  
13          as to whether it might also be engaging these  
14          additional non-Toll-like receptors.

15                      So we tested this using cell lines  
16          which over expressed our RIG-1 or MDA5 with a  
17          reported gene. And you can see that, indeed,  
18          that yellow fever vaccination could induce NF-  
19          kappa B activation in response to engagement  
20          of either one of these receptors expressed on  
21          the cell line.

22                      So this was again some new insight

1           that we had acquired through this kind of  
2           unbiased sort of approach.

3                        So now some of you might be asking  
4           well are these changes in the gene expression  
5           that you see in vivo really bonafide induction  
6           of genes within a certain cell type or does it  
7           simply reflect changes in the cellular  
8           composition of the peripheral blood  
9           mononuclear cells -- migration, exit of cells,  
10          and so on, okay?

11                      Well, this is a very difficult  
12          question to answer.  But what we did was to do  
13          a poor man's experiment to address this.  So  
14          we simply took PBMCs from a healthy  
15          individual, a couple of healthy individuals in  
16          vitro, previously unvaccinated with the yellow  
17          fever vaccine, and then just dumped this  
18          yellow fever vaccine in vitro.  And then did  
19          a microarray at a couple of different time  
20          points.

21                      And what we see is that the  
22          signatures that are induced in vitro are

1           remarkably similar to what we had seen in vivo  
2           in response to yellow fever vaccination  
3           suggesting that much of the changes that we  
4           are seeing are most likely due to de novo  
5           expression of genes rather than any  
6           alterations in the cellular composition, okay.

7                         But if you recall, the original  
8           purpose of doing this experiment was to see if  
9           there were signatures that would predict the  
10          CD8 response or the antibody responses.

11                        And so as much as we had hoped  
12          that the Type 1 interferon signature would be  
13          one such signatures, it apparently is not  
14          because we did not see any correlation between  
15          people who had a high level of expression of  
16          these Type 1 interferon genes and the  
17          magnitude of the T or B cell responses.

18                        So we resorted to a second  
19          approach to select for genes that would  
20          correlate with the antibody response or the  
21          CD8 T cell response. Again, we can talk about  
22          this but I'm going to skip this in the

1 interest of time.

2 And if you did that, from your  
3 chip, which has about 25,000 genes, you can  
4 come up with the signature of about 200 genes  
5 with which you can nicely segregate the  
6 vaccinees into two groups -- the yellow group  
7 and the red group, okay.

8 So you segregate them based on  
9 your signature that you have selected, okay.

10 And what you see is that the folks here in the  
11 yellow group have a CD8 T cell response that  
12 is less than three percent whereas the folks  
13 in the red group have a CD8 T cell response  
14 that is greater than three percent, okay.

15 So this was encouraging, showing  
16 that this kind of approach can be useful in  
17 delineating a subset of genes that seem to  
18 correlate with the magnitude of the CD8  
19 response.

20 But really the real test of such a  
21 signature is to ask to what extent it can  
22 actually predict, not simply correlate, but

1 predict immune responses in a completely  
2 independent trial. And that's what we did.

3 And working together with some  
4 bioinformatics folks at Georgia Tech who had  
5 developed a model called the DAMIP  
6 classification model -- Discriminant Analysis  
7 via Mixed Integer Programming.

8 What it is is that it is an  
9 algorithm that can sift through tons of data  
10 and recognize patterns in this data, okay.  
11 So, for example, it can look at the 200 genes  
12 and then it can begin to classify them in  
13 vaccinees, okay.

14 So using this model, we were able  
15 to come up with a set of about ten genes or so  
16 and a number of predictive rules. So we had  
17 obtained the signature using Trial 1. And  
18 then we were using a second trial of  
19 independent vaccinees to see whether we could  
20 predict the magnitude of the CD8 responses  
21 there.

22 And what we see is that with just

1 a couple of genes here, for example the solute  
2 carrier family member 2 or 6 and then this  
3 gene, eukaryotic initiation factor alpha  
4 kinase 4, you can predict in Trial No. 2, with  
5 up to 80 percent accuracy, the magnitude of  
6 the CD8 response.

7 And you can generate a number of  
8 predictive rules of this type. For example,  
9 Rule 1, Rule 2, Rule 3, and so on. And you  
10 can do the opposite. You can actually derive  
11 the signature from Trial 2 and then use it to  
12 predict in Trial 1.

13 What is very interesting to us is  
14 that many genes, for example this gene,  
15 eukaryotic initiation factor 2 alpha kinase is  
16 multiply represented in very many of these  
17 signatures, okay.

18 So this now begs the question of  
19 what some of these genes might be doing. And  
20 if, indeed, they are so important in  
21 controlling the CD8 T cell response. And so  
22 this is exactly what we are doing now.

1                   So basically it turns out that  
2           that gene, EIF2AK4, which is multiply  
3           represented in these signatures is also called  
4           GCN2 and it plays an absolutely key role in  
5           the so-called integrated stress response.

6                   Now as many of you know, the  
7           integrated stress response is launched in  
8           response to various cellular stresses. For  
9           example, recognition of viral infection  
10          through protein kinase R or oxidative stress  
11          through HRI, or stresses in the endoplasmic  
12          reticulum perhaps due to a viral infection,  
13          through PERK, and also in response to  
14          proteasome inhibition.

15                   The combination of all these  
16          stress response pathways is the  
17          phosphorylation of this molecule called eIF2  
18          alpha, okay. And it turns out that this  
19          molecule plays an absolutely key role in the  
20          translational shutdown, global translational  
21          shutdown in cells, okay.

22                   And one thing that happens when

1           that -- when the translation is shut down is  
2           that you have formation of what is called the  
3           stress granules, okay. So now this raises the  
4           question of whether this yellow fever vaccine  
5           might be trigger the stress response pathway.  
6           And if so, whether this has a link to the  
7           adaptive immune response, notably the CD8 T  
8           cell response.

9                         So we have begun to do some  
10           biology here. So, for example, you can see  
11           that yes, indeed, that in PMCs, or in the cell  
12           line, the exposure to yellow fever vaccine  
13           does result in the phosphorylation of EIF2  
14           alpha.

15                        Secondly, consistent with this,  
16           you do find the formation of these stress  
17           granules. These are dense aggregates of  
18           proteins and RNA that appear within the cell  
19           when it is under stress. And the function of  
20           this stress granule is thought to be to  
21           protect untranslated message RNA from  
22           degradation, okay.

1                   So what influence does this  
2           pathway, does the induction of this and the  
3           formation of stress granules have on the CD8  
4           T cell response mechanistically? This is an  
5           area that we are actually investigating using  
6           mice that are deficient in these various  
7           stress response genes.

8                   But our early indications are that  
9           GCN2 activation is key in the induction of CD8  
10          T cell responses to yellow fever vaccination.

11                   Now I've talked a lot about CD8 T  
12          cell responses but we should remember that  
13          this vaccine works mostly -- or is thought to  
14          work mostly through neutralizing antibody  
15          responses. Can we predict neutralizing  
16          antibody responses?

17                   And, again, the answer is yes. So  
18          we can actually do the same kind of approach.  
19          We can come up with these two clusters that,  
20          in fact, distinguish vaccinees based on the  
21          antibody titers and then we can do the DAMIP  
22          model.

1                   And what is very interesting is  
2                   that this gene, tumor necrosis factor receptor  
3                   superfamily 17, here is present in every  
4                   single DAMIP signature, either going from  
5                   Trial 1 to Trial 2 or vice versa.

6                   What is that gene? So it turns  
7                   out that this gene is also called the B cell  
8                   maturation protein, BCM, BCMA, CD269. And it  
9                   is a receptor for BAFF BlyS, which we know to  
10                  be absolutely key in the induction of antibody  
11                  responses.

12                 So here is another example of a  
13                 gene, that we've come up a through completely  
14                 unbiased sort of approach, that seems to be  
15                 one of the best predictors for the  
16                 neutralizing antibody responses 90 days after  
17                 the initial vaccination, okay.

18                 So, again, this provides one an  
19                 opportunity to delve into the biology of this  
20                 and to ask well, how is the yellow fever  
21                 vaccine inducing this gene? Is it through a  
22                 TLR dependent, TLR independent pathway and so

1 on and so forth.

2 So to summarize what I think we  
3 are seeing is that this vaccine, one of the  
4 best vaccines in the world, is engaging  
5 multiple pathways. It is engaging TLRs on  
6 multiple subsets of DCs and which downstream  
7 of that is the emptor-dependent regulation of  
8 interferons.

9 It is also engaging non-TLRs, for  
10 example RIG-I MDA5. In addition, some of  
11 these other genes like protein kinase R,  
12 oligoadenylate synthetase 1, 2, 3, and L,  
13 which are involved in innate immunity. And  
14 TRIM5 alpha and its complement component IqB,  
15 BAFF, BLyS -- I didn't have time to talk about  
16 some of these.

17 But most intriguingly, it seems to  
18 be activating many genes involved in the so-  
19 called integrated stress response. And I  
20 mentioned EFl2ASK4. I mentioned  
21 phosphorylation of eIF2 alpha and stress  
22 granules. But there are also these other

1 genes.

2 So the question is in what way do  
3 any of these modules link to the ultimate  
4 immune response and then ultimately to  
5 protection?

6 So this is exactly what we are  
7 beginning to understand now using various  
8 animal models and knockout models. But the  
9 long-term goal of this kind of approach we  
10 would like to think is that this might be  
11 beneficial in predicting the efficacy or the  
12 immunogenicity of new and emerging vaccines of  
13 questionable efficacy.

14 But also, in the context of this  
15 workshop, in perhaps predicting the potential  
16 adverse reactions that might develop from  
17 vaccinations.

18 So thank you for your attention.

19 And I'd be happy to take your questions.

20 Thank you.

21 (Applause.)

22 PARTICIPANT: Bali, I really

1           enjoyed your and Troy's paper. I've got two  
2           quick questions. One is that in that first  
3           figure you can kind of separate out CD8  
4           responses and antibody responses.

5                         And when I was in Atlanta, I got  
6           17D and I can tell you that from about Day 9  
7           to Day 16 or 17, it was a really bad week. Do  
8           those adverse events correlate with broadly  
9           CD8s or antibody?

10                        DR. PULENDRAN: Are you talking  
11           about adverse events meaning the adverse  
12           events that sometimes develop in response to  
13           this yellow fever vaccination?

14                        PARTICIPANT: No, just  
15           overwhelming rough flu-like symptoms.

16                        DR. PULENDRAN: Okay, okay, that's  
17           interesting. We haven't -- you know that is  
18           a good question -- we haven't done this  
19           analysis directly to see whether there is any  
20           correlation between the type of response and  
21           these mild adverse events.

22                        What I can tell you is that there

1           was an individual last year who developed very  
2           serious adverse events. He almost died  
3           unfortunately but then fortunately he  
4           survived.

5                           And it turns out that in this  
6           individual -- and we had expected that perhaps  
7           the reason why this person almost died was  
8           because his immune system was compromised and  
9           there was very weak adaptive immune responses.  
10          But on the contrary, we saw the opposite.

11                           In fact, there seemed to be an  
12          exacerbated CD8 response and tremendous  
13          neutralizing antibody titers that persist for  
14          a very long time. But interestingly this Type  
15          1 interferon canonical signature was absent in  
16          that individual, basically completely  
17          diminished. So, yes.

18                           DR. SUTKOWSKI: Okay. I guess it  
19          is time for lunch then.

20                           Dr. Slater, do you have some  
21          announcements?

22                           (No response.)

1 DR. SUTKOWSKI: Okay, I guess  
2 we'll plan to be back at two-thirty please.  
3 Thank you.

4 (Whereupon, the foregoing matter  
5 went off the record at 1:23 .m. to  
6 be reconvened in the afternoon.)

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1                   A-F-T-E-R-N-O-O-N   S-E-S-S-I-O-N

2                   2:32 p.m.

3                   DR. SHEVACH:   Okay, I'd like to  
4                   welcome you all to Session 3, which is  
5                   entitled Preclinical Safety.   And our first  
6                   speaker will be Marion Gruber from the FDA,  
7                   giving us a brief overview of current  
8                   nonclinical testing requirements for adjuvant  
9                   and adjuvanted vaccines.

10                  DR. GRUBER:   Yes, good afternoon.  
11                  Yes, welcome to the session.   In this  
12                  preclinical Session No. 2 we are going to be  
13                  discussing current and perhaps novel  
14                  approaches to preclinical safety assessments  
15                  of adjuvanted vaccines and adjuvants.

16                  And whereby we don't really want  
17                  to restrict the discussions to animal models  
18                  but we also want to look at potential  
19                  alternative technologies to really at least to  
20                  support or supplement safety studies in animal  
21                  models.

22                  So the focus, at least how I would

1 see it, is really discussing the current  
2 nonclinical testing requirements for adjuvants  
3 and adjuvanted vaccines and we are going to  
4 give you the perspective from the U.S. FDA.  
5 And then we will hear from our European  
6 counterparts on their thinking about  
7 recommendations for preclinical safety  
8 assessments.

9 And at the same time, I think what  
10 we all should -- or what we would want to do  
11 is to perhaps challenges some of these  
12 approaches and see are they still relevant  
13 when we look at preclinical safety assessments  
14 of adjuvants or adjuvanted vaccines.

15 And in this regard, we have  
16 formulated a number of questions, nonclinical  
17 issues that should be discussed and they will  
18 be subject for tomorrow's Roundtable No. 1.

19 Dr. Baylor had showed you some of  
20 these questions this morning. And as I'll  
21 give the overview here on nonclinical testing  
22 requirements for adjuvanted vaccines, I'll

1 point out some of the issues that we want to  
2 discuss and hopefully improve upon.

3 So I think the goal should not  
4 necessarily be how can we do more but how can  
5 we improve the current methodologies that are  
6 available to us so that we can better inform  
7 clinical development.

8 This was pointed out this morning  
9 but I'd just like to remind everybody that the  
10 majority of vaccines, and it doesn't matter if  
11 they are adjuvanted or not, they are given to  
12 healthy subjects, including healthy children  
13 and that does place significant emphasis on  
14 their safety.

15 And it is especially critical in a  
16 time where, at least in developed countries  
17 where the immediate benefit of a vaccine in  
18 terms of preventing infectious disease, may  
19 not be immediately obvious because of relative  
20 absence of the disease in developed countries.  
21 And, therefore, the risk-benefit is looked at  
22 on the individual level and the perception of

1 risk by getting a vaccine may outweigh the  
2 perception of the benefit.

3 So it is crucial that vaccines  
4 really undergo a rigorous pre-licensure,  
5 preclinical, and clinical safety assessment.  
6 And thus increased focus has been given to  
7 nonclinical safety assessments, including  
8 toxicity studies in animal models, to support  
9 proceeding to clinical studies.

10 Now safety is always primary but  
11 safety is relative. It is not absolute. So  
12 in determining whether a vaccine product is  
13 safe, one has to look at the indicated target  
14 population, the nature of the product, the  
15 indication, and the circumstances under which  
16 the vaccine will be used.

17 And even if you read through the  
18 definition of safety in the Code of Federal  
19 Regulation, it will tell you that safety is --  
20 or the definition of safety here is relative.

21 I don't really want to go over  
22 this here in detail. We have heard this

1 morning about the potential safety concerns as  
2 they relate to adjuvants and adjuvanted  
3 vaccines.

4 And because of safety concerns,  
5 the law in the Code of Federal Regulations  
6 under the IND regulations requires that  
7 adequate information about the pharmacological  
8 and toxicology studies that have been  
9 conducted or that should be conducted for the  
10 vaccine or adjuvanted vaccine need to be  
11 available.

12 And on the basis of these studies,  
13 the sponsor has to conclude that it is  
14 reasonably safe to conduct a proposed clinical  
15 investigation.

16 However, the law provides us with  
17 flexibility here in that it also states the  
18 kind, duration, and scope of animal and other  
19 tests that are required will vary with the  
20 duration and the nature of the proposed  
21 clinical investigation.

22 Dr. Sutkowski stated this morning

1           that adjuvants are considered constituent  
2           materials under 610.15. And, again, to remind  
3           you, the law also states that an adjuvant  
4           shall not be introduced into a product unless  
5           there is satisfactory evidence that it does  
6           not effect adversely the safety or potency of  
7           the product.

8                         Now what are the goals in  
9           nonclinical safety evaluations? Well, first  
10          of all, nonclinical safety evaluations should  
11          help to support entry into clinical trials  
12          where human safety, of course, is ultimately  
13          evaluated.

14                        So at least given the limitations  
15          of currently available animal models, and  
16          hopefully we will be discussing this this  
17          afternoon a little bit, one has to be mindful  
18          of the fact that certain toxicities, that is  
19          rare toxicities or perhaps toxicities that  
20          only occur in certain human subpopulations,  
21          may only be addressable in humans.

22                        But given the limitations of

1 animal safety evaluations, such testing may  
2 help to determine a safe dose to be evaluated  
3 clinically and to also identify any potential  
4 or unknown toxicities or toxicities on certain  
5 target organs. So if we do toxicity  
6 evaluations in animal models, we are really  
7 looking for unexpected effects.

8 The current guideline that the FDA  
9 is referring to in terms of their guidance and  
10 recommendations for preclinical safety  
11 assessments of vaccines, including adjuvanted  
12 vaccines, is the WHO guidance that has been  
13 published in 2003.

14 And it is really a document that  
15 tries to harmonize the recommendation and  
16 requirements for nonclinical safety  
17 evaluations for vaccines across the regulatory  
18 agencies. And as such, it is recognized by  
19 the U.S. FDA as well as by the EMEA.

20 The toxicology studies for  
21 adjuvanted and adjuvanted vaccines that are  
22 typically conducted are local tolerance

1 studies and repeat dose toxicity studies  
2 whereby the term repeat dose toxicity study is  
3 really loosely used here because it doesn't  
4 really follow the testing paradigms as you may  
5 know it for the typical chemical drug  
6 entities. And I'll get to the design issues  
7 in a couple of minutes.

8           There is another form of toxicity  
9 assessment that is frequently required in  
10 these developmental toxicity studies, in  
11 particular if a product is indicated for a  
12 target population that would include females  
13 of childbearing potential. But I'm not going  
14 to be discussing that at this point.

15           Just a few words, toxicity studies  
16 need to be conducted in compliance with good  
17 laboratory practice regulations. Those are  
18 specified in 21 CFR 58. And the test article  
19 or the vaccine lot that is used in animal  
20 studies should be from a lot or from lots that  
21 are manufactured with the same production  
22 process as those lots intended for clinical

1 use. That is the ideal situation.

2 Now in terms of the animal model,  
3 we have a couple of questions framed for  
4 discussion in tomorrow morning's roundtable,  
5 and that is really what is a relevant animal  
6 model and how do we choose animal models for  
7 these types of studies.

8 In general, what we have been  
9 recommending to date is that one species is  
10 sufficient. So we would not require toxicity  
11 evaluation in two different animal models in  
12 general. And we are recommending that a  
13 species is chosen in which antigen is  
14 immunogenic and in which the odd adjuvant  
15 augments the immune response.

16 Now we have to perhaps discuss  
17 this a little bit further in tomorrow's  
18 roundtable discussion to see what are really  
19 the limitations here. Do we have to redefine  
20 the relevant animal model, in particular since  
21 we heard this morning, you know, about the  
22 species specificity of the immune response,

1 the different distribution of Toll-like  
2 receptors, et cetera. So that is certainly an  
3 issue that we should be reevaluating.

4 If the species is sensitive to a  
5 pathogen, so that is if the animal model would  
6 allow challenge, that would be ideal. But  
7 that is not a current requirement of one  
8 animal model to be chosen for a tox study.

9 And, of course, there are a couple  
10 of other issues. There needs to be a  
11 sufficient number of animals per sex for  
12 groups and the number, of course, it is not  
13 really set in stone. And it depends somewhat  
14 on the animal model also that is chosen for  
15 the tox study.

16 Toxicology studies are usually  
17 conducted as combination safety activity  
18 studies whereby toxicological endpoints and  
19 immunogenicity endpoints are both evaluated in  
20 one study and we are recommending that to  
21 really preserve the use of animals.

22 Of course, it is important to

1 include relevant controls such as the saline  
2 placebo. For adjuvanted vaccines there is a  
3 recommendation to really also include an  
4 adjuvant-only study. And, of course, it is  
5 important to evaluate the vaccine-adjuvant  
6 combination that you want to study in clinical  
7 trials.

8 There is also an option to submit  
9 a document that we call a master file for the  
10 adjuvant only, which would typically include  
11 chemistry, manufacturing and control  
12 information of the adjuvant, and toxicology  
13 assessments that may have been performed on  
14 the adjuvant only.

15 A few words regarding the study  
16 design, our currently recommended study  
17 design. We have been recommending to  
18 administer at least one full human dose to the  
19 animal model. And that should not be scaled  
20 for body weight or surface area wherever that  
21 is feasible.

22 Of course, it is recognized that

1 sometimes, especially if you have a small  
2 animal model like a mouse, it may not be  
3 feasible to administer the full human dose.  
4 And in that case, there is a scaling usually  
5 or typically based on body weight.

6 Now this recommendation really  
7 comes from the discussions that we had on how  
8 to best do toxicological studies for vaccine  
9 antigens. And we were saying at that time --  
10 and that was in 2002 -- since the dose -- the  
11 immunogenicity of the vaccine in the human  
12 should really sort of drive the dose, we  
13 thought it was sufficient to really do one  
14 dose only.

15 So there is no requirement for  
16 dose ranging study. But that, of course, is  
17 another issue that we are going to be  
18 discussing tomorrow in the roundtable for in  
19 terms of evaluating toxicity of adjuvants  
20 should there be a recommendation to perform  
21 dose ranging studies.

22 Since vaccines are administered

1 clinically as episodic dosing, episodic dosing  
2 and not daily dosing is also recommended for  
3 these preclinical safety studies whereby  
4 sufficient time between vaccinations should be  
5 allowed so that the host immune response can  
6 be developed.

7 And we typically recommend that at  
8 least one additional vaccination should be  
9 done in the animal model relative to the  
10 clinical trial so if the vaccine is given as  
11 three doses in the clinical trial, then four  
12 doses should be evaluated in the animal model.  
13 And we refer to this as the so-called end plus  
14 one rule.

15 We usually recommend for the same  
16 route of administration to be used as is  
17 planned for the clinical trial. And if there  
18 is an intention to use the vaccine-adjuvant  
19 combination with a delivery device, then that  
20 should be evaluated preclinically as well if  
21 this is possible. Sometimes, of course, the  
22 animal model chosen prohibits use of a certain

1 delivery device that is proposed to be used  
2 clinically.

