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WORKSHOP ON ADJUVANTS AND ADJUVANTED PREVENTIVE AND THERAPEUTIC VACCINES FOR INFECTIOUS DISEASE INDICATIONS

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WEDNESDAY
DECEMBER 3, 2008

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The workshop convened at 8:00 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.

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Roundtable Discussion:

CARL ALVING, M.D., Walter Reed Army Institute of Research
BRUCE BEUTLER, M.D., Scripps Research Institute

MARTIN FRIEDE, PhD, World Health Organization (WHO)
NATHALIE GARCON, Pharm.D., Ph.D., GlaxoSmithKline Biologics
HANA GOLDING, Ph.D., Division of Viral Products, CBER, FDA
SARAH GOULD, Ph.D., Sanofi Pasteur
MARION F. GRUBER, Ph.D., OVRR/CBER/FDA

EMMANUEL HANON, GSK Biologicals
EUGENE MARASKOVSKY, Ph.D., CSL Limited
DEBORAH NOVICKI, Ph.D., Novartis
DEREK O'HAGAN, Ph.D., Novartis Vaccines and Diagnostics, Inc.
FABIO RE, Ph.D., University of Tennessee Health Science Center

ROBERT SEDER, M.D., Vaccine Research Center, NIAID
ELIZABETH SUTKOWSKI, Ph.D., Co-Chair, CBER/FDA
GEERT VAN den BOSSCHE, DVM, Ph.D., Bill and Melinda Gates Foundation
JAN WILLEM VAN der LAAN, Ph.D., National Institute for Public Health and the Environment, The Netherlands
WILLIAM WARREN, Ph.D., VaxDesign Corporation

Session 4: Clinical

JAY E. SLATER, M.D., Co-Chair, CBER/FDA
W. RIPLEY BALLOU, M.D., Bill and Melinda Gates Foundation
GIOVANNI della CIOPPA, M.D., Novartis

CHARMAINE GITTLESON, M.D., CSL Limited
STEVEN REED, Ph.D., Infectious Disease Research Institute
HEATHER DAVIS, Ph.D., Pfizer
GARY DUBIN M.D., GSK
GREG GLENN, M.D., Intercell, USA
MARTINE DENIS, Ph.D., Sanofi Pasteur

OFER LEVY, M.D., Ph.D., Children's Hospital Boston and Harvard Medical School
RINO RAPPUOLI, Ph.D., Novartis

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Session 5: Roundtable

W. RIPLEY BALLOU, M.D.
HEATHER DAVIS, Ph.D.
GIOVANNI della CIOPPA, M.D.

MARTINE DENIS
GARY DUBIN, M.D.
MARTIN FRIEDE, Ph.D.

CHARMAINE GITTLESON, M.D.

GREG GLENN, M.D.

THOMAS HOLDICH, MBBS, ATL

OFER LEVY, M.D., Ph.D.

RINO RAPPUOLI, Ph.D.

STEVEN REED, Ph.D.

DAN ROTROSEN, M.D., NIAID/NIH

FLORIAN SCHODEL, M.D., Merck

JAY E. SLATER, M.D.

THOMAS VERSTRAETEN, M.D., M.Sc., GSK

Session 6: Wrap-Up

HANA GOLDING, Ph.D.

CHUCK HACKETT, Ph.D., NIAID/NIH

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P R O C E E D I N G S

(8:01 a.m.)

MODERATOR SLATER: Good morning, everybody. As you work your way to your seats, I will ask the participants in the roundtable to please work your way up to the front to our not-quite-round table.

Just again a couple of brief comments. You all notice that we are in the bigger space. That is not a mistake. The group that was supposed to take one of the rooms canceled at the last minute, and so we benefit from that.

Those of you who parked in the parking lot, please make sure to get a parking voucher today again.

I have had several questions about the slides and whether they would be available for distribution. I will give the answer that I have given to everybody, and the answer is maybe. I have not secured permission from any of the speakers to make their slides public

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and disseminate their slides, which I will attempt to do in the day or two following the conference.

Once we have received an answer from each of our speakers, then those presentations for which we have received permission will be posted. What I would suggest is that you go back to the website on which you registered for this meeting and check, and there will be a link there, my guess is, in about a week for those presentations for which we have secured permission.

At that, I will turn this over to Dr. Gruber, and have a good roundtable.

DR. GRUBER: Well, good morning, and welcome to the second day of this workshop. We will begin the discussions with the nonclinical issues.

As I was saying yesterday when I presented the current approach to nonclinical testing requirements for adjuvants and

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adjuvanted vaccines, some of the approaches were really devised from recommendations that stand for testing of vaccine antigens, and we didn't really focus on adjuvant-specific issues.

Thus, I had mentioned yesterday that some of the approaches and parameters may need to be revisited to really make the nonclinical testing approach fit the adjuvant-specific issues.

That is actually the purpose of the roundtable discussion this morning. What I would like to do is to start with a series of questions. Now I think some of these may be a little bit ambitious, and we are probably not going to get to discussing them all in detail. I think, therefore, it may make sense to just prioritize, and starting perhaps with the most practical concerns and considerations.

Let me just go through the questions. Then I am going to be circling

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back to what I thought are the issues that we need to be focusing on this morning.

So question number 1 was: If the current approach to adjuvant toxicology testing is sufficient or should it be revised? Is it sufficient to test only the highest 1x human dose of the vaccine-adjuvant combination, as we do currently, as well as the adjuvant alone, or should dose ranging studies be conducted on the adjuvant alone?

Should additional parameters such as cytokine levels or other biomarkers, C-reactive protein or fibrinogen levels be also assessed? And what about other aspects of the current study design?

I mentioned yesterday that the route of administration should mimic the clinical dose, and what about the dosing regiment? To remind you, we are using episodic dosing in toxicology studies to mimic the proposed clinical dosing regiment. Is it adequate for adjuvant testing as well? Should

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there be more frequent dosing?

I think one of the issues that I personally would like to really touch on a little bit this morning is really the animal species and the animal models. Well, that is challenging because, I guess, even now we already tried to get at this issue a little bit in 2002 in the workshop we had then on nonclinical testing of vaccines.

At that point, it was thought that there were perhaps some animal models that would allow testing in special subpopulations, but that the series really hasn't moved, and I guess we may want to revisit this a little bit this morning.

The first question is whether it is sufficient to test in only one animal species, as the current recommendation is; and then again, what really constitutes a relevant animal model?

To remind you again, we consider a relevant animal model as a model that is able

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to mount an immune response to the vaccine antigen and whereby the adjuvant would enhance the immune response to the vaccine antigen. But how do we get our arms around the specie specificity of the innate immune response, and also the mechanism of action of the antigen and adjuvants in that context.

Then again, should toxicology studies be conducted in specific animal models to support the safety of adjuvant in special subpopulations? So if you develop a vaccine specifically indicated for the pediatric population, should toxicology assessment be conducted in a juvenile animal model, for example?

Additional questions get at the issue about the immunologic parameters that should be evaluated. Again, should it be the vaccine antigen-specific response only or should we now also consider the adjuvant-specific responses?

How can we, and how do we, best

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incorporate in vitro assays into nonclinical safety assessments to supplement safety assessments in animal models?

Then here are a couple of additional questions. I am not optimistic that we maybe even get to this this morning, but at least I wanted to put them up, because of these issues that the regulatory agencies are grappling with.

That gets at the issue of what to do with combination adjuvants. So as we have heard yesterday, some of these adjuvant systems include a variety of adjuvants, such as QS21 MPL, for instance.

So the question is: If it is adequate to assess only the combination when assessing a combination adjuvant, so the adjuvant system in its totality, or should toxicity studies -- and I said here dose ranging studies -- be conducted on each separate component?

Then what additional tox studies

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should be conducted? There may be some concerns about an adjuvant system to either cause or exacerbate preexisting conditions such as autoimmunity or inflammatory disease. Should this be evaluated a priori?

Of course, there is the issue about do we have adequate animal models to assess that. Then what about additional studies such as genotox or chronic toxicity studies that are currently not required in vaccine toxicology assessments?

The reason about chronic toxicity studies or long term evaluation is coming from the fact that some of the vaccines that are currently in clinical development are those that may be given as repeated doses over a long period, such as the adjuvanted influenza vaccines that are currently in development.

So an individual would get every year seasonal influenza vaccine, and that would result in exposure to perhaps even multiple types of adjuvants that are either

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concurrently administered over multiple years. How can this be studied? Should it be studied?

That is the overview of the questions, and I think that is quite loaded, that program. So let's go back to the first issue, because I think that may be the one that we can tackle this morning, and that is: Looking at the current approach and the testing paradigm, should it be revised or should we keep it, as we have done it for the last couple of years?

So the first sub-bullet that I have put up here is: Is it sufficient to test only the highest 1x human dose, if that is feasible in the animal model, of the vaccine/adjuvant combination or should we include dose ranging studies here or should dose ranging studies only be conducted on the adjuvant alone?

So whoever wants to take that first question -- Dr. Van der Laan? Thank

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you.

DR. VAN DER LAAN: Thanks, Marion, for this question, for this opportunity to discuss.

When we first were thinking about the guideline for vaccines, it was just in the period that vaccines became under a normal regimen in Europe, and there was a normal toxicity to be done on the final product, and should we replace the normal toxicity testing, at least a test of the final product, in an animal species.

That is why we have thought about just the human dose, the human formulation, and a vaccine is not a simple formulation. It is not a drug product. It is just a complex formulation. It is not easy to halve -- It might be easy to halve the dose as a type of -- just half the volume. But it is not easy to increase the dose because of the volume, and that is why, just for my first practical reason, the human dose is the highest dose.

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Of course, we can think about other approaches, although just for the last few years also, starting with revising toxicity approaches for biologicals, we are more focusing on the pharmacological effect than on the toxicological effect, far away from the human dose.

So in my view, the approach should be handled with some flexibility, but is in general okay. For an adjuvant, of course, you should have your developmental studies: What is the optimum in the dose of an adjuvant? But that is more proof of concept than toxicity.

DR. GRUBER: Thank you very much for this comment. Are there any other comments from the roundtable on this issue?

MR. ACKLAND: Jim Ackland, independent consultant. I guess my question that I have been grappling with is why do we need to change? What is it that the regulators are seeing that means that we

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should change our existing toxicology assessment of new vaccines with new adjuvants?

So is there something that needs to be changed, needs to be fixed, and that might help us say how we should change it. So what is being seen in the clinic that is not being seen in the preclinical studies that we need to be looking for?

DR. ALVING: I could make a comment on that. I am not a regulator, obviously, but there are some instances when in human trials there have been clear toxic effects that have occurred, systemic effects, not life threatening, but --

So the question is whether we want to be able to pick up those potential toxic effects at an earlier stage in an animal model. I would really wonder about that myself, actually -- I think this is an excellent question -- because in all of the studies which I have seen in which there have been comparisons of different adjuvants, the

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conclusion, I believe -- maybe I'm wrong; if somebody would correct me, I would appreciate it -- that all of the adjuvants that were tested were considered safe, even if there was some degree of reactogenicity.

So I would say, actually, the whole vaccine per see, including the adjuvant, is a more appropriate thing to look at rather than focusing only on the adjuvant, just based on the historical apparent lack of clinical problems that have been observed.

DR. NOVICKI: So I guess some of these questions are so broad, it's a little bit hard to get my head around it. But one comment I would like to make is that toxicology studies are not the only opportunity to gain safety information.

When one is conducting studies where you are dose ranging in pharmacological studies looking at the immune response, to build in some parameters there is an opportunity to capture some information.

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So I think, in a way, we have to think more creatively around the nonclinical package perhaps than just zeroing in on the GLP toxicology studies.

I think that some of the questions that we are asking are somewhat limited by the type of material that we are dealing with. So if you've got a liquid emulsion, in order to do -- and it is set at a certain concentration and physical characteristics, etcetera -- you are not going to be able to keep the dose constant and increase the concentration of components. So you are changing it already.

So your dose is going to be limited by how many times you want to poke an animal, and that might be a completely different situation than if you are trying to incorporate an immunologically stimulatory biologic into a PLG microsphere.

So I think that there is no way to make the guidelines or guidance cover all of the different situations that the people in

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this room are working on. I think that it has to be broad enough to give people general guidance on what to do, but then an individual developer of the product has got to think rationally about what they really need to know about the molecule in order to safely test it.

So it's just some thoughts.

DR. SCHODEL: Hi. This is Florian Schodel from Merck. I've had a question that sort of I have been thinking about since yesterday.

We are mostly concentrating on the acute responses, and the tests are mostly concentrating on what happens in the acute phrase reactions, and they are really not an issue; because that is what you see quickly in your Phase I studies. That is where you have very good instruments. They are also frequent. I can very easily figure out whether they do that and, as the colleague before me said, the toxicology armamentarium that we currently have, I think, answers these

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questions quite adequately, LPS responses, fever, pyrexia, those kinds of things.

Now what I was hoping for a little bit yesterday -- maybe that is a question I would like to ask the panel: Are there any tests that could be used in preclinical work that would actually help us grapple with the much more difficult to answer questions in the clinic, such as, for example, this suspicion that there might be autoimmune responses generated. That could have negative consequences, which you can't test in the clinic, because they are too infrequent.

So you see one case, and then you would have to test millions, as somebody -- I think it was you -- laid out yesterday, in order to get a clear clinical answer.

So are there mechanistically based animal tests that we could use to exclude a mechanism -- basically say, if we put the adjuvants in a preclinical test and it doesn't show that mechanism, then we don't have to

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suspect that we will really need to test for it in the clinic; because those acute things, as I said, are frequent, and they are easy to deal with.

I don't know whether there is an answer to that, but that is the question I would like to get an answer to.

DR. FRIEDE: Okay. So let me try and just give some thoughts which covers that and a lot of what we heard yesterday.

So I would like to begin with the observation that we have actually been giving adjuvants to people for the last 100 years. We have been giving wholesale pertussis vaccines to most of the people in this room, and that contains a lot of Toll4 agonists.

We have been giving IPV to most of the people in this room. That contains a Toll3 agonist. We have been giving meningitis vaccine, which is a Toll2, a bit of Toll5 in there.

So we actually have a tremendous

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clinical background of administration of adjuvants to people, and we haven't picked up in any post-market surveillance any evidence of these vaccines which contain very potent immunostimulatory molecules having any correlation with autoimmunity. And certainly, there has been a significant study looking at this with the Hepatitis B vaccine, multiple sclerosis and a number of other things.

So I think, just to get the pendulum swinging back in the right direction, we need to set up an environment where we actually facilitate adjuvant development, not impede it. So we must remember that we have this background of having administered adjuvants, many of them in large quantities and relatively impure, for the last 90 years or so.

So then to move forward from this, I think looking for autoimmunity in animal models is going to be extremely complex, because if you look for something, you will

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find it. If you inject into an animal model that is susceptible to autoimmunity, be it lupus, be it the studies we saw yesterday, immunostimulants, I am sure that you will be able to trigger something. But the test would be that we actually administer all of the vaccines we already have used, which we know that there is no correlation of autoimmunity, and I'm sure that in this animal models these vaccines would induce such responses.

So I think those animal models are actually inappropriate.

PARTICIPANT: I would like to amplify on the comment just made about how there are vaccines out there that have a lot of TLR adjuvants. Another, of course, is Bacille Calmette-Guerin or BCG that is given around the globe at birth and has Toll2, Toll4 and other innate immune adjuvants as well. So you might add that to the list.

DR. GOULD: I think those are good points. I just want to take us back maybe to

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the question that we are trying to deal with first, because, obviously, that is a question that, I think, is important to come back to in the autoimmunity. But here we are trying to look at whether one human dose is sufficient with vaccine and adjuvant alone or whether we should be looking more at dose ranging.

I guess it depends what we are trying to achieve. I come from a background from the pharmaceutical industry where I've spent 10 years dealing with small molecules, and there you push the dose. You want to check toxicity. There you are looking at chronic dose often, and you are trying to ind a signal, and we know for sure that there is a lot of time that drugs get into the clinic and then eventually fail because of some toxicity or other, which actually hasn't been picked up in the preclinical.

The vaccines, yes, they have a very safe record, and we have been testing adjuvants and vaccine adjuvants, and on the

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whole we are not seeing any major issues, and we have been pushing adjuvants up because of the safety concerns.

I gave the case history yesterday of why don't we push the dose up, just to see, well, what could we really induce if you really pushed the dose up; and we didn't see anything.

Now you can talk about endpoints and autoimmunity. Okay, we weren't looking for that. So that would only be detected by normal parameters we were picking up. But we pushed the dose, and we didn't see anything.

So I'm not sure that there is much value in pushing the dose, because it depends on what your adjuvant is, because there's new adjuvants coming onto the field. So is there going to be something coming onto the field that we don't understand?

DR. GRUBER: Yes, and I think that is a good point, and I think that is why, at least from the FDA perspective, we wanted to

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bring up this issue about should dose ranging studies on the adjuvant alone really be incorporated into toxicology assessment?

You can look at history and say so far we haven't seen any red flags. I also want to make the point that, by no way, do we want to link all of these questions that we have put up here to the concern for autoimmunity.