3 And I already mentioned the  
4 importance of including appropriate control  
5 groups. Placebo as well as recovery groups,  
6 so that is usually one study of animals that  
7 is allowed to recover. That means it is  
8 followed up somewhat longer to really evaluate  
9 if the rare adverse events or adverse effects  
10 are noted are they reversible.

11 I already spoke to the number --  
12 to the sample sizes here.

13 And also -- and I mentioned that  
14 at the beginning of the talk -- if we do these  
15 tox studies, we seldom are really after a  
16 certain adverse effect. What we want to look  
17 at is really unexpected effects.

18 Are there potential toxicities  
19 that we can be made aware of by doing these  
20 safety studies in the animal models? And,  
21 therefore, it is important to really include  
22 a broad spectrum of measures and parameters to

1 be evaluated such as in-life procedures that  
2 include daily clinical observations, weekly  
3 body weights, feed consumption, as well as  
4 physical examinations of the animals.

5 There should be an assessment of  
6 local reactogenicity and, of course, clinical  
7 chemistry, hematology, and immunological  
8 assessments after initial vaccination as well  
9 as scheduled necropsies.

10 Terminal procedures are conducted  
11 typically one to three days after final  
12 immunization and then, of course, after a  
13 number of weeks in the recovery group.

14 And there is an assessment, a  
15 histopathological assessment of the injection  
16 site and necropsy and histopathology on select  
17 tissues. The select tissues are usually the  
18 pivotal organs, those that may be primarily  
19 effected by vaccine administration. And also  
20 a histopathology on the immune organs.

21 When vaccines are adjuvanted, we  
22 have been recommending that the full tissue

1 list be evaluated. And when I say full tissue  
2 list, I am referring to the tissue list that  
3 is included in the WHO guideline on  
4 nonclinical safety assessments for vaccines.

5 So that, in a nutshell, was the  
6 current overview on approaches to nonclinical  
7 safety assessment of vaccines and adjuvanted  
8 vaccines. And as you can see, there are  
9 probably multiple issues that we are going to  
10 be discussing.

11 I'm not going to really put up all  
12 these questions. We're going to do this  
13 tomorrow at the beginning of the roundtable  
14 discussion. But I think what we really should  
15 focus on is really, again, what does the  
16 current approach look like, how can we improve  
17 upon this so that we are perhaps going to be  
18 in the position that preclinical safety  
19 information can inform clinical development.

20 And I'll stop here. And if there  
21 are no pressing questions, I think we can  
22 introduce the next speaker.

1 DR. SHEVACH: Well, we can take a  
2 couple of questions if there are any.

3 (No response.)

4 DR. SHEVACH: Nope? Okay. We'll  
5 go on to the European perspective, Dr. van der  
6 Laan.

7 DR. VAN DER LAAN: Thanks for  
8 invitation organizers, FDA and the NIH, for  
9 being here to speak for this unexpected large  
10 audience on adjuvants. I'm from the National  
11 Institute of Public Health in the Netherlands.  
12 But also representing the EMEA and the Vaccine  
13 Working Party.

14 I will first start just with a  
15 remark. In the EU, we have a guideline on  
16 adjuvants but my personal opinion is that  
17 guidelines are only guidelines and not the  
18 law.

19 So if you want to apply  
20 guidelines, they are meant to help you and not  
21 to block development. So please think before  
22 you apply.

1                   When writing the first guidance on  
2 vaccines in 1997, the adjuvants has only this  
3 small paragraph. Adjuvants were included in  
4 other aspects and other exepients and more was  
5 not written. At that time, adjuvants were not  
6 very strong.

7                   But later on there was a much  
8 stronger discussion. And for me it was very  
9 helpful to think about adjuvants as to make a  
10 differentiation between the type of signals  
11 they give.

12                   You can differentiate along  
13 several other criteria but for me it was most  
14 helpful that you can take an adjuvant, what is  
15 really the purpose of the adjuvant and the  
16 mechanism of action? And Virgil Strands from  
17 the veterinarian company in the Netherlands  
18 has worked on it.

19                   And, okay, we now know that most  
20 of the adjuvants are engineered to target the  
21 antigen-presenting cells, the key players in  
22 the innate immunity. And that is what we are

1 working on.

2 But in the EU, we have just looked  
3 at the alum as the traditional adjuvant and as  
4 it is so long on the market, we indicate that  
5 for alum, of course, I think you would still  
6 think about is it relevant to use it but we  
7 have no further strong requirements for that.

8 But for all other new adjuvants,  
9 the same applies, in fact, for the adjuvants  
10 as well as for the vaccines as a whole.  
11 Ideally, enhanced protection against the  
12 disease, that should be the final purpose of  
13 the adjuvant and you should test that also in  
14 this way.

15 In use by infectious agents, that  
16 is the ideal situation. But we have to admit  
17 that the fact that there a lot of human  
18 diseases specific for human and there are no  
19 animal disease models available, in that case,  
20 surrogate markers, for instance adequate  
21 responses in the immune system, might be used.

22 But, in fact, they have to be

1           validated in a sense, at least evaluated as  
2           what are the right surrogate markers and  
3           antibodies are not primarily, for each and  
4           every disease, the right surrogate markers.

5                        So the actual situation is  
6           although there is a guideline now written a  
7           few years ago, the scientific research on  
8           adjuvants is still based on trial and error.  
9           And those words have been on the screen  
10          already several times this morning.

11                       And the research is not directed  
12          to requirements for marketing authorization.  
13          They are in a lot of cases carried out by  
14          small specialized companies or university  
15          laboratories. And if they were successful,  
16          they will be taken over by the bigger  
17          companies to sell out their dream.

18                       From a pharmacological point of  
19          view, we feel, as the European authorities,  
20          that there is a lack of knowledge of mechanism  
21          of action. There is a lack of dose response  
22          relationships. A lot of studies are done with

1           only one, maybe two dosages, a lack of  
2           combination studies with different endpoints.

3                       And most is the focus on  
4           immunological effects and there is hardly any  
5           idea about cardiovascular or CNS effects. You  
6           can imagine that if you have a vaccine leading  
7           to the release of cytokines, that there might  
8           also be cardiovascular effects, the safety  
9           pharmacology.

10                      There are a lot of difficulties.  
11           There are no clear systematic data on high  
12           dosages of adjuvants. For some products,  
13           there might be historical data present. But  
14           sometimes fine distributed over the literature  
15           and difficult to find.

16                      There are combination products fro  
17           different types of adjuvants with different  
18           types of quality of course. A very important  
19           point, as Marion Gruber already mentioned, the  
20           children is an important population. But are  
21           we -- do we know what is the effect of an  
22           adjuvant in very young children where their

1 immune system not very mature. And should we  
2 then test this in juvenile animals and at  
3 which time of juvenile animal?

4 So what to do with specific human  
5 diseases if there is no animal disease model,  
6 should we go for another disease, a similar  
7 disease? We have seen that for HPV that you  
8 can use a type of disease that is specific for  
9 dogs or other and use that as a type of animal  
10 model.

11 And under specificity regarding  
12 the antigen, an adjuvant is combined with an  
13 antigen but are there coexisting antigens at  
14 the same time in humans? And, of course, also  
15 in men. And is there any interference with  
16 that? Or do we know what adjuvants are doing  
17 at the same time in humans for all other  
18 antigens that are present?

19 So the guideline on adjuvanted  
20 vaccines was presented in July 2005. And I  
21 will give a very short overview. It is just  
22 focusing on the proof of concept.

1                   There are a lot of different  
2 mechanisms of action. And we have seen a lot  
3 of them this morning already. So there are a  
4 lot of possibilities.

5                   One of the aspects of the use of  
6 adjuvants is that they are combined with  
7 subunit vaccines to get a sufficient approach.  
8 And just in our experience, that might also be  
9 the rationale for such an adjuvant.

10                  We have done a lot of work on  
11 influenza that last few years because of the  
12 threat of a pandemic. And if you look at  
13 influenza immunization, there is a type of  
14 gradients. You can see that there is a  
15 maximal protection by a full infection  
16 experience. It is, of course, not complete  
17 but at least it gives a maximal protection.

18                  There is broad cross-protection  
19 with live attenuated vaccines. However, most  
20 of the seasonal vaccines are just using whole-  
21 virion vaccines or just subunit vaccines and  
22 maybe the rationale for the addition of an

1           adjuvant might be to combine it with low  
2           immunogenic vaccines to gather more robust  
3           protection but also a broader cross-protection  
4           if we are now thinking about just the  
5           development of the pandemic vaccine.

6                         And we are now discussing how  
7           broad is the protection against the mutual  
8           mutation shifts. Then also the use of  
9           adjuvants might be relevant in that respect.

10                        The increased immunological  
11           response should be shown in a relevant animal  
12           model. Are the cells of the innate immune  
13           system really triggered? And to what extent  
14           are humoral and cellular immune responses  
15           activated? And is that relevant for the  
16           protection?

17                        Of course, data from combinations  
18           of the adjuvants other antigens can be used as  
19           supportive evidence but nothing is the same.  
20           The adjuvant or antigens are different from  
21           each other as we have also seen this morning  
22           the difference between influenza and smallpox.

1                   Public literature can be used as  
2                   supportive information for the proof of  
3                   concept.

4                   So this gives the first aspect  
5                   what I have told thus far was on just the  
6                   proof of concept ideas. Of course, safety is  
7                   an important aspect but it should be seen in  
8                   relation to the efficacy.

9                   If we look at the safety of the  
10                  adjuvants and you can see -- you have to put  
11                  it in the framework of how broad is the effect  
12                  of an adjuvant, that's why we have the  
13                  emphasis in Europe to also to test the  
14                  adjuvant alone whereas the methodology should  
15                  follow the pattern of use of the vaccine.

16                  There might be a differentiation  
17                  between the stimulation of the non-specific  
18                  resistance to infections, the innate immunity,  
19                  and increasing the immune response to the  
20                  specific antigens.

21                  The intended action is to induce  
22                  long-lasting changes in the immune system by

1 influencing the sensitivity to the defined  
2 antigen but as was said already, but to  
3 emphasize it here, what about the increased or  
4 decreased sensitivity to the unknown or  
5 unintended antigens?

6 Also with respect to the adjuvant  
7 alone, you should test it in two species  
8 unless you can justify that it is only  
9 sensitive in one. And preferably also in a  
10 non-rodent as we -- and maybe I can emphasize  
11 this as this morning we discussed mice lie.  
12 Mice can lie indeed.

13 Some adjuvants might exert a high  
14 level of species specificity and I think we  
15 also would -- what has been said about the  
16 specificity of the Toll-like receptors, we  
17 have to take into account that some animal  
18 species might be less responsive.

19 And ideally the selected species  
20 should be the same in which the proof of  
21 concept has been studied to see the  
22 differences. Of course, it might be difficult

1 if there is not that much experience.

2 But even for ferrets, if you have  
3 the handbooks of animal toxicology, you see  
4 large chapters on ferrets to be tested in  
5 toxicology not only for influenza vaccines but  
6 also for other purposes.

7 Toxicity endpoints, local  
8 tolerance is the first one but also  
9 hypersensitivity and anaphylaxis.

10 Pyrogenicity is, in my view, a type of adverse  
11 effect or toxicological effect and not  
12 belonging to quality.

13 And under systemic toxicity, we  
14 require a full histopathology of primary and  
15 secondary immune organs and maybe also other  
16 organs if it is a new product. Of course you  
17 can limit yourself and the risk you take if  
18 you have just focusing on local applications.

19 Just a short word about  
20 reproductive testing, there is an FDA  
21 guideline but just -- and because of the facts  
22 that in the ICH S6 for biopharmaceuticals, we

1 are discussing about the relevance of  
2 reproductive toxicity studies with antibodies.

3 We have found that the placental  
4 transfer of antibodies is very low during  
5 organogenesis. And so focusing on the  
6 antibodies as an important endpoint of  
7 exposure during the whole part of the  
8 pregnancy is for me now questionable.

9 And I think it might be more  
10 important to think about the placental effects  
11 of the transfer of cytokines or cytokines and  
12 interferons. That might be difficult to do  
13 that in rodents. I think that that needs some  
14 further discussion.

15 We can be very short about  
16 genotoxicity and carcinogenicity. For the  
17 toxicity for the combination of adjuvant and  
18 antigen, we focus on local tolerance. And I  
19 would support the idea of the repeat of what  
20 has been said already about the repeated dose  
21 toxicity studies.

22 It is focusing on the immune

1 response as a type of phenomenon as we should  
2 think about the rationale of the why using  
3 that specific adjuvant in combination with  
4 that specific antigen. And I have to say that  
5 at least in the scientific advice procedure in  
6 Europe, there is a lot of improving concepts  
7 are now being developed.

8 What is on the market in Europe?

9 We have accepted MPL as MPL/alum, ratio 1:10  
10 AS04, and Fendrix, a hepatitis B vaccine, and  
11 Cervarix, as the HPV vaccine, and also MF59 in  
12 Focetria pandemic influenza vaccine. This is  
13 just a short listing of what was in the EPAR,  
14 the European Public Assessment Report, on the  
15 website of the EMEA.

16 For Fendrix, there is a specific  
17 remark that the MPL is completely absorbed at  
18 the element. And it is a reflection of the  
19 knowledge at that time that that was an  
20 important aspect. I'm not sure that we would  
21 all think that we know today that we should  
22 emphasize that too much today.

1           The immunogenicity was not done in  
2 relevant animals. Hepatitis B vaccine, there  
3 is no -- those animals are not representative  
4 of the immunogenicity. There are also  
5 reproductive toxicity study and the rabbit  
6 study that repeats the dose. You can question  
7 the relevance of those studies but at least  
8 they are in the dossier.

9           There is a safety pharmacology  
10 study but no species is mentioned in the  
11 report. And there are some toxicity studies.

12           Just with Cervarix, the same  
13 adjuvant system, there is an extension of the  
14 dossier with immunogenicity data in rhesus  
15 monkeys. Now the safety pharmacology is  
16 spelled out in rats and dogs for MPL alone.

17           There is some pharmacokinetics for  
18 MPL but that's not related to the activity of  
19 the MPL itself. There are also reproduction  
20 toxicity studies in rats for over the whole  
21 spectrum.

22           For the MF59, we have only one

1 product, the Focetria. It is a pandemic  
2 influenza vaccine with special regulations for  
3 the guidance on pandemic influenza vaccines.  
4 And so that specific concentrations indicate  
5 that limited evidence for the support of the  
6 safety and the efficacy of this adjuvant is  
7 accepted because of the threat of the  
8 pandemic.

9 There are some proof of concept  
10 data in ferrets but there was no control  
11 without an adjuvant so it is not fully clear  
12 from that data whether MF59 really stimulates  
13 the immune response.

14 And all other aspects on MF59 are  
15 shown only in mice. There is a safety  
16 pharmacology study in dogs for the only local  
17 tolerance. You see very limited supportive  
18 datasets but it was accepted because of the  
19 character of the vaccine.

20 So in conclusion, the EMEA  
21 guideline on adjuvants is reflecting the state  
22 of the art for the moment. We can discuss

1           whether we would have more and new data. As  
2           the EU is opened to receive new applications,  
3           we see the discussions in the scientific  
4           advice procedure and we have something a bit  
5           more than only alum in our licensing  
6           procedures.

7                         Thank you.

8                         (Applause.)

9                         DR. SHEVACH: Any pressing  
10           question? Thank you. Oh, there's one.

11                        DR. FRIEDE: Martin Friede, World  
12           Health Organization.

13                        So, Jan Willem, I was a little bit  
14           surprised to see the statement on MF59 being  
15           accepted because of its application in  
16           pandemic vaccine. And it was accepted because  
17           of that.

18                        Because in Europe, especially in  
19           Italy, MF59 was accepted in a national  
20           licensing procedure prior to the EMEA. So  
21           there is 20, 30 million cases of human  
22           administration of MF59. So how does the EMEA

1           then position the approval process for MF59  
2           separate from what this historical data we  
3           have of a licensed vaccine within Europe?

4                       DR. VAN DER LAAN:   Yes.   Maybe  
5           that's a reflection of the fact that I am  
6           Dutch.

7                       (Laughter.)

8                       DR. VAN DER LAAN:   And I will  
9           explain to you that the product that you refer  
10          to is the seasonal vaccine, Fluvad, with also  
11          MF59.   That's indeed on the market in Europe.  
12          But not in all countries.

13                      We have in the European Union, and  
14          that's not wholly Europe but at least the main  
15          part, 27 countries.   And as far as I know --  
16          but maybe someone can correct me -- the Fluvad  
17          is on the market of 15 of the 27.   And it is  
18          not accepted in all.

19                      So that's why the European  
20          position is only because of the guideline on  
21          the vaccines and not -- there's no, at the  
22          moment, no full acceptance of that vaccine.

1 DR. SHEVACH: Okay. Thank you.

2 We'll move on.

3 Dr. Alving, from Walter Reed, Use  
4 and Limitations of Animal Models.

5 DR. ALVING: Well, thank you very  
6 much.

7 If we accept that the vaccines are  
8 initiated, that the principle that initiates  
9 the immune response in vaccines is the  
10 antigen, then the adjuvant's function to  
11 amplify the immune response or to channel the  
12 immune response in a particular direction, for  
13 example Th1 or Th2 -- and we haven't mentioned  
14 about a lot about other sites such as mucosal  
15 sites yet -- increase the duration of the  
16 immune response, and actually help to overcome  
17 tolerance when necessary. For example, for  
18 cancer antigens this might be important.

19 So the question that I'm going to  
20 address is can the functions and the safety  
21 parameters of adjuvants for humans be  
22 predicted either qualitatively or

1           quantitatively by utilizing animal models.

2                       Now I want to make a caveat here.

3           This is a pretty complicated topic. There are  
4           lots of different kinds of adjuvants and I'm  
5           going to focus mainly on adjuvants where  
6           certainly one of the major mechanisms is  
7           thought to be a depot effect, either a depot  
8           of the antigen together with the adjuvants or  
9           a depot of the adjuvant alone.

10                      So with that caveat in mind, the  
11           answer to this question is, in some cases yes,  
12           and in some cases not. And I'm going to give  
13           two examples in the next three slides that  
14           illustrate both of these, the first being with  
15           respect to safety. And the second being with  
16           respect to efficacy.

17                      So I want to start first with the  
18           safety. Now the first -- as we have been  
19           mentioning here, one of the most commonly used  
20           adjuvants at the present time is Lipid A.  
21           Well, Lipid A is the pyrogenic factor that is  
22           present at the -- is the anchor site of the

1 bacterial level polysaccharide.

2 And there was a series of  
3 wonderful studies done by Sheldon Greisman at  
4 the University of Maryland in the 1960s. And  
5 what he showed was absolutely astonishing in  
6 my view. It's very, very interesting.

7 If you look here, these are the  
8 doses. This is the endotoxin, the  
9 lipopolysaccharide given to rabbits. And  
10 exactly the same lipopolysaccharide given to  
11 humans. And these are increasing doses.

12 So that at the lowest dose -- and  
13 this highest dose up here is a tenth of a  
14 nanogram, there is no response in either one  
15 of them. But when you go to the higher level,  
16 which I believe here is one nanogram to 1.4  
17 nanograms, suddenly in each case here you get  
18 an immune -- sorry, a pyrogenic response.

19 This is an increase in  
20 temperature. And the -- actually what he  
21 shows in the study is he shows the subjective  
22 response that the human actually had at the

1 same time. And so they were looking at this  
2 in some detail. Now this is based on  
3 milligrams per kilogram, injecting  
4 intravenously into rabbits.

5 You get a slightly different  
6 response if you look at the total LPS that was  
7 actually injected into the rabbits. So that  
8 in this case, you do get some pyrogenicity  
9 when you look at the total, not on the  
10 milligram per kilogram basis in this. And so  
11 there are some discrepancies. But even in  
12 general, it looks like a pretty good  
13 representation.

14 So in this case, this is an  
15 excellent example where the adjuvant that  
16 we're talking about, and we'll get into some  
17 of the pyrogenicity studies of the  
18 monophosphoryl Lipid A actually, as I go along  
19 here, too, a little further. And this is  
20 actually for the total dose.

21 Now we have -- there are ways to  
22 influence this and actually we've done a

1        number of studies, Phase I trials in humans,  
2        where we have actually done pyrogenicity where  
3        we put the Lipid A into a carrier, the  
4        liposome carrier, and this is where we are  
5        looking at the -- this is the total Lipid A  
6        injected on a microgram per kilogram basis  
7        into the rabbits.

8                    And so this is the free Lipid A  
9        right here. And as you can see, it is highly  
10       pyrogenic even at this dose here which is  
11       0.022 micrograms per kilogram.

12                   However, when you put the material  
13       into liposomes, and this is simply different  
14       amounts of Lipid A that were incorporated into  
15       the liposomes with an increasing dose, you get  
16       a pyrogenic response at a much higher dose.

17                   In fact, when you look at the  
18       difference, for example, between what looks to  
19       be approximately the initial place where you  
20       get a pyrogenic response here, it is a 55-fold  
21       difference in the pyrogenicity.

22                   If, in contrast, you go to the

1 chemical test for lipopolysaccharide  
2 endotoxin, the Limulus amebocyte lysate assay,  
3 this is a much more sensitive test. And this  
4 is a chemical test. This is obviously in an  
5 intact animal. This is in a tube.

6 And in this particular instance,  
7 when you put the endotoxin -- increasing  
8 amount of endotoxin, the Lipid A into the  
9 liposomes, despite the fact that there is  
10 endotoxin there, it is not detected as being  
11 any higher than the liposome lacking the Lipid  
12 A.

13 So that the Limulus assay doesn't  
14 necessarily prove the absence of Lipid A if it  
15 is there. So you can actually get liposomes  
16 that are Limulus negative and liposomes that  
17 are Limulus positive. So there is a hundred-  
18 thousand-fold difference there.

19 So the second example that I want  
20 to give you is really quite dramatic. It was  
21 the first circumsporozoite protein antigen  
22 that was developed by GlaxoSmithKline in

1 collaboration under a cooperative research and  
2 development agreement with the Walter Reed  
3 Army Institute of Research. It was initially  
4 tested in mice and rabbits and found to be  
5 efficacious but it failed in humans.

6 So the question that arises then -  
7 - at the final question at the bottom, what  
8 are some of the variables that influence the  
9 predictability of adjuvants in humans versus  
10 animals?

11 Well, one of the obvious things  
12 that could predict this is the differences in  
13 sizes between the different animals. This is  
14 actually from this paper by Freireich, et. al,  
15 in 1966, where they are comparing the body  
16 weight of these different species of animals  
17 and the surface area.

18 And this is the -- the km factor  
19 is the body weight over the surface area. And  
20 if you look at this, the human -- the adult  
21 human is 1,000 times heavier -- or 3,000 times  
22 heavier than a mouse. However, in contrast,

1 the surface area of the human is only 242  
2 times greater than the mouse.

3 Now if you take the idea that you  
4 are looking at the internal organs, than maybe  
5 these great difference occur in the internal  
6 organs. After all, the volume, if you look at  
7 the internal peritoneum as a sphere, the gut  
8 volume goes up as the cube of the diameter,  
9 while the surface area goes up as the square  
10 of the diameter.

11 So the -- now let's look at the  
12 monkey. The monkey is -- the humans are 20  
13 times heavier than a monkey and yet the  
14 surface area is only 6.7 times heavier than  
15 the monkey. So it is clear that this  
16 conceivably could be an effect. Here's the  
17 body weight over the surface area compared to  
18 the mouse versus the monkey compared to the  
19 human.

20 So the monkey is three times more  
21 than the human -- well, the mouse -- or the  
22 human is three times more than the monkey

1 compared to more than 12 times higher in the  
2 mouse.

3 While these may be factors, what  
4 could be the implications of this? This is a  
5 study -- I apologize that this was actually  
6 published in a very difficult to obtain thing  
7 if anybody would like a reprint.

8 But it was where we actually did -  
9 - and I think you'll see in a moment why we  
10 did this -- published it here -- the depot  
11 effects of liposomes absorbed to alum -- these  
12 are liposomes, I believe, containing  
13 monophosphoryl Lipid A. Yes, they were.

14 And what we did is we put  
15 phospholipids that were fluorescent -- there  
16 were two different kinds of fluorescent --  
17 rhodamine and fluorescein dye attached to  
18 phosphatidylethanolamine.

19 These liposomes were then absorbed  
20 to aluminum hydroxide and the injection site  
21 was in the gastrocnemius muscle of the left  
22 rear limb of the mouse. Is the gastrocnemius

1 mouse 1/3,000th the gastrocnemius muscle of  
2 the humans? I mean here you are injecting 50  
3 microliters into a mouse. Are you going to  
4 inject 3,000 times that into a human? I don't  
5 think so.

6 But what we found was really quite  
7 extraordinary. Over a period of six days,  
8 there was apparently no change at all in the  
9 amount of material that was present at the  
10 injection site. In fact, when we looked at  
11 the -- we took a variety of mice, we let the  
12 pathologists look at the fluorescence blinded  
13 and grade the degree of fluorescence and so  
14 forth.

15 The first thing -- and if you look  
16 here at the intramuscular injection, the first  
17 was at 24 hours there was a little bit that  
18 appeared in the spleen but there was no  
19 detectable amount in the spleen at all after  
20 that. In contrast, in the lymph nodes it was  
21 continuously appearing through the whole time  
22 in the lymph nodes.

1                   So the conclusion there is that  
2                   there could be an idea that there may be --  
3                   the differences in the sizes of the animals  
4                   may have -- there may be adjuvant effects that  
5                   occur in the entire animal that would be  
6                   similar that would be coming from a mouse and  
7                   a human.

8                   But the effect on the whole animal  
9                   -- that is the rate of release may be similar.  
10                  But it getting from the site of injection in  
11                  a mouse, let's say, to the spleen of the mouse  
12                  might be a lot different than getting to the  
13                  arm of a human into the spleen of a human in  
14                  terms of the distances and so forth that the  
15                  material has to traverse.

16                  Now I just want to now switch  
17                  topics to another type of adjuvant, incomplete  
18                  Freund's adjuvant. It is a water-in-oil  
19                  emulsion in a light paraffinic mineral oil of  
20                  low viscosity called Drakeoil that is  
21                  stabilized by an emulsifier consisting of  
22                  Arlacel A, which is isomannide monooleate.

1                   Since water-in-oil emulsions  
2                   require relatively low energy input for  
3                   emulsification, the shearing forces obtained  
4                   by pushing oil through a small orifice by  
5                   connecting opposing syringes is usually  
6                   sufficient. And the antigen is generally  
7                   included in the water phase of the emulsion.

8                   Incomplete Freund's adjuvant is  
9                   one of the most potent adjuvants ever devised.  
10                  The question is why is it not routinely used  
11                  in humans.

12                  Well, in 1964 to 1965, 900,000  
13                  people in U.K. received an influenza vaccine  
14                  adjuvanted with incomplete Freund's. Forty  
15                  persons developed local nodules. And of  
16                  these, nine developed a cyst that required  
17                  local surgical aspiration or incision. The  
18                  cysts were viewed as a toxic reaction similar  
19                  to cysts sometimes seen in mice.

20                  A tetanus toxoid vaccine in New  
21                  Guinea and cholera vaccine in the Philippines  
22                  containing IFA reportedly had higher levels of

1 local reactions. IFA induces tumors. This  
2 was the killer actually in the United States.  
3 It induces tumors in male Swiss mice. However  
4 it is very infrequent in female Swiss mice.  
5 And it does not induce tumors in Balb/c or C57  
6 black mice. This was the death knoll for  
7 incomplete Freund's adjuvant.

8 IFA is known as a potent agent for  
9 induction of autoimmune arthritis in mice, a  
10 condition also known as adjuvant arthritis.  
11 It's all sounding bad. It is feared that IFA  
12 may cause cysts, cancer, or autoimmune  
13 arthritis, or other diseases in humans.

14 How does all this stand up to  
15 scrutiny? Well, the first thing that was done  
16 was to actually take a mouse and inject the  
17 mouse with incomplete Freund's adjuvant. Then  
18 let it go. I believe it went for 270 days  
19 actually.

20 And then the same incomplete  
21 Freund's was put in a bottle and put on the  
22 shelf in the laboratory. And then the mouse

1           was sacrificed at the end. Here is the dorsum  
2           of the mouse. And as you can see, you get  
3           what appears to be a separation here that  
4           looks very similar to the separation that you  
5           see in the bottle. And this is just a walled  
6           off cyst that occurs here.

7                           And so this is actually something  
8           that -- the separation that you see where you  
9           get clear oil that occurred in the cyst, these  
10          were not infectious cysts. They were chemical  
11          cysts that occurred. It could simply be  
12          drained off it need to be. But anyway, it was  
13          problem.

14                          Jonas Salk looked at the long-term  
15          safety of incomplete Freund's adjuvant. He  
16          was one of the greatest advocates for  
17          incomplete Freund's, particularly for the  
18          initial polio vaccine. He was forced to not  
19          use the polio vaccine with incomplete Freund's  
20          adjuvant and as a result of that, instead of  
21          having a single injection polio vaccine, he  
22          had to go with multiple injections to get the

1 same immune response.

2 So in the initial study, Salk, in  
3 collaboration with the Army, immunized 18,000  
4 recruits with influenza vaccine emulsified in  
5 IFA at Fort Dix. Cyst-like reactions observed  
6 in some but were eliminated by purifying the  
7 Arlachel A.

8 Subsequent to this, there were no  
9 cysts that were observed. It appeared to be  
10 a degradation product of the Arlachel A that  
11 was responsible for these cysts.

12 Because these were military  
13 individuals, they had a long-term follow up in  
14 the military medical system, a nine to ten  
15 year follow up of the Salk cohort.

16 Cyst-like reactions required  
17 hospitalization treatment in 0.1 to 0.6  
18 percent and outpatient treatment in 1.2 to 4.1  
19 percent. Otherwise, no significant  
20 unexplained problems, no effect of vaccine on  
21 the incidence of mortality.

22 There was then a 16- to 18-year

1 follow up. There were no adverse correlations  
2 with diseases or death.

3 And then finally, this is another  
4 difficult one -- I have an original copy of  
5 this journal, Vaccine Research. It is now  
6 out of publication. It doesn't exist anymore.

7 But this is a wonderful study that  
8 was done by Abe Benenson actually in which he  
9 did a greater than 35 year follow up on these  
10 same individuals. There were no adverse  
11 correlations with 74 different diagnostic  
12 categories, including arthritis and autoimmune  
13 disease.

14 Decreased mortality was observed  
15 in five disease categories, significantly  
16 reduced mortality. A p of 0.01 was observed  
17 with respect to cancers of the digestive  
18 tract.

19 So that it appears that in the  
20 mouse, for example, the adjuvant arthritis and  
21 the cancer and so forth may, in fact, be  
22 diseases of mice. This is the thing that you

1 really have to worry about the possibility  
2 that there may be diseases of mice that will  
3 not occur in humans. But in any case, this is  
4 an example of a long-term study that was done.

5 Now I want to switch to another  
6 adjuvant and this is the MF59. This is with  
7 the Herpes simplex vaccine in which gD2 and  
8 gB2 were used in combination with MF59 as  
9 we've hear earlier.

10 This was one in which there was  
11 published a series of studies in which the  
12 adjuvant activity of MF59 was compared with  
13 the adjuvant activity of alum. And so the  
14 ratio of MF59 to alum are the numbers that are  
15 shown on this table with all of these  
16 different kinds of antigens here.

17 And as you can see, it goes all  
18 over the place -- five, 122, two, 42, and so  
19 forth. But if you look at the Herpes simplex  
20 material, the guinea pig, which was the  
21 primary model for looking at the Herpes  
22 simplex efficacy in the animal models, it

1 looked as if it had the best activity in the  
2 guinea pig right here.

3 But actually they should have  
4 looked at the baboon up here, which is a  
5 sevenfold lower activity because when they  
6 actually went to the human trial in the Phase  
7 III trial, there was no significant --  
8 actually, it was very interesting. This  
9 appears to be a female vaccine. And there was  
10 a tendency towards efficacy in the females but  
11 it was not significant and there was no  
12 efficacy in the males at all.

13 In contrast, when what is now  
14 known as the AS04, the MPL absorbed to alum  
15 was utilized, then there was distinct efficacy  
16 in the females. So it did appear that the  
17 animal studies had not given exactly the type  
18 of thing.

19 Really I want you to just remember  
20 all of those differences in the adjuvant  
21 compared to the alum. And we'll get into that  
22 in this study right here. This is a study

1 with a malaria antigen, MSA-2. And this is  
2 where actually five different adjuvants were  
3 tested: Freund's complete/incomplete,  
4 Alhydrogel, SAF-1, which is an oil-in-water  
5 emulsion with this thionyl muramyl dipeptide  
6 in there, Montanide ISA 720, which is a water-  
7 in-oil material that looks pretty much -- that  
8 uses mannide monooleate -- it is pretty much  
9 similar to incomplete Freund's adjuvant, and  
10 liposomes containing Lipid A that we supplied.

11 And if you look at the -- this is  
12 the same thing. This is the adjuvant activity  
13 in sheep, rabbits, and mice of these five  
14 different types of things. These are two  
15 different experiments right here.

16 And you can see there are huge  
17 differences. For example, this 33 versus 155,  
18 60 versus 466. If you look down here in the  
19 liposomes, it is a tenfold difference in the  
20 rabbit and the mouse.

21 It didn't seem to be that there  
22 was any rational way to figure out exactly

1           what was happening in these different animal  
2           species.

3                         And actually what Glaxo did, again  
4           in collaboration with the Walter Reed Army  
5           Institute of Research and the Naval Medical  
6           Research Institute, now known as the Center,  
7           and Glaxo through a cooperative research and  
8           development agreement, there were studies done  
9           in Phase I studies with three different types  
10          of adjuvants in humans.

11                        Now the first adjuvant is the AS04  
12          that worked so well in the Herpes simplex  
13          vaccine. And that actually gave a slightly  
14          less response right here.

15                        The AS03, which is simply the  
16          emulsion containing RTS,S in an oil-and-water  
17          emulsion looked pretty good. Actually it  
18          looked very good. These were very good  
19          results in terms of antibody levels.

20                        And then finally the AS02, which  
21          was also shown there.

22                        These individuals were then

1 challenged with malaria to determine the  
2 protection. And all three of these gave some  
3 protection. With the AS04, there was one  
4 individual protected. In the AS02 -- what is  
5 it -- the AS03, there were three protected.  
6 But in the -- so it is six out of seven were  
7 protected with the AS02.

8 So it looked as if the human  
9 results were giving the correct answer and  
10 this actually was a comparative adjuvant trial  
11 in humans.

12 So the summary of this is is that  
13 WRAIR and GSK initiated a rigorous comparative  
14 preclinical safety and immunogenicity  
15 evaluation of six GSK proprietary formulations  
16 of RTS,S in rhesus monkeys.

17 And during preclinical studies in  
18 mice, a synergistic effect was observed  
19 between QS21 and MPL. Nathalie Garpon has  
20 talked about these studies actually earlier  
21 today. And the combination of oil-in-water  
22 emulsion, MPL, and QS21 was selected based on

1 the results from the monkey studies.

2 And she actually showed this same  
3 slide. The purple numbers right here, this is  
4 the dramatic number right here which showed  
5 the effect that the AS02 gave that turned out  
6 to be the most protective one of all.

7 What I would emphasize is that --  
8 oh, here is another example where we used  
9 liposomes containing Lipid A together, again,  
10 in collaboration with GSK at Walter Reed. And  
11 this is in monkeys where we are increasing the  
12 -- each one is an individual monkey,  
13 increasing the amount of Lipid A MPL that was  
14 in the liposomes and we got quite dramatic  
15 immune results here.

16 Based on that, we went to a human  
17 study of this last formulation that I just  
18 showed you. And this is where we actually --  
19 each bar represents a single individual human.  
20 And this is the antibody levels. This is  
21 micrograms of IgG per milliliter that were  
22 observed -- very high levels here but it was

1 at a very high level of MPL that was done  
2 there. This is a dose ranging study.

3 And this is compared, for example,  
4 with the original work that was done with alum  
5 where the very poor results that were  
6 obtained. And that was the subject of the  
7 Washington Post article that I showed you at  
8 the beginning.

9 Actually it is my belief that an  
10 adjuvant researcher should actually receive  
11 the treatment that he actually gives to his  
12 children and to other people. And this has  
13 actually been a tradition in the Army.

14 Rip Ballou, over there, he  
15 received the same thing. I believe you got  
16 challenged and you got malaria though, did  
17 you, Rip. Yes, sorry.

18 (Laughter.)

19 DR. ALVING: But anyway, this is a  
20 very interesting thing here is the side  
21 effects. Now we had had monophosphoryl Lipid  
22 A here. We did the original pyrogenicity

1 study on this in rabbits. And it was  
2 negative. It was nonpyrogenic in the rabbits.  
3 But it was positive in the Limulus studies  
4 assay. It was strongly positive in the  
5 Limulus assay. But negative in the rabbits.

6 And what we did is the vaccine was  
7 essentially nonpyrogenic and nontoxic even  
8 though at the highest dose, which is shown  
9 right here, which is Group 5, the volunteers  
10 received 2.2 milligrams of monophosphoryl  
11 Lipid A.

12 This actually was about 12 times  
13 higher than the previously established maximal  
14 safe dose of MPL when given intravenously.

15 I'd just like to point out that most vaccines  
16 are not given intravenously. And this was,  
17 therefore, felt to be safe and potent.

18 Despite this, my wife has expressed some  
19 reservations about how safe it was in view of  
20 my subsequent characteristics after receiving  
21 this vaccine.

22 (Laughter.)

1 DR. ALVING: So actually what I  
2 want to emphasize is that the animal studies  
3 may not give you the proper results. And you  
4 may have to resort to doing Phase I trials.

5 This is perhaps a little more  
6 expensive but what you should be looking at  
7 when you do sequential Phase I trials with  
8 different adjuvants is you should be looking  
9 for knockout results. Nobody wants to go  
10 around doing statistics actually. You want to  
11 do eyeball statistics.

12 And if you can't see it, it's just  
13 not worth looking at. And so you can see  
14 through the years, it looked as if it there  
15 was -- this is actually the last trial that  
16 was shown here -- this is a paper by Dan  
17 Gordon who was, actually, the first person  
18 ever protected by a synthetic malaria vaccine  
19 as part of this program.

20 However, this is not foolproof  
21 because -- okay, so based on this, the  
22 approximately theoretical minimum protective

1 value -- some people may quibble with this --  
2 but it is somewhere in this range around here  
3 where some people would get protected against  
4 the malaria.

5 But this is not a foolproof thing  
6 because it was subsequently shown by Steve  
7 Hoffman in a subsequent study using the Detox  
8 formulation that when he repeated this with a  
9 second Phase I trial that the results were not  
10 quite as dramatic.

11 So the conclusions from what I've  
12 discussed here is that the rabbit pyrogenicity  
13 assay is a reasonable predictor of endotoxin  
14 pyrogenicity in humans.

15 The Limulus amebocyte lysate assay  
16 may be less useful in this respect simply  
17 because it is stunningly sensitive. Even the  
18 liposomes in the example I showed you, even  
19 the liposomes lacking Lipid A gave some  
20 reactivity.

21 Pyrogenicity, in turn, is also a  
22 reasonable predictor of many types of

1 mediators such as IL-1, IL-6, TNF, et cetera,  
2 induced by endotoxin. Certain toxic effects  
3 induced by adjuvants in mice, such as adjuvant  
4 arthritis, autoimmune disease, and adjuvant-  
5 induced cancer have not necessarily been  
6 observed with the same adjuvants in humans.

7           The weights and surface areas of  
8 different animals might influence the  
9 predictability of safety and efficacy of  
10 certain types of adjuvants in humans. Perhaps  
11 because of this, nonhuman primates may be  
12 better than rodents, including mice, guinea  
13 pigs, and rabbits for predicting efficacies of  
14 adjuvants that rely mainly on depot effects.

15           Animal models are useful for  
16 investigating mechanisms of certain types of  
17 adjuvants but do not always predict safety or  
18 efficacy in humans. Comparisons for down  
19 selection for human vaccines of different  
20 types of adjuvants, especially those that rely  
21 mainly on depot mechanisms, are probably best  
22 evaluated in nonhuman primates or, better yet,

1 in sequential Phase I trials in humans.

2 And finally, the effects of  
3 adjuvants or adjuvant combinations that rely  
4 on different mechanisms such as depot effects,  
5 recruitment of antigen-presenting cells, TLRs,  
6 secretion of cytokines, or combinations of  
7 these cannot necessarily be reliably predicted  
8 by any given animal model when looked at prior  
9 to actually doing the experiments.

10 Thank you.

11 (Applause.)

12 DR. SHEVACH: Thank you.

13 We have time for one very quick  
14 question.

15 DR. GARPON: Yes, I have a  
16 question on what you were saying about MF59  
17 and the guinea pig model for Herpes and the no  
18 correlation of what was seen in guinea pigs  
19 versus efficacy in the clinical trial.

20 DR. ALVING: Yes.

21 DR. GARPON: What is the question?

22 The animal model or the readout that was used

1 as a correlate?

2 DR. ALVING: The readout, are you  
3 meaning the ratio over the alum?

4 DR. GARPON: Yes.

5 DR. ALVING: Well, this is -- the  
6 ratio of the adjuvant effect over the alum has  
7 been widely used. But I was just pointing  
8 that out. It may be the readout. You are  
9 right. It could be the readout. There could  
10 be other ways of looking at that.

11 DR. GARPON: But there was an  
12 antibody response, right?

13 DR. ALVING: There was what?

14 DR. GARPON: There was the  
15 antibody response you used as a ratio?

16 DR. ALVING: That was the antigen  
17 response, exactly. Yes.

18 All right. Thank you.

19 DR. GRUBER: Okay. It is a  
20 pleasure to announce the next speaker before  
21 the coffee break. And this is Dr. Ethan  
22 Shevach, my co-chair from the NIAID, who will

1 speak about long-term effects of adjuvants,  
2 consequences on the adaptive immune response.  
3 Thank you.

4 DR. SHEVACH: Well, thanks for  
5 having me. Thank the organizers for inviting  
6 me.

7 I have to also mention I'm sort of  
8 the odd man out here in that I'm the only one  
9 really interested in suppressing the immune  
10 response when all the rest of you are  
11 interested in enhancing the immune response.  
12 But perhaps something can be learned from  
13 someone who is interested in immune  
14 suppression rather than immune activation.

15 My only tie to adjuvants is a  
16 historical one. I've been a member of the  
17 Laboratory of Immunology of NIAID for 40  
18 years. The Laboratory of Immunology at NIAID  
19 was founded by Jules Freund some 51 years ago  
20 for those of you that remember.

21 I'm going to talk about one of the  
22 key cells in the immune response that plays a

1           role in absolutely everything. It hasn't  
2           really been mentioned here. It is the  
3           regulatory T cell.

4                         This is the way I view the immune  
5           response. It is always imbalanced with our  
6           attempts to activate the immune response.

7           This is why autoimmune disease or allergy or  
8           immunopathology or graft transplantation -- I  
9           don't have immunization with vaccines up here  
10          but it is in this column. And everything is  
11          balanced by regulatory T cells.

12                        And it is nice when there is an  
13          even keel. But, of course, any time you are  
14          going to immunize an animal or man, one has to  
15          overcome the effects of these regulatory T  
16          cells.

17                        Too much regulatory T cell action  
18          can lead to chronic infection. The  
19          overabundance of regulatory T cells is a  
20          feature of cancer.

21                        What are regulatory T cells? So  
22          someone showed a slide this morning with a

1 number of papers referring to TLR receptors.  
2 I think the number of papers over the last ten  
3 years referring to regulatory T cells -- maybe  
4 I'm exaggerating a little bit -- is probably  
5 a log higher from 1997 to the present time.

6 Almost every single T cell subset  
7 has been endowed with regulatory function.  
8 For the sake of time, I'm not going to go  
9 through all these subsets. I don't think all  
10 these other ones are terribly important but  
11 one subset is definitely important.

12 And these are the so-called thymic  
13 derived Foxp3 positive regulatory T cells.  
14 Some probably come from the thymus. Some may  
15 be generated in the periphery. And we will  
16 address this concept.

17 This is a new idea. We used to  
18 think that all the regulatory T cells that  
19 expressed Foxp3 arose in the thymus and came  
20 out in the periphery. More recent studies  
21 have suggested that regulatory T cells can  
22 also be generated in the periphery, in the

1        gut, in lymphoid tissues, perhaps in response  
2        to certain kinds of immunizations.

3                    Overall, in both mouse and man,  
4        and everything is the same in this field,  
5        about ten percent -- eight to 12 percent of  
6        CD4 positive T cells express the transcription  
7        factor Foxp3 -- we'll come back to that in a  
8        minute -- which appears to be the real lineage  
9        marker of this subset of cells.

10                   We used to call these CD4  
11        positive, CD25 positive T cells. That's how  
12        they were originally discovered by Shimon  
13        Sakaguchi. That's what we called them for  
14        many years.

15                   But the real marker -- not  
16        necessarily the most perfect marker -- is the  
17        expression of Foxp3. And this population of  
18        cells controls the immune response to  
19        everything.