I think we all realize that may be one potential concern, but I don't want to be misunderstood to mean that the overriding concern here was adjuvants as an autoimmune induction. That really is not where we are coming from.

When we were saying to look at the possibility to include dose ranging, it is because sometimes if you are stuck with one dose -- and I have seen final study reports; my colleagues have seen it, that you have a signal, and you don't know what to do with that. If you would have dose ranging studies

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in different study arms, you are able to explain it, and you can say, okay, I see it perhaps at higher dose but not at lower doses, not at a lower dose, and it doesn't compare well with the clinical dose; so let's not be too concerned about it.

If you just have this one dose, you don't really have anything to compare it to, and then it makes data evaluation somewhat complicated. So, therefore, we brought up the point, where feasible -- and we realize that some adjuvant systems do not allow dose ranging because of concentration issues and things like that, but where feasible, should the recommendation be made, because it does help and facilitate data evaluation and interpretation at points.

DR. VAN DEN BOSSCHE: So I would like maybe to add a little bit of complexity to the discussion.

First of all, I think, when we are talking about adjuvants, we should really be

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specifying what type of molecules we are talking about. Personally, I think adjuvants is not the right definition even, because I would prefer to talk about adjuvanticity. Why is this? Because we know that, for example, antigens could have adjuvant effect as well.

On the other hand, we know that adjuvants are going also to impact presentation and one of antigens. So I think basically the discussion here is the difference between small molecule adjuvants where we are afraid of systemic distribution, where we know that this is happening. That is the reason why we try to change these molecules in order for them to be more targeted.

The other type of molecules or the other type of compounds that have adjuvanticity effect are -- this could be fibrous particles, inactivated, attenuated, whatever. These are the more complex super-molecular, macro-molecular surrecia, which we

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know that they are less likely to distribute systemically.

These are the type of compounds where we have definitely less problems. These are the compounds that are going to be very targeted, have a local effect.

I think the discussion should really be more focused on how do we make sure -- unless we think, we do think that we should be using adjuvants as drugs. I don't think so. I think it is fundamentally different.

If we agree upon this, that not only adjuvants but vaccines in general should have a local and targeted effect, then we should, first of all, stay away of these drug-like molecules and use them as such, which I think is one of the major problems and the major issues of discussion.

I also don't understand why we need to test adjuvants alone. It is very clear that the antigen will impact or may impact on the effective of the adjuvants, if

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you are talking, for example, tolerance or breaking tolerance.

It is also very clear that the adjuvant is going to impact on the presentation of the antigen, on the processing of the antigen. Both of these things go together, and you may be observing completely different effects if you don't use them together and if you test them separately.

So I am really sorry, but I think these are the type of things we need to discuss first. What do we really expect the adjuvant to do? Should it be a kind of systemic effect? Should it be a localized effect?

If we think -- If we agree that the vaccine should induce a generalized effect through, first, local triggering of immune competent cells and then by expanding through the lymph nodes, T cells, B cells and so on, then we may be thinking about what is the best way for these molecules to be administered.

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I don't think it is in a drug-like form like small molecules, which will be readily distributed and into circulation and which then may elicit this type of questions, autoimmunity, breaking tolerance, immune pathology and so on.

DR. VAN DER LAAN: May I comment on that also from practice. Although I agree that there is something to say to support the feeling that vaccines should be handled more locally, the final effect of a vaccine is that it is a complete systemic protection of the body. So the definition of local is a bit difficult.

With respect to adjuvants, whether or not adjuvants are drugs or non-drugs or should be tested alone, there are some Toll-like receptor agonists such as imiquimod for TLR-7 or CpG for TLR-9 that are used and administered separately from the antigen in, for instance, a cancer vaccine study.

That is why we in Europe have

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defined that specific remark on what is an adjuvant, what is an immune therapeutic. The current practice is that in some clinical studies CpG is given much more frequent and with much more repetitively than only once with the antigen.

So it is not a final solution to have adjuvants not as handled as drugs, and I am not discussing about the legal aspects. then we have also to change the laws, but that is not a scientific issue.

DR. GRUBER: I would like to make one more point to ask a question to the panel before we perhaps go to the next question.

That is: Mention was made that there doesn't seem to be a clear reason why adjuvants should be tested by themselves. First of all, to clarify, from a regulatory perspective, of course, we strongly feel that the adjuvant system needs to be tested in the context with the vaccine antigens.

So the final clinical formulation

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that is administered to the human subject will need to be studied on the toxicity study. However, we have felt from a regulatory perspective that there is value in terms of studying a novel adjuvant at least by itself to tease out potential adverse effects that you may see with the adjuvant alone to sort of explain what the signal or the adverse event would be, realizing that certain synergistic effects, of course, could take place and would lead perhaps to an adverse outcome. But we felt that trying to discern the, if you want, reactogenicity between the vaccine adjuvant or the adjuvant alone would be helpful. I would like to hear some comments from the panel on this.

DR. VAN DEN BOSSCHE: Well, sorry. Again, I would like to make the same kind of comment. I understand the logic behind this. The only comment would really be what we are testing there, according to my opinion, doesn't make sense, because it is not going to

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be relevant for the action of the adjuvant once you have it in the formulation, once it is in the presence of the antigen.

The action will be different, as well, the intrinsic activity of the adjuvant as its distribution, for example. We know that this is the trick, is to formulate. It is to put it into particles, if these are small molecules, for example, in order to change the distribution, in order to change the uptake by the cell, in order to change the processing of the antigen, and so on.

So testing the adjuvant alone -- I understand the logic behind, but again I think we need to get away of this kind of perception, that adjuvants are drugs. I mean, we want to use them as vaccines. What are we going to do with all this complex formulations like VLPs, virosomes, where everything is integrated? Do we consider them being adjuvants?

As Martin just pointed out, they

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do contain adjuvants. Are we testing them separately? What are we going to do about these guys? So I really don't know. I mean, if we want to follow this drug-like approach, there is no way we can test them.

DR. O'HAGAN: I think we end up with that problem if we talk about adjuvants, and there are so many different kind of formulations, of materials, of components. Fundamentally, I think the argument, to me, is if it is something that is really novel that we have not seen before, that we have not utilized as an adjuvant at all, then it seems appropriate to investigate the inherent potential for toxicity of that compound.

So if there are issues that are going to arise, you would like to know early. Ultimately, it is really about the safety of the vaccine product you would make with the adjuvant formulated into, which is the GLP standard toxicology, etcetera. But for the new compound and new agents and new approach

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where there isn't a solid background, I would say it is wholly appropriate to investigate the inherent potential for toxicity of a compound.

DR. NOVICKI: I would just add that I think that to do entire, full blown programs of long term studies with an adjuvant alone, I don't think, is appropriate. But to understand the fundamental basically hazard identification in the early stages when you want to understand what are the potential risks, are there special studies that you might see that are indicated by some early signal, or do you get a very sort of flat kind of signal, no concerns, and then you do -- I mean, every study that we do with a vaccine containing an adjuvant, we incorporate very frequently adjuvant alone and then also a saline control.

So in every study where we are looking at the product, we are also incorporating these other control groups. So

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historically, if it is an adjuvant platform that a company is developing, then you are continually collecting data on that compound over time with more and more antigens.

So being able to build that kind of a history with it is really important. It is a little bit more challenging if it is a company where you've got a one-up adjuvant, it is only going to be used with one indication, and then doing an entire program for that one shot is perhaps onerous for a smaller company. But I think some fundamental information that shows you what you need to be looking for in subsequent studies can be very helpful.

MR. BALLOU: I would like to comment as a clinician who has had to -- who has worked with many adjuvanted vaccines over the years.

I have found the preclinical toxicology -- and I read those reports in depth before we start a clinical trial -- have been very helpful in helping me understand how

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to design a clinical program. I think the value of having the adjuvant control and looking at that histology and understanding what the adjuvant is doing in terms of local or systemic reactogenicity in an animal model helps you guide what you are going to do in the clinic.

What I have been very impressed with is the fact that sometimes the fixed dose that one proposes from preclinical is not, in fact, the dose that you end up using in the clinic, and that can only be determined through a proper trial design and actually asking these questions.

So I have found the current testing process to be very helpful in helping us guide clinical development, but not highly predictive about where we are going to end up with in terms of a clinical dose.

Certainly, I have very clear examples of where there is a difference when the adjuvant is added with an antigen versus

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the adjuvant alone, and sometimes it is very unpredictable how that happens.

Where I do have concern, I think, echoing Derek's comments, is for completely novel adjuvants where we don't have a track record yet, I think it is very important to do pretty careful dose ranging studies on this, particularly for adjuvants where it is difficult to disassociate the antigen dose from the adjuvant dose, and we know that there are adjuvants being proposed where the two are linked, for example.

My final comment is: I do not like the idea of breaking down adjuvant systems into component parts. We know they behave differently, and Qd QS21 by itself is a very different molecule than when it is quenched in liposomes, and I think you can get completely misleading results by breaking them down and trying to tease out individual toxicities when, in fact, what you are testing is a compound designed to give you a

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particular outcome.

DR. GRUBER: I think we will take you first. I don't know who came up first.

DR. WARNER: So I want to reiterate some of the things that I just heard. I am glad to hear those comments. This is Garvin Warner from Wyatt.

We got to remember what these studies are really designed to do. They are designed to give the clinician some guidance about what target organs are. Now those are traditional tox endpoints, right?

I am not talking about pushing things to an MTD necessarily, but having the appropriate safety margin for a novel, a new chemical entity or a new biologic entity is useful information to know that you don't have a catastrophe.

Now I do use, and I like having in terms of regulatory guidance 1x the human dose, but I got to admit, I often go to 2x to give me some margin, because of body weight

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issues with infants and things like that.

I guess my point is here that for a novel, a new biologic entity, new chemical entity, there is some advantage to making sure that you have pushed the dose so that you can identify potential target organs.

Some of the things we are working with are very potent biologic agents, locally or systemically, indirect systemically, but I think there is some advantage to early on at least understanding whether you have a catastrophe and you have some reasonable dose multiple over a body weight basis or a millimeter squared basis, just to help instruct the clinic and help the clinical program.

I don't think dose ranging in animal studies is very useful for either an efficacious dose, but again identifying target organs and coming up with those potentials is a useful thing.

DR. GRUBER: Thank you. I think

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we will take one more comment, and then we will move on to the next point.

DR. CHEN: This is Bob Chen from CDC. Most of my work has been vaccine safety in the post-marketing setting, and I would like to, hopefully, bring some of that experience to the discussion here.

So I would like to first address Martin's comment, that while in the past certain vaccines have been used a lot, and the fact that we didn't see certain problems -- is that adequate by itself to say that things are okay?

I would say that perhaps not in the sense, for example, the yellow fever vaccine or smallpox vaccine have been used forever, and it is really only in the last five or six years that we know this yellow fever vaccine associated with viscerotropic and neurotropic disease as well as the smallpox vaccine myopericarditides as problems really emerged, because we now have the

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surveillance systems that look at these things carefully.

That being said, these true, rare, serious associations are probably most likely genetically mediated, and it makes sense. We are introducing a relatively large exposure to a distribution of kind of biological genetic background, and so it is probably the tail of the curve, and that is why kind of the post-marketing surveillance is really when you are likely to see that.

Therefore, the issues of autoimmunity are probably akin to that. So it would be very difficult in the pre-licensure, in the animal model necessarily to detect that unless you know of a specific way to study that.

The concern that I would like to raise to the group, however, is really the thimerosal lesson, and that is the problem we got into with thimerosal is that we looked at each issue and each vaccine by itself, but we

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didn't recognize that in real life what happens is that the child or the adult frequently gets multiple vaccines, and that when you add up the thimerosal dose across that schedule, that is when you run into trouble.

So then, given the previous comment that, if we are introducing kind of new adjuvants that attack -- or kind of induce different parts of the immune system in kind fairly strong ways, is there an animal model way in which we want to look at that before we actually move forward with a schedule?

DR. GRUBER: Yes. I think that is a good point, and it was a question that comes later on. We will see if we can get to this, to evaluate this a little bit more.

I just wanted to finish up on at least this part, considering the time. In toxicology studies for vaccine antigens, one of the parameters that we are looking at is the antibody response, and then in terms of

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other parameters that we checked they are looking at clinical chemistry, hematology parameters.

Recently, because of the formulation of vaccine antigens with novel adjuvants, recommendation has been made to also evaluate additional parameters such as CRP and fibrinogen levels.

I would like to hear a little bit thoughts from the podium from the roundtable on this. And again, I wanted to stress the point, just because this is in a question doesn't mean that the agency is making this a requirement or says this because of some safety signal. These are just things that we thought about to perhaps -- You know, if we look at these parameters, could it lead us to a more comprehensive evaluation of the safety of the adjuvant component and, if not, well, we are happy to hear your comments and concerns on that issue.

So should other parameters such as

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cytokine levels or additional biomarkers be assessed? Who wants to take that question? Okay, we will take Deborah first and then Jan.

DR. NOVICKI: I think that it is fairly straightforward to look at things like CRP or fibrinogen, and I mentioned yesterday, if there's people in the audience who weren't here yesterday, we routinely measure fibrinogen along with the other coagulation parameters in our tox studies.

So adding CRP, it may give you a slightly more sensitive measurement perhaps or a slightly different time course post-dose than measuring fibrinogen, but they are probably telling you about a similar aspect of the biology.

As far as looking at cytokine levels, my favorite species, because I can give the clinical dose by the clinical route, etcetera, is the rabbit whenever it is appropriate and there is no reason not to use it, and reagents are not readily available for

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doing cytokines and cell sorting, etcetera, in rabbits.

Now is it an area of interest for me, for companies or something, to start to work on these kinds of things? Yes. That would be a really good tool. Then you could actually make a better link.

We do a lot of our preliminary work in mice. We ultimately end up in people where a lot of companies now are starting to take translational medicine approaches and generating some similar data in humans. Then you've got the tox species sitting in the middle where you don't look at some of the parameters that might bridge from the mouse to the man.

So I would be very interested. It is an area that I think we would have to work on, though.

DR. VAN DER LAAN: Thanks. For the biomarkers, there are numerous cytokines and other endpoints possible. I think that

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you have to make a reasonable choice for that. It should have any relationship. Why are you just studying a type of cytokine? Is it to different shapes and type of response? I think that should be clearly indicated, and why is that then chosen as a type of biomarker? What should it tell you?

Then if it is to tell you, for instance, comparability between species, what is the most relevant species? I think that that is very important. Do mice, or can be used also other species. But is there any relationship between a biomarker and a final effect? Is the studying of CRP or fibrinogen then a response indicator for a clinical effect? That is an important issue. You first think for a blind.

Toxicology is just trying -- and I'm happy with the discussion that, from the clinical point of view, toxicology is indeed mainly -- and you see that in WHO documents -- mainly to guide the study design for the

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clinic. All other effects are later on and just highlighting some aspects, but some aspects in animal studies can be done at a higher dose and might be more -- give more feeling of what can happen in an organism; whereas all that type of studies cannot always be done in humans.

So we have to be careful in this type of using of biomarkers. What is the real meaning of these type of biomarkers?

DR. GRUBER: Thank you very much for this comment. We will take one more, I think, on this issue. then we will move on.

DR. VAN DEN BOSSCHE: Just a very short comment. I think we may be discussing this question not that much in terms of what cytokines exactly. I think we should be thinking about what are really cytokines, depending on the animal species we are testing that are, for example, relevant for local inflammation.

I think the parameters that are

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there, I guess, in most of the animals are relevant, the CRP, fibrinogen, for example, and also which are the parameters could be markers, not necessarily biomarkers but markers, for systemic distribution.

These are really the two types of phenomena we are concerned about, local inflammation, systemic distribution. So to the extent that the parameters we are testing are relevant for the animal species we are doing the testing in, I think this would make sense, yes.

DR. LEVY: Just a quick comment. This is Ofer Levy, Harvard Medical School in Boston Children's Hospital.

It is something we have struggled with, and I think some of the members of the panel touched on it, which is it is easy enough to do to measure these cytokines, but does it correlate in any way? Is it at all predictive of toxicology or other downstream problems? I think that is very important to

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keep an eye on.

It would be nice if we had a body of literature that indicated the level of cytokines for the already approved vaccines that are induced in humans, so that you have a backdrop on what you are comparing to.

My concern is that, if you start measuring for a lot of things with novel high sensitivity assays and pick up trace production of certain cytokines and then somebody pulls out a paper from Journal of Immunology and says, well, cytokine X has been associated with encephalitis in a certain model, the next thing you know somebody takes that as proof that your vaccine is going to cause autoimmunity. That is very weak kind of thinking.

So, obviously, we need to proceed in a thoughtful way and with some caution. I think it is good to measure these, but in a thoughtful way, and I think it is complicated.

PARTICIPANT: I don't know if I

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need to say anything more than what was just said, because just adding things on is regulatory creep. Even CRP -- you know, we have been asked -- We do fibrinogen, and then, hey, why don't you do CRP? Well, that's not going to help us understand anything better. It is just another marker.

Cytokines? I can see -- you know, after the TeGenero event, I can see everybody freaking out about some cytokines. If I am working with many of the adjuvants that I work with, I expect to see a lot of cytokines. So -- systemically, whether it is happening locally or systemically or whatever.