20                   If there are any nonbelievers in  
21        the audience that these cells are important,  
22        I'm going to show you one simple experiment,

1 a really adaptive experiment that Fiona Powrie  
2 and Bob Coffman did a long time ago. If one  
3 takes the CD4 positive T cells that don't  
4 express Foxp3 and inject them into a RAG  
5 knockout mouse, almost 100 percent of the mice  
6 develop severe inflammatory bowel disease as  
7 can be seen from this section of the colon.

8 If one injects regulatory T cells  
9 along with the Foxp3 negative cells into this  
10 immunodeficient mouse, the regulatory T cells  
11 completely protect against IBD. So regulatory  
12 T cells are real. They are a definite  
13 population. You can't argue about them. They  
14 exist.

15 So most of these cells were said  
16 to come from the thymus until about five years  
17 ago when a number of groups published that one  
18 could induce Foxp3 expression by merely  
19 culturing conventional CD4 positive T cells in  
20 the presence of TGF beta and and a TCR  
21 stimulus.

22 And indeed this works every time.

1 If we take a population of cells that  
2 expressed zero to no Foxp3 positive cells,  
3 culture them in the presence of TGF beta, a  
4 TCR stimulus, and a high concentration of  
5 Interleukin 2 -- and Interleukin 2 is  
6 absolutely required in this -- we can generate  
7 a population where you can see now 90 percent  
8 of the cells express Foxp3.

9 And these cells have all the  
10 properties, including the in vivo-suppressive  
11 effects of the thymic-derived regulatory T  
12 cells. TGF beta is an absolute here.

13 The three percent you see here is  
14 real. If we put in anti-TGF beta to the  
15 culture, we knock it down to zero. And there  
16 is a little bit of TGF beta course presence in  
17 the fetal calf serum we use for culture. So  
18 this is in vitro.

19 Does it occur in vivo? Can Foxp3  
20 negative cells be converted to Foxp3 positive?  
21 And there are a couple of different ways of  
22 showing this. I'm going to show you a drastic

1 example but it is the proof of the concept.

2 Several laboratories have  
3 generated Foxp3 as a transcription factor so  
4 it can't be used as a cell surface marker  
5 obviously for regulatory T cells. But GFP,  
6 the fluorescent dye has been knocked into the  
7 Foxp3 locus. So now that all Foxp3 positive  
8 cells are green, which is very convenient --  
9 and you can see about ten percent of these CD4  
10 cells are green.

11 We're not interested in those  
12 cells. We're interested in the cells that  
13 don't express Foxp3. So we sort the GFP  
14 negative cells. Again, we inject them into a  
15 RAG knockout mouse that has not T cells or B  
16 cells. And we just leave the mouse on the  
17 shelf for two to eight weeks.

18 And you can see gradually the  
19 development of Foxp3 positive cells both green  
20 and stained with antibody as well over a  
21 period of eight weeks. So by eight weeks,  
22 roughly 25 percent of the CD4 cells in this

1 particular mouse express Foxp3.

2 We can sort these cells, get them  
3 out of the mouse, and show they have most of  
4 the suppressive properties of the thymic-  
5 derived cells. So this occurs in the  
6 periphery. And this is a rather important  
7 idea.

8 And it raises the issue of what is  
9 stimulating these cells. Is it TGF beta in  
10 vivo? That's not really been proven.

11 Obviously if they can occur and  
12 develop spontaneously in this mouse,  
13 presumably they can develop normally in your  
14 peripheral tissues.

15 And I bring this up because we  
16 have to ask the question where do T  
17 regulatory cells come from in the adult man?  
18 Thymic output stops at puberty. Perhaps all  
19 the regulatory T cells hang around, divide  
20 very slowly.

21 Alternatively, regulatory T cells  
22 are constantly being generated under

1 conditions that we don't understand in our  
2 peripheral lymphoid tissues. And that in the  
3 80 year old, which has exactly the same number  
4 of Foxp3 positive cell as a 20 year old, most  
5 of these cells may be derived in the  
6 periphery.

7 And that brings us to a sort of  
8 scary question. It used to be thought that  
9 the TCR repertoire of the Foxp3 positive  
10 cells, most of those generated in the thymus,  
11 is biased toward the recognition of self  
12 antigens. That's proven by sort of esoteric  
13 molecular approaches that I'm not going to  
14 show you.

15 Other studies suggested that  
16 pathogen-derived antigens could also be  
17 recognized by T regulatory cells. So there  
18 was nothing special about the repertoire  
19 really of T regulatory cells. Perhaps a self  
20 bias but a very diverse repertoire that could  
21 recognize anything.

22 And then you people have to ask

1 the question could a vaccine adjuvant  
2 preferentially expand a population of antigen-  
3 specific regulatory cells resulting in vaccine  
4 failure? That's sort of a caveat to  
5 understanding how vaccines can work.

6 And a few of the notions I heard  
7 this morning were sort of low dose, very  
8 little danger. That is really the ideal kind  
9 of vaccine that you would want in certain  
10 respects. Those are the kinds of conditions  
11 that people propose would induce regulatory T  
12 cells rather than boost up an immune response.

13 Of course my colleagues in the  
14 autoimmunity field would be rushing to patent  
15 this vaccine if it was specific for an auto  
16 antigen. So it really depends on how you look  
17 at everything what conclusions you draw.

18 They would love to have a vaccine  
19 to a tissue-specific antigen, to an auto  
20 antigen that would boost up a population of  
21 regulatory T cells and would function as a  
22 vaccine for autoimmunity.

1           So this is my sort of bad Chinese  
2 restaurant menu slide where you have one from  
3 Column A and one from Column B. How do  
4 regulatory T cells work? I've been in this  
5 field for about 13 years.

6           And first of all, it used to be  
7 considered that the CD4 positive regulatory T  
8 cells would act on CD4 responders and CD8  
9 responders. That was a nice concept. It's  
10 still possible.

11           But over the years, papers --  
12 reasonably good papers I have to say -- have  
13 appeared that regulatory T cells can act  
14 directly, I have to say, on every other  
15 possible cell type in the immune response.  
16 Most recently mast cells, if you look at the  
17 current issue of Immunity and also a paper  
18 published that I am a co-author on in the  
19 Journal of Immunology over the past year.

20           So regulatory -- how can they do  
21 it? How can one cell type suppress all these  
22 other cell types? Well, that's where you get

1 to Column B.

2 Column B are the proposed  
3 mechanisms by which these regulatory T cells  
4 function, some sort of easy to understand.  
5 They could secrete suppressive cytokines, IL-  
6 10, or TGF beta. The latest fad cytokine they  
7 might secrete is IL-35 -- really the  
8 publications of only a couple of laboratories.

9 It said they can do all kinds of  
10 other things. They express the antigen CLTA-4  
11 on their cell surface. Perhaps that interacts  
12 with dendritic cells, with CD80/86 on  
13 dendritic cells, and induces the production of  
14 IDO or by some other means inactivates  
15 dendritic cell function.

16 I won't go through all of these.  
17 None of these have been -- these are largely  
18 the products of single laboratories or one or  
19 two laboratories, certain membrane molecules  
20 may be involved in their suppressive function.

21 My own view is we really don't  
22 know how they work. I wouldn't say they use

1           one mechanism. Perhaps they pick a mechanism  
2           depending on the nature of the environment  
3           they are in.

4                        If they have to suppress  
5           inflammatory bowel disease where there is lots  
6           of inflammation, they probably have to make  
7           IL-10 most of the time. But not all the time.  
8           Is that all they do? We don't know.

9                        So I think these are important  
10          research questions for the future. How  
11          exactly do regulatory cells work? And if you  
12          don't know how they work, it is hard to  
13          understand how you could manipulate their  
14          functions, which is really the goal.

15                      If you want to stop regulatory T  
16          cell functions or inhibit it, how do you do it  
17          if you don't exactly know their mechanism of  
18          action or even their target cell?

19                      So adjuvants, I was forced to  
20          think about adjuvants, having been invited to  
21          give this talk, and there are several papers  
22          suggesting that adjuvants, primarily TLR

1 ligands, might function by acting directly on  
2 regulatory T cells.

3 And regulatory T cells in both  
4 mouse and man probably express most of the  
5 TLRs, although that hasn't been done I'd say  
6 with the greatest care, but there is enough  
7 data to suggest that regulatory T cells, along  
8 with every other cell type including CD4  
9 effector cells, express TLRs.

10 So adjuvants, and I'm going to use  
11 TLRs as my model here, might conceivably act  
12 directly on regulatory T cells to do something  
13 to them or, alternatively, act on another cell  
14 type, the antigen-presenting cells -- and this  
15 makes more sense -- that adjuvants, say CpG  
16 acting on an antigen-presenting cell would  
17 induce the antigen-presenting cell to make  
18 certain cytokines -- the examples I'll give  
19 are IL-6 and TNF alpha -- that would then act  
20 on effector T cells and somehow render the  
21 effector T cells resistant to the suppressive  
22 effects of the regulatory T cells. And that

1 is another way adjuvants can influence  
2 regulatory T cell function. So direct and  
3 indirect effects can be manifest.

4 I hate to show a list of papers  
5 that have been published in a field but this  
6 is a perfect example that really reflects our  
7 ignorance. So don't look at any of these  
8 things except the enhances, reverses,  
9 enhances, reverses, reverses, enhances,  
10 reverses, okay.

11 (Laughter.)

12 DR. SHEVACH: I'm not convinced by  
13 any of these papers, frankly.

14 The first paper claimed that LPS,  
15 for example, would enhance mouse T regulatory  
16 cell survival and suppressor function. That's  
17 definitely true. I tried extremely hard in my  
18 own laboratory to repeat that. It's not going  
19 to get published, of course, because I  
20 couldn't repeat it. It doesn't work.

21 So this is a mouse Treg study.  
22 Toll-2 Pam3CS K reverses mouse Treg function.

1           Somebody in my laboratory has been trying to  
2           repeat those two papers from two different  
3           groups. We can't repeat one piece of data in  
4           them.

5                        So a word of caution about  
6           believing anything on this slide. And the  
7           only thing I'll mention is the famous Pasare  
8           and Medzhitov paper that everybody loves to  
9           quote.

10                       But this basically was what was  
11           shown on the previous slide -- that CpGs  
12           stimulated APCs to make IL-6 and probably  
13           something else that functions as growth  
14           factors for T effector cells.

15                       And under those conditions, the T  
16           regulatory cells fail to manifest their  
17           suppressive effects. The regulatory cells  
18           were driven to expand by IL-6 and something  
19           else that was never defined in that original  
20           paper. So that is an indirect effect. And  
21           that's quite understandable.

22                       But direct effects of TLR agonists

1 on regulatory cell function, none of these  
2 papers are very convincing. But it is  
3 something, of course, to worry about because  
4 you are all about to administer TLR agonists  
5 as adjuvants.

6 I'm going to finish up and talk  
7 about what happens when you don't have  
8 regulatory T cells because I was given the  
9 task of sort of defining the real dirty secret  
10 of adjuvants, which is could they cause  
11 autoimmunity, which is what some of you are  
12 worried about.

13 So if you lack regulatory T cells,  
14 humans that lack regulatory T cells develop  
15 something called the IPEX syndrome, which  
16 stands for immune dysregulation,  
17 polyendocrinopathy, enteropathy, X-linked.

18 And they developed essentially  
19 every single type of autoimmune disease. Many  
20 of the boys born with this disease develop  
21 diabetes in utero. It is the most severe  
22 autoimmune disease known to mankind.

1                   They also develop IBD, they have  
2                   tremendous lymphadenopathy,  
3                   hepatosplenomegaly, most of them die at age  
4                   two to three unless they get a bone marrow  
5                   transplant. But even bone marrow  
6                   transplantation is very, very hard to manage  
7                   in this particular disease.

8                   Fortunately very rare and I also  
9                   should point out that the mothers of these  
10                  children are completely normal even though  
11                  they have had half of their X chromosomes  
12                  inactivated, those cells that have the normal  
13                  Foxp3 allele function normally in a trans-  
14                  fashion and suppress their potentially auto-  
15                  reactive cells.

16                  And mouse and man are exactly the  
17                  same in this field. There is something called  
18                  a scurfy mouse. The scurfy mouse has a  
19                  mutation in Foxp3 so it is a two base pair  
20                  insertion in Foxp3 resulting in deletion of a  
21                  big domain of the transcription factor.

22                  And they develop a disease that

1 looks just like the human disease, exactly  
2 like the human disease lymphadenopathy,  
3 hepatosplenomegaly. They have an exfoliative  
4 dermatitis that I'll show you. And they also  
5 die.

6 And unfortunately if you are a  
7 male mouse, a male scurfy mouse that has no  
8 regulatory T cells, you die at about three  
9 weeks of age of flagrant, exuberant  
10 inflammation and autoimmunity.

11 So this is just to show you that  
12 regulatory T cells can really function. And  
13 in this particular experiment, we used  
14 regulatory T cells from a normal black 6 mouse  
15 that we induced in culture as I showed you on  
16 that early slide.

17 We took non-T regulatory cells and  
18 induced them to express Foxp3 and this is one  
19 of their in vivo functions. They can actually  
20 rescue the scurfy mouse. So this is a wild-  
21 type mouse. This is a scurfy mouse. And it  
22 is pretty evident when you give the scurfy

1 mouse these induced T regulatory cells on Day  
2 1 of life and this is looking at about Day 30  
3 of life, you've rescued this mouse from this  
4 runting syndrome.

5 And it is not just injecting any  
6 old population of T cells into this mouse.  
7 It's not just putting lymphocytes in because  
8 if you put into the scurfy mouse lymphocytes  
9 that have been expanded up in the presence of  
10 anti-TGF beta so there are no Foxp3 positive  
11 cells in your prep, the mouse is still runted.

12 And this is what a scurfy mouse  
13 tail looks like. There is a unique infiltrate  
14 into the skin of the tail. This is ear skin.  
15 It's what we usually look at the  
16 histopathology.

17 They have lymphocytic infiltrates  
18 everywhere: lung, liver, heart, pancreas. I'm  
19 just showing you a little small example of  
20 this. If we put these induced T regulatory  
21 cells in, the tail is completely normal, the  
22 ear is normal, and the lung, liver, or what

1           have you, are completely normal for as long as  
2           we keep the experiment going which, in this  
3           case, is about 30 days. But this is only one  
4           single shot of these induced T regulatory  
5           cells.

6                         Why am I showing you this  
7           particular model? Why is it interesting?  
8           Because this is the ultimate test of an animal  
9           that has the propensity to develop  
10          autoimmunity. This animal develops  
11          autoimmunity to every single organ that it  
12          has.

13                        So it is sort of like a person who  
14          has the ultimate -- you know you are all  
15          worried that a vaccine adjuvant might induce  
16          autoimmunity in someone of the right genetic  
17          background that has a propensity to develop  
18          say Type 1 diabetes. These animals besides  
19          having a cellular infiltrate also have high  
20          titers of autoantibody. In this case it is  
21          autoantibody to skin.

22                        So I'm going to show you what -- I

1 was sort of given the challenge to discuss --  
2 and I'm just going to do it on this slide  
3 rather briefly -- how would you test whether  
4 and adjuvant could induce autoimmunity? And  
5 I gather that is a major issue in this field.

6 And I began to think about that  
7 problem and it wasn't so easy for me to come  
8 up with something that would really address  
9 the issue and give an answer.

10 And I'm a mouse organ-specific  
11 immunologist, so to speak. I'm interested in  
12 all kinds of organ-specific autoimmune  
13 diseases in the mouse. But, for the most  
14 part, their induction requires the use of  
15 Freund's adjuvant.

16 And there are very, very few  
17 spontaneous models of organ-specific  
18 autoimmune disease. But probably the best  
19 example is the NOD mouse which develops a  
20 disease like diabetes. And perhaps you could  
21 test functions of the -- the ability of a --  
22 the potential ability of an adjuvant to induce

1 autoimmunity or autoimmune diabetes but that's  
2 only one disease. If that's not what the  
3 adjuvant really works on, you wouldn't see  
4 anything in other diseases.

5 Most of the mouse models are  
6 systemic autoimmune diseases. The NZB mouse,  
7 the MRL mouse, the GLD mouse are very slow to  
8 develop, mostly due to -- they obviously have  
9 helper T cell dysfunctions but most organ  
10 damage is primarily due to immune complexes.

11 And most of them have been  
12 characterized in molecular defects, for  
13 example, in the Fas or Fas ligand deficient,  
14 is characterized and that really isn't a  
15 characteristic of any human autoimmune disease  
16 for the most part. There are patients that do  
17 have mutations in Fas and Fas ligand.

18 The scurfy mouse, I think, is sort  
19 of the most interesting one. It develops  
20 immune pathology everywhere so it has the  
21 potential to develop autoimmune disease in  
22 every single target organ.

1                   It looks just like the IPEX  
2           disease in man. It is the same disease. So  
3           I began to think about how I would test an  
4           adjuvant.

5                   And I haven't done this  
6           experiment. I'm only showing you half the  
7           experiment which is sort of the control that  
8           I have done. And I'm not showing you how I  
9           would test an adjuvant but I will predict one  
10          result.

11                   So you can't do this experiment in  
12          a scurfy mouse. The scurfy mouse is going to  
13          develop unbelievable autoimmune disease unless  
14          it gets a transfusion of regulatory cells.  
15          But what we've done is to take cells from a  
16          Day 13 -- ten to 13 day old- or even a week  
17          old-scurfy mouse.

18                   So these are cells that have  
19          already been activated, in part, have the  
20          potential to recognize autoantigens because  
21          they have never been exposed to regulatory  
22          cells. And we transmit them to a RAG knockout

1 mouse.

2 And what we see is a  
3 recapitulation eventually of the disease in  
4 scurfy mouse. And I'm going to show you that  
5 data. And we do these experiments -- we can  
6 do these experiments in seven days. We can  
7 take these scurfy cells, transmit them to the  
8 RAG knockout and begin to see manifestations  
9 of autoimmunity by seven days of life.

10 Let me show you the experiment and  
11 let me show you what I would have done if I  
12 had worked for TeGenero. Thank God I didn't.

13 So this is taking scurfy cells and  
14 injecting them into a RAG knockout mouse and  
15 we do this either with purified CD4 cells or  
16 actually Eva Heuter, who did these  
17 experiments, did this with total scurfy cells.  
18 She just took everything from the spleen of a  
19 scurfy mouse and injected it into a RAG  
20 knockout mouse.

21 And this is histology scores at  
22 about 14 days after transfer. Skin, lung,

1 liver, you begin to see infiltrates. You begin  
2 to see lymphadenopathy. You begin to see  
3 increases in T cell numbers in every organ you  
4 look at.

5 And this is the control study  
6 where we -- this is why she did the  
7 experiment. She wanted to show that Foxp3  
8 positive regulatory cells, in this case ones  
9 we induced in vitro, would prevent this  
10 disease in a co-transfer model -- something  
11 that we could control.

12 We can control the number and type  
13 of cells we inject. We can control the number  
14 of regulatory cells that we inject. And,  
15 indeed, when she injected these induced T  
16 regulatory cells, if you look at the middle  
17 bars, the triangles, she really completely  
18 suppressed the development of autoimmunity.

19 The other -- if we inject non-T  
20 regulatory cells, they seem to potentiate the  
21 disease.

22 So here is a model where we have

1       some effector cells that have been injected  
2       into the mouse. And the mouse will -- the  
3       recipient mouse will eventually develop organ-  
4       specific autoimmunity everywhere.

5               So one might ask the question what  
6       would happen if at this time of cell transfer  
7       of the effector population alone, one  
8       administered an adjuvant? Would it enhance  
9       the ability or increase the frequency and the  
10      intensity of the autoimmune response? Could  
11      you use this kind of model -- and I'm only  
12      showing this as a kind of model that I thought  
13      about.

14              And I can tell you one experiment  
15      which hasn't been done. But I can predict the  
16      result. So if one took the agonistic anti-  
17      CD28 antibody that was used by the TeGenero  
18      Company in humans -- and I think they actually  
19      had the mouse-equivalent antibody that had the  
20      same affinity for mouse CD28 as the human  
21      antibody had for human CD28, if one injected  
22      that antibody at the same time one transferred

1           these cells, this mouse would have been dead  
2           seven days after transfer of the effector  
3           cells because it would have really activated  
4           these effector cells and brought this score up  
5           to six and death rather rapidly.

6                         So this is a kind of model where  
7           one could test. And that would be an  
8           experiment.

9                         Even in the presence of Foxp3  
10          positive cells where regulation is taking  
11          place, one could ask the question would an  
12          adjuvant be so strong that it would overcome,  
13          either by acting on the regulatory cells or by  
14          hyperactivating the effector cells, overcome  
15          the suppressive effects of the regulatory  
16          cells. And one would begin to see autoimmune  
17          disease under those conditions.

18                        And it doesn't matter because  
19          conceivably you could only see autoimmune in  
20          the pancreas in this kind of model and you'd  
21          be protected against everything else. But  
22          that would still tell you that the adjuvant or

1 the agent you were using would have an effect  
2 in enhancing the immune response to  
3 autoantigens.

4 So let me just finish up very  
5 briefly. Multiple types of regulatory cells,  
6 but the most important one, the Foxp3 positive  
7 ones -- and these could be generated both in  
8 the thymus and in the periphery. In the  
9 periphery conceivably they could be generated  
10 in response to antigens, to exogenously  
11 administered antigens including vaccines.

12 The cancer people are very worried  
13 about this concept because they think that the  
14 regulatory cells really have a predisposition  
15 to see autoantigens. But I think this holds  
16 for every kind of antigen.

17 Unfortunately, what we'd really  
18 like would be an animal that is normal that  
19 doesn't have regulatory T cells. That would  
20 be really nice to have. You could immunize  
21 it. You could see what role regulatory T  
22 cells played in response to all kinds of

1 antigens.

2 It is impossible to make such an  
3 animal. If one completely depletes regulatory  
4 T cells from mice, they rapidly develop  
5 autoimmunity and are dead within 18 days.  
6 They look like a scurfy mouse. And this has  
7 been done using a Foxp3 controlling the  
8 diphtheria toxin receptor and giving the  
9 animal the diphtheria toxin to deplete the  
10 Foxp3 positive cells. So you can't have that  
11 kind of situation.

12 It would be really nice to know  
13 how these cells worked because if you really  
14 knew the molecular mechanism they worked, one  
15 might use drugs or antibodies to transiently  
16 inactivate them and then give the animal a  
17 vaccine.

18 There have been attempts to do  
19 this with a drug. It is a combination of IL2  
20 and diphtheria toxin marketed by Ligand  
21 Pharmaceuticals. I haven't been impressed  
22 that it really depletes regulatory T cells

1           very effectively. And, of course, would only  
2           deplete those that express IL2 receptors.

3                       Lastly, T regulatory cells do  
4           express TLRs. Perhaps TLR ligands can  
5           influence regulatory T cell function in  
6           certain ways. I'm unimpressed by what has  
7           been published in the literature as being  
8           real.

9                       And one thing I think all of you  
10          have to consider, to conclude, is that any  
11          kind of test of an adjuvant effect really has  
12          to include some form of evaluation of the role  
13          of the adjuvant on regulatory T cell function,  
14          be that a direct effect of the adjuvant or an  
15          indirect effect of the adjuvant on another  
16          cell type with something happening to  
17          regulatory T cells.

18                      Thank you.

19                      (Applause.)

20                      DR. GRUBER: Yes, thank you very  
21          much for this very interesting presentation.

22                      I think we have time for one --

1 for two questions.

2 DR. SHEVACH: He, I know. You can  
3 ask the question.

4 DR. MALONE: Okay, as an empirical  
5 vaccine developer, it seems to me that inbred  
6 mouse strains with their own unique  
7 immunogenetics are going to be relatively  
8 poorly predictive of outbred human responses.

9 And it seems to me that the only  
10 way that we are really going to be able to  
11 assess this is if we get a little more  
12 sophisticated in how we assess our safety  
13 signals in our human clinical trials rather  
14 than having a preset, prescreened tox assay  
15 that we have to employ in order to get into  
16 the clinic.

17 DR. SHEVACH: Ideally you are  
18 right. But let's say you had an agent that  
19 really made that mouse I put a few effector  
20 cells into much worse, would you use it?

21 DR. MALONE: But is that going to  
22 be predictive of outbred humans? I think

1           we're going to have a small -- I think we're  
2           going to have a signal. I think there is a  
3           reasonable chance that we're going to have a  
4           signal in, you know, is it one in 10,000?

5                     DR. SHEVACH: Well, I gave a mouse  
6           that could develop anything. That's why I  
7           picked it. Not, you know -- we can talk about  
8           it.

9                     DR. MALONE: I'm just concerned  
10          about at this stage of development of Treg  
11          biology employing a tox screen threshold in  
12          our assessment of a vaccine or adjuvant  
13          candidate prior to entry into the clinic. I  
14          mean --

15                    DR. SHEVACH: No, you are right.  
16          My talk is only -- it was only meant to make  
17          you think. I succeeded.

18                    DR. MALONE: And I love Treg  
19          biology. Thank you.

20                    DR. GRUBER: So one more question.

21                    DR. SHEVACH: Bob, you can ask me  
22          later.

1 DR. SAHNER: Hi, David Sahner, NES  
2 Medical Consulting.

3 This actually plays off of the  
4 prior question quite nicely I think.  
5 Basically what you would propose with this  
6 experiment would be evaluating for  
7 exacerbation of an underlying autoimmune  
8 model.

9 Key to interpreting the data would  
10 be some sort of sense of what the threshold  
11 for significance might be potentially. And we  
12 already know from humans the mothers of the  
13 IPEX neonates that they can tolerate some  
14 relative deficient of Foxp3 cells compared  
15 with the rest of the population.

16 So knowing that having merely a  
17 subset of or a smaller quantity of what is  
18 typically found in the circulation of Foxp3  
19 positive cells in these mothers, knowing that  
20 this smaller quantity of cells is up to the  
21 task of preventing these horrific autoimmune  
22 complications in humans, I think it becomes

1           very important to be sure in an animal model,  
2           if one is put forth, that we have a clear  
3           sense of the threshold.

4                         And, of course, I have to agree  
5           with the first questioner. I think it is  
6           going to come from clinical data -- that the  
7           insights will come --

8                         DR. SHEVACH: Well, let me give  
9           you one example that is different from the  
10          mothers. In one of the papers using the Toll-  
11          2 agonist Pam3CS, Eddie Lu's laboratory  
12          claimed -- and I can't reproduce this myself  
13          I must say -- that it decreased the level  
14          globally of Foxp3 positive -- of Foxp3 in all  
15          regulatory T cells by about 50 percent.

16                        And there is a paper from  
17          Blovell's laboratory where he had a mutation  
18          that they discovered really by accident where  
19          the regulatory T cells expressed, on a per  
20          cell basis, all of them were 50 percent of the  
21          normal level. And that mouse developed a Th2-  
22          mediated autoimmune disease.

1                   So a little hard to predict but  
2                   good question.

3                   DR. GRUBER:   Okay.   I think we'll  
4                   take an extreme quick coffee break.   And we  
5                   will reconvene at 4:20.

6                   (Whereupon, the foregoing matter  
7                   went off the record at 4:14 p.m.  
8                   and went back on the record at  
9                   4:28 p.m.)

10                  DR. GRUBER:   So I'm very pleased  
11                  that I can announce the next two speakers for  
12                  this session that will give us an industry  
13                  perspective on the design and challenges of  
14                  conducting preclinical toxicological studies  
15                  to support safety of adjuvanted vaccines to  
16                  allow proceeding to clinical studies in  
17                  humans.

18                  And the first speaker is Dr. Sarah  
19                  Gould.   She's the head of Nonclinical Safety  
20                  at Sanofi Pasteur.

21                  DR. GOULD:   Thank you, Marion.  
22                  And thank you for inviting me today.

1                   So we've seen some very  
2           interesting talks. And I'm a toxicologist and  
3           have been for a number of years.

4                   And I'm not sure how many  
5           toxicologists are here in the meeting. Now  
6           there's one hand that's gone up. So there are  
7           some. Not many. So maybe as a toxicologist  
8           we have a slightly different perspective.

9                   A lot of it is about risk  
10          assessment. The models that we use may not be  
11          100 percent predictive. Can you hear me now?  
12          Sorry about that. But they are models and the  
13          aim is to be looking for signals and to be  
14          supporting to move forward into the clinic.

15                  So the outline of my presentation  
16          today, I'm going to -- some of my presentation  
17          has already been picked up on already -- I'm  
18          going to look a little bit at the background  
19          and some considerations.

20                  I'm going to give two case  
21          histories in brief. And then I'm going to  
22          leave you with a what-about because I'm going

1 to stimulate, I hope, some thoughts. I'm  
2 going to ask some questions. And I don't  
3 necessarily have all the answers. And that's,  
4 hopefully, one of the reasons for this meeting  
5 here is, and particularly tomorrow, that we  
6 can hopefully sit down and go through some of  
7 those questions.

8 So as you've already seen clearly  
9 presented this morning, there are mainly two  
10 guidelines when we are looking at the safety  
11 evaluation of adjuvants. There is the EMEA  
12 guideline, which was specifically written to  
13 give advice on going into humans with  
14 adjuvants. And there is the WHO guideline,  
15 which does discuss moving forward with  
16 adjuvants.

17 In 2007, there was a DIA meeting  
18 in which EMEA, FDA, and industry got together.  
19 And there were many questions raised. There  
20 was a clear message given by the FDA and the  
21 EMEA. It's case by case. And I think that is  
22 correct.

1           But, of course, that does bring  
2           its problems. And I think there is a clear  
3           industrial need to consider this further --  
4           this subject further. And to evolve.

5           So these are some of our tools as  
6           a toxicologist that we sort of use. So  
7           there's the general toxicity studies. This is  
8           assessing systemic toxicity. We also include  
9           local tolerance in this. We try to include it  
10          within the same studies. We also have to  
11          consider the ethical use of animals.

12          There's reproductive and  
13          developmental toxicity studies. There's  
14          safety pharmacology studies, genetic  
15          toxicology, juvenile studies. There's also  
16          other specific toxicity studies that we may  
17          conduct, immunotoxicity, investigational  
18          studies, mechanistic toxicity studies. I  
19          haven't mentioned them all.

20          And there's also -- I can mention  
21          the paragenecity studies and hypersensitivity  
22          and anaphylaxis studies, which I'm going to

1           come back to in our what-about.

2                         So when we're considering a  
3           toxicology assessment, the first question is  
4           what are we testing? What is the adjuvant?  
5           Now there can be a lots of different types of  
6           adjuvants which you have seen this morning in  
7           the presentations, giving you some ideas of  
8           the types of adjuvants out there.

9                         So how do we define it? Do we  
10          define it as a new chemical entity? As an  
11          excipient? As a biological? So you really  
12          need to try and know what you are testing.

13                        Then we need to consider are we  
14          assessing this adjuvant alone? And there's  
15          this master file concept. And we also must  
16          assess the adjuvant with the antigen.

17                        Again, we've already seen this  
18          morning the discussion about when you use  
19          adjuvants. It is with a vaccine. You can  
20          administer this, vaccines and adjuvants, via  
21          various routes. Today I would say the most  
22          commonly used route is intramuscular and

1 subcutaneous. But obviously other routes are  
2 being developed: ID, IV, patch, nasal, oral,  
3 et cetera. Again, the list goes on. I  
4 haven't mentioned everything.

5 And the dosing schedule -- the  
6 dosing schedule of vaccines is usually short  
7 term. I put usually there because I'm not  
8 sure how we define flu vaccines which are  
9 given yearly or something like some of the  
10 immunotherapies for cancer or HIV.

11 So moving forward a little bit  
12 more to discuss about the toxicological  
13 assessment, looking more about the designs,  
14 and asking some questions. What studies do we  
15 do?

16 Well, our main interest is in the  
17 systemic toxicity and local tolerance for  
18 which we usually conduct either single or  
19 repeat general toxicity studies. And here we  
20 have to start asking some questions. What  
21 species? How many? What's the study design  
22 going to be like? What dosing schedule? How

1 many doses levels? And I'm going to take you  
2 through some of these points.

3 When we consider the number of  
4 species, let's just have a look at some of the  
5 guidelines. So for a new chemical entity, if  
6 we're looking at general toxicity, assessing  
7 systemic toxicity, we require two species, a  
8 rodent and a non-rodent. Now as you've seen,  
9 animals are not always predictive so we use a  
10 rodent and a non-rodent to try and help that.

11 For a biological, we tend to try  
12 and use a one relevant species. If we can't  
13 find a relevant species, then you might want  
14 to consider two.

15 So what do we want to use for an  
16 adjuvant? One species or two? Again, it's  
17 case by case.

18 In the EMEA guidelines, it should  
19 be tested in two species unclear otherwise  
20 justified. And ideally at least one species  
21 selected should be the same as that used in  
22 the proof of concept studies.

1                   So if an adjuvant has no species  
2                   specificity, say like an oil emulsion, perhaps  
3                   you want to consider using two species. If an  
4                   adjuvant exerts a high level of species  
5                   specificity, e.g., like some of the cytokines,  
6                   then perhaps one relevant species is enough.

7                   Now looking at the dosing  
8                   schedule, when you are assessing the adjuvant  
9                   alone -- now how are we going to consider  
10                  this? Are we going to consider it as a  
11                  vaccine or a new chemical entity?

12                  Well, the EMEA supports that a  
13                  vaccine-like administration. The WHO is less  
14                  specific. It refers to the ICH and excipient  
15                  guidelines.

16                  So, for an example, if we have a  
17                  look at the sort of typical doses that we  
18                  might administer, a clinical schedule, and  
19                  then add one, it is the N+1, which was talked  
20                  about earlier about Marion, giving one dose  
21                  maybe every two to three weeks, looking at  
22                  when we do necropsy, Day 2, and then maybe

1 give a non-dose period, maybe 14 days after  
2 the last period.

3 Use the same route as the clinic,  
4 for example, intramuscular. And what dose  
5 level? The human dose? By volume? By mg/kg?

6 So how many dose levels? And what  
7 about the MTD? That's the maximum tolerated  
8 dose. So, again, when you are looking at new  
9 chemical entities, then the guidelines suggest  
10 that you are looking for a dose relationship  
11 should be established and you should reach  
12 maximum tolerated dose. You really need to  
13 understand what this small molecule might do  
14 if you are looking at a small molecule.

15 The adjuvant guidelines for the  
16 WHO is not specific detail. The EMEA suggests  
17 that a dose relation should be established,  
18 range of doses may be relatively low, reflect  
19 the clinical dose, and maximum tolerated dose  
20 is not needed.

21 And how many doses? If we're  
22 setting the adjuvant alone and developing a

1 master file, this adjuvant may actually be  
2 used in more than one vaccine. So if it's  
3 being used in more than one vaccine, there may  
4 be more than one clinical schedule. So one  
5 vaccine may be one dose, another vaccine could  
6 be three.

7 And when you are supporting the  
8 clinic and you don't know what will the clinic  
9 do. Is the dose in the Phase I trial fixed?  
10 And then I think maybe tomorrow some of this  
11 will be discussed, the companies and projects  
12 may have a very different approach.

13 So there are some possibilities.  
14 Just administer one dose, a HEMO clinical  
15 dose. You have to make the assumption,  
16 therefore, that this is going to be a no-  
17 effect dose level. Or you administer more  
18 than one dose and, if so, what information are  
19 we looking for?

20 The MTD is not needed. Maybe it  
21 is going to help us understand the dose  
22 response, that is if we're seeing any

1 toxicity. Maybe it's going to be used to  
2 investigate possible toxicity, in fact like  
3 push the dose up a little bit or to make sure  
4 we do get a no-effect dose level.

5 Again, about the doses, are they  
6 based on volume or mc/kg? And are you going  
7 to use this data for dose setting in the  
8 clinic? Or is the clinic going to assign the  
9 dose before you go into the clinic?

10 Genotoxicity, do we assess  
11 genotoxicity? Again, it may depend on the  
12 classification of your adjuvant. So for NCEs  
13 and excipients versus a biological, it might  
14 be different. For an oligonucleotide, we  
15 might want to consider DNA integration.

16 So for the EMEA, it states if it  
17 is considered a nonchemical entity, that we  
18 follow the ICH2a guidelines. I just put a  
19 little comment here that genotoxicity tests,  
20 as mentioned in the ICH2a guidelines, are  
21 conducted at the highest maximum tolerated  
22 dose.

1                   So hopefully you can see that's  
2                   opposite to what we were trying to achieve  
3                   with our adjuvants where we are saying we  
4                   don't have to achieve a maximum tolerated  
5                   dose.

6                   These guidelines are currently  
7                   being changed so this actually might change.  
8                   But it is just something that is of interest  
9                   at the moment, specifically for a toxicologist  
10                  as to how we answer the question.

11                  So now I'm going to take you  
12                  through two case studies. And the first one -  
13                  - I've named them Adjuvant X and Y for IP  
14                  reasons.

15                  This adjuvant is currently in  
16                  Phase I clinical trials and it is an emulsion.  
17                  And the idea here was to create a master file.  
18                  So for the toxicology assessment to move  
19                  forward into Phase I, we conducted a general  
20                  and genetic toxicity study with the adjuvant  
21                  alone.

22                  We conducted a repeat dose

1 toxicity study and we used two species, a  
2 rodent and a non-rodent. So here we used the  
3 rat, which is a preferred rodent species --  
4 the mice are okay but you can't get as many  
5 samples out of them so we prefer the rat --  
6 and the rabbit as a non-rodent species.

7 Again, how you select the species  
8 can depend on a number of factors. And I  
9 don't think I'm going to go into that here  
10 because of I haven't got enough time.

11 We tested two groups, an adjuvant  
12 alone group and a saline control. And here we  
13 looked -- we did more than the human dose. We  
14 did three dose levels because we decided we  
15 were just going to push the dose up. We  
16 weren't aiming for maximum tolerated dose but  
17 we were going to just have a look to see what  
18 potential effects there may be partly due to  
19 because of some of the safety concerns that  
20 adjuvants have.

21 But we fixed our volume at -- the  
22 human volume or which is the normal human

1 volume that we tend to use is not .5 ml or for  
2 the rat, for which you can't inject the human  
3 dose, we gave two doses of 250 microliters.

4 Now as we said, at this time we  
5 are creating a master file here so the  
6 clinical regime is unknown. So we have to  
7 sort of guess what might be the worst case  
8 scenario here.

9 So we looked at -- if you look  
10 across at the schedule of vaccines, on the  
11 whole, five to six is the maximum you'll ever  
12 see in our experience although you may have  
13 different experiences, so we decided that we'd  
14 dose five times and every three weeks.

15 The study design which, again, I'm  
16 not going to go into detail but it's based on  
17 the WHO, EMEA, and ICH standard guidelines so  
18 we were looking at various endpoints such as  
19 clinical signs, local reactogenicity, body  
20 weight, food consumption, clinical pathology,  
21 ophthalmology, histopathology, and organ  
22 weights. And we included a recovery period of

1 14 days after the last dose.

2 Because this was an emulsion and  
3 not a biological, we decided that we would  
4 just test the potential genotoxicity. So here  
5 we followed the standard ICH2a guidelines and  
6 we tested in an in vitro Ames and mouse  
7 lymphoma assay. And an in vivo mice/mouse  
8 micronucleus assay.

9 And as I said earlier, for this  
10 assay we have to really dose to the maximum  
11 tolerated dose. So, in fact, we did do a  
12 preliminary study. It's just a single dose  
13 that is given here. But we did a dose ranging  
14 finding single study to assess the maximum  
15 tolerated dose and then we did the pivotal  
16 study.

17 And, again, without going into  
18 major details, this is standard ICH design.

19 Now as we said, before you can  
20 move forward into the clinic if you are  
21 testing -- you must test your adjuvant with  
22 the vaccine. This was a prophylactic vaccine.

1 And now the clinical plan is known.

2 So for this we supported this with  
3 a general toxicity study. And we supported in  
4 the rabbit as we believe this to be the most  
5 appropriate species. With the antigen that we  
6 were developing, we got an immune response in  
7 the rabbit, for us is a good model.

8 So we dosed the rabbit with the  
9 human dose, just the human dose, not .5 ml.  
10 We went in via the clinical route,  
11 intramuscularly, and we followed the clinical  
12 regime, N+1 -- dosing once every three weeks.  
13 And the study design was as I've discussed  
14 previous.

15 So that was what we did for  
16 Adjuvant X. And as I said, it's in the  
17 clinic.

18 So moving on to Adjuvant Y, which  
19 is a biological, and this is currently in the  
20 Phase I -- now actually I have to say that we  
21 didn't do the toxicological assessment so I  
22 can't give you some of the rationale behind

1           what was done.

2                       But the idea here is to point out  
3           that there are some slight differences that  
4           you will see. So for the general toxicity  
5           study, they chose to do an acute study and a  
6           repeat dose toxicity study. And here they  
7           chose the mouse.

8                       For the route, they chose two  
9           routes to assess, subcutaneous and  
10          intramuscular.

11                      Now the acute study, they dosed at  
12          2,000-fold the human therapeutic dose. And  
13          for the repeat dose toxicity study, they chose  
14          three dose levels at five-, 15-, and 45-fold  
15          of the human therapeutic dose. They included  
16          a Trif buffer control and they dosed five  
17          doses weekly.

18                      They also conducted a local  
19          tolerance study. So some people do conduct  
20          these studies separately. And for this, they  
21          chose the rabbit. The mice isn't so good for  
22          assessing local tolerance. And, as you've

1 heard, the volumes in the mouse, you can't  
2 inject the same volume. So in this case, the  
3 rabbit is a better model for assessing local  
4 tolerance.

5 Again, they chose the routes  
6 subcutaneous and intramuscular and they gave  
7 a single dose of 10-fold the therapeutic dose.

8 They also conducted a genetic  
9 toxicity test, an in vitro, just an in vitro  
10 Ames, and they did a range of doses from three  
11 to 3,160 micrograms.

12 And as I said, this is currently  
13 now in the clinic.

14 So now just two last slides -- the  
15 what-abouts. So there are a lot of things  
16 that I haven't covered. There are still a lot  
17 of questions.

18 In these studies are we really  
19 investigating the appropriate endpoints?  
20 We've heard some of the appropriate endpoints  
21 in terms of potential autoimmunity. How are  
22 we going to test for that?

1                   What biomarkers are we testing?

2                   Well, we aren't really adding any additional  
3                   biomarkers to what is classically used at the  
4                   moment.

5                   Safety pharmacology, I haven't  
6                   discussed.

7                   Developmental and reproductive  
8                   toxicity studies, at the moment, we've only  
9                   supported the Phase I clinical trial. We're  
10                  slowly moving forward and these questions are  
11                  coming up in which now we need to turn and  
12                  consider the same questions, number of  
13                  species, doses, schedule, et cetera.

14                  I haven't covered toxicokinetics  
15                  and there are questions there. And what about  
16                  biodistribution, particularly for biologicals  
17                  such as the oligonucleotides?

18                  Pyrogenicity tests and the PAS and  
19                  ASA tests which are mentioned in the EMEA  
20                  guidelines, so these aren't always routinely  
21                  conducted.

22                  And I think with pyrogenicity

1 tests, we have to be careful as to when we  
2 measure the temperature and telemetry is  
3 probably really the best way. So I've  
4 certainly see studies where they've measured  
5 for pyrogenicity and it has come up negative  
6 and actually it is possibly because of the  
7 study design. And, again, species is  
8 important.

9 The PAS and the ASA tests, well,  
10 I'm not sure this is being used at the moment  
11 or that it is thought of as particularly  
12 predictive.

13 I haven't discussed  
14 antigen/adjuvant ratios. I haven't discussed  
15 about combination adjuvants, whether we can  
16 just considered it as one entity or we test  
17 them as two separate entities. And what about  
18 concomitant vaccines where you are giving more  
19 and lots of different adjuvants, et cetera?

20 What about the pediatric  
21 population? And I know this has already been  
22 mentioned by Jan Willem? And species can

1 effect margins. So a rabbit is approximately  
2 the same weight as a baby. So what happens to  
3 our margin when we're dosing using the rabbit  
4 as our model? And what about juvenile  
5 toxicity?

6 And I think that's me. So thank  
7 you. Any questions?

8 (Applause.)

9 DR. GRUBER: Thank you very much,  
10 Sarah.

11 We have time for a couple of  
12 questions.

13 DR. TIM SULLIVAN: Could you  
14 remark please on -- you mentioned the  
15 biologic, the Adjuvant Y species activities  
16 and choice of the toxicology, you said it  
17 wasn't yours -- you didn't develop that.

18 DR. GOULD: Yes.

19 DR. TIM SULLIVAN: But does that  
20 come into play? Or can you make some general  
21 remarks on how that would come into play if it  
22 had been your project to run?

1 DR. GOULD: I think it is a good  
2 question actually and it depends on the  
3 product as to what you'd be looking for in  
4 terms of your species being relevant.

5 And in terms of relevant species,  
6 I think I have some general questions there  
7 that makes it very difficult to answer,  
8 particularly with adjuvants.

9 So it might depend on the  
10 mechanism of action. And sometimes, you know,  
11 you just have to say there isn't a relevant  
12 species. And then there's questions.

13 DR. TIM SULLIVAN: And not to make  
14 too big of a deal out of it but I'm Tim  
15 Sullivan from Idera and our product line is  
16 oligonucleotides. And I don't know how they  
17 are regulated in Europe but in the United  
18 States, they are not considered biologics. So  
19 that would be within CDER.