So I don't see much value in the safety. If the biology requires you to do it or there is some reason -- we are making this adjuvant because it doesn't produce IL-6 systemically -- well, there's a good reason. It is not necessarily there's a "check the box" safety endpoint.

If you had an extended acute phase

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response, you would see that in other ways. You would see that in histopath in the liver. You would see it everywhere.

DR. NOVICKI: I don't want to be misinterpreted and having anybody think that I am proposing to measure all of these materials in large clinical trials. That wasn't the point at all.

Really, what I was thinking about was trying to grapple with some of the disconnect between what we see in animals -- I mean, vaccines -- The vaccines that we have worked with, we almost see -- We see very few signals, and actually seeing a reversible elevation in fibrinogen is one of the only things that we see with a lot of our products that are adjuvanted with MF59.

So for me, in a way, it is a marker that something is happening that I am seeing an effect that I expect to see, and seeing that its reversibility is happening in the appropriate time frame that I am used to

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seeing is helpful from the standpoint of evaluating that the biology is similar to things that we have seen before, and it is not something that is persisting for much longer, which might be an indication of a longer systemic reaction.

So I'm not saying that we should measure fibrinogen necessarily in clinical trials even. I am just saying that for our purposes that is a helpful marker for our adjuvant.

I think that -- I was also thinking a little bit more in an investigative mode when I was thinking about trying to bridge between mouse, rabbit and man. So I think, when we -- A mouse can't complain about malaise. A rabbit doesn't tell us that it's got a headache. So some of the adverse things that we capture in clinical trials, if we are trying to select, say, internally amongst a panel of adjuvants, some of this sort of information could be helpful from a selection

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standpoint to try to -- you know, if you can find a profile that is predictive of immunogenicity without as much systemic inflammation, that might be a better choice to investigate than another that has a slightly different profile.

So I was not strictly thinking about GLP and clinical trials as much as areas where you might want to be a little bit more investigative.

DR. GRUBER: One more comment.

DR. GOTTESDIENER: Yes. Keith Gottesdiener, Merck. I am fairly new to the vaccine area. I am a clinician who actually grew up in the small molecule area.

To me, the issue really is how predictive is -- The previous speakers had said, how predictive are the things we measure in the animal tox studies to help us in the clinic I don't see any other purpose, really, of doing these things.

On the other hand, I share the

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concerns of other speakers that just measuring these things without understanding their meaning is really worthless and will cause a lot of anxiety.

This reminds me, actually, a lot of the discussions that happened with the agency a couple of years ago about genomics testing and clinical trials where the agency actually took -- in the U.S. and in the EU took really a leading role at forming a safe harbor type of approach.

What they said is we would like to collect that data. We are not going to make interpretations upon that data today, because we have no basis upon which to make those interpretations, but we are going to begin collecting a database so, as we take those things into the clinic, we can begin slowly over time -- maybe it will be a 10-year plan -- to sort how valuable those things are going to be.

At the moment, if we measure these

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things and we don't have that safe harbor, I think many companies are worried that those tests will be misinterpreted.

On the other hand, I think everybody would agree that trying to understand prospectively how valuable things are in tox studies to eventually predicting in the clinic would be a laudable goal.

So I actually challenge the agencies to think about ways where they could actually be the mediator of our process, where we could collect the information without detriment to the present and yet still build plans for the future.

DR. GRUBER: So we decided we are going to advance to the next slide, because we wanted to actually get a couple of discussions going on the animal species on the point on how in vitro assays should -- or can they be -- incorporated into nonclinical safety assessment, and then the issue that was brought up by the CDC on how to evaluate and

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test potential combinations of different cytokines that are formulated with different vaccine antigens and may be given concurrently.

So this is basically -- For me, this is maybe the most difficult issue to answer, and that is the question: What constitutes a relevant animal model?

Perhaps we can actually take these first two questions. Is it sufficient to test in only one animal species, and what constitutes a relevant animal model, together; because in my view at least, it is very difficult to really get your arms around to get even one animal species that you may consider relevant.

That is the reason, I think, at least why from a regulatory perspective we have made the recommendation that it is sufficient for vaccines to test in only one animal species. However, the question was raised again: Is that sufficient when you

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look at the safety assessment of adjuvants?

I would like to make the point that this issue was not necessarily raised by the regulatory agency. So I would like to receive some comments on the issue about do we redefine what is a relevant animal model? Hana, you wanted to make a comment? Thank you.

DR. GOLDING: Yes. I think this is -- Again, I am just expressing my sort of personal thoughts, not as a representative necessarily of the regulatory agency. But the more I am thinking about this whole development of novel adjuvants -- and I really want to echo what Derek was saying -- especially when are starting to look at novel adjuvants, the more we know about them, the better.

I would like to really propose that what we need to think as a group is what I would call progressive testing, and this progressive preclinical testing or animal

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testing may start during the discovery period. It clearly has to be tailored to the type of adjuvant.

If it is a TLR agonist, clearly you want to test it in the best animal model that is appropriate in terms of specie specificity, but sometimes that is not available immediately.

So I don't think we should have to think right now what are the tests to do in the rabbit. Rather, we have to think how to really match our evaluations of a novel adjuvant to the product itself, and what additional testing one can do.

Right now when we look at immune response to a novel adjuvant in combination of vaccine, really, we are measuring the immune response, namely the antibody response, the CTL responses.

We haven't actually started to look about are we inducing any changes to the Treg. Are we using any changes to the level

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of T17. Some of those novel type of subtypes of T cells that are so important to keep the balance of effect versus autoimmunity.

Maybe this is the time during the discovery period when you are using an adjuvant with a known biological activity that, on the one hand you introduce it to increase the type of antigen specific immune response. What other type of disturbance overall to the immune system may be induced?

I think this kind of sort of stepwise approach doesn't necessarily mean that animal studies stop when the clinical studies start. Very often, we really did not learn anything or did not find any safety signals in the rabbits or the preclinical studies, moved into the clinic, and all of a sudden we see reactions which we did not expect.

There is nothing wrong of saying, okay, now based on these signals in a small number of people, can we go back and find the

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appropriate animal model? It might be nonhuman primate. It might be another model that will help us to understand this particular reaction.

So I think we shouldn't look at the sort of preclinical studies or animal studies as a sort of stand-alone, one-time talks. You do it. You finish with it. Whatever you get, you don't have to revisit. I would like to see it more as a sort of progressive approach that is tailored to the type of the adjuvant and continue even in parallel with the clinical trials.

DR. SEDER: So I would like to follow up a little bit on that. There is important species differences in the expression of Toll-like receptors between mice and primates and humans.

So most of the studies that have been done with adjuvants have always looked at antibody. You really can't assess T cell responses in rabbits, and you will get very

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different results in the case of, say, CPG from what you would see in a mouse, from what you would see in a nonhuman primate.

So if you are after T cell responses, you are likely going to have to do trials in nonhuman primates to try to predict whether Toll-like receptor ligands or other adjuvants would be effective.

You have reagents that exist in primates similar to human to measure such responses that don't exist in rabbits. So at least in terms of understanding immunogenicity for cellular immunity, it is likely you are going to have to use primates. They also represent an outbred species. So you can get some idea of the type of repertoire you get that wouldn't sometimes be predictive in the mouse, because they wouldn't express -- because they are restricted by certain HLA haplotypes.

So I think it is a problem, because primates are expensive, and they are

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limiting. But in terms of going forward with more novel types of Toll-like receptor adjuvants for T cells, you are likely going to have to enter into doing primate studies, and it will give you a lot more information than what currently exists.

The other thing that came up on this slide was using in vitro predictability with Toll ligands really would mislead you. So Toll-7 and 8 is a small molecule that gives you very robust in vitro responses, but in vivo, unless it is formulated, would give you very poor responses.

By contrast, poly IC is very robust in vivo, because it acts on a lot of cells that you are not testing from peripheral blood. Yet in vitro, it gives you a relative modest response.

So in using those type of screening assays, you will be very misled again, unless you go in vivo and do these in primates.

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DR. FRIEDE: Okay. So just to maybe combine the two slides, considering in vitro analysis with animals. There is the risk of high degrees of polymorphism in the receptors of some of these Toll-like receptor agonists.

So the relevant animal model would be an animal model that displays a receptor which recognizes Toll-like receptor agonists. It would be very important to use human cells and verify that the animal that you are going to use actually is able to recognize in a similar manner to humans, and this will also then enable you to design the clinical studies in a maybe more relevant way, because you may see not only polymorphism between the animals and people but also between people and people, especially between populations.

For many of the vaccines which you are trying to make, malaria, TB, HIV, we will be going across multiple populations. So being aware of receptor polymorphism at an

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early stage will be important to design these clinical studies, because this could affect toxicology as well.

DR. GRUBER: I'm sorry. We have Dr. Alving, and then Dr. Van der Laan, and then Dr. Warren.

DR. ALVING: (Off microphone comment) -- The facial palsy was observed in humans.

I have a question. Could this have been picked up with another animal model? Would it have been -- Clearly, when you are giving intranasal administration, you might get different results if you give it to a mouse than if you give it to a baboon, for example.

The question -- but you might get the same receptor binding characteristics that might cause toxicity that would cause neurological effects that might have been observed more easily in some other animal.

So what I would say in a

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circumstance like that where everybody knows that e. coli enterotoxin or cholera toxin bind to gangliocyte GM1 like a covalent bond, and that you might get retrograde travel into the neurological system, causing a facial nerve palsy by giving it intranasal, is there some way that that could be looked at?

I would advocate a more intelligent thing, looking at what is known about the particular adjuvant, and how can you perhaps address a specific circumstance.

DR. GRUBER: We will have Dr. Garcon commenting on this very comment first, and then it is your turn, Jan.

DR. GARCON: I just wanted to point out that that was identified, actually, and there was -- we saw that in mice. When you do give intra-nasal in mice, you do have retrograde transport in the passage in the olfactory bulb. So that was defined and seen, and that is the reason why we didn't move forward, actually, with clinical trials with

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intranasal vaccines.

DR. VAN DER LAAN: Thanks. Yesterday I had also given my view on what is a relevant animal model. Also for adjuvants, of course, I can describe what work has been done.

What we have done in Europe is a small study on the applicability of pigs and especially many pigs in this respect. That might be a very good alternative to the high use of nonhuman primates, which is politically, at least in Europe, highly under pressure. So many pigs are -- pigs are a very good track device, and many pigs are immunologically not different from the land raised pigs, and there are a lot of reagents available. That is at least one.

That brings me also to the point of the special populations, elderly and pediatric. One of the concerns that was expressed last week in the Vaccine Working Party when we prepared this adjuvant workshop

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from a regulator point of view is that the adjuvants -- the response to adjuvants is not well known, whether it depends on the age, and especially as a lot of vaccines are given very early in life, we do not know what is the effect in small children on long term imprinting in the immune system.

There are effects -- There are studies, for instance, on pertussis vaccination in Brussels indicating that early vaccination has indeed important consequences in inducing changes in the immune system, the very early immune system.

DR. WARREN: I just wanted to make a few comments as well on some of the other speakers. Hana Golding made a very good comment in the fact that perhaps we should be a little bit more progressive in terms of not necessarily thinking that animal in vitro studies stop when the clinic begins.

In fact, in many cases we have -- I think it is important to look at clinical

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samples in in vitro assays to help understand why things did go wrong and understand why in that subset of the population things may have gone wrong. I think that her idea of these more progressive studies is a very good idea.

Then I wanted to, in the art of being controversial, go back to some of the things that Bob Seder had said. Sorry, Bob, I figured that this is part of the fun up here, is the fact that he made sort of a blanket statement of, you know, in vitro models have not been as predictive and go to the nonhuman primate. But we have actually seen examples in our lab where the nonhuman primate has actually been incorrect in in vitro assays, have been correct when going to the clinic.

In fact, I could probably come up with more examples where the nonhuman primate model has not been predictive for human responses. You could just go to every HIV and cancer trial and come up with examples.

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I think the idea there is that when making comments about in vitro studies, you have to sort of take into account not every in vitro study is the same. Are you using just PBMC cells? Are you using one cell type? Are you looking at cell lines or primary cells?

I think that not every in vitro assay is alike, just like not every animal model is alike as well.

DR. SEDER: Can you just give me any examples of what you are talking about where it wouldn't be predictive and what you have done, and what trials you are referring to?

DR. WARREN: I'm not at liberty to say right now, but we did indicate -- I did indicate it, but I'm not at liberty to say. And I didn't mention T cells. It was more toxicology.

DR. GRUBER: I was going to take two comments from the floor here. Go ahead.

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DR. GUPTA: Rajesh Gupta from CBER. I think the adjuvant situation is so complex that you cannot generalize that one species or more than one species. I think you have to leave flexibility, depending upon the relevance of the model, that if you show that the animal model is relevant with the appropriate receptors and all that, one species may be enough. But if it is not appropriate or a relevant model, maybe you have to go for two species.

They may not be even relevant, but still you have more chances of picking up something, if you are doing more species. Similarly, with the in vitro, I think, assays also.

I think we can keep on criticizing saying that they don't matter or they matter, but doing more, if you show relevance with your particular adjuvant system, I think it makes sense. So I would say that we should have flexibility of more than one animal model

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in addition to the in vitro assays also.

DR. LEVY: Ofer Levy, Boston Childrens Hospital. I just wanted to amplify on the importance of different species. The topic today is adjuvants, and a lot of these adjuvants engage the innate immune system, and it is known that the innate immune system is hyper-variable between mammalian species. It is one of the regions of greatest variability between mice and humans, for example, and several of the speakers on the panel spoke to that.

So we are going to have to all be very thoughtful as to what animal models we look at.

The other point I wanted to bring up is similar to the point I made about the cytokines. I think the new subclasses of T cells that have been found are very important biologically, and probably important clinically, but I still don't think we are collectively smart enough or knowledgeable

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enough to know how an in vitro assay of those cells corresponds or not to any toxicity.

So if we are looking at T regulatory cells, we know that they can suppress adaptive immune responses, and we know that, likely, a transient and local reversal of T reg function is probably a feature of most effective vaccinations.

So now we have T cell phenotypes that we can test for by flow that we weren't even aware of 10 or 20 years ago, and probably what is happening when we use the vaccines that we already have approved, is that there is a transient reversal of T reg suppression.

So once again, I am just saying it is good to gather this information, but I hope we don't jump from saying, well, this adjuvant can cause a local transient T reg reversal, therefore it is going to lead to a massive autoimmune catastrophe.

DR. GRUBER: One more comment from the floor.

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DR. PETROVSKY: So I don't know the answer to this, but I guess an interesting ethical issue has been raised here, and I think Nathalie sort of alluded to this.

What happens when companies identify toxicity issues and stop development, but then watch other companies developing similar or the same product, perhaps because they haven't done the same due diligence? Is there an ethical issue there about disclosing that information, either to regulators or to the public or to the scientific community, to alert that there is an issue that has been identified? And then maybe you avoid a disaster in the clinic.

DR. GARCON: I would like to answer that. First, 1AT is not the next. So I can't comment from other that has been used. The data we generated were disclosed to the regulatory agencies.

DR. PETROVSKY: Can you comment on how they handled that in terms of approving

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the other clinical studies?

DR. SEDER: Can I just say one -- This is not one size fits all. If you look at adenoviral vaccines, what you get in the mouse to the primate to the humans in terms of different serotypes is always predictive. Thirty-five is the weakest; 26 is better, and ad-five is the best.

So right from a mouse you can kind of predict what will happen in the human. That is very clear. Most of us in this room know that, when you do DNA vaccines in mice, it works beautifully. Then you go to primates, to humans, it's much less.

With Toll ligands I would argue that it could be just misleading based on the differences in biology. So if you were talking about adenoviral vaccines, the mouse would be a perfectly good model to predict probably what you will get.

So it depends on what you are looking at. Since this room is focused on

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specific adjuvants, the only point was that you need reagents in the species especially related to T cells. These antibodies are easy to measure across multiple different antibodies, but that is not really the issue going forward.

We have plenty of adjuvants for antibodies. What we desperately need are much better adjuvants for TH1s and CD8 cells.

DR. GRUBER: Yes. So, Becky, you have a comment to make? Okay. Then we wrap this up. Go ahead.

PARTICIPANT: Thank you. So the question that Carl brought up that Nathalie has provided us information, I think is very instructive, and I wondered, Nathalie, whether this problem that you discovered was actually discovered in the course of doing your discovery work and non-GLP studies or if it was only discovered when you actually moved forward into formal GLP toxicology studies, or can you disclose that?

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DR. GARCON: So we have looked at intranasal vaccination and valued adjuvants to be used internasally, and that includes the adjuvant system we have. During the course of those evaluations, we do look preclinically at the safety profile of what we are using, and that can be different assays and most of them being in the European guidelines.

Intranasal vaccination is a different aspect, which is not covered today by guidelines, and we did look indeed at what was the effect of immunomodulator when given intranasally in the mouse. That is how we saw that.

PARTICIPANT: But was that a GLP study or was that an earlier sort of pilot study?

DR. GARCON: That was pre-GLP, yes. That was before going into human.

PARTICIPANT: Okay. So I guess my point is I want to reiterate or sort of reinforce -- I think Debbie was the one that

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said this earlier -- that perhaps a lot of these safety issues -- In these animal studies, we can only look for frequent or sort of severe things.

We are not going to find the things that Bob Chen is looking for in these preclinical animal studies. Only when you have large databases are you going to find those sorts of problems.

So I think that a lot of the animal studies that are done in discovery work and in immunogenicity testing actually could be very informative for finding the kinds of things that the regulators are trying to find with these studies.

I think the drug toxicology studies -- often by the time you get to that point, you have already identified your starting dose. You have done that in your immunogenicity studies, which were non-GLP studies.

You are going forward into the

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toxicology study with that dose you have already decided you were going to start with. So I think by then it is almost too late to be defining, quote, "your safe starting dose."

Also, Debbie pointed out the difficulty in bridging between -- You do these studies in the animals even measuring the same parameter, let alone the fact that there are many parameters you can't measure in the animal, that you ask the human do you have a headache, do you have malaise, do you have myalgia. But even when you look at the same parameters, something like ALT, when you measure it in the animal, you are doing -- you are comparing group means between the control arm and the treated arm, and you are looking for a signal based on statistically significant differences between group means.

When you do your Phase I study and you look at ALT, you are looking at the individual, and you are comparing that individual's result to a normal range and

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deciding whether or not that it had an adverse event based on a toxicity scale that says it is so many times the upper limit of normal.

So you can't even bridge the tox GLP data to the human data, by and large. So I think it is very difficult to expect these toxicology studies to do what I think the regulators want done for vaccines, and even for adjuvanted vaccines.

So I think we need to be more mindful about looking for things in the course of even the non-GLP studies, immunogenicity studies, etcetera, and looking to those studies more for our safety parameters, because I think it is very difficult. These toxicology studies really aren't serving the purpose that, I think, we need.

DR. GRUBER: Thank you for this comment. I don't think I fully agree with that, but we are going to go ahead and hear Bill, and then we are going to spent the last 10 minutes discussing yet another question.

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Go ahead, Bill.

DR. EGAN: Thank you. Looking at these questions, they are very, very complicated and difficult, and if we look, for example, about just one versus two animal species, I think it is very likely that one answer does not fit all and that it depends on the type of adjuvant that is being looked at and the mechanism of action of that adjuvant, at least to the extent that it is known.

I think it also depends on the questions that you want answered. For example, if an adjuvant is a TLR agonist, do you want to animals to measure cytokine responses, and then what do you do with that data? Are you looking for unexpected responses or interactions with other TLRs or for something else or organ pathology?

Also, designing what to look at versus the populations for which the vaccine is intended or for which the adjuvant will be used, elderly or pediatric or

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immunosuppressed.

I think, as we answer these questions, it is necessary to remember that the issues with, for example, a CPG oliogo versus the e. coli labeled toxin versus, say, an oil and water emulsion with squalene are very, very different and difficult to address with one single prescription.

So I think we are going to have to come down to something that is more tailored to the particular adjuvant.

DR. GRUBER: Yes. I thank you for these comments, and I think they are very well taken.

I think what we have heard, really, from this discussion is that we really have to allow flexibility. We have to look at the compound under study. We have to see what is the perceived mechanism of action, if there is an animal model available, if there is no animal model available, and depending on that, I think, we will have to allow flexibility to

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build in other outcome measures to perhaps help us to make an informed decision.

That can be the pre-toxicological assessments that Becky referred to, what we often refer to as pilot immunogenicity and mechanism of action studies, together with approaches to look at in vitro models, as pointed out by Dr. Warren.

I think, for these novel compounds, we really have to sort of keep thinking out of the box, if you will. That, of course, is complicated by the fact that, if you really want to do a toxicity study, your animal model has to lend itself also for this type of evaluation, which is why we usually use the rabbit or the rat or the mouse.

There is a historical control database. There is a lot of experience with that, and getting into issues such as the nonhuman primate -- I mean, the agency or the Office of Vaccines has always taken the approach that we are using these models only

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when absolutely necessary, because we also have to be mindful of the refinement, replacement and reduction of animal models, and then coming in saying, well, we have to use the nonhuman primate, because we don't have any other animal model available to us.

I think we have to think about that very carefully and maybe have this as a last resort after we look at all other options.

So I would like to actually come to the perhaps last issue to be discussed, and I am going to get up and flip forward here.

I think we have heard some comments about testing or not testing the individual components in an adjuvant system. We are going to skip this.

I wanted to get back at something that was brought up by Dr. Chen, I think it was, about -- Is this working? It's not working, right? I'll go back to my place.

I wanted to actually talk a little

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bit about the -- I think it is the second bullet perhaps combined with the first, and it speaks to the potential exposure to multiple types of adjuvants, either concurrently administered or over multiple years.

So let's take a theoretical example. You have a vaccine that is indicated for adolescents, and it is a novel vaccine. It is combined with an adjuvant. The adolescent population also is supposed to receive a second vaccine that is also combined with another adjuvant.

So it is getting at testing of concurrent vaccine combinations that are combined with novel adjuvants, and it gets at the fact, what if some of these vaccines have to be given or administered over multiple years such as adjuvanted influenza vaccines?

Can we even get our arms around that in the preclinical setting or is that something that should best be addressed in the clinical arena?

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So if I can hear some comments on this issue.

DR. FRIEDE: Since nobody else is doing it, I may get myself into trouble.

I think this is very difficult to do at the preclinical level. My gut -- shooting from the hip, I would say, that initially we would have to manage this at the clinical level, but I would suggest that research is undertaken to actually to try and examine whether at the preclinical level we can pick up anything which is interesting.

I think we are still so far away from this that we just don't know. So for the moment, I would say clinical, but we should be doing some research to see whether there are animal models that could help us identify this.

PARTICIPANT: I had a comment on this. Probably, your pharmacological data in the -- both in the clinical aspect as well as preclinical data in developing this can

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facilitate that, in that you are looking at clearance from the injection site and activation of the immune system, and clearance of the material. So that you are not going to get a cumulative effect, as was brought out for the thimerosal comment.

If things are allowed to clear out in sufficient amount of time, you won't get this, say, multiple effect from different adjuvants and different antigens being administered at the same time.

DR. ALVING: I just want to point out one thing, and that is that, unless you are talking about one of Darwin's tortoises that lives more than 100 years or something, when you are talking about exposure over multiple years, the life span of a mouse, for example, is about two years.

I actually have done injections of Lipid A and lipisomes containing Lipid A sequentially over the entire lifespan of mice, and I actually published that. Actually, what

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I found was injecting normal saline had a devastating effect on the mice in the sense that all of the mice -- or I would say most of the mice, actually, all had tumors when they were at the end of their lifespan.

So the question is, when you are talking about giving adjuvants over multiple years in animals, what is the animal species that we are talking about here? This is a really important question.

DR. VAN DEN BOSSCHE: Long term studies, animals or humans, I think there are -- Just to pick up the last question, there are for some exogens long term studies with monkeys for 10 years and ducks for seven years, but those are exceptional studies.

Later this week I will have a talk on carcinogenicity, and I would like to get rid of the two-year mouse and two-year rat study, because of all that spontaneous tumors. They do not indicate anything. So we should not go into that direction.

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On the one hand, we have limitations in our animal studies, and there are important aspects in human studies. So what type of endpoint should be studied in humans, and how should we monitor?

I think it is important, therefore, to have advantage of the request for risk management plan in the regulatory field and to ask companies to have a very good monitoring for the first 10 years for specific aspects that cannot be reasonably studied in animals.

So we should not -- We cannot over-ask our animals, and what is the most appropriate timing? I don't know. We have discussed that last week with several clinicians, and some people said, yes, and all the immune reactions should be public within 10 years and, if it is not, okay. Then it should be okay, but at least there should be a careful follow-up.

I am not sure whether that will be

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discussed in the remaining part of the day.

DR. SUN: Wellington Sun from CBER. I am relatively new to the vaccine regulatory field, but from what I have seen, these questions posed, I think, maybe should be posed in a different way.

I don't think there should be much argument in terms of looking at long term effects of these adjuvants and the cumulative -- potential cumulative toxicities, but the way the vaccines are developed in this country is by companies, and companies have their own adjuvants, and some of them are proprietary.

So the question, to me, is not whether these studies should be done, but by whom. I think in developing a product many companies will not be looking at adjuvants of other companies and looking at how that would affect toxicity. Even if we had good animal models to predict those kind of toxicities, that won't be done by the private sector, I think.

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So I think there is some responsibility by the public sector to address these questions, but I am not sure how in this context right now.

DR. GRUBER: Thank you very much then. Are there any additional comments from the podium here?

If that is not the case, I would like to conclude this roundtable discussion. I thank you very much. I think it was very helpful, very stimulating, and I think we are going into the next -- the clinical session of this workshop. Thank you very much again.

(Applause.)

MODERATOR SLATER: Thank you all very much. We are going to go ahead and start the next session right away. So please take your seats. There will be a break at 10 after 10. Please take your seats.

Everyone, take your seats. We are starting Session 4. Thank you very much. Take your seats, please.

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If you will look at the schedule, Session 4 is actually quite long. It goes from now -- you still can't hear? No? I'm hearing an echo. Okay.

Session 4 begins now. We are continuing for about four and a half hours, but that is not so bad, because we actually have lunch and two coffee breaks. Thank you very much.

I would like to introduce my co-chair, Dr. W. Ripley Ballou. He is the Deputy Director for Infectious Disease Development and Global Health at the Bill and Melinda Gates Foundation.

He is going to introduce this session. Just a note for all of you and for the speakers. The timing today is somewhat tighter than it was yesterday. For one, we have only scheduled a one-hour lunch. So we can't shave that down too much.

Second of all, there is some other event, maybe a wedding or something, tonight

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in this room. So we actually have to be physically out of here by 5:15. That's good news in some ways, bad news in other ways, but we are going to need to stay to a good schedule for the rest of today.

I also want to in advance thank Dr. Ballou. He was really involved in the planning process for this whole meeting right from the get-go and just about at every twist and turn, and there were many twists and turns. He was really very constructive, very helpful, and a good force and influence in putting together this session.

So thanks very much, Dr. Ballou, and I will turn the session over to you.

DR. BALLOU: Thank you very much, Jay, and thank you for the opportunity to co-chair this session. It is great to see so many friends and colleagues in the audience.

Just to give you a brief agenda. We will have this introduction, which I will keep to less than 10 minutes, then 20-minute

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well controlled time presentations from the vaccine developers focusing on their clinical experience, a 90-minute roundtable with audience participation, and we ask, so that we can maintain the schedule, that we hold the questions between speakers for the roundtable.

Now the clinical goals for vaccines with new adjuvants are, of course, to optimize vaccine efficacy, and we believe we do this by increasing the optimal -- by identifying the optimal formulation. That will give us an increase in the magnitude and breadth of the immune response, but the flip side of the coin is that we are also trying to maximize safety and, inherently, one approaches this by trying to use the lowest amount of adjuvant that you need and the fewest doses that you can deliver to reduce the risk that you will have an issue with safety.

The challenge, of course, is early detection of possible safety signals.

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Clinical trials must be designed that demonstrate the need for the adjuvant, that determine the optimal adjuvant dose, that down-select between different adjuvant formulations when that is a situation, and it commonly is, that characterize short-term safety and reactogenicity profiles, that allow you to appropriately dose range across different age groups, and to assess long term vaccine safety.

I don't think there is a lot of debate about whether these are important parts of the clinical development program for new adjuvants. The issue is how do we do this in a cost and time effective fashion.

When one looks at assessing local and systemic adverse events, which we refer to in the vaccine community as reactogenicity, there are issues about methodology, and it has been very difficult historically to compare reactogenicity of various adjuvanted vaccines across platforms and across companies.

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I think there has been a lot of constructive work done in the last several years to try and standardize approaches to this, and hopefully, as we move forward, there will be better comparability across platforms.

One of the things that I have been impressed with is that at least with some adjuvant formulations, there really are age-specific reactogenicity. For example, in some of the adjuvants that I have worked with, we have seen the greatest reactogenicity actually in healthy young adults and with the same formulations having considerably less reactogenicity in the elderly and in young children.

Is reactogenicity a predictor of long term safety? I think this is a question that is not answered, and in my mind, it really does -- It has been a confusing issue in the clinic, because frequently early on in clinical development when you are still looking at the proper dose, and maybe not even

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in the target population, you do see local reactogenicity with some of these adjuvants.

Whether these are, in fact, going to be predictors or not, I think, is an issue, but it slows down clinical development as you debate that question.

How much detail is enough to collect in clinical trials? Are biomarkers an appropriate adjunct for reactogenicity or safety measures? And as we begin to have more and more access to complex immunological tools, a logical and direct consequence of this is it is driving up the cost of doing clinical trials, which is an issue that, I think, concerns everybody.

If you are monitoring for rare events, it is obviously an issue to be able to detect a doubling over background incidents, and this assumes that you know or can measure these background incidents, and that is obviously an issue.

There is a little bit of an issue

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on my slide here, but we saw these figures yesterday for incidents rates of diseases like intussusception with rotavirus like SLE or Guillain -Barre Syndrome where the background rates are very, very small. It takes very large clinical trials to be able to detect a doubling of these background rates.

How can we better design studies to assess the risk of rare, serious adverse events that can be real issues for vaccines as classes?

What do we know about the clinical experience with new adjuvanted vaccines? There is a handful of vaccines for which there is now considerable clinical experience, in particular, the seasonal influenza vaccine that is adjuvanted with MF59 where there is certainly well more than 10 million doses. Individuals have received these vaccines over a number of years.

The HPV vaccine that is adjuvanted with ASO4 is probably at least 500,000

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individuals. H5N1 with a variety of adjuvants is in the order of 10,000. Malaria vaccines adjuvanted with AS02 are in the multiple thousands, HSV with AS04 in the multiple thousands. But this represents less than a third of all the vaccines that are being looked at with new adjuvants, and the vast majority of these are -- the human experience to date can be measured only in the hundreds.

So there is a large amount of data that will have to be collected around these other vaccine candidates as we move forward in order to be able to say something about the safety of these adjuvanted vaccines in the future, and this represents a challenge.

So as we go through the discussions and as the presenters come through today, I would like to give you a highlight of what we are going to be addressing in the roundtable.

There are three or four classes of questions: How can we design studies that

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will detect (a) specific differences in adjuvant responses, that provide long term safety information, that provide dose ranging data on adjuvants as well as antigens.

How can we design studies that will incorporate safety information obtained from preclinical data?

How can we design studies that will incorporate information obtained from previous clinical trials using the same adjuvant?

These are some of the questions that I, hopefully, will have addressed by our series of speakers over the next hour and a half.

So I will stop there and invite Giovanni della Cioppa.

DR. DELLA CIOPPA: Well, good morning, and first of all, thank you, the organizers and the chairpersons, for giving me the opportunity to be here with you and present our clinical data.

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I am going to focus on MF59, as mentioned a minute ago. Indeed, we have a considerable body of evidence with this adjuvant, especially because it has been -- it is a component of the seasonal influenza vaccine Fluad which had been on the market since 1997.

Fluad is marketed in 26 countries, the U.S. not being one of them, but it is marketed in Germany and in France and Spain, and in Italy and New Zealand, in Australia, in many other countries. Therefore, there is a substantial amount of clinical experience with this adjuvant, over 40 million doses distributed worldwide.

There is also a substantial amount of clinical trial data. We tested MF49 in various permutations in over 33,000 subjects. This will be the object of the talk, because on request of the FDA and as part of a drug master file that we recently submitted, we have embarked in the big effort of generating

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the pooled analysis on all clinical trial evidence we have.

I am going to tell you a little bit about the overall objectives. Then I am going to focus briefly on the way we have measured and defined the outcomes, a few words on the methodology, the population, and then I will dive into the results.

Fundamentally, this large pooled analysis was carried out to address the following questions: Compared with non-MF59 containing vaccines -- so not in absolute terms but in relative terms -- do MF59 vaccines increase the risk of nine outcomes: local reactogenicity, system reactogenicity, all adverse events, autoimmune diseases, cardiovascular diseases, all serious adverse events, new onset of chronic diseases, hospitalizations, and death?

So the first thing you have to do when you have in front of you a task like this is to be quite precise on the definition of

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these outcomes, and I apologize for this busy slide, but it is important for those of you who are in the course of clinical trials that these definitions are rigorous, are predefined, and are agreed with the regulator before you do the exercise.

So the first two, of course, are what we call reactogenicity. They are solicited, which means that in the case record form the investigator and the subject is asked whether or not a certain thing happened.

There's a number of them, but with regard to local reaction, the most important ones are ecchymosis, erythema, induration, pain, swelling and tenderness.

With regard to systemic ones, the most important ones that we have looked into are arthralgia, chills, fever, headache, malaise, myalgia, and nausea.

Outcome number three are all adverse events. These are unsolicited events, though there is no specific question in the

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CRF, which may be occurring at anytime between the moment the patient, the subject, entered the study, and the moment the subject exits the study.

The all important outcome of autoimmune disease is actually defined as a subset of the previous one, of the adverse events. So the full AE dataset was coded using the MedDRA version 10-1, and autoimmune diseases were identified using the 34 preferred terms that are listed on the right side of this slide.