20 DR. GOULD: I didn't hear the  
21 question. Sorry.

22 DR. TIM SULLIVAN: It wasn't a

1 question so much as a comment.

2 DR. GOULD: Oh, all right, okay.

3 DR. TIM SULLIVAN: You had listed  
4 oligonucleotide as a biologics. And I would  
5 consider that still a chemical rather than a  
6 biologic.

7 DR. GOULD: You're saying it is  
8 not a biologic? I can't hear you.

9 DR. TIM SULLIVAN: Yes, not a  
10 biologic. It's just the CDER.

11 DR. GOULD: Okay.

12 DR. GRUBER: I think we need to  
13 clarify this a little bit. I had a little bit  
14 of trouble understanding but, you know,  
15 oligonucleotides or DNA oligonucleotides or  
16 DNA vaccines, in the U.S. FDA are actually  
17 regulated as biological products. But perhaps  
18 you can clarify. I may have misunderstood.

19 DR. TIM SULLIVAN: Well, as a  
20 vaccine certainly as an adjuvant.

21 DR. GRUBER: Well, you know, the  
22 point is -- I mean -- so you're talking about

1 things like CpG motifs and -- well, you know,  
2 the point is -- and that's what we tried to  
3 get at this morning is, you know, the way you  
4 define an adjuvant.

5 It's really not an active  
6 ingredient. And as such, you know, we  
7 wouldn't really call it, in that case,  
8 biological product. It's like we go by the  
9 regulatory definition as being, you know, in  
10 the U.S. FDA, we call it constituent  
11 materials. The EMEA, I think, defines it as  
12 excipients.

13 So the term biological product, I  
14 think, is really reserved for what we call the  
15 active ingredient, that is the vaccine  
16 antigen, for instance.

17 DR. TIM SULLIVAN: Yes. You've  
18 got a good point. And I'll step back and say  
19 from the point of view of doing like a master  
20 file or something on the oligonucleotide by  
21 itself, apart from the vaccine application  
22 which obviously clearly would be a biologic.

1 DR. GRUBER: Yes. Liz, can you  
2 comment on this in terms of the master file  
3 issue? I mean I think we --

4 DR. SUTKOWSKI: It would all  
5 depend why you are completing a master file  
6 and what you plan to do with it, right?

7 I mean we certainly would have --  
8 we would consider master files for  
9 oligonucleotides for use as adjuvants and we  
10 would consider -- we would expect to see the  
11 same kind of information come in as we would,  
12 you know, for other biologic components.

13 It's the same CMC-type  
14 information. It would just depend on what it  
15 is used for, I think. But I think his concept  
16 is probably very different from what we are  
17 talking about if it is something in CDER,  
18 possibly as a therapeutic, it might. I just  
19 don't know what else to say, you know.

20 DR. GRUBER: Okay. If there are  
21 no more questions, I think I'll go ahead and  
22 introduce the next speaker. And I'll do this

1 -- I'll remain seated by doing so.

2 The next speaker is Dr. Deborah  
3 Novicki from Novartis. And she is going to be  
4 presenting some of the company's experience in  
5 performing toxicological studies. And she is  
6 raising, I think, also some very interesting  
7 questions.

8 Thank you very much for being  
9 here.

10 DR. NOVICKI: Thank you for  
11 inviting me.

12 Let's see. Here I am. Thank you.

13 Okay, so we've heard a lot of  
14 different points of view today. Just -- I'm  
15 going to focus on earlier in this session and  
16 I'm going to try to not be redundant because  
17 it is almost unavoidable that there is some  
18 continuing theme that is running through these  
19 talks.

20 We heard about the U.S. and EMEA  
21 approaches. We heard about some limitations  
22 of animal models, some potential long-term

1 effects of adjuvant treatment, some of the  
2 programs that have been done by Sanofi for two  
3 of their experimental adjuvants. And I'm  
4 going to focus on MF59 adjuvant, which Derek  
5 O'Hagan spoke to this morning and described  
6 some of the research and mechanism of action  
7 and proof of concept-type of work. I'm going  
8 to focus on the toxicology program for this  
9 adjuvant.

10 Just to put the picture back up  
11 there to remind you, it is an oil-in-water  
12 emulsion and the squalene oil component is a  
13 natural compound and is highly metabolizable.

14 The current status of the file is  
15 a master file submitted in the U.S. We had a  
16 little discussion earlier today about Flud  
17 being approved in several European countries  
18 but not all. It is -- my latest is 23  
19 countries so I'm not sure. It changes, you  
20 know, month by month.

21 This vaccine is licensed for  
22 greater than 65 years old and younger age

1 groups are being studied.

2 Focetria was mentioned as a  
3 pandemic vaccine that is approved in the EU.  
4 And Derek showed some of the data that we have  
5 with nonclinical studies with a variety of  
6 different antigens and a variety of different  
7 species.

8 And tomorrow in the clinical  
9 section, there will be two presentations by  
10 Drs. Della Cioppa and Rappuoli about clinical  
11 aspects of MF59. So that will be something  
12 that will be a nice bridge from what you are  
13 hearing today on the nonclinical parts.

14 I'm not going to reach each of  
15 these WHO and EMEA points. I kind of put it  
16 up here to remind myself about the point that  
17 I wanted to make. Sarah did a very nice job  
18 kind of pointing out what the expectations are  
19 as well as each of the individual speakers  
20 presenting the U.S. and the EMEA perspective.

21 So from the pharmacodynamic  
22 standpoint, I think there was a lot of

1 discussion about that this morning. What does  
2 the vaccine do? What does the adjuvant do?  
3 What are the target cells? What is the  
4 species specificity? All of these things play  
5 into the design of the pharmacodynamic and  
6 proof of concept studies.

7 So the studies that we've done are  
8 primarily mechanism of action studies in mice  
9 and studies with mouse and human cells to kind  
10 of bridge from mouse to human. And then a  
11 wide variety of immunogenicity and challenge  
12 studies that are actually oriented toward  
13 supporting the various vaccines that have been  
14 examined or are being developed with the MF59  
15 adjuvant as a platform.

16 We touched on safety pharmacology  
17 studies. We did not do classic safety  
18 pharmacology study which would normally be  
19 something like continuous monitoring  
20 immediately post dose for a specified period  
21 of time, instrumented animals.

22 What we did -- and these studies

1           were done quite a while ago -- they were dog  
2           tolerability studies basically, not classic  
3           toxicology. And animals were treated with a  
4           saline control or MF59 adjuvant.

5                        They received three injections  
6           that were spread apart approximately by two  
7           weeks. And at the end of the study period,  
8           one week post last dose, the animals were  
9           assayed for cardiovascular and for  
10          neurological function. So this was a week  
11          after the last dose.

12                      So not your classical safety  
13          pharmacology but at least following three full  
14          doses to dogs. There were no effect on  
15          cardiovascular parameters. And  
16          toxicologically or tolerability-wise the  
17          vaccine was well tolerated in these same  
18          animals.

19                      Pharmacokinetics, I think that  
20          we've had a brief discussion this morning that  
21          we do not do classical pharmacokinetics with  
22          either antigens or with adjuvants. But there

1           certainly was the point raised of being  
2           interested in understanding where is your  
3           adjuvant going. Is it local? Does it go  
4           systemic? And sometimes you find out that it  
5           is going systemic because you actually find  
6           systemic effects that you may not have been  
7           prepared for.

8                         With MF59 -- and these are quite  
9           old studies, 1995, 1999 -- it was mostly  
10          focused on seeing how long did the MF59  
11          persist at the injection site and where did it  
12          go. So there were radiolabeled studies done  
13          in rabbits and radiolabeled or fluorescently-  
14          labeled studies done in mice.

15                        And basically MF59 clears fairly  
16          rapidly from the injection sites but these  
17          studies did not include a full assessment of  
18          all the different tissues and the different  
19          specific activities in these tissues. These  
20          were more mechanistically oriented.

21                        Local tolerance has been discussed  
22          repeatedly. It is something that we do in

1 every study. We look at the injection sites  
2 from every study and we build this into our  
3 toxicology studies.

4 We don't do standalone local  
5 tolerability because really if you are doing  
6 a repeated dose study, there is not reason not  
7 to look at the injection sites from that  
8 study.

9 We routinely look at MF59 alone or  
10 the antigens plus MF59. A point, as I was  
11 reviewing the guidelines myself, that was  
12 pretty interesting -- the WHO guidance talks  
13 about sites inadvertently exposed, for  
14 example, eye exposure. And we never have done  
15 any kind of testing like this. And it's not  
16 something that really would have occurred to  
17 me.

18 But we did think about skin  
19 sensitization in a manufacturing setting. And  
20 so a dermal sensitization study was done in  
21 guinea pig, a classic Magnusson-Kligman Guinea  
22 Pig Test.

1                   I wanted to continue on talking  
2                   about local tolerance. And this is in an  
3                   experiment with New Zealand white rabbits  
4                   using the clinical dose, the clinical volume,  
5                   the clinical route of administration, which  
6                   was intramuscular, the N+1 number of doses  
7                   that were mentioned by Marion was done, dosing  
8                   on Days 1, 15, and 29, so episodic but  
9                   compressed dosing relative to routine clinical  
10                  dosing.

11                  Doses were alternated between hind  
12                  limbs and necropsies were conducted two and 14  
13                  days following the last dose.

14                  And it might be difficult for you  
15                  to see this and I don't have any clever red  
16                  boxes and only one pointer. So let me do --  
17                  I need new glasses. If you just focus on this  
18                  column here, which is the MF59 alone, you can  
19                  see that where there were animals with  
20                  injection site findings, and this is  
21                  histopathology, the findings were generally  
22                  scored as minimal to mild, with an occasional

1 moderate.

2 This is the right-hand injection  
3 site. So this site had received two  
4 injections, one on Day 1 and one on Day 29.  
5 So we're looking at the injection site either  
6 two days post last dose or 14 days post last  
7 dose.

8 And if you just look down here,  
9 you can see that the number of observations  
10 are decreasing and the severities of the  
11 findings are approximately the same.

12 If we go to the left-hand  
13 injection site, which was only injected on Day  
14 15, a single administration, you can see in  
15 the same column, which is the MF59 alone  
16 group, there are no observations left after 28  
17 days, whereas two days post, there were the  
18 similar findings to the right injection site.

19 So basically minimal to mild types  
20 of effects in the muscle and reversible over  
21 time. And there's no association with  
22 granuloma formation with MF59.

1                   The induction of hypersensitivity  
2                   and anaphylaxis, we did not conduct wither the  
3                   PCA or the ASA test. I did mention that we  
4                   looked at skin sensitization with the  
5                   Magnusson-Kligman. And if we had seen a  
6                   signal there, it's likely that we would have  
7                   investigated further. But we didn't so we  
8                   didn't.

9                   There is the mention in the EMEA  
10                  guideline that one may examine the adjuvant-  
11                  induced increase of IgE against the antigen.  
12                  We have not done this. So for the folks in  
13                  the audience who are responsible for doing  
14                  nonclinical programs, I would be interested to  
15                  hear tomorrow who does, if any of us.

16                  One thing I can say is that in  
17                  repeat dose studies in various species, we've  
18                  never had any signs or symptoms of  
19                  hypersensitivity or anaphylaxis. And I think  
20                  we will be able to say the same for people  
21                  when you hear the clinical presentations  
22                  tomorrow.

1                   Pyrogenicity was also mentioned as  
2                   an endpoint that is important to assess. We  
3                   test body temperatures in animals that are  
4                   given the vaccine in our routine toxicology  
5                   study.

6                   And then as far as a release  
7                   criterion, we don't test MF59 because it is  
8                   not a product by itself. It is always  
9                   formulated with an antigen so we do our  
10                  pyrogenicity testing in the final vaccine  
11                  product -- the pyrogenicity test that is the  
12                  type for release of the vaccine.

13                  For the reproductive and  
14                  developmental toxicity, it is clear that by  
15                  the time a vaccine is going to get to the  
16                  market, if it is being used in women of  
17                  childbearing potential, one would choose to  
18                  investigate reproductive toxicology.

19                  So MF59 alone we did pilot testing  
20                  in rabbit through the C-section with 0.25X and  
21                  half the clinical dose with dosing  
22                  continuously on gestation Day 6 through 28.

1           And then the definitive study was actually  
2           done with the MF59 plus antigen, the actual  
3           vaccine candidate versus a saline control.

4                       We also did a full pre/postnatal  
5           development tox student in rodents, rats, with  
6           five or six injections that were administered  
7           over the time before, during, and up until the  
8           C-section and postnatal evaluations.

9                       The individual MF59 adjuvanted  
10          vaccines are testing for repro tox if they are  
11          going to be used in women of childbearing  
12          potential, including adolescent populations.  
13          So if they are indicated for old people, like  
14          for Fluad, we didn't do it.

15                      For genotoxicity, we did Ames  
16          testing and we did the Mice Micronucleus test  
17          in vivo and we followed the very standard  
18          paradigm that one would follow for a new  
19          chemical entity.

20                      We went up to the 5,000 milligram  
21          per kilogram, which is the max dose specified,  
22          and in the Ames assay up to 5,000 micrograms

1 per plate. I guess you can assume that if I'm  
2 not saying that there was a positive result  
3 that it was negative.

4 Carcinogenicity, we have done no  
5 testing for the carcinogenicity of MF59  
6 adjuvant or any of our preventive vaccines.  
7 We haven't done it and we don't plan to.

8 So I know that there is a comment  
9 in one of the guidelines that addresses  
10 carcinogenicity and that, perhaps, is  
11 something that we should discuss a little bit  
12 tomorrow.

13 And it may be more pertinent in  
14 the context of therapeutic vaccines if it is  
15 a vaccine that we would be giving for a long  
16 time -- and I know people have raised the  
17 issue where you get a flu vaccine every year.  
18 And so it is something that I think is a  
19 discussion point for tomorrow.

20 There is a lot of information in  
21 the guidelines on various aspects of the  
22 systemic toxicity assessments. And Sarah

1           actually did a very nice job covering a bunch  
2           of the points.

3                        I wanted to focus in for the  
4           ending of my talk with looking at the  
5           multiples that we achieve in some of our  
6           programs. So this is just showing for MF59  
7           alone this is really an NCE-like program.

8                        We've got repeat dose toxicity,  
9           embryo/fetal development, AMES, Mouse,  
10          Micronuc, Magnusson-Kligman, and embryo/fetal  
11          development. But the place that it is  
12          different is in the repeat dose toxicity and  
13          in the embryo/fetal and developmental tox,  
14          we're only using low to X multiples of the  
15          clinical dose. So that is different.

16                       And this is another slide just  
17          showing some more studies that have been done.  
18          This is the dog tolerability that I mentioned  
19          earlier. These are a bunch of different  
20          rabbit studies. There are repeat dose  
21          studies, single dose up to three to six  
22          administrations of MF59 adjuvanted antigens

1 and MF59 alone. And, again, various multiple  
2 but tending to the low side.

3 Just overall, the toxicological  
4 findings with MF59 plus and minus adjuvant  
5 tend to be effects on white blood cells. In  
6 most studies, there are some elevations but  
7 sometimes you can see some decrease, depending  
8 on the exact time point when you take it if  
9 things have marginated.

10 But everything is reversible in a  
11 very short time frame. We do -- I think  
12 Marion had said in one of her -- or it is in  
13 the questions -- should we be measuring  
14 fibrinogen or C-reactive protein or things  
15 that might be associated with acute phase  
16 reactions?

17 We routinely measure fibrinogen  
18 because it actually is a nice little marker to  
19 show us that something is happening in  
20 rabbits. We tend to see up to a doubling up  
21 to two to three days post dose. And then it  
22 rapidly declines back to baseline.

1                   Normally this is not associated  
2 with any sort of effect on prothrombin times.  
3 Very occasionally, we might see a slight  
4 shortening of PT.

5                   Along with antigens certainly we  
6 see an elevation in globulins and changes into  
7 the Ag ratio because it is calculated. And I  
8 mentioned before and showed you the data on  
9 the injection site histopathology with MF59.

10                   But we do evaluate a full panel of  
11 other things. And basically we have -- it is  
12 very well tolerated.

13                   And one of the things that I'd  
14 like to point out that we have monitored over  
15 many years is we take a sample of bone,  
16 including the articular joint, to look at the  
17 cartilage and in case there is any sort of a  
18 signal for the adjuvant-induced arthritis.  
19 And we have not see a signal in any of our  
20 studies.

21                   And we also have looked at uvea  
22 because historically uvea was an area where

1           some findings had been observed. Not with  
2           MF59, with other things.

3                        So now I'm going to come to a  
4           comparison of exposures. And these are not  
5           your classic sort of pharmacokinetic  
6           exposures. That is why it is in quotes.

7                        What we did was look at the  
8           smallest population that we think we would  
9           ever administer an adjuvant to. And so we  
10          based it on a six-month-old human infant. And  
11          we used the CDC growth charts.

12                      The body weight, surface area --  
13          sorry -- the body surface area calculation was  
14          done with a formula from John Current's paper.  
15          And then we used the animal BSAs from the Mike  
16          Derelenko's Toxicologist's Pocket Handbook.

17                      So if my math is correct -- and  
18          you don't have to look at every number here --  
19          looking across a panel of studies -- and this  
20          rat study is the embryo/fetal development. So  
21          this isn't a classic tox study. But I wanted  
22          to put the multiples in here just so we could

1 see if you take a clinical dose, you put it  
2 into a rat, what does it look like.

3 So if you look across these three  
4 rabbit studies, you can see that based on body  
5 weight, it is true. You are basically having  
6 a XX to a XXXX multiple of your clinical dose  
7 that is being used in your toxicological  
8 study.

9 The rat, of course, it is smaller.  
10 And you end up with a higher multiple. But  
11 one of the things I thought about as I was  
12 thinking of what does the exposure really  
13 consist of is that in the tox study, you are  
14 really giving more doses, a shorter period of  
15 time in between doses. So I also looked at  
16 what the cumulative dosing would look like.

17 And so if we look at a study where  
18 we gave 14 daily doses, we end up with  
19 multiples that look a little bit more  
20 reasonable. They're not tremendously high.  
21 But, you've definitely got a multiple there  
22 based on cumulative dosing. And I think you

1 can see that there is a reasonable set of  
2 multiples across these studies.

3 Then looking at body surface area,  
4 I went through the same exercise. And you can  
5 see on a single dose basis it's not  
6 tremendously different because the infant is  
7 so small. So this is what you get if you are  
8 looking at animals, rats and rabbits, using a  
9 clinical dose or close to a clinical dose and  
10 comparing it to a human infant.

11 So now we come to the really hard  
12 stuff. The toxicity of components to  
13 classical target organs I think is fairly well  
14 understood from the standpoint of drug  
15 development. But when we come to trying to  
16 look at things like the autoimmunity  
17 discussions that we've had today, I think that  
18 this is an area that we are probably going to  
19 spend a lot of discussion tomorrow.

20 The first dose cytokine response,  
21 some of the cytokine storm, hypersensitivity,  
22 there are certain areas where we may be really

1 limited on what the animal models can tell us.  
2 And I think that this is a place that I would  
3 certainly like to hear people's ideas  
4 tomorrow.

5 One of the things that I was  
6 thinking about is what can we really expect  
7 from our animal studies and one of the  
8 questions from the last or the second to last  
9 session kind of touched on this point which is  
10 the diversity of people.

11 And this table just shows how many  
12 subjects you've got to study if you want to  
13 see a doubling of a rare event. So by the  
14 time you are looking for two out of a million  
15 instead of one out of a million, you have to  
16 be looking at 50,000 subjects.

17 So one of the things that I feel  
18 is pretty limiting is the ability for us to  
19 use a quantity of animals. It's not  
20 reasonable to think that we're going to be  
21 able to detect really, really rare events in  
22 animal models. So that's something that we

1 all struggle with.

2 And I think this is my second to  
3 last slide. I wanted to, again, provoke for  
4 tomorrow some discussion about are  
5 immunomodulators or things that are given  
6 separate from the vaccine really the same as  
7 an immunostimulant, an adjuvant, whatever you  
8 want to call it that you give at the same  
9 time.

10 I'd like to also acknowledge that  
11 the program of MF59 studies is a cast of  
12 hundreds. And if you include all of the human  
13 volunteers that have participated in our  
14 trials, thousands and thousands. So I just  
15 would like to thank all of those people and  
16 just acknowledge a great team that worked to  
17 bring this compound forward.

18 And thanks for the invitation and  
19 for listening.

20 (Applause.)

21 DR. GRUBER: Thank you, Dr.  
22 Novicki.

1 Are there questions?

2 DR. PETROVSKY: Nick Petrovsky,  
3 Australia.

4 One of the issues of toxicity, I  
5 think, is the interaction between the  
6 administered compound and the genetics of the  
7 person receiving it. And obviously a lot of  
8 these preclinical studies are being done in  
9 inbred models where all the animals have the  
10 same genotype.

11 So obviously if the rare side  
12 effect is a relationship of a particular  
13 genotype with a compound, then you knock the  
14 chances of picking it up even if you study the  
15 million mice or rabbits of the same genotype  
16 is zero.

17 DR. NOVICKI: Toxicology studies  
18 generally are done with outbred strains or  
19 outbred animals. But they are not as outbred  
20 as we are.

21 DR. PETROVSKY: Yes.

22 DR. NOVICKI: All right?

1 DR. GRUBER: Okay. Thank you.

2 Okay, I think we're coming to the  
3 last two speakers of this afternoon's session.

4 As we mentioned this morning, in  
5 terms of thinking about improving and  
6 optimizing preclinical safety assessments of  
7 vaccines, what alternative methodologies can  
8 be used to supplement the currently ongoing  
9 nonclinical safety assessment programs?

10 And so it is a pleasure to  
11 announce today two speakers, the first of them  
12 Dr. Hana Golding from the Center for Biologics  
13 who is going to be talking about her research  
14 on the use of human cell lines for  
15 quantitative preclinical evaluation of vaccine  
16 adjuvant safety.

17 Thank you for being here, Hana.

18 DR. GOLDING: Thanks, Marion. And  
19 this is sort of a very special moment for me  
20 to be able to be a part of CBER and also to  
21 share with you some of our thoughts on how we  
22 move forward in this very important field of

1 developing and testing new adjuvants.

2 And I think Dr. Fauci put it very  
3 well as well as Dr. Goodman. There's no  
4 question that there is a need for novel  
5 adjuvants to improve the immunogenicity of  
6 very challenging vaccines against emerging  
7 diseases and pathogens.

8 And clearly the development of  
9 adjuvants are an iterative process. And we  
10 heard a little bit from Derek O'Hagan about  
11 their screening program. There are other  
12 biotech companies that are involved in active  
13 screening of novel adjuvants.