Now in order not to miss any, for 7 preferred term, the search went broader and also related preferred terms as defined by the standard MedDRA queries were also included. For instance, for aplastic anemia, in order not to lose anything and to be as considerate as possible, we also included the related terms according to the MedDRA standard queries such as leukopenia, thrombocytopenia, and so on. So the most -- a very conservative

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approach.

The fourth outcome, cardiovascular diseases, is also a subset of all adverse events.

Then we have serious adverse events, all of them, unsolicited, occurring at anytime during the trial, the definition being the classical one in the clinical trial.

Outcome number 7, new onset chronic diseases, was defined as a subset of serious adverse events, where new onset was defined as a condition which was not recorded in the medical history of the subject, and chronic was defined as no complete resolution within 30 days of onset.

Important to note is that excluded from this outcome were infectious diseases, diseases associated with congenital structural abnormalities, malignancies with first diagnosis earlier and three months after the last study injection.

Finally, the last two were

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hospitalization and death, and here again the point to make is that these were solicited. So they were a specific question in the case report form. Of course, there is a big difference between solicited and nonsolicited events in the way they are then captured.

The population: The main population of the meta analysis is the one you see on the far left of the slide, all indications, all studies. This was then divided into two subpopulations, the flu trials to give a more homogeneous idea of how the adjuvant could behave, which included seasonal and pandemic flue trials, and the non-flu trials that we have -- We have conducted trials in five indications, cytomegalovirus, Hepatitis B, Hepatitis C, HIV and Herpes simplex.

Each of these populations was then analyzed by age with four age categories: All ages, children, adolescents less than 18 years of age, nonelderly adults 18 to 65, and

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elderly 65 and over.

In this presentation I will focus on three populations, the main one, the primary one, all indications, all ages; then flu, all ages; and because of the importance of adjuvants for the elderly, I have decided to also touch upon flu, elderly.

A few words on the approach, the statistical approach. We have looked at the comparison between the group receiving MF59 and the group non-receiving MF59 in terms of risk ratio. Now for events that occurred in fixed time windows such as reactogenicity, we use a weighted risk ratio based on the pooled Mantel-Haenszel type estimator weighted by size of study; whereas, for events occurring at anytime during the study, the majority of them, such as unsolicited AEs, we used an adjusted risk ratio based on Poisson regression model adjusted for the number of days in the study and the number of vaccination.

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Important to keep in mind is that, at least for flu studies, the number of vaccination is an indirect adjustment for age, because the children get more vaccination get the adults and the elderly.

Now the population: A very large population, over 33,000 subjects in total, of which almost 28,000 were flu studies and about a bit less than 6,000 were non-flu studies. Of the flu studies, the vast majority of studies were conducted in elderly, with almost 20,000 subjects. So the database is large.

Let's go now to the results. This slide, which is the P slide in this presentation, gives you the results for the primary analysis.

This is the so called forest plot, and I'm sure many of you are familiar with this kind of graphic expression of the risk ratio. The bottom line is that each of the nine events has kind of a branch with a dot in the middle and two whiskers on the side. The

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dot is the point estimate of the risk ratio, and the two whiskers give the 95 percent confidence interval.

If the whole branch is totally on the left or on the right of the vertical line, then there is a significant difference in favor of one of the two groups.

So this slides gives a number of, I think, very interesting hints on the safety of MF59 and beyond. First of all, if you look at the first two outcomes, there is a marginal but statistically significant increase in the MF59 group in local and systemic reactions.

So reactogenicity is increased. We knew this. This is confirmed by this meta analysis, by this pooled analysis. It is also true that the risk ratio, the marginal increase in risk in the MF59 was small.

Now if we now skip to the fourth outcome, and this is autoimmune disease, we see that the confidence intervals are very broad, and they cross the vertical line, which

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means that there was no significant difference between the MF59 group and the no-MF59 group in terms of autoimmune diseases.

The rest of the outcomes came as a surprise to those of us who have done this exercise. If you look at the third outcome, all AEs, you see that the risk ratio is .75, and the confidence intervals are all on the left side of one, .71 and .80, which means that the group who received MF59 was overall at a lower risk of adverse events compared to the group who did not receive MF59.

If you go down the list with the other remaining five outcomes and you look at cardiovascular diseases, serious adverse events, hospitalizations, and death, you see the same pattern. You see that for all four of them and marginally also for new onset chronic diseases, you have a significantly lower risk in the group that received MF59. So there were fewer cardiovascular events, fewer SAEs, fewer hospitalizations, and fewer

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deaths in a statistically significant fashion.

I think what is particularly interesting is the cardiovascular disease. You look at the risk ratio of .46 with 95 percent confidence interval of .38 to .56.

Now what happens if we move from all comers, all indications, all ages, which again was the primary outcome to flu? Here is a much more homogeneous indication. This is all flu, all ages, 28,000 subjects.

Again, the pattern is very similar. You have a marginal but significant increase in reactogenicity. You have no significant difference in autoimmune disease. The point estimate switches from the left side to the right side, but that is not really relevant.

There's very few events. There's 10 in total. So one more or less makes the point estimate fluctuate, but the important thing is that the confidence intervals are very broad, and they cross the vertical line

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of risk ratio of one.

You see again then for new onset chronic diseases, cardiovascular diseases, serious adverse events, hospitalizations and death, there is a significant decrease in the risk in the group receiving MF59 compared to those not -- the group not receiving MF59 in flu trials for all ages.

Again, a similar pattern is repeated again when we restrict the population even further to an even more homogeneous population. Here we have all flu, but only elder. Of course, the -- and the numbers go down, but they are still significant.

Again, significant increase of local and systemic reactogenicity, no significance difference when it comes to autoimmune disease, and significant decrease of adverse events, serious adverse events, cardiovascular -- not hospitalization. Hospitalization and deaths here are marginal.

Because of the importance of

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autoimmune diseases for this discussion around adjuvants, we have conducted a number of sensitivity analyses to see whether the outcomes were affected by changing a little bit the rules of the game, so to speak.

So what you see here is, for both all indications and for flu, three additional analyses we have conducted to test the robustness of the primary analysis.

The first sensitivity analysis was done by adding a very large trial. It goes under the code of V7P35, which actually had 30,700 subjects, but was not included in the original analysis in the original database, because the collection of safety was incomplete.

In this study, only AEs were collected, necessitating a physician's visit and occurring only during the first week, and then SAEs and hospitalization and death occurring throughout the study were collected. When you add this study, you obviously

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increase considerably the database.

The second sensitivity analysis was done by removing the subjects with a history of autoimmune disease, and the third sensitivity analysis was done by having both combined, by adding this large study, V7P35 and removing the subjects with a history of autoimmune disease.

What you see is that for all indications there is a very minor fluctuation with these sensitivity analyses, whereas for flu the risk ratio goes down from about 2 to 1 and then to less than one as you conduct the sensitivity analyses. But overall the signal -- the direction of the signal doesn't appear and doesn't change.

As usual with these large efforts, there are a number of problems. I will not even try to address them, but obviously here we have a heterogeneous population. There is a different observation period, different number of vaccinations, different study

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designs, different health condition at baseline. In flu, the subjects were healthy, and in non-flu the subjects weren't.

A very difficult thing was to merge the extension studies to the original studies in order not to double count events, and also we had to include, and we did include, studies with a second adjuvant where, of course, the safety profile of the second adjuvant, which often was much worse than the one of MF59, kind of contaminated, in a way, the outcome.

Before giving you a final slide, I just want to show you what we are trying to do now to follow up and to confirm the observations that we have done in this large meta analysis.

We are conducting a large prospective observational study, which goes under the acronym of LIVE, which stands for Lombardy Influenza Vaccine Effectiveness study. It is a prospective observational

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study.

I would like to propose maybe for the discussion later that this can be a very useful tool to assess long term, with the kind of numbers that were mentioned before, the safety of a vaccine.

This study is done in the population of elderlies. It is done in one region of Italy, Lombardy, in different local health units, and again it is comparing an MF59 containing trivalent influenza vaccine with the equivalent without MF59.

It is done over three influenza seasons, and again this can be an interesting and useful maybe methodological suggestion for when you need to do such large efforts. You don't have to do it all in one season. In fact, we are doing it over three seasons, last year's season, this year's season, and next year's season, so that we can reach a sample size of at least 150,000 subjects.

The goal, the main goal, is to

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compare MF59 to no-MF59 influenza vaccine for the risk of hospitalization and for influenza related diseases, diagnosis of influenza and pneumonia. So real bottom line, real effectiveness, real hard core stuff that justify the use or non-use of an adjuvant.

This is a number of secondary endpoints that go in the same direction: Overall mortality, cardiovascular mortality, risk of hospitalization, direct cost, cost of antibiotics, and so on.

It is interesting how this thing was set up. Of course, all subjects -- this is a prospective study, observational -- had to sign an informed consent. The vaccinations were delivered by the district health care providers. The outcomes were collected to the hospital databases, and the link of the outcome to the vaccination was done through the Social Security number of the subjects.

In the first year we enrolled almost 44,000 subjects. In the second year we

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are over 50,000, and we expect to reach the mark of 150,000 by 2010.

So to conclude, and this is my last slide, going back to the meta analysis. What kind of answers do we have to the questions that we have started with?

Is there an increased reactogenicity? Yes. it is marginal, but it is significant.

Is there increase of autoimmune disease? No.

Is there an increased risk of AEs, cardiovascular disease, all SAEs, new onset of chronic diseases, hospitalization and death? No. In fact, there seems to be an overall trend for fewer events in the MF59 group which, of course, will have to be addressed and studied in different contexts and confirmed by different trials and, hopefully,. by different manufacturers.

This is the end of my presentation. Thank you very much.

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(Applause.)

DR. BALLOU: Thank you very much. Are there any questions for Dr. della Cioppa?

PARTICIPANT: Giovanni, just a very quick one. A very interesting presentation.

Is LIVE randomized and blinded?

DR. DELLA CIOPPA: There are about 100 trials. About 60 percent were randomized, and the remaining were uncontrolled. Of the randomized, most of them were observer blind.

PARTICIPANT: I was speaking specifically of the prospective study, the LIVE.

DR. DELLA CIOPPA: Oh, it is an observational study. So it is not.

DR. CHEN: Two questions. First is that one of the challenges in the safety field is that safety cannot be measured directly. It can only be inferred indirectly from looking at the routes of absence of multiple different adverse events.

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One of the challenges in the safety field is that, if we take ourselves back in history, that in the field of physics and chemistry, without the establishment of a standardized periodic table of elements, that field cannot move forward scientifically.

So with safety, until we start to standardize which case definitions and how we look at the adverse events across different trials, it becomes very difficult for us to make sense of the data in a truly meaningful way.

There is a collaboration called the Brighton Collaboration that has been established to try to standardize that. For those of you in the audience who are not familiar with that, I would encourage you to go to that website so that, as you conduct your trials, your data could be collected in a more standardized format.

I was curious. Did you guys think about using the Brighton Collaboration case

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definitions in your study?

DR. DELLA CIOPPA: We are very familiar with the Collaboration, and we are all -- we are actually collaborating, and we are using it. Clearly, in this exercise we used studies that went back up to 15 years. So, clearly, we had to use what we got, but in fact, you raise a very important point.

Standardizing outcomes is critical, and equal critical is to predefine outcomes. That is why I tried to kind of define them for you. I would go beyond that. Standardizing measurements is equally critical, because one of the most difficult things that happens when you do a meta analysis, when you have different ways of measuring the same thing in different studies.

If you have even the most innocent looking thing, such as race, if you have in one study three races and in another study seven races, you have to create an algorithm to combine them, and you can multiply this by

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a billion. Then you are going to have the level of complexity that you are facing with these pooled analyses.

As you said, the more complicated they become, the less reliable the results are. So standardization is actually a key to these efforts. So it is a welcome effort that you are doing.

DR. CHEN: The second comment is: Kind of one of the most provocative findings was the relative difference in deaths in the trials. I was wondering, is there a way to kind of go back and adjust for seasonality, etcetera, and look to see if there are any differences in characteristics of the MF59 flu vaccine versus others to see if that might be a real finding?

DR. DELLA CIOPPA: These are very new data. They are actually unpublished, and we are seeking publication for them. We will indeed do that. An important thing, however, is to warn against over-interpretation and an

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attempt to over-choreograph the data. But we will certain look at individual cases to see whether there is any lessons learned.

PARTICIPANT: As far as autoimmune disease, could you comment on the follow-up, because oftentimes flu studies only go to 28 days or sometimes six months, but long term follow-up in your flue studies is sometimes uncommon. So that would be one question, just exposure time.

Then the other question would be: In Hepatitis B, typically, there's more vaccinations in a single vaccination. So did you find anything in the subset of Hepatitis B, and how long were they followed for safety. I think I'll stop there.

DR. DELLA CIOPPA: Right. With regard to the second question, I don't know exactly. I would have to get back to you on the Hepatitis B. But I do have the data on the overall, the duration of follow-up, which is actually quite long.

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The mean duration of follow-up for the primary population was 234 days, about slightly over eight months in the MF59 group, and 188 days, slightly over six months in the control group.

So was that enough? No. I mean, if you want to see long term down the road, 10 years down the road, you need different tools. I would venture to recommend to the regulators that prospective observational studies are the only tool to address that kind of question, because if you do this in the course of a clinical trial, above and beyond the incredible amount of money that this would cost, you have to face the problem of dropouts, and sometimes the dropouts negate the value of randomizing subjects.

So it is a complicated matter, but I would suggest that prospective observational studies are the way to go.

PARTICIPANT: Right. I think the other question would be: Flue vaccinations

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are recommended yearly. So in your LIVE studies or other studies, are you re-vaccinating and following for --

DR. DELLA CIOPPA: We are, because these are elderly subjects, and so they had to be revaccinated. But for the study population, the 150,000 subjects are not 50,000 subject revaccinated three times. Every year you have a new cohort that comes in.

DR. BALLOU: Last question?

DR. VERSTRAETEN: Tom Verstraeten from GSK. Very nice presentation, and very reassuring that your results are similar to what we will be showing in a minute from a similar analysis we did.

I had the same question as the previous one on the exposure time, but linked to that, since you know your exposure time, did you try to assess the number of cases you should have seen, some kind of observed-to-expected analysis, to assess the completeness

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of your capture?

DR. DELLA CIOPPA: We haven't done that, and we will do that.

DR. VERSTRAETEN: Thanks.

MODERATOR SLATER: Thank you. A 20 minute break. Let's reconvene at a little before 10:30.

(Whereupon, the foregoing matter went off the record at 10:09 a.m. and went back on the record at 10:29 a.m.)

MODERATOR SLATER: Welcome back. I will do some more housekeeping while you are going to your seats.

First of all, just to clarify, we will -- Because of the time, we will entertain questions in this session only if we can do so within the time constraints for each speaker. So if your speakers, as did the last two speakers, not only met their time constraints but stay within them, we have plenty of time for questions.

If, as is totally reasonable,

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people reach their time constraints, then we will save the questions for that speaker until the roundtable discussion, which is coming very soon. So that, I don't think, should be a problem, but we do want to stay on schedule so everyone can get an hour's lunch.

Talking about lunch, because there are other meetings going on on the floor, the hotel has decided that, instead of having our three lovely stations placed right here, we are now going to have a single but larger station downstairs in the White Oak Room where I have not been, but I am told there is seating there, and they should be able to accommodate -- I don't know how many people it will accommodate, but anyway, that is where lunch is in terms of the concessions.

There are, of course, the same restaurants and local concessions that you may have used yesterday. They should all be there today. So, hopefully, everyone will be fed during lunchtime.

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We now go on to hear from Dr. Charmaine Gittleson, the head of Clinical Safety at CSL Limited. Dr. Gittleson.

DR. GITTLESON: So, firstly, I would like to thank the organizers for inviting us to this meeting. We have come a long way, all the way from Australia, along with some others.

So what I am going to do today is to try and give you a sense of what the clinical development challenges are that we have considered whilst developing various programs with our adjuvant, ISCOMATRIX adjuvant.

This is not a presentation where I am going to go through a lot of data from the various clinical studies, but I will use some data to try and illustrate what we have tried to do and what we have considered as we have gone through our programs.

So just as a reminder, the adjuvant that I am talking about is ISCOMATRIX

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adjuvant, the small cage-like structures based upon the saponin complex with cholesterol and phospholipid that, when combined with an antigen, forms what is known as ISCOMATRIX vaccine. This is what was presented yesterday as part of Dr. Maraskovsky's presentation.

So what I will do today is talk about some of the development considerations. I will give you an overview of what the clinical exposure is with ISCOMATRIX adjuvant. i will talk about the challenges that we have addressed in looking at how we interpret the immune response, and then the bulk of my presentation will focus on the evaluation of potential safety signals, something that already has been discussed at length today.

So, really, all of us are very aware of the need to have a look at the benefit versus risk parameters when developing a vaccine or any program, and where CSL has really tried to concentrate is where we could show additional benefit.

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So we are very aware of the sensitivity to perceived or potential risk with a novel adjuvant, and so we have had a look at where we could bring additional benefit to patients, for example, with therapeutic vaccines, and that has been a large part of our focus, or where we can have a look at patient populations in whom the response is suboptimal perhaps already to marketed vaccines.

So we have concentrated a lot on the elderly population and in diseased patients. So as examples of the experience, the ISCOMATRIX adjuvant has been now administered to approximately 1,300 patients. Now these are in completed or ongoing studies and with CSL programs or partner programs. So that number of 1300 is a moving target.

A lot of the evidence has come from healthy adult studies which really represents the Phase I programs. And as I have mentioned, we have done work with elderly

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patient populations, in some cases where we have compared elderly and younger adults within the same program.

We do have some data from HCV and HIV infected, and while this workshop is really about vaccines for infectious disease, I do mention that there are some studies done in the oncology sphere. As mentioned, we have worked with prophylactic and really concentrated as well on therapeutic vaccines.

Now this is not the total sum of the exposure. There was an early development program with ISCOMATRIX vaccine, and this brings us to one of the first challenges that we had to face.

So the early adjuvant formulation that was being used in the late 1990s, for the 798 subjects who were exposed in eight completed studies to at least one vaccination. Really,. the work we did there was really to proof of concept to demonstrate that we were eliciting strong hemo responses, and we did

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some earlier exploratory T-cell work.

What we did find in that program was that there were patients that were withdrawing due to AEs, and the AEs most commonly noted that were causing withdrawals were local injection site pain and a flu-like syndrome of fatigue and myalgia.

Looking at the risk profile, we felt that this was unacceptable, looked back at our formulation, and tried to understand what we could do to improve upon this tolerability profile.

Some of the work that was done in that reformulation work was really to try and improve the purity of our vaccine, to remove some of the components of animal origin and to remove some of the -- to further remove bark impurities from the saponin, and also to have a look and see whether we could remove fractions of the saponin that we felt were not essential for eliciting the immune response.

In the program that I just showed

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you on the preceding slide where we have used the optimized version of the adjuvant, we now don't see these withdrawals due to adverse events. Sure, we do see reactogenicity, but we don't see patients withdrawing.

So let me move on to what we have done to have a look at the immune response in our programs. One of the things that has been raised already this morning is what do we need to do to justify the use of ISCOMATRIX adjuvant.

Do we use the adjuvant alone? Do we, obviously, use the combination vaccine? Do we compare against the antigen? Do we have saline controls, and what value can be seen out of those? This is a topic of debate even within our own company.

I am going to use an example of a study which is not in a vaccine for infectious disease but comes from the oncology program, because I think it does illustrate some interesting points, and this is from early on

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within the program.

What we were able to do with this study design was, firstly, we were able to dose escalate our adjuvant, and looking at the lefthand side of the graph, this is looking at antibody responses, and participants with an NYE subpositive minimal residual disease, patients with a history of melanoma and breast cancer predominantly.

What one sees on the lefthand side of the graph is that this was during dose escalation with the antigen and the adjuvant and showing that at low doses of the adjuvant, whilst we had some patients, small patient numbers -- some patients eliciting an immune response, but what was really most interesting is that, when we compared using the antigen alone with adding the adjuvant, we were able to show and justify the value of adding an adjuvant to this program.

It raises the question as well, though, of do we need to have a look at the

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adjuvant alone, and we have had debates this morning already about the value or not of looking at adjuvant alone in the preclinical programs, and does that really translate into the clinical program, and we would like to suggest that not as a regulatory requirement but more as understanding one's own novel adjuvant, that one could consider in the early development stages of one's program -- so in Phase I -- having an adjuvant-alone arm and having a look at certain parameters, that would allow you to describe the effects of one's adjuvant, perhaps affect some of the mechanisms, and perhaps be able to use at a later stage to link back to some of the clinical indications, but what to measure, we will discuss later on, is really a challenge in how predictive that is of further signals is equally challenging.

So looking at the immunogenicity assays, we started off using -- Because looking at therapeutic vaccines, really

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looking at CD8 responses, and started very nonspecific assays looking at DTH, and this has evolved for us, as we have realized that we really wanted to have a very much more specific look at what kind of effect function there was at our CD8 responses.

These are very complex assays to do, very difficult to do with large clinical programs and require a lot of work to set up labs. So one of the things that we have also done is had a look at developing taking a validated registered assay such as QuantiFERON and looking at interferon gamma ELISA methodologies to have a look at CD4, CD8 responses.

There are a lot of challenges in these evaluations. These are not currently validated immune correlates with the clinical endpoints. So if you are using them to make assessments of vaccine dose and of adjuvant dose, which immune correlate does one use, and how might that translate to your clinical

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outcome?

Standardization is not yet attained. It is difficult across our own programs to be able to compare from one study to the other, but even when we have a look at what work other people are doing and try to compare adjuvant efficacy, very difficult to do. What we did realize is that the more you look, the more you find, and it is worthwhile digging and doing the additional assays.

So what this shows is the ability to increase efficiency of detection to tweaking of one's assay method. This is using a therapeutic protein and having a look at interferon gamma on an ex vivo CD8 assay, intracellular cytokine staining assay, where we had a look at using individual peptide pools on Pool A and Pool B and saw a certain standard response. We used HLA-2 restricted peptides and saw a certain response.

Then what we did was we used overlapping peptides, and where we used

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overlapping peptides we saw an increase in the response. So again, where do you stop in making these decisions?

We can move on to the meaty stuff, and that is the evaluation of potential safety signals. So the question, really,. that is on all of our minds is will adjuvants alter clinical risk? I am going to focus on this aspect: Whether it be chronic inflammation, whether it be acute effects in Guillain-Barre, more organ-like toxicities such as multiple sclerosis, more systemic events, and then hypersensitivity and vasculitis toxin events.

Now, obviously, I am not touching on it today, but obviously, we have looked at reactogenicity and done a lot of work, and are now starting to try and see how we could predict which patients might have greater local reactogenicity.

We are doing some work trying to link back to see patients who come into studies with higher antibodies labels at the

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start of a study. Does that predict that they will have more severe reactions or patients who mount a more robust immune response. What kind of immune local reactions do they have?

Just concentrating on the more rare events, there are a lot of challenges. How do we assess association with the adjuvant per se?

We know that we need to take into account background population prevalence. For example, just the background population prevalence of autoimmunity sits at five percent. How does that impact on our ability to interpret what we are seeing within our own clinical programs?

Patients may develop some markers of autoimmunity just as a result of having an infection, and that may happen concurrently with exposure to our vaccines. Within our own programs, patients may receive our vaccine as well as other vaccines, vaccines on the markers. How does that all impact on the

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epidemiology?

Are predictive markers feasible, and are they even valuable? How do we actually determine whether patients have had a specific case of interest? Someone mentioned earlier the Brighton Collaboration. Can we standardize certain case definitions. As yet, Brighton Collaboration doesn't have standardized case definitions for autoimmune conditions.

How do we evaluate whether this is the vaccine per se, the antigen effect with the adjuvant, or what is the contribution of the adjuvant alone, and how does that impact when you are developing one adjuvant for multiple different programs?

So what have we done? Well, we have had a look to see are we actually inducing cytokines, for example, because we do want to see some cytokines. This is an ex vivo assay looking at T-cell responses with a therapeutic vaccine where we have had a look

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at a time point after vaccination.

We do see that we induce TH1 cytokines that we were specifically looking for in association with a CD8 response. We have looked more broadly than this. This is the data that I am demonstrating today.

What we really were interested in, though, is did we get sustained chronic increase in cytokines? So we have taken some of our programs, and just some of the data that I have brought to show today is where we had a look at nonspecific -- the previous slide I showed is antigen-specific.

This is serum showing nonspecific cytokine levels, and what we did was looked at post the third dose of a vaccine regiment with a therapeutic vaccine.

What we noticed was that we did not see sustained levels of cytokines, and when we compared the two yellow lines, compared looking at some of the pro-inflammatory cytokines, IL-1 beta, looking at

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GCSF, IL-6, that these were not sustained and were in the same levels as what we were seeing with the placebo.

This raises questions. What does one compare to? Is saline placebo adequate? Should we be comparing to a licensed vaccine?

We have also had a look to see whether we could see any markers of allergy, chronic inflammation or autoimmunity. This is a study where we have had a look at older adults as well, with a licensed vaccine that contains an antigen and then used an ISCOMATRIX vaccine containing the same antigen, and we had a look at pre-dose, post-dose, post-dose, and then looked at whether there were any treatment emergents on new post-dose events.

We didn't see any new post-dose markers, IgE, CRP or any of the markers of autoimmunity. What is interesting to note is that there is pre-dose markers within the patient population.

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Now some of these may precede clinical diagnoses. The point I want to make here is that it is possibly really important to collect serum in your study and bank it, because if later on you do have a diagnosis of an autoimmune disease and there wasn't anything in the history, you would probably want to be able to go back and have a look and see whether there are any pre-dose markers present.

One of the other things that we have tried to have a look at is intensive systemic toxicity. We have had a look at laboratory evaluation and, certainly, one of the things we have had a look at is liver function tests to see whether there is anything from more systemic immune stimulation.

This is again looking at that licensed vaccine with the same antigen that was then combined with ISCOMATRIX, and this is showing -- it's a bit difficult to see -- ALT

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on the lefthand side and bilirubin on the right, and what we see is that patients -- there is very little variability between pre and post-dose, and this was having a look at Day 7 post-dose.

So what are we trying to do to see whether we can tease out what the clinical signals of safety are for ISCOMATRIX vaccines in our programs?

Well, what we have done is established an adjuvant based clinical data repository. This is a data repository holding all of the clinical data that allows us to have a look at all the adverse events data and all the lab data. It is not just the SAE database.

It's a lot of work involved in doing this, and it requires excellent collaboration between your biostatisticians and your data management vendors and your clinical safety physicians.

What we have done is having a look

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retrospectively at our data using MedDRA tools at this stage, similar to what the previous speaker spoke about. We are having a look using high level group terms to try and capture it more broadly and not miss various potential diagnoses across those that I have shown you here, autoimmune diseases specifically, and we have also combined that and having a look at standard MedDRA queries looking again for some more interesting topics.

What we have not demonstrated is signal, looking at any of this data. There are a lot of challenges in setting up and maintaining such a database. One, we have multiple vaccine programs within CSL. Secondly, CSL works with a number of partners who have their own vaccine programs, and one of the biggest challenges that we face is how do we standardize AE definitions across our various programs in the absence of such guidances from people like -- from places like

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the Brighton Collaboration, and how long do we collect the data for? What meta data do we collect around it?

It is really important to know what other vaccines patients get, what other infections they may get, what their baseline medical history is, and to link all of that together to be able to interpret the data is really quite challenging.

It is really important for us when we do this to go back and look at individual cases and to be able to challenge the sites, if we are able to get back to those investigators and ask more about those patients.

How do we present and use this adjuvant data? One of the approaches we've taken is we have put together an adjuvant -- ISCOMATRIX adjuvant investigator brochure.

So each vaccine has its own investigator brochure, but we have done this as well for the adjuvant where we have

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concentrated on the safety signals, where we have looked to integrate data within various programs, and it allows us to have a look at whether we are seeing anything different between age populations, diseased versus healthy populations, and different vaccines.

What we would like to suggest moving forward is a prospective type of analysis where we set up before we start and determine how we could analyze for rarer events using a meta analysis, where we prospectively define the events of interest and set standardized case definitions for all the clinical programs within our own programs and possibly with partners.

It would require setting prospective statistical analysis plans where we can have a look at trials for a particular product, but we can also look across a particular product with an adjuvant and then look across various products with the same adjuvant.

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We feel that that may facilitate and encourage us to have more standardized approaches to our studies, and it would allow us to have a look at some subgroup evaluations.

So in conclusion, we think that the whole concept of looking at dose ranging one's adjuvant, of trying to determine whether we have a successful adjuvant, using the immune correlates in the ways that are measured really require further development.

We acknowledge that predictive safety biomarker development is very challenging, and we are just taking exploratory looks at our data at this stage, but really are grappling with what does it mean if we do see something there.

We think that there is value in evaluating the safety of the adjuvant technology itself by having a more integrated approach to looking at the adjuvant across a number of different vaccine programs, but we

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acknowledge it needs to be done alongside the development of a vaccine itself, and that one has to do the benefit/risk analysis for that vaccine itself, but that part of one's thinking may be influenced by what you are seeing by looking across the adjuvant.

We really do believe that meetings like this are of value, because the ongoing engagement between the scientific community and the agency and the collaboration that is required for us to further the development of these adjuvants is optimal.

Thank you.

(Applause.)

MODERATOR SLATER: Thank you very much. Are there any questions? Thank you, Dr. Gittleson.

Our next speaker is Dr. Steven Reed. He is the head of research and development at the Infectious Disease Research Institute.

DR. REED: Thank you, Jay, and

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thank you, Rip, for inviting me.

I want to talk about our experience with MPL in a stable emulsion for development of a therapeutic vaccine against Leishmaniasis.

Leishmaniasis is a parasitic disease caused by a wide number of species of Leishmania. Many of you probably haven't been exposed to these parasites, even in a philosophical or practical sense, but they are transmitted by a sandfly, and they are widespread, and they have a lot of different forms, cutaneous, mucosal, visceral and so on.

So there is quite a challenge to develop a vaccine, either a therapeutic or prophylactic, for these organisms.

These are the form that are transmitted by the sandfly, and these are the forms that multiply within the mammalian host. So in this regard, they are very interesting, because they are obligate intrasiter organisms that prefer to replicate in a macrophage. So

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in this regard, they are like toxoplasma, for example.

The good thing about developing a vaccine for Leishmaniasis is that many of the species share antigens, and they have common antigens. So you can actually develop a vaccine that will cross-protect against many species.

This is a clinical form of Leishmaniasis caused by L. donovani. So it is the visceral form characterized by hepatosplenomegaly. This is a severe mucosal Leishmaniasis. These are pictures from the World Health Organization website. This is caused by L. braziliensis, very destructive; and this is the most common form, which is cutaneous Leishmaniasis.

So all these are caused by different species, but as I mentioned, many of them have similar antigens.

The ideal vaccine that we are trying to produce here is, obviously, safe but

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also one that induces effective T-cell responses and long term immunity that we can use both to prevent and treat, ideally, and has broad cross-reactivity between the species.

Of course, at our Infectious Disease Research Institute we are a nonprofit, dedicated exclusively to diseases of developing countries. We have to make vaccines that are cost effective and that will be actually adopted by the countries that can afford them.

So one thing I want to point out in this slide -- and this is the only animal study I will show -- is that it is very important when you are trying to develop a vaccine that works in any of the animal models to have a formulation that is effective. This is basically a mouse footpad model measuring the lesion size, and all you really need to see is that all the black lines are not protected. On the bottom, solid orange, is

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protected.

Now what is the difference? That is our vaccine antigen, which comprises of three recombinant proteins fused together as a single molecule, and this molecule, this tri-fusion protein one, when formulated in MPLSE is quite protective in hundreds of experiments, hundreds of animals, not only in mice but in nonhuman primates, and we have done a lot of dog vaccine studies and so on.

What is interesting about this slide, though: If you formulate MPL in an aqueous solution, you get almost no protection. So the MPL itself isn't intrinsically protective. You have to formulate it in this stable emulsion.

By the way, the stable emulsion is an oil and water emulsion. So in that regard, it has similarities to MF59 and to GSK's ASL3. Similarly, though, the emulsion alone does not protect. So if you look at the antigen plus the emulsion, it will not give you the Th1

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response that you want or protection against this parasite.

So these are all the controls that show that you really can't protect with antigen. You can't protect with adjuvant alone. You can't even protect with antigen and the emulsion or with MPL if it is not properly formulated. So that is one thing that is quite important.

By the way, I didn't really point out, but Leishmaniasis in this model is the classical CD4 mediated immunity that we are trying to induce. This is one of the systems that Bob Coffman worked out, a seminal contribution of immunology of Th1, Th2 responses, and so we pretty much know in this model what we are trying to achieve, both immunologically and, of course, in protection.

We have done several trials. We have three open INDs from the FDA for both therapeutic and prophylactic indications. Our first study was done in the United States,

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showing good safety, some injection site reactions, no SAEs or chemistry problems.

Usually, on the third injection we would see some local reportable adverse event, but nothing severe.

Immunogenicity: We saw most people given this interferon response, everyone converted to immunoglobulin as specific for the parasite. I think the responses were a little lower than they really are, just because of the assay we were using at the time. So I would expect a little higher percentage of interferon gamma but, nonetheless, it gave us an indication of the dose range of protein that we should be using.

In these studies, the MPL dose was kept constant at 25 micrograms of MPL, which is on the low side from what most formulations include. Then, as I mentioned, we did 20, 20 and 40 micrograms of antigen, and we found that more was not better in terms of immunogenicity.

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Now we went then to therapy trials, and several of them were done in Brazil, Peru, and several others are ongoing. I'll just talk about a couple of examples.

What is interesting in the Brazil trials again, we got no higher amount of adverse events. These were individuals that are infected with Leishmaniasis, and why we do these trials is because the standard of care, which is pentavalent antimony, is quite toxic.

All individuals received standard of care. However, in Brazil in this particular area they like to give a lower dose of antimony. That gave us a little more of a window to compare drug with drug plus vaccine and actually get some indication of potential tendency toward efficacy.