14 My talk, and I think of the  
15 following talk, will kind of address the  
16 questions of what new tools we may need in the  
17 early screening, the early development of  
18 novel adjuvants that may give us a hint of  
19 what could be safety signals in vivo and will  
20 help us in the sort of screening and selection  
21 process.

22 So the rationale for the studies

1           that I am going to describe have actually been  
2           described before. We know that novel  
3           adjuvants may cause pyrogenicity and other  
4           short-term local or systemic toxicity. But  
5           also in the back of our mind is that  
6           immunomodulating activities or adjuvants may  
7           also promote unintended long-term  
8           consequences.

9                         What we already heard today is the  
10          fact that adverse reactions observed during  
11          clinical trials of adjuvanted vaccines may not  
12          always be detected in preclinical studies in  
13          small animal models due to species' viability  
14          and pattern recognition receptors, including  
15          gene sequences as well as tissue distribution.

16                        So we thought it would have been  
17          nice to concentrate on human-derived cells.  
18          And we have initiated a program for rapid  
19          evaluation of novel adjuvants and vaccine  
20          delivery systems based on what we would like  
21          to term human detector cell lines.

22                        And I want to emphasize this is

1 very much a work in progress. And I'm going  
2 to share with you some of our plans and  
3 preliminary data.

4 So the couple of tests that I'm  
5 going to cover today is we're trying to  
6 develop an in vitro assay to measure  
7 proinflammatory cytokines such as IL6, IL1  
8 beta, TNF alpha, and IL8 as a predictor of  
9 systemic toxicities in vivo.

10 We also would like to develop an  
11 assay to measure prostaglandin E2 because we  
12 know that this could be a very early mediator  
13 of temperature increase due to its ability to  
14 cross the blood brain barrier and work on the  
15 pre-optic interior hypothalamus and induce  
16 increasing temperature even in the absence of  
17 cytokines or before cytokines are induced.

18 Another sort of long-term goal is  
19 to develop an assay to measure elevation of  
20 intracellular calcium in astrocytes as a  
21 potential biomarker of indirect neurotoxic  
22 potential.

1                   And the fourth assay that I'm  
2           going to discuss with you is that assay to  
3           detect bacterial endotoxin in vaccine  
4           formulations containing novel delivery systems  
5           such as nanoparticles which are shown to  
6           interfere with the LAL test. And I'll give  
7           you a preliminary result on that as well.

8                   So as far as the proinflammatory  
9           cytokines, of course one has to select the  
10          right type of cells and not one cell line is  
11          going to answer all the questions.

12                   We started off with a cell line  
13          that had been described before, the MM6 ELISA,  
14          which is a promonocytic cell line with a known  
15          spectrum of TLRs. And we decided to try and  
16          use it to quantitate the levels of  
17          proinflammatory cytokines released in the  
18          presence of adjuvants.

19                   Of course it is very important in  
20          order to quantitate it to have the right  
21          comparator. And we know from the literature  
22          that LPS in rabbits at a dose of .5 EU per ml

1 was defined as the pyrogenic threshold, namely  
2 this dose led to induction of increase in body  
3 temperature of more than .6 Celsius.

4 Therefore, in all of our assays, as a positive  
5 control, we are using a USP reference  
6 endotoxin, the EC6 Lot G, and we run a  
7 complete dose response.

8 Now in order to sort of generate  
9 what I would call a proof of concept for the  
10 usage of this approach, it was important to  
11 start with several adjuvants that have been in  
12 the clinic, preferably in significant number  
13 of people, and had a known clinical safety  
14 profile.

15 And one can then ask well, how do  
16 these adjuvants behave in your own assays?  
17 Can you see a similar correlation in vitro to  
18 what was found in vivo?

19 So you heard a lot today about  
20 alum, which, you know, is licensed in both the  
21 U.S. and Europe, the mechanism of action in  
22 both antigen deposit and injection site

1           inflammasome induction, as you heard earlier  
2           in the talks, and it has a very excellent  
3           safety record to date.

4                        You also heard quite a lot about  
5           MF59 water-in-oil emulsion that has been  
6           licensed in Europe, again, the mechanism of  
7           action has been unraveled. It includes APC  
8           maturation and antigen uptake. It has a good  
9           safety record as well.

10                      The saponin QS21, which we also  
11           heard about earlier today, as you know it has  
12           been widely used in animal vaccines. It has  
13           been evaluated in several human trials as well  
14           but it has a mixed safety record including  
15           studies that have been interrupted due to  
16           adverse reaction.

17                      So we thought this would be a nice  
18           sort of starting panel of adjuvants to test in  
19           the system. And as you can see -- and this is  
20           an example of how the system is done -- we are  
21           generating a dose response using different  
22           amounts of the endotoxin. And what is shown

1 here in circles is the threshold of the .5 EU  
2 in blue circles and then in rectangular, this  
3 is the amount of cytokine proinflammatory that  
4 was produced.

5 So, for example, in this  
6 particular slide we are looking at IL6. And  
7 we are comparing different dose to different  
8 adjuvant. As you can see, at the various  
9 doses that we have used, the MF59 and the alum  
10 were basically inert.

11 And in with the QS21, we are  
12 starting to see an increase in IL6 production  
13 at the highest dose that was used in this  
14 experiment, which is the 20 microliter per ml.

15 However, I want to emphasize in  
16 both of these cases, even this IL6 production  
17 did not reach the level and the TNF alpha on  
18 the right did not reach the level that was  
19 seen with the LPS at this threshold. So we  
20 would not consider that an unsafe production  
21 of either TNF alpha or IL6.

22 On the other hand, when we looked

1 at other proinflammatory cytokines, especially  
2 IL1 beta and IL8, now if you compare the three  
3 adjuvants to the dose response with the LPS,  
4 you can see that definitely in the case of the  
5 IL8, the amount that is produced in response  
6 to QS21 exceed that that was found with the  
7 threshold dose of LPS. And also in the case  
8 of IL1 beta, it is very close to the  
9 threshold.

10 So just looking at those three  
11 adjuvants with clearly different clinical  
12 profiles, we found that one can see a  
13 differential proinflammatory cytokine produced  
14 whereby the QS21, which is the more  
15 reactogenic in the clinic did give elevated  
16 levels of at least two out of the four  
17 proinflammatory cytokines that are expected to  
18 be beyond the pyrogenic threshold of LPS.

19 With this kind of initial proof of  
20 concept, we are now starting to looking at  
21 different groups of novel adjuvants. First it  
22 was interesting to compare a mineral salt. In

1 addition to aluminum phosphate, calcium  
2 phosphate, you heard before, has been in the  
3 clinic before. And one would expect that it  
4 may have a similar mechanism to aluminum  
5 phosphate and the safety profile.

6 As you can see in this slide, on  
7 the other hand, the findings were quite  
8 different. So while alum is really inert,  
9 this is, again, the LPS dose response  
10 indicating the sort of threshold of the .5 EU  
11 response at either IL8 or IL1 beta.

12 And you can see that unlike  
13 aluminum phosphate, calcium phosphate actually  
14 generates much higher levels of both of these  
15 cytokines, suggesting, again, that this  
16 particular compound may be more reactogenic in  
17 vivo. But of course this needs to be  
18 corroborated.

19 We heard a lot about TLR agonists  
20 and, you know, a multiple of them. What we  
21 have started to do in this so far is to test  
22 several, the FS3, the TLR26 agonist, the

1 Pam3CS, which is the TLR12 agonist, Flagellin,  
2 which is a TLR5 agonist, and MPL. We've all  
3 heard a lot about about MPL today. The cell  
4 lines that we are currently using are not very  
5 appropriate for the TLR7,8,9 agonists.

6 So what did we find? Again, on  
7 the left, we are looking -- here we are  
8 looking at either IL6 or IL1 beta, the LPS  
9 dose response with the sort of threshold dose  
10 of LPS.

11 And as you can see, actually after  
12 different TLR agonists that we have tested so  
13 far, the PAM3, the Flagellin, and the FSC, all  
14 actually generated proinflammatory cytokines  
15 about the LPS threshold while MPL, which was  
16 the detoxified Lipid A, has been actually very  
17 inert in both of these assays, suggesting  
18 again that not all TLR agonists behave the  
19 same. And actually one would expect those  
20 three agonists, the TLR2 and the TLR5 to be  
21 much more reactogenic maybe than MPL.

22 So this is just sort of a general

1 summary of what I did show you and some data  
2 that I have not shown you. So I showed you  
3 the difference between alum and CAP in our  
4 system. The MF59 was very inert.

5 On the other hand, the QS21  
6 definitely gave a signal that would be  
7 expected of more reactogenicity. Of the TLR  
8 adjuvants, those are the three that gave  
9 relatively strong signals.

10 We already tested a large number  
11 of delivery systems, including liposome,  
12 dendrimers, PLG, and colloidal gold, all of  
13 them were actually very inert in that  
14 particular cell line. And we started to test  
15 a few adenovirus vectors as well.

16 I also want to just share this.  
17 This is even more preliminary data in our  
18 attempts to quantitate PGE2 production. We  
19 are using a nice kit which involves FRET  
20 measurement whereby the donor is this molecule  
21 which is conjugated to anti-PGE2 at 620  
22 nanomolar and the acceptor molecule, which is

1 linked to PGE2 at 665.

2 So you can have a very nice dose  
3 response comparing the emission at 665 over  
4 620. And then you can now add a supernate  
5 from cells that had been activated with  
6 different adjuvants and determine whether they  
7 have any PGE2 in terms of inhibition of this  
8 particular dose response.

9 And I just want to show one  
10 preliminary data. The cell line that we have  
11 started using have been reported to be a good  
12 cell line for that purposes.

13 It is a U937 that had been  
14 activated with PMA, which then induced the  
15 differentiation into a macrophage adhering  
16 cell line. And if you now add LPS, it's a  
17 threshold .5 EU, you can now see that you can  
18 measure a significant amount of PGE2.

19 And we, of course, are going to  
20 extend the studies now to measure the complete  
21 dose response of LPS and other adjuvants and  
22 TLR agonists.

1                   I just would like to then  
2           summarize this part of the talk. So what we  
3           have shown so far is that measurements of  
4           multiple proinflammatory cytokines released by  
5           human cell lines compared with LPS standard  
6           could be used as a first screen of novel  
7           adjuvants for predicting possible toxicity in  
8           vivo.

9                   We would like to eventually  
10          develop some sort of algorithm that will be  
11          called the safety score -- the number of  
12          proinflammatory cytokines the are induced and  
13          the levels.

14                  We also are in the process of  
15          developing a test of PGE2 production in  
16          macrophage-like activated U937 cells which may  
17          provide an add-on information on potential  
18          cytokine-independent toxicity of adjuvants.  
19          And, of course, all of these studies need to  
20          be corroborated initially in rabbits,  
21          including measurement of circulating cytokines  
22          and PGE2 which are underway to provide

1 correlation between the safety scores that we  
2 have generated in vitro and hopefully we will  
3 connect them to the in vivo system.

4 The last part of my talk will  
5 actually have to do with something that is  
6 Carl Alving already described and that's the  
7 potential problem with LAL testing of new  
8 adjuvants. And we know that the LAL test had  
9 been approved as a substitute for the rabbit  
10 pyrogenicity test in 1983.

11 However, since then several  
12 factors have been identified that could  
13 actually be interfering with the endotoxin  
14 measurement by LAL.

15 And those involve chemical  
16 inhibitors, physical inhibitors, and more  
17 recently it was found that nanoparticles,  
18 including liposomes, gold particles, and  
19 dendrimers may actually interfere with  
20 sensitivity of the LAL assay. Both  
21 enhancement and inhibition were recorded.

22 So when we became aware of that,

1 we thought it will be actually nice to see  
2 whether some of the cell-based assays that we  
3 have developed could be an additional  
4 approach.

5 This is just a very simple sort of  
6 depicting -- this slide depicts sort of the  
7 principle of the LPS detection in the Limulus  
8 amoebocyte, lysate, and LSA whereby a proenzyme  
9 is converted to coagulate in the presence of  
10 the gram negative bacterial endotoxin. And  
11 this coagulate can then lead to the clotting -  
12 - to the self-association of coagulin.

13 So on the other hand, if one  
14 thinks of the way LPS is detected in mammalian  
15 cells, it is really binding to the TLR MD2/CD14  
16 as was resolved recently by the crystal  
17 structure.

18 This is sort of a diagram of the  
19 LPS and most important of the lipid A, which  
20 is detected in the LAL assay. And the  
21 possibility that we wanted to test is that are  
22 the different parts of the lipid A, the core

1           versus the isolated lipid cytokines may be  
2           acting in the LAL versus the TLR MD2 system.

3                         And in order to do that, we first  
4           wanted to make sure that our both MM6 ELISA  
5           and the LAL are very reproducible and you can  
6           see the cell assay that were conducted over  
7           one year and we got a very similar dose  
8           response. This is IL6. And also in the LAL  
9           we get a very good coefficient variation in  
10          most of the doses.

11                        So now one can actually conduct  
12          what we call spiking experiment where  
13          different amount of LPS are added in either  
14          the LAL or the MM6 ELISA in the presence of  
15          different nanoparticles. And here we are  
16          looking at the colloidal gold at 59 nanomolar  
17          or DPPC liposomes, a very commonly used  
18          liposome in vaccine adjuvants.

19                        And just to show you here,  
20          actually the colloidal gold can get an  
21          enhancement of the LAL assay, which will  
22          basically generate a false positive results.

1           And in the case of the liposomes, many types  
2           of liposomes, especially cationic liposomes  
3           but also neutral can actually cause  
4           interference in the LAL assay.

5                       And that was, I think, discussed  
6           briefly recently whereby low levels of LPS may  
7           be missed in the presence of liposomes. As  
8           you can see here, using the MM6 ELISA was much  
9           less sensitive to interference by either DDPG  
10          lysosome or colloidal gold.

11                      And at this point, this is a very  
12          early stage of this research. We just wanted  
13          to summarize by saying that the presence of  
14          nanoparticles in biological product can  
15          significantly the ability of endotoxin to  
16          activate the clotting enzyme cascade in the  
17          LAL assay.

18                      A cell-based assay may then  
19          provide another approach. Prior to evaluation  
20          of products containing nanoparticles such as  
21          liposomes for endotoxin contamination, it may  
22          be important to test the ability of the

1 nanoparticles to interfere with LAL or with  
2 other biological assays by actually using  
3 spiking with LPS at multiple concentrations.

4 So this was kind of just a summary  
5 of our work in progress. And I really wanted  
6 to emphasize that all of the studies were  
7 supervised by Marina Zaitseva in our group.  
8 She is leading our adjuvant program.

9 Most of the work that you've seen  
10 was generated by Tatiana Romantseva and Oksana  
11 Blenova has recently joined the group and is  
12 working on the PGE2.

13 We are very thankful also to  
14 Marina at the NCL who works with us on the  
15 nanoparticles and Anu Puri and robert  
16 Blumenthal on liposomes.

17 We also were collaborated with the  
18 Canadian group on archaeosomes, which I  
19 haven't showed you. QS21 was provided by  
20 Antigenics. Novartis, especially Derek  
21 O'Hagan was very forthcoming in sharing with  
22 us several Novartis adjuvants, which I've

1           showed you the results. And we have also  
2           established collaboration with the VRC and  
3           GenVec to look at different adenovirus  
4           vectors.

5                           Thank you.

6                           (Applause.)

7                           DR. GRUBER: Well, thank you,  
8           Hana.

9                           I think we have time for one or  
10          two questions. Yes, go ahead.

11                          DR. SAHNER: Hello, again, David  
12          Sahner.

13                          One comment and one question. The  
14          comment is obvious. Proinflammatory cytokines  
15          are a double-edged sword here obviously in the  
16          sense that they may correlate with toxicity  
17          but also may be integral to the mode of action  
18          of an adjuvant. So there is just that element  
19          of caution, obviously, and I'm sure you've  
20          thought about that quite intensely.

21                          But secondly, given that, what is  
22          the -- can you comment on dose selection or

1 concentration selection for your experiments  
2 because the concentrations locally within the  
3 interstitial fluid at the site of injection  
4 obviously are quite critical and may be very  
5 high and incite the sort of local  
6 proinflammatory response that one wants to see  
7 to enhance the endogenic response whereas the  
8 peripheral concentrations may be negligible.  
9 So there may be no meaningful impact on  
10 systemic levels of proinflammatory cytokines  
11 like TNF alpha and IL1 beta and so forth.

12 So can you comment on the dose  
13 selection and then perhaps on my comment as  
14 well.

15 DR. GOLDING: I think both your  
16 comment and your question are right on target.  
17 There's no question. And that is something  
18 that we are actually, you know, struggling  
19 with when we designed the experiment.

20 There is not question that there  
21 are many ways to evaluate in vitro the  
22 activity of adjuvants. And we specifically

1           actually shied away from using the traditional  
2           way of looking at dendritic cells that  
3           generate all the good cytokines, the IL12, the  
4           interferon alphas and so forth.

5                        We tried to focus on cells that we  
6           think are involved in the more sort of  
7           proinflammatory that can lead to the  
8           reactogenicity of adjuvants. And of course we  
9           have to be very cognizant of the fact that  
10          some of the cytokines that are produced may be  
11          part of the normal immune response to these  
12          adjuvants.

13                      And, therefore, we had a big  
14          effort of really including in our system an  
15          important comparator. Up until to now we  
16          worked mainly with LPS because at least for  
17          that particular TLR agonist, sort of the  
18          maximum tolerated dose is known.

19                      And we are now in the process of  
20          establishing a similar type of maximum  
21          tolerated dose for other types of of TLR  
22          agonists.

1           Of course this is still, you know,  
2           work in progress. And computation will  
3           ultimately be very important. And we will  
4           have to then corroborate any finding that we  
5           find in vitro with some in vivo studies in  
6           animal models such as rabbit although we  
7           started out the project acknowledging that the  
8           animal models may be limiting.

9           As far as the doses that were  
10          used, this is an even more difficult problem.  
11          So first of all whenever we start with any new  
12          adjuvant, we are actually determining in our  
13          cell system what is the highest tolerated dose  
14          that does not effect the viability of the  
15          cells. And then we go down from there.

16          Now do we exactly -- in some cases  
17          in many of the adjuvants, we are actually able  
18          to work in a dose range that was not so  
19          different from what was used in the clinic,  
20          assuming that the local, the initial local  
21          concentration we tried to sort of mimic in  
22          vitro. But in other cases, of course, the

1 doses were not identical.

2 So this is not going to be  
3 something that will become part of any  
4 guidance documents anytime soon. Rather, we  
5 want to offer that as another way to start  
6 thinking of what we can use as a research tool  
7 and in the very early development, a screening  
8 of novel adjuvant.

9 And by making it at least  
10 standardized and quantitative with the  
11 appropriate type of internal control, we would  
12 hope that it will reach a stage that it will  
13 give us some predictive value for in vivo  
14 toxicities and, therefore, it can then be used  
15 if you really -- you know, as part of this  
16 iterative process of screening multiple  
17 adjuvants or even modification to adjuvants to  
18 screen out those that are the most  
19 reactogenic.

20 DR. GRUBER: Thanks, again, Hana.

21 At this point, I'd like to ask the  
22 last speaker of today or for today to come to

1 the podium. That is Dr. William Warren and he  
2 is from VaxDesign.

3 Yes, Dr. Warren, thank you very  
4 much for accepting our invitation.

5 And Dr. Warren's title of his  
6 presentation is VaxDesign's in vitro mimic of  
7 the immune system for evaluating adjuvants.

8 Thank you for being here.

9 DR. WARREN: Thank you very much.

10 For the last talk, Jay Slater gave  
11 me complete authorization to talk about  
12 anything that I wanted and to say whatever I  
13 wanted, right Jay?

14 Anyhow, thank you very much for  
15 the invitation to come.

16 I'd like to talk to you a little  
17 bit about some of the work that we have been  
18 doing on using our in vitro -- what we call a  
19 MIMIC system to look at various adjuvant  
20 studies.

21 The benefits to something like  
22 this are pretty clear compared to an animal

1 model. Among other things is the fact that we  
2 can capture diversity in the population. We  
3 have a fully robotic platform to look at  
4 hundreds, if not thousands, of individuals to  
5 look at their various responses.

6 And some of the benefits that we  
7 get are, of course, this is robotics so we can  
8 get a high throughput evaluation of what is  
9 occurring to look at multiple numbers of  
10 donors.

11 But also the thing that this  
12 system really affords is that one surrogate  
13 human really can contain all the controls  
14 whether it be no antigen, no adjuvant,  
15 vaccine, adjuvant alone, pathogen, et cetera,  
16 et cetera.

17 So one human or surrogate human  
18 can do this entire thing. And, of course, as  
19 we talked about, what we're hoping to get is  
20 faster cycle time in terms of understanding  
21 mechanistics and the mechanisms of action as  
22 well.

1                   In terms of an overview of the  
2                   MIMIC system, there are four main steps to  
3                   this process. The first step is really blood  
4                   collection from the donors that we get. We're  
5                   located about -- a few minutes away from  
6                   Florida's blood center, which is the fourth  
7                   largest blood center in the U.S.

8                   We've worked out all of the  
9                   protocols for freezing and thawing the cells  
10                  as well as purifying the PBMCs from the  
11                  apheresis products that we have.

12                 The next step is that we can take  
13                 these PBMCs and then put them into the first  
14                 module. So our system is very modular. And  
15                 that we can dissect the mechanisms of immunity  
16                 if we're looking at innate immunity, adaptive  
17                 immunity, and then functional analysis as  
18                 well.

19                 So the first one is we talk about  
20                 putting something into what we call the  
21                 peripheral tissue module. This is really  
22                 mimicking sort of skin or peripheral tissue.

1 In this case we can look at innate immune  
2 responses such as reactogenicity as well as  
3 immunotoxicity.

4 This is where we pulse the  
5 antigen-presenting cells and we can take these  
6 and then put them into the second module,  
7 which we call the lymphoid tissue equivalent  
8 module, which is a culture of T cells, B  
9 cells, as well as follicular dendritic cells  
10 in which we can look at adaptive immune  
11 responses to look at antigen-specific T cells  
12 responses as well as B cell responses to  
13 either create CD4 help, CD8 cytotoxic T cells,  
14 or antibody responses.

15 We can then take these out of that  
16 module and put them into the third module,  
17 which is really a functional assay where we  
18 can look at hemagglutinin inhibition assays,  
19 microneutralization assays, or cytotoxic T  
20 cells assays.

21 So the MIMIC system compared to  
22 normal physiology is really shown in this

1 slide. It is fairly straightforward on why  
2 we've developed these various modules which  
3 can be put into a 96-well format. And all be  
4 robotically controlled.

5 And this way we can dissect, you  
6 know, the innate responses from the adaptive  
7 responses and also look at the entire system  
8 at the same time.