These are immunogenicity studies in the Brazil trial. Quite a few things going on here, but focus on the interferon gamma to the parasite antigen that we call 111F or to interferon gamma-2, what we call the soluble

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Leishmania antigen, the crude protein.

What you will see is we have in a dose dependent manner increased responses in the interferon gamma to the parasite antigen, as well as to the -- sorry, the specific antigen, as well as to the whole parasite itself. This is a log scale.

So this points out a couple of very interesting things. Even though these patients are infected and they have active lesions, they do not recognize strongly the antigens in the vaccine prior to immunization. They do recognize them post-immunization in a dose dependent way, and the vaccine actually leads to a greater response in interferon gamma to the whole parasite, but not to Th2 type cytokines, which is in the blue.

So we know that the vaccine then could induce the recognition of new antigens, and the recognition was characterized by a Th1, not a Th2, response.

In terms of efficacy, remember

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that everyone got drug, the standard of care, even though in this case the standard of care was a lower dose than what is typically used -- - this is 10 milligrams per kilogram of the antimony -- but we saw a tendency again toward a higher cure rate in the individuals that received vaccine plus drug versus drug alone or adjuvant alone, and at this particular time we haven't seen relapse, at least in two of the dose groups, 10 and 20 micrograms.

Just parenthetically, from all the trials we are doing, we think that 10 micrograms is probably within the optimal range. We don't think we need 20, and five may be a little bit too low.

The other interesting thing about this Brazil trial is, as the investigators saw, a tendency toward more rapid cure. Here we see individuals receiving vaccine. This is percentage cure on this axis. You'll see a little higher, statistically significant higher individuals that were cured at the AD-

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4, our first observation point, in the individuals receiving the combination as opposed to drug alone.

So again, a very small trial, nine patients per group, per arm, and not statistically significant in all parameters, but at least a good tendency toward increased cure. And the investigators noted that the individuals not only tended to cure more rapidly, but leave no scarring. That is probably not too unusual, because the more rapidly you cure, probably the less amount of time the lesion persists, and so the less scar that you have.

I will point out that the other reason to do these kinds of studies is because this cutaneous form that we see in Brazil has a tendency to progress to mucosal Leishmaniasis, very destructive, very difficult to treat. So it is another reason you want a very complete and comprehensive therapeutic approach in these patients, and we

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think that drug plus vaccine is probably the best option.

In Peru, we treated mucosal Leishmaniasis, that destructive form that does progress in some of the patients I just described, and again a dose escalation study, 5, 10 and 20 micrograms, keeping the MPL standard.

Again, you see a good response to the antigen after vaccination and not prior to vaccination -- we will point some of that out in a minute -- and again a good cure rate. Nothing really dramatic here, because in this case we used high dose of antimony. So most of the patients with antimony alone and receiving placebo cured quite well, as well. But we are quite happy, because as you can imagine, when you have a very strong immune response, as you do in these cases with mucosal Leishmaniasis, you want to make sure that your vaccine doesn't exacerbate or have any toxicity, and we did find that in the Peru

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trial.

We don't know yet about long term follow-up whether there will be a lower amount of relapse in the vaccine individuals yet, but as in the Brazil study we did see a tendency again toward a faster rate of cure that excited the doctors that were working on this. It was a blinded study, and they were very happy, because they rarely see people curing clinically before three months, and here we at day 84, a slightly higher number of people curing as compared to placebo alone.

Rhea Coler in the lab did some nice immunological studies, and this is just an example of a flow cytometry in a patient that did very well, a cured patient, with immunochemotherapy.

These are looking at CD4 responses, interferon gamma, TNF and IL-2. So as Bob Seder pointed out recently, these are the three cytokines that are most closely correlated with correlate of protection in

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Leishmaniasis, and we see this individual making good responses to all these three cytokines after immunization but not before. So again, quite interesting. Strong immune response to Leishmania as a whole, but no immune response to the vaccine antigen, which could explain why some of these people just aren't doing well.

This is a similar assay from an individual who did not cure, and here either before immunization or after we see no increase in the cytokine responses.

This is the kind of exam they have to do. This is a subjective exam, but it is the lesion of mucosal Leishmaniasis, and again why the individual investigator is very excited, because we see some people responding as early as four weeks after the beginning of immunization, which he had really never seen before. That was a vaccine and drug treated individual.

So in summary, with all these

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trials it is good to point out that the vaccine was safe and well tolerated, quite immunogenic even in a patient with an active immune response. Did not exacerbate disease which, of course, you always want, but I think that that is not a given; and we have seen, by the way, safety and efficacy in mouse models at therapy as well as in dogs, dogs that have visceral Leishmaniasis, which is a problem in the Mediterranean area and Brazil.

So we really think that the ability to reverse active disease with a therapeutic vaccine may be possible and that Leishmaniasis may be one of the models in which that is achievable.

Several other trials are ongoing or about to start, including visceral Leishmaniasis in India and Sudan, post-kala-azar dermal Leishmania in Sudan. This one is ongoing now, a diffuse cutaneous Leishmaniasis in Venezuela.

These are patients that are like

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the Balb/c of the Leishmania world. They do not respond with a Th1 response. They have a lot of antibody, and they cure with drug, but then they relapse. These are very pathetic kids, because they just keep going their whole life with recurring Leishmaniasis. Our goal here is to use drug plus vaccine and convert their response so that they will have durable response to drug.

Then, of course, we are doing another CL trial in Brazil.

A special thanks: Thank you very much, Rhea Coler who is here in the audience, for all the preclinical studies in the clinical immunology. Anna Marie Beckman is also here, head of regulatory, that made these all possible, and our clinical investigators, Alejandra Lianos and Evaldo Mascemento.

Funding has been going on from NIH for many years and the Bill and Melinda Gates Foundation. Thank you very much.

(Applause.)

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MODERATOR SLATER: Thank you, Dr. Reed. Are there any questions for Dr. Reed?

DR. ALVING: This is Carl Alving. When you -- In the military, there are a lot of cases of cutaneous Leishmaniasis that occur, particular in the Middle East and South America and so forth.

When they get treated with antimonial drugs, my understanding is that the lesion disappears, but the organism is still there. Is it still there after you find what you call cure?

DR. REED: Carl, the antibody levels decrease to the point where it is very difficult to say. The individuals, however, will persist with a positive skin test. So like latent tuberculosis, I would expect the answer is probably yes, but that is a very interesting question, and it is relevant to whether we can use such an approach to reduce the skin parasites so the humans won't act as a reservoir.

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By the way, one of our goals is working with Allen McGill and the military to replace Pentostam with a vaccine, because as he tells you and you know -- he tells us and you know that this is not a pleasant treatment for the soldiers.

PARTICIPANT: Steve, when you are doing these trials on several different forms of Leishmaniasis, you are dealing with patients that have preexisting both antibody and T-cell levels of a variety of sorts. For example, particularly in the DCL patients you will have, as you know, Th2 polarized response.

Are you seeing any evidence of skin reactivity, let's say allergic sensitization or anaphylaxis, in the DCL patients or other forms of skin reactivity reflecting recall responses to the vaccination?

DR. REED: Yes, that's a great question. Thanks. I should have pointed this

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out. Both in DCL -- We haven't seen that in DCL, but I will say the trial has only been going on for six weeks. But mucosal Leishmaniasis also is somewhat related to allergy.

These patients have IgE, and they have more of a Th2 response. It's a mixed response, but what I am thinking of in this particular setting, that our therapy, especially in that setting of mucosal Leishmaniasis, is more akin to a desensitization for allergy.

We are seeing a shift away from Th2 response. The Th1 doesn't necessarily go up much, because they are already very strong, but we do see a decrease in IgE, IL4, IL5. So I think that is what we are really doing, is down-regulating this Th2 response, and it makes a lot of sense that MPL would do that, because MPL is used in allergy desensitization in Europe. So that is why, I think, our choice of the adjuvant was very good.

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Hi, Carter.

DR. DIGGS: Hi, Steve. If I read the slide right, it looked like that in your adjuvant alone trial, you had a higher instance of AEs that you thought were probably vaccine related.

So this reflects back to this morning's roundtable and the issue of testing adjuvants alone. Could you comment on that, and particularly with respect to the association of antigen and your emulsion.

DR. REED: Right. So in the adjuvant alone arm that we did in Peru, the AEs -- There were no SAEs. The AEs were not significantly different with vaccine or with adjuvant alone.

We thought it was important to include an adjuvant alone, because the patients already have organisms. So it is -- If you want to see efficacy in the long run, it's nice to have the adjuvant alone. Maybe you don't need the vaccine.

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What we did not see in that group, and I didn't show the immunology, is any conversion to a Th1 response to the parasite. So we expect that that group won't do as well, but as far as the AEs, there is no really statistical significantly different level.

Those are mainly injection site reactions on the second or third immunization, a slightly sore arm. It's just how we put in the reporting.

By the way, I should mention this. We gave our vaccine subcutaneously, and we are thinking of switching over to intramuscularly, the intramuscular injections, which we think might help a little bit with the local reactivity.

MODERATOR SLATER: Well, thank you very much. I would like to ask Dr. Heather Davis to come up. Dr. Davis is from Pfizer Global Research and Development and Coley Pharmaceutical, a Pfizer company.

DR. DAVIS: Thank you very much.

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I greatly appreciate the opportunity to speak to you today.

I will be summarizing the clinical experience with CpG 7909 as the vaccine adjuvant in studies that were carried by Coley Pharmaceutical Group, which is now part of Pfizer, as well as by some of Coley's partners.

First of all, what is CpG 7909? It is an agonist for TLR9 which is found within the endosome of human B cells and plasmacytoid dendritic cells. TLR9 normally recognizes molecular patterns that are found in viral and bacterial DNA and not mammalian DNA; hence, it is recognized as a pathogen associated molecular pattern, and these are known as CpG motifs.

TLR9 can also be activated by synthetic oligonucleotides that contain such CpG motifs.

The desirable features that CpG oligonucleotides offer as a vaccine adjuvant -

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- first of all, with respect to chemistry and manufacturing, they are fully synthetic. They are easily characterized, at least the ones we have taken into clinic, and they are very stable. Bulk drugs can last for decades, and finished drugs, certainly, for years.

With respect to pharmacology, TLR9 has the most restricted distribution of all the TLRs in humans, just on the B cells and plasmacytoid dendritic cells. So as long as it works, then this could be a highly desirable feature, since there is no need to activate more than you require. In animal studies it is shown to enhance both antibody and T-cells with Th1 biased responses.

CpG 7909, which was also known as Vaximmune when it was used by Coley is a 2 20 former oligonucleotide. It contains three copies of the CpG motif, the GT-CG-TT, that we had found to be highly effective in humans, and it is effective in virtually all species. One notable exception is rabbit, which seems

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to be TLR9 deficient. So it is not just this oligo it doesn't respond to well. It is all CpG oligos.

It is a B class, which means it is monomeric and remains linear, no higher ordered structures, which makes it very easy to do the QC, and it is synthesized with a wholly phosphorothioate backbone, which makes it nuclease resistant. So it doesn't have to be encapsulated in any way for protection.

This slide summarizes the clinic development history of CpG 7909 as a vaccine adjuvant. There have been a total of 37 vaccine clinical trials since the year 2000. The first ones were carried out by Coley, and the approach was to add mix but with an approved vaccine just for proof of concept.

Three trials were carried out, two with Engerix-B Hepatitis B vaccine, one in normal, healthy volunteers, one in HIV infected patients, and another trial with a trivalent split flu vaccine in healthy

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volunteers.

A number of trials have been carried out by our commercial partners under license. Emergent, which was BioPort at the time, conducted a trial with their anthrax vaccine. GSK and Novartis -- at that time, Chiron -- have also carried out a number of trials in either the infectious disease space for both of them or oncology for GSK.

As well, Lou Miller's group at the NIAID, the Malaria Vaccine Development Branch, has conducted four Phase I trials in U.S. and Mali in adults. The Ludwig Institute has used CpG 7909 with their tumor antigens, and they have conducted a total of 10 Phase I or Phase I/II trials, and an additional 10 trials have been conducted by academic investigators, either in the infectious disease or oncology space.

I will now summarize the immunogenicity and safety findings for the Coley studies, as well as some of our partner

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studies where we have access to all of their data, and I will start with the immunogenicity findings.

In Coley's very first trial, we added CpG 7909 to Engerix-B Hepatitis B vaccine and found that it greatly enhanced both the kinetics and the magnitude of the antibody response.

This graph shows, with the blue bars being the groups receiving CpG, that the proportion of subjects which achieved a seroprotective titer of 10 million International Units per mil or higher at two and four weeks after a single dose was 58 percent and 75 percent respectively, and this is in contrast to zero percent and eight percent for the commercial control vaccine.

The actual antibody titers after the first and second doses were ten to fiftyfold higher with the CpG added. The responses at the lowest dose, which is shown in green, the 125 micrograms, were suboptimal

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but still highly effective; whereas, the .5 and 1 milligram doses gave equal immunogenicity and efficacy.

A second trial was carried out, also adding CpG 7909 to Engerix-B. In this case, it was conducted in HIV infected patients, half of whom had previously failed to respond to a normal course of vaccination with the commercial vaccine.

The subjects received three doses of vaccine which were given at zero, four and eight weeks, thus an accelerated schedule. As in the healthy volunteers, both the kinetics and the magnitude of the antibody response was enhanced, and in the CpG group, which is shown here as pink bars, you can see that the proportion of the subjects which attained and sustained seroprotective titers remained significantly higher all the way up to five years after vaccination.

In these same subjects lymphoproliferative responses were evaluated.

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This is a rather crude assay for T-cell, but that is what was done at the time, and you can see that the CpG groups have had significantly enhanced proliferative responses all the way out to four years after vaccination.

In another study that was carried out by Coley and Emergent with DARPA funding, CpG 7909 was added to the commercial anthrax vaccine. The antibody response, both the total IgG as well as its neutralization activity, had enhanced kinetics as well as magnitude with the CpG added. That is shown in green, and is very similar to what I just showed you with the Hepatitis B surface antigen trial.

As well, the Malaria Vaccine Development Branch has carried out four Phase I trials which are outlined here. They have had a total of 11 volunteers -- or, sorry, 111 volunteers who have received CpG 7909 with one of two different malaria antigens adsorbed alum, the AMA-1C1 or the MSP-1.

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The adult subjects were located either in the U.S. or in Mali, and they received two or three doses of either the CpG adjuvanted vaccine or the control vaccine, which was the same minus CpG, as indicated in the table.

The next slide summarizes their immunogenicity results. With the AMA trials, of which there were three, in the U.S. adults they found an 11 to 14-fold higher titer in the CpG groups after the second vaccination, and a five to sixfold higher after the third vaccination, all highly significant and virtually identical to what we saw with the Hepatitis B surface antigen.

In the Malian adults, the responses were significantly less. They were only about twofold higher in the CpG group.

For the MSP trial, which was carried out on U.S. adults, there was about tenfold higher titers which were significantly higher with CpG than without, and the figure

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that is in the lower right shows that data. So they tested two different antigen doses at the low antigen dose, a 40 microgram, compare black with no CpG to red with CpG, or the higher dose, 160 micrograms of antigen, compare blue, no CpG, to green, with CpG.

In a trial that was carried out by Daniel Speiser of the Ludwig Institute, T-cell responses to a Melan-A peptide vaccine was tested in melanoma patients, and he found that the T-cell responses were enhanced in the CpG group but not in the group where the peptide had only been combined with incomplete Freund's and adjuvant, incomplete Freund's in both groups.

So I am going to show you a single mouse data slide to help put this in context with the next data I am going to show you.

This shows that we have found strong synergy between CpG and other adjuvants, especially those that have a delivery or depot type function, and this is

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presumably because they keep the CpG together with the antigen and ensure delivery of the CpG to the same sites in the node, same cells, presumably.

All of the previous clinical data I have shown you had either alum -- that was in every one of the infectious disease vaccines -- or incomplete Freund's. That was in the Ludwig oncology vaccine. So the question is: What happens in humans when CpG is used on its own, and two such studies have been carried out.

In the first study, which was a Coley study, CpG was added to a single dose of a trivalent split influenza vaccine, and in this case the enhancement of the antibody that could be attributed to the CpG was only seen in subjects who already had some preexisting immunity. In this case, they had been screened this way, and it was for A/Sydney, and you can see that on the left.

On the other hand, the subjects --

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all of the subjects were negative for A/Beijing and B/Harbin, and in that case there was no effect of the CpG on the antibody titers.

Nevertheless, when we looked at interferon gamma secretion from PBMCs that had been restimulated ex vivo, an increase in interferon gamma was noted for all three serotypes regardless of whether or not the subjects had preexisting immunity.

The second trial was conducted by GSK, and in this case they added CpG 7909 to Hepatitis B surface antigen without the alum that is normally found in the commercial vaccines.

In the upper right, you can see that the antibody level was enhanced over what the antigen would have done alone, but it was not as strong as what we had seen in our earlier study where we had alum present, nor was it was strong as the three other formulations that they tested. But it is

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noted that these are all adjuvant combinations. The three top lines are not single adjuvants.

In the bottom right you can see that the CTL assay was not detected with the CpG on its own with no further formulation, but it was with the other three adjuvant combinations.