9 So in terms of the outline today,  
10 first I'll talk about the adjuvant studies  
11 that we've been able to look at in the PTE  
12 module and then we'll start to give an  
13 indication of how we would look at this in  
14 vitro system to dissect the mechanisms of  
15 action by looking at inflammation versus DC  
16 activation, looking at increased diapedesis  
17 that may occur across an endothelium, DC  
18 activation versus true antigen presentation in  
19 an antigen-specific way. And then even  
20 thinking about TH polarization as well.

21 We'll talk about how we can look  
22 at naked antigen versus adjuvanted and then

1 immunotoxicity of biologicals.

2           So in thinking about innate  
3 responses, the first one that we will talk  
4 about is the peripheral tissue equivalent  
5 module. In this case we can put PBMC -- what  
6 we have is we co-cast a collagen matrix inside  
7 of a 96-well format and grow a confluent and  
8 quiescent endothelium on top which sort of  
9 mimics blood vessels to first order.

10           We put PBMCs on top of the  
11 endothelium for about an hour and a half. And  
12 during that time monocytes primarily  
13 extravasate through the endothelium and then  
14 will spontaneously differentiate into antigen-  
15 presenting cells just like that occurs in  
16 vivo.

17           And what happens is these will  
18 differentiate into macrophages as well as  
19 dendritic cells. A large fraction of these  
20 dendritic cells will reverse transmigrate  
21 through the endothelium again, a process that  
22 mimics crossing the lymphatics on the way to

1 the lymph node. And this is typically where  
2 we will pulse these APCs with these dendritic  
3 cells with the adjuvant, the cosmetic  
4 formulation, the antigen, or the vaccine.

5 One of the things that we have  
6 been able to do is show that these cells are  
7 identical to dermal explants by doing  
8 extensive phenotypic analysis.

9 And by doing that, we found that  
10 there's primarily three types of antigen-  
11 presenting cells that come out of the  
12 peripheral tissue-equivalent module.

13 One is a subpopulation which is  
14 sort of immature CD14 positive dendritic cell  
15 precursors. The other one is an immature DC  
16 and the third type is a mature dendritic cell.

17 The ones that remain in the  
18 collagen matrix end up having more of a  
19 macrophage phenotype. And, again, we've done  
20 extensive phenotypic analysis and these are  
21 essentially identical to human dermal  
22 explants.

1                   Now when we're looking at the  
2                   effects of various adjuvants, what we wanted  
3                   to do -- in this case we are looking at the  
4                   effects of alum at various concentrations on  
5                   the three types of dendritic cells, that  
6                   reverse transmigrate out of the endothelium.

7                   And you see that no matter -- when  
8                   we have no treatment alum at various  
9                   concentrations, the relative numbers remain  
10                  about the same. But you see that we're not  
11                  changing the APC phenotype by the addition of  
12                  alum, which is consistent with what is found  
13                  in the literature.

14                 When we looked at alum -- and of  
15                 course we can assess the supernatants to look  
16                 at the generation of proinflammatory  
17                 cytokines, and you can see that alum does  
18                 indeed generate proinflammatory cytokines at  
19                 lower concentrations.

20                 As we increase the concentration  
21                 significantly, we begin to see toxicity  
22                 effects and the cells are beginning to die and

1 the cytokine production begins to diminish.

2 With alum, one of the things that  
3 came up is looking at the effects of alum  
4 versus alum with an antigen. And that's what  
5 this slide shows is when we have alum alone,  
6 we saw no change in the APC phenotype.

7 But when it is with an antigen, in  
8 this case it is with a plague vaccine where  
9 its fusion between the F1 and V antigens, you  
10 can clearly see by with different doses of the  
11 vaccine, we clearly see a change in the APC  
12 phenotype where it is changing to a more  
13 mature phenotype with the addition of alum  
14 with the antigen for this vaccine formulation.

15 So it brings up to what a lot of  
16 talks were about earlier today is looking at  
17 the adjuvant alone is important but just as  
18 important, looking at the adjuvant with the  
19 antigen is incredibly important as well  
20 because you get a different mechanism of  
21 action -- or you can.

22 With MF59, one of the things that

1 Hana Golding just showed in her talk and other  
2 talks by Dr. O'Hagan, mentioned the MF59 dose  
3 change. Indeed, the maturation state of the  
4 APCs, we see that in our in vitro MIMIC system  
5 as well.

6 Clearly you can see that by adding  
7 in MF59 at around 25 percent weight per weight  
8 is what is physiologically equivalent of what  
9 goes into a human, you see that we are getting  
10 more of a mature phenotype out of the  
11 dendritic cells.

12 And then with CpG, just looking at  
13 a total Aquaceptor 9 agonist, you can clearly  
14 see that we're getting a change in the APC  
15 phenotype as well. With the addition of CpG,  
16 we're seeing, again, a more mature phenotype  
17 at the expense of immature and DC precursors  
18 coming out of this peripheral tissue  
19 equivalent model, all consistent with what is  
20 in the literature.

21 And if we just look at relative  
22 comparisons between various types of cytokines

1 and chemokines that come out of -- that arise  
2 in the supernatant of the PTE module, you can  
3 clearly see that MF59 is relatively non --  
4 does not secrete a lot of chemokines nor  
5 cytokines. Alum is somewhere in between. And  
6 CpG -- and this is on a log scale -- generates  
7 the largest concentration of proinflammatory  
8 cytokines and chemokines.

9 And when you put the whole story  
10 together, each of the mechanisms of action in  
11 and of themselves between MF59, alum, and this  
12 is aluminum hydroxide, I apologize, and CpG  
13 act quite a bit differently.

14 CpG, you get a large change in DC  
15 development as well as cytokine production.  
16 MF59, you are seeing large changes in DC  
17 development but very little change in cytokine  
18 generation. And alum, very little change in  
19 DC development but sort of middle-of-the-road  
20 -- sort of middlish cytokine generation.

21 So now just after giving some of  
22 these datasets with this in vitro system, I

1 wanted to start thinking about further  
2 dissecting the mechanisms of action of  
3 inflammation versus DC activation.

4 So this one, one of the things  
5 that we can do in the assay, and we just  
6 wanted to show, is that what we try to do is  
7 make this endothelium very quiescent to begin  
8 with.

9 And the way that we test this is  
10 by with neutrophil migration assays because  
11 typically you should only see about one  
12 percent of the neutrophils cross through the  
13 endothelium. And we can see that.

14 And we can also add in different  
15 types of proinflammatory cytokines on this to  
16 induce inflammation and induce neutrophil  
17 migration.

18 And that is really what this slide  
19 is showing is that we can have an inflammation  
20 model here as well where we can artificially  
21 make the peripheral tissue equivalent inflamed  
22 and, of course, we always want it quiescent

1           when looking at adjuvants.

2                       But when you are thinking about  
3           autoimmune diseases such as rheumatoid  
4           arthritis or other inflammatory diseases, this  
5           can be a useful inflammation model.

6                       And one of the things that we  
7           typically do is well, for quality control, is  
8           that we can really show, in this case what  
9           we're looking at is the up-regulation of E-  
10          selectin by looking at the surface marker of  
11          CD62e, you can clearly see that when it is  
12          inflamed, we see an up-regulation of the  
13          surface marker on the endothelium.

14                      And when at the control, you know,  
15          the types of error bars that we have, that we  
16          can get a very quiescent endothelium to begin.  
17          And, of course, the cytokines productions that  
18          come about when inflamed versus quiescent as  
19          well. And here we are artificially inflaming  
20          it, as I said, with a cytokine cocktail.

21                      And one of the things we wanted to  
22          show is what MF59 is doing as just an example.

1           And what we are finding is that MF59, in and  
2           of itself, does not induce a lot of  
3           inflammation nor toxicity in this peripheral  
4           tissue equivalent module by looking at various  
5           different types of cytokines that are  
6           generated from the control versus MF59 at  
7           various concentrations from physiologically  
8           equivalent to below.

9                        The next one is thinking about  
10           increased diapedesis because what you would  
11           anticipate is that if it is inflamed, you  
12           might open up the vasculature a little bit and  
13           you'll see increased diapedesis.

14                       And so here is an example -- we're  
15           just giving a few examples here of MF59. And  
16           what we're showing here is when we have the no  
17           treatment.

18                       So one of the things that we  
19           talked about is we put PBMCs on top of the  
20           endothelium. Mostly monocytes extravasate  
21           through but we're also getting some residual  
22           T and B cells as well as NK cells

1 transmigrating through the endothelium as  
2 well.

3 But what you can see is by -- with  
4 the addition of MF59, we're seeing a  
5 significant increase in diapedesis across the  
6 endothelium as well. So we can really start  
7 to dissect what may be occurring for a  
8 mechanism of action.

9 Last one is thinking about DC  
10 activation versus antigen presentation, and  
11 this one is just a small little cartoon that  
12 sort of shows that, you know, part of the fun  
13 of what we're doing is activating this DC and  
14 really getting it jazzed up and ready to go.

15 But really this DC means nothing  
16 if it can't find the right receptor match  
17 within the repertoire -- oh, it died. Oh, my  
18 gosh. I hope the computer didn't die. No, it  
19 didn't. Okay.

20 Sorry. This is really an R-rated  
21 movie. And given that we're near Washington,  
22 D.C., it is probably good that we stopped it

1 at G-rated.

2 (Laughter.)

3 DR. WARREN: Jay, I apologize for  
4 not showing the R-rated movie like you wanted,  
5 okay.

6 So anyhow, one of the things we  
7 wanted to do is show that we can pulse these  
8 antigen-presenting cells with the antigen and  
9 show specific T cell responses both to recall  
10 as well as primary T cells.

11 And here what we did is we looked  
12 at -- we're looking here at antigen-specific  
13 responses by looking at up-regulation of CD40  
14 ligand as well interferon gamma through  
15 intracellular cytokine staining.

16 And we looked at standard PBMC  
17 assays. Standard PBMC assays were even added  
18 in DCs to sort of artificially get it going  
19 versus what we see when we put it in the MIMIC  
20 model.

21 And you can clearly see in the  
22 MIMIC model, whether we're looking at

1 secondary responses for influenza or tetanus  
2 toxoid, that the MIMIC model is giving  
3 significantly greater responses. And for  
4 primary responses, we looked at recombinant-  
5 protective antigen of Bacillus anthracis as  
6 well as MSP1 merozoite surface protein for  
7 malaria.

8 And clearly you can see that we  
9 are able to get antigen-specific T cell  
10 responses to both naive and recall responses.

11 Wanted to show that we can do it  
12 for multiple types of naive antigens, showing  
13 some of the hard core data. So those of you  
14 who don't believe pie charts can look at this.

15 And here we're looking at  
16 MSP1/AMA1 for malaria, KLH and GP120 from HIV.  
17 And we look at the culture and target  
18 conditions on top with no antigen, no antigen  
19 controls all the way to the far right where we  
20 have the specific antigen and the culture and  
21 the target as well.

22 You can clearly see we are above

1 the noise, any near noise floor, and looking  
2 at antigen-specific T cell responses in the  
3 MIMIC culture.

4 And we can also look at live  
5 attenuated vaccines. There was an excellent  
6 talk -- several talks today on yellow fever.  
7 And here just shows how we can look at live  
8 attenuated as well as inactivated vaccines as  
9 well.

10 And clearly we're seeing signals  
11 when looking at up-regulation of 154 and  
12 interferon gamma for antigen-specific T cell  
13 responses as well.

14 And just to give you an idea of  
15 the types of inter-donor variability that we  
16 see in the assay -- just wanted to show you  
17 for representative ten donors, the types of  
18 variabilities that we get within the assays.

19 Next we want to just talk about  
20 Th1/Th2 polarization because once this is in  
21 the lymphoid tissue equivalent, we can also  
22 assess the types of cytokines that are being

1 generated as well.

2 And here just wanted to show for  
3 yellow fever vaccine, well-known vaccine to  
4 generate a Th1 -- a balanced Th1/Th2 response,  
5 we are indeed showing this by the up-  
6 regulation of Th1 cytokines such as IL2 and  
7 interferon gamma as well as Th2-type cytokines  
8 such as interleukin 13 and IL5.

9 Next wanted to show differences  
10 between naked antigen versus adjuvanted  
11 antigen. In this case what we did is looked  
12 at RecombiVax, which is alum adjuvanted,  
13 versus the naked protein.

14 The thing to really note here --  
15 and this is kind of a busy slide and I  
16 apologize -- but what we're looking at are the  
17 HPV -- or hepatitis B surface antigen-specific  
18 antibody responses, IgG via ELISPOT.

19 And we're testing it for  
20 RecombiVax. And note that antigen  
21 concentration is 50 nanograms per mil with the  
22 RecombiVax versus the naked antigen alone,

1           which we had to go to five to ten micrograms,  
2           essentially a thousand times higher in  
3           concentration of the naked antigen, to get  
4           similar antibody responses, really showing  
5           that we can look at dose sparing issues as  
6           well as look at naked, you know, antigen and  
7           adjuvanted-antigen as well.

8                         And last we'll talk about  
9           immunotoxicity of biologicals. We had two  
10          great presentations earlier in this session  
11          from Sanofi and Novartis talking about  
12          toxicity and how the -- and now we'll talk  
13          about how the MIMIC model might be used for  
14          this.

15                        So what we wanted to do is look at  
16          in vitro reactogenicity and immunoregulatory  
17          or really immunomodulatory effects of various  
18          types of compounds. And we looked at various  
19          immunopotentiators such as Imiquimod. We've  
20          also looked at Gardiquimod, CpG, CpG control.

21                        We've looked at different  
22          immunosuppressants such as cyclosporine

1           methotrexate as well as dexamethasone. And  
2           then we've also looked at monoclonal antibody  
3           therapeutics such as OKT3, which isn't really  
4           on the market any more, anti-CD154, CTLA-4,  
5           and anti-TNF-alpha.

6                           And one of the things I just  
7           wanted to show really quickly the types of  
8           datasets that we can generate with the MIMIC  
9           system. Here we're looking at maturation of  
10          the dendritic cells in the model for the  
11          various different compounds that we looked at.

12                           And you can see that the  
13          immunopotentiators generally led to up-  
14          regulation of maturation, which makes sense.  
15          That is what you would expect.

16                           And CTLA-4 is really a human  
17          fusion protein that blocks the CD80/86 marker  
18          with respect to the interaction with the T  
19          cell. And you can clearly see that it is  
20          down-regulated which is, again, what you would  
21          anticipate to see.

22                           We've looked at T cell

1 proliferation as well as B cell proliferation.  
2 And, again, it comes as no surprises that, you  
3 know, things such as Imiquimod and CpG will  
4 induce B cell proliferation and  
5 immunosuppressants such as cyclosporine  
6 methotrexate would decrease it.

7 So we're seeing everything that is  
8 very consistent with the literature and  
9 expectations to sort of give proof of concept  
10 that the model is indeed behaving correctly.  
11 And, in fact, if we put the entire table  
12 together, and we have all the data to support  
13 this but just wanted to give it in a table  
14 form.

15 You can see for the -- like things  
16 such as CpG and Imiquimod, it's known to be  
17 more of an adjuvant, the MIMIC activity, it as  
18 showing as an immunopotentiator, cyclosporin  
19 methotrexate, immunosuppressants, and we're  
20 seeing immunosuppression of all the different  
21 types of aspects that we're seeing.

22 And for various of the monoclonal

1 antibodies we are seeing things that are  
2 anticipated from the literature as well as  
3 from in vivo studies as well within this in  
4 vitro model.

5 And earlier today, Dr. O'Hagan  
6 from Novartis showed a slide and we talked  
7 with Jeff Ulmer, who also works at Novartis,  
8 and we asked him if we could modify the slide  
9 slightly to sort of fit our hypothesis or our  
10 thesis and where the MIMIC model would fit in  
11 to a lot of idea of adjuvant discovery when  
12 thinking about the various diverse libraries  
13 where we could do really rapid screens for  
14 immunogenicity.

15 And then find out if we have good  
16 hits, then follow on with immunotoxicity or  
17 toxicity in general. And then go on to  
18 understand structure/function relationships  
19 with this in vitro model.

20 And the nice thing is is that we  
21 can dissect things very easily. There was an  
22 earlier talk about the influence of Tregs.

1 We've looked at the influence of Tregs when we  
2 can pull them out of our assay or put them in  
3 to look at specific antigen-specific responses  
4 as well and we see marked changes going on  
5 there, too.

6 So in terms of conclusions, we  
7 understand that right now we believe that the  
8 MIMIC model does appear to replicate or at  
9 least be a biomimetic of human immunity. But  
10 we are also quite cognizant that validation is  
11 an ongoing process.

12 And each of our customers has a  
13 different way of validating the system. So it  
14 really is an ongoing process.

15 But the data does appear  
16 encouraging. Even though we've talked mostly  
17 about adjuvants today, we've done a lot of  
18 vaccine responses as well as immunotoxicity as  
19 well. And we see that some of the  
20 applications for adjuvants can be, with  
21 respect to optimizing formulation, thinking  
22 about QA/QC, dissecting the mechanisms of

1 action, as well as looking at immunotoxicity  
2 and immunoregulatory effects as well.

3 And with that, I'd like to thank  
4 the audience for staying for the very last  
5 talk as well as our funding agents and Amgen  
6 and Novartis for their respective works.

7 Thank you.

8 (Applause.)

9 PARTICIPANT: Yes, the T cell/B  
10 cell data looks quite good. I'm having a  
11 difficult time with the dendritic cell data  
12 with respect to the CpG effects because my  
13 understanding is in the human system, the TLR9  
14 is really only expressed on human B cells and  
15 plasmacytoid DCs. And the type of DCs you are  
16 looking at are monocyte derived, from what I  
17 can understand in the system.

18 So I was just wondering have you  
19 been able to demonstrate TLR9 expression on  
20 those dendritic cells that are reverse  
21 transmigrating out of the endothelial layer  
22 because --

1 DR. WARREN: Yes, that's a very  
2 good question. The thing that I forgot to  
3 mention is that in our model, we have indirect  
4 evidence that there are plasmacytoid DCs as  
5 well.

6 PARTICIPANT: Okay.

7 DR. WARREN: So it's not just  
8 monocytic-derived DCs. There are -- we don't  
9 have hardcore evidence because there are so  
10 few but we do have indirect evidence that  
11 plasmacytoid DCs are included in the assay as  
12 well.

13 PARTICIPANT: It's just that the  
14 facts, if you look at the conversion of those  
15 graphs, like 90 percent of the cells are up-  
16 regulating CD80. So it can't just be  
17 plasmacytoids that are doing it unless it is  
18 an indirect effect.

19 DR. WARREN: Yes. And as I  
20 mentioned -- so the other thing, which was not  
21 -- I didn't mention it as well as I could have  
22 is we do have a small number of residual B

1 cells that transmigrate into the endothelium  
2 or are stuck on the endothelium as well. So  
3 we do have the inclusion of B cells as well as  
4 plasmacytoid DCs in the model as well. And  
5 that can possibly explain some of the effects  
6 with CpG.

7 PARTICIPANT: Thank you.

8 DR. WARREN: Good observation.

9 Thank you.

10 PARTICIPANT: Yes, I was quite  
11 excited to hear -- I'm right here.

12 DR. WARREN: Oh, okay. Sorry.

13 PARTICIPANT: I was quite excited  
14 to think of when you people came to visit us  
15 and also with the whole approach of VaxDesign  
16 to try to predict responses because we know  
17 how expensive it is to get in the clinic. And  
18 we'd like to be able to predict which thing we  
19 have that would work better for our particular  
20 antigens.

21 But I must say so far what you  
22 presented and what I've read, I'm not -- I

1 don't know whether you have really convinced  
2 me anyway. Others may be quite convinced that  
3 your system will really predict for us what we  
4 need and whether you have done any critical  
5 studies that will give that answer.

6 I heard your talk. I heard all of  
7 your presentations. But I'm not, at this  
8 point, convinced.

9 DR. WARREN: Thank you for the  
10 comment even though I didn't want to hear  
11 that.

12 (Laughter.)

13 DR. WARREN: But I think that we  
14 always like the naysayers around because of  
15 the fact that part of our mission is to  
16 convert. But we can't show you all the data,  
17 unfortunately, because of the fact that the  
18 data that we can show is ones that we've  
19 conducted ourselves.

20 All of our data is owned by the  
21 customers. And they do not give us liberty  
22 many times to show the data.

1                   But I think that if we are ever to  
2                   unveil a lot of the datasets -- because each  
3                   customer comes in -- because they all come in  
4                   like you, which is very fair, is like hey, let  
5                   me give this system a shot. I don't quite  
6                   believe it. Let me test it.

7                   And they come in with ten  
8                   different compounds. And they want us to  
9                   evaluate it, sometimes blinded, sometimes not.  
10                  And then we have to sort of prove ourselves to  
11                  each and every customer through a pilot  
12                  program and work out way through that.

13                  And, unfortunately, we're not  
14                  allowed to publish a lot of those pilot  
15                  projects because of the fact that sometimes  
16                  they are formulations from the customers.

17                  So it is a fair comment and fair  
18                  observation. But, unfortunately, I think it  
19                  is like most things. That you have to put  
20                  your finger into the pudding and test it  
21                  before you really can appreciate whether it is  
22                  true or not. Or whether or not it tastes

1 good.

2 DR. GRUBER: Well, thank you very  
3 much. I found this presentation actually very  
4 stimulating.

5 I think there may be hope that we  
6 are on the way to perhaps define and develop  
7 alternative methods and methodologies to  
8 include in safety assessments of these vaccine  
9 products.

10 I wanted to ask Dr. Slater is  
11 there anything else? I wanted to give you the  
12 final word for this evening. He's coming up  
13 to the podium.

14 DR. SLATER: No. Just to wrap up  
15 more with housekeeping comments.

16 Please remember everybody,  
17 tomorrow morning we start at eight o'clock.  
18 If you are speaker at tomorrow's session, I  
19 would encourage you, if possible, to load your  
20 talks on to the computer either now or  
21 tomorrow morning a few minutes before eight  
22 o'clock.

1                   Once we start, we will have a  
2 morning break and we will have a lunch break.  
3 But we will go directly from the first  
4 roundtable discussion into Session 4. So it  
5 would make things a little bit smoother if you  
6 get here a few minutes early and load your  
7 talks on.

8                   Aside from that, it has been a  
9 long day. It's been an outstanding day.  
10 Thank you to all of the speakers today and  
11 thank you to all of the co-chairs.

12                   Tomorrow promises to be just as  
13 long a day and probably more work because of  
14 the roundtable discussions which will involve  
15 a lot more give and take. So get a good rest  
16 tonight and we'll see you tomorrow morning.

17                   (Applause.)

18                   (Whereupon, the above-entitled  
19 workshop was concluded at 6:05 p.m.)

20

21

22

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