Moving on to clinical safety: As an overview for all the trials where we have tested CpG either as a sole adjuvant or combined with alum -- I am leaving out the incomplete Freund one, because it has quite a few AEs associated just with the incomplete Freund's, and we also -- because we had not done those trials, we don't have all of the data.

In these sets of trials, there has been no serious adverse events related to vaccination. The common adverse events that were seen are similar to those seen with vaccines in general, largely local and

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systemic reactogenicity, local adverse events being pain, erythema and induration, systemic largely falling under the flu-type symptom category, namely, headache, body ache and fatigue. These are generally of mild to moderate severity, and of short duration, namely, one to two days.

This table summarizes the effects of the safety of CpG 7909 in four different vaccine trials. I've introduced all of these to you earlier. So you should recognize them from the left column.

In some cases, the frequency and severity of either the local and/or the systemic adverse events was exactly the same as with the control vaccine, and these I have highlighted in green.

In other cases, the adverse events were of the same intensity but more frequent, and that is shown in yellow; and in the anthrax vaccine, both local and systemic adverse events were more frequent and more

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intense.

So I am going to show you a single example. That is the second one down or the Engerix-B in the healthy volunteers, to give you some idea of what that data looks like when we make these general conclusions.

First of all, local tolerability: You can see that in this case the -- this is the Engerix-B tested in healthy volunteers. In this case, local adverse events were of increased incidence for the two highest dose groups. That is pink and green. But the severity was not increased. They were all in the mild to low moderate level.

It should be noted that all of those three doses had been highly effective from an immunogenicity point of view. So this shows that it isn't necessary to have enhanced reactogenicity in order to obtain enhanced immunogenicity.

The systemic adverse events were not more severe, and there is no clear pattern

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for incidence. The two lowest groups of CpG appear to have a higher frequency, but the highest dose group didn't. So it is very hard to make any conclusion from this one, but definitely not clear evidence of increased systemic adverse events.

Other safety issues that we have seen or considered: Neutropenia, a transient grade 1 or 2 neutropenia is frequently noted on the second or third days after vaccination, and this returns to baseline by Day Three.

We carried out extensive animal studies, and the results from those studies suggest that this is due to cellular redistribution to the periphery and the lymph nodes rather than a true neutropenia. In the other words, the cells were out seeking the danger signal that we have injected in the intramuscular space.

The second is more of a hypothetical risk that we have been acutely aware of, because we are injecting as DNA, and

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that is the presence of anti-DNA antibodies, and whether or not those might induce autoimmune disease.

Anti-single stranded DNA is a very common observation in the vaccine studies, more in the 50 percent of the subjects, especially if there is more than one vaccine dose, will present with anti-single stranded DNA antibodies, and these are transient.

It is very similar to what can occur after any infection. We have anti-single stranded DNA antibodies that elevate under different circumstances in our life, including infections, and these are considered to have no clinical significance.

Anti-double stranded DNA moves more into an area where you might say is there a concern. These were rare. Less than one percent of the subjects in these trials presented with anti-double stranded DNA, again were transient.

In these cases, these few cases,

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they were never associated with an elevated ANA, which would be perhaps a true danger signal, and there was no evidence of any clinical autoimmunity.

So to summarize, CpG 7909 has been administered in 37 vaccine clinical trials, and it is for immunogenicity. The best adjuvant effects are clearly when it is combined with a delivery system type adjuvant, and Derek O'Hagan spoke about some of the reasons behind that yesterday.

Antibody -- I have shown you some but not all of this data -- shows enhanced kinetics magnitude as well as avidity and duration, T-cell responses, enhanced magnitude and duration.

The safety profile is similar to vaccine alone, generally well tolerated with no SAEs. Mild injection site reactions and flu-type symptoms are frequent. In some cases, these are of increased incidence or severity to the AEs seen with the control

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vaccine, but they still remain in that mild to moderate category.

I am just going to end on this last slide which responds to a question that was raised yesterday about oligonucleotides being biologics. So this shows why oligonucleotides on their own -- obviously, a vaccine is a biologic, but on their own are not.

They are, first of all, very small compared to plasma DNA which is, I think, where the thoughts came from. They don't code for anything. They are totally synthetic. They bind to a receptor in the body, and signaling through that receptor then they activate a normal cellular function; and contrary to what was reported yesterday, they cannot integrate. They are too short.

In contrast, plasmas which are used in DNA vaccines are very large. They are double stranded DNA. They do encode a foreign gene. They are manufactured in a biological

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system. They are expressing that foreign gene in the nucleus of one of your cells, and integration is theoretically possible.

So they are very, very different, even though they are both DNA, and that is why when they are used alone, they fall under drugs. Thank you.

(Applause.)

MODERATOR SLATER: Thank you, Dr. Davis. Any questions?

DR. PETROVSKY: Heather, you mentioned 37 studies. Can you comment on the total number of subjects in those studies in total?

DR. DAVIS: I wish I had the total number, but I don't. I think the maximum in one study would have been 60, but some of the oncology ones are as few as five or six subjects. So I'm sorry, I don't have the total number, Nikolai.

DR. PETROVSKY: And also we heard this morning with a meta analysis. Have you

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ever sort of contemplated trying to do a meta analysis by combining the different study results together?

DR. DAVIS: It is something we are starting to work on, but we sort of have to group them by the other adjuvants that might be there. So we will be doing that, certainly from a safety point of view, and I have given you a high level summary of that. We will do that with more granularity.

For immunogenicity, when it is an alum CpG, very, very similar results have been shown with five different antigens now. And interestingly enough, it is almost the same degree of enhancement seen in mice, even though they have a different TLR9 distribution.

PARTICIPANT: Yes. The anti-single stranded DNA antibodies that you saw -- were they directed against phosphorothioates or normal phosphodiaster linked?

DR. DAVIS: They would recognize

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any DNA. We tested them against calf thymus DNA as well as the oligo of our sequence, oligos of other sequences. It was equal rate across the board.

PARTICIPANT: Thank you.

DR. MALONE: What is your working hypothesis for the decreased responsiveness in Mali population?

DR. DAVIS: I am going to -- I know the group from the Malaria Vaccine group is here. Can one of you perhaps answer that question? Ruth?

DR. ELLIS: Hi. I am Ruth Ellis from MVDB. There may be some down-regulation of TLR9, particularly in Mali in adults, due to all the cumulative particular malaria exposure.

We are hoping to go to Mali in children and look for immunogenicity there. That is our target population.

MODERATOR SLATER: We will take one more quick question.

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PARTICIPANT: I thought that one of the points that you were making in your general slide was that there were more -- there was more reactogenicity in the anthrax study, for example, than in the Engerix.

I wasn't able to really quite deceive why that was, whether there was a difference in dose or whether you feel that there is a difference in the antigen CpG interaction. I wonder if you could comment on that.

DR. DAVIS: It is a more reactogenic vaccine, to begin with, than the other ones that were tested, and that is one of the reasons that emergent is working with CpG as a way to try to be able to reduce antigen dose and reduce number of doses that are required for that.

So possibly under those circumstances, adding the CpG tipped it up a little bit more than the other, because that was the only one where we did see both

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increased frequency and increased severity. That is the best I can come up with.

The dose of CpG was the same as used in the other trials. The alum was alhydrogel, the same. So I think it has to be an antigen related situation, and perhaps with dropping that dose of antigen, that wouldn't have happened.

MODERATOR SLATER: Thank you. Next is Dr. Gary Dubin from the Prophylactic Vaccine's Clinical Development at GSK. Dr. Dubin.

DR. DUBIN: Good morning, everyone.

Yesterday many of the presenters in the first session talked about the benefits of using adjuvants and adjuvant systems in terms of factors linked to target populations or targeted pathogens. So I won't cover this slide, which I think was already reviewed yesterday.

What I would like to do in the

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next few minutes is use some concrete examples of clinical development programs for vaccines where we at GSK Biologicals have actually taken different adjuvant systems into the clinic and used these to illustrate some points about clinical development of adjuvanted vaccines.

Now yesterday we also reviewed what we mean when we refer to adjuvant systems, and the design principle that we have used at GSK is to combine a vaccine antigen with an adjuvant system. An adjuvant system is defined as a combination of a classical adjuvant -- for example, aluminum salts, emulsions or lipisomes -- and an immunomodulatory molecule like MPL, QS-21, CpG or alpha-tocopherol.

The goal of using an adjuvant system is to try to induce a tailored immune response to achieve sustained and enhanced protection.

So the three examples of clinical

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development programs that I will describe in the next few minutes are programs that are either supporting vaccines that are licensed in some countries or large development programs where we have accrued a fair amount of clinical data.

So the first example I would like to cover is pandemic influenza. I think, as known to this audience, in an influenza pandemic the global population will be largely naive toward the pandemic strain that ultimately emerges, and this will necessitate a high hemagglutinin content and a two-dose vaccine regimen, largely because non-adjuvanted inactivated H5N1 vaccines are poorly immunogenic, even when used at high hemagglutinin content.

So this is one of the challenges, I think, which we believe use of an adjuvant system can help overcome.

Now a pre-pandemic strategy has several potential advantages in terms of being

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able to induce protection before a pandemic is declared and ensuring the population is at least immunized, because as I think is also known to this audience, the time window between declaration of a pandemic and significant morbidity/mortality would really be too short to fully protect the entire population. But there are a few requirements that we think a pre-pandemic vaccine needs to have.

One is that it should elicit immunity to drifted strains, and the second is that it should be antigen sparing, because potentially the population that might be targeted with a pre-pandemic vaccine would be broad.

Now the formulation that we have evaluated is a pandemic vaccine and is a pre-pandemic vaccine, as shown on the slide. It essentially combines H5N1 hemagglutinin in antigen with an adjuvant system that we refer to as AS03, which is a combination of an

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immunomodulator, alpha-tocopherol and oil-in-water emulsion.

So this is actually data from a Phase II study conducted in adults 18 to 60 years of age, and I think Dr. Fauci referred to this data yesterday in his opening session.

In this study, adults were immunized with H5N1 antigen, either adjuvanted with ASO3 or unadjuvanted, and there was a dose range used in the study which included a lowest dose of 3.8 micrograms of the H5 antigen and the highest dose of 30 micrograms.

As you can see on this slide, when the adjuvanted vaccine was administered even at the lowest dose, the 3.8 microgram dose, after completing a two-dose series, shown here, the immune response induced -- in this case, as indicated by seroprotection rates -- achieved the criteria that had been established by CBER and by CHMP; while the highest dose of the unadjuvanted vaccine failed to achieve that same criteria.

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The same results apply to actual quantification of hemagglutinin inhibition titers, geometric mean antibody titers. So I think this really helped establish proof of principle, indicating that the use of an adjuvant system in this case could convert what is regarded as a relatively poorly immunogenic antigen into one that is highly immunogenic, and it can be used in a lower dose to achieve acceptable levels of seroprotection and geometric mean antibody titers.

Now in this same study, a subset of subjects were evaluated for induction of heterologous neutralizing antibody, and I think this is one of the other important criteria that we think that is important in terms of consideration for a pre-pandemic vaccine.

So you can see in this graph that shows reciprocal neutralizing geometric mean antibody titers individuals that received

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adjuvanted vaccine, and these lines each show the subset of subjects receiving the lowest dose, the 3.8 microgram dose, of the adjuvanted vaccine achieved increases in neutralizing activity, not only to the homologous virus, which was A/Vietnam, but also to drift variants which were clade 2.

Seroconversion rates for these drift variance range from 75 to 86 percent. Now it is not shown on the graph, but it is indicated here at the bottom. The unadjuvanted vaccine groups for all clade 2 viruses failed to have -- or failed to induce responses. So these individuals that were vaccinated with unadjuvanted vaccine did not have detectable neutralizing responses to drift variant virus.

Now the next example I would like to turn to is the GSK HPV vaccine. This is a vaccine that has been in development for the last 10 years, and by way of background, I wanted to say a few things about the natural

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history of HPV.

So HPV is now clearly identified as the necessary cause of cervical cancer, and there are two genotypes of HPV, HPV-16 and 18, which are responsible for the majority of cervical cancers. About 70 percent of cervical cancers are caused by these two HPV types, but we believe protection is important beyond HPV-16 and 18, because, obviously, there is a full 30 percent of cervical cancers that are not caused by these types.

The target of universal vaccination programs in countries that have introduced HPV vaccination is primarily pre-teenage girls. So we believe it is important that vaccination also induce long-lasting protection, because girls are likely to be at risk of acquiring HPV infection throughout their sexually active life.

The composition of the HPV vaccine that we have developed includes virus-like particles, VLPs from HPV-16 and 18, combined

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with another adjuvant system, ASO4. ASO4 is a combination of monophosphoryl lipid A, MPL, combined with aluminum hydroxide.

In our early development program of this vaccine, we conducted Phase II studies looking at different adjuvant formulations, and this is a summary of some of the Phase II data that emerged from those early studies.

In this study, individuals were vaccinated with three doses of HPV vaccine, either containing the ASO4 adjuvant that is shown in pink or aluminum hydroxide adjuvant, same antigens, different adjuvant. That is shown in green.

Then individuals were followed for 48 months, and neutralizing antibody titers were assessed against each of the two VLP components. What you can see here is that for both HPV types, HPV-16 and 18, we saw consistent differences in the level of neutralizing antibody induced, with higher titers observed in the subjects receiving

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ASO4.

I would also like to point out that the peak response, which was seen one month after completion of the three-dose series, predicted what we saw when we looked at the long term follow-up four years out. So higher titers at month seven predicted higher titers at month 48.

Now based on the results from those early studies, we initiated a large Phase II-B and Phase III study using the ASO4 adjuvanted HPV vaccine. The results that I show on this slide are results from our first efficacy study. So this is human efficacy data.

In this study, we vaccinated 1100 subjects with the ASO4 adjuvanted vaccine compared to an aluminum hydroxide control, and have followed these subjects out through 6.4 years. These are efficacy results for a number of HPV-16 and 18 endpoints over that extended follow-up period.

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You will see that we measure as endpoints protection against incident infection -- that's detection of HPV-16 or 18 -- in previously uninfected individuals at a single time point. We also assessed protection against persistent infection that is consecutive detection, the same virus type, either at a six-month interval -- that's six-month persistence -- or 12-month persistence was another endpoint.

Then we have also assessed the efficacy of the vaccine in protection against some of the histologic consequences of persistent HPV infection, cervical intraepithelial neoplasia Grade 1 or worse or Grade 2 or worse. These are recognized as surrogates for cervical cancer.

So what you will see in this study is that we observed a high level of protection against the majority of these endpoints out through the entire 6.4 year follow-up period. In fact, in this study there were no

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breakthrough cases of persistent infection, CIN1+ or 2+ in subjects receiving the HPV vaccine.

The follow-up in this study continues. We have now entered another extension phase to this study. So we hope to be able to continue to demonstrate the duration of protection through another three years at least in this longer term follow-up.

Now in this study we also assessed the ability of the vaccine to induce protection against infection with phylogenetically related HPV types, at least types that are phylogenetically related to the vaccine types.

So HPV-45 is the third most common HPV type associated with cervical cancer and is phylogenetically related to HPV-18, and HPV-31, the fourth most common type globally associated with cervical cancer, is phylogenetically related to HPV-16.

Over the six and a half-year

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follow-up period, we assessed protection against incident infection with HPV-45 and 31, and observed significant protection against each of these two types.

Now this is using incident infection as an endpoint, which is not a very robust correlate of cervical cancer, but we have extended these results with recent publication of a Phase III study which has shown protection against six-month persistent infection with these two types.

In fact, the recently published Phase III data coming from a large efficacy study that has enrolled about 18,000 subjects confirms the high level of efficacy against HPV-16 and 18, CIN2+ as well.

So we think the ASO4 adjuvant used in this vaccine is an important determinant of immunogenicity. That is very clear from our early studies, and we think or at least hope that this will translate into long term protection to be demonstrated with longer term

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follow-up in our ongoing studies.

The third example that I would like to turn to is the example of a malaria candidate vaccine. Malaria is a very serious medical problem, especially in Subsaharan Africa. There are about 300-500 million cases of malaria each year and about 1-3 million deaths attributed to malaria. Most of these occur in young children.

Although there are currently available interventions, these are not highly effective. They have effectiveness, but they are not highly effective. So that there is clearly a need for malaria vaccine.

The vaccine candidate that has been under development combines an antigen which we refer to as RTS, S. So this is a circumsporozoite protein, a proportion of that protein, fused to Hepatitis B surface antigen, combined with another adjuvant system which we refer to as ASO2.

This is a combination of

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immunomodulators, MPL and QS21, in an oil and water emulsion.

Now the malaria program has actually been a relatively longstanding program in collaboration with Walter Reed Army Institute of Research, and I think research on this vaccine goes back at least 20 years. But in 1996, there was publication of what I consider a very important study, at least at establishing the proof of principle of the difference adjuvants can make.

In this study, three doses of the adjuvanted RTS,S antigen were administered with three different adjuvant systems. Two weeks following the third dose, adults were challenged with infectious mosquitos, and then the readout here was protection against malaria.

So the three different adjuvant systems that were used in this study were the ASO4 adjuvant, the one that I just talked about used in cervix, the ASO3, the one that

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I spoke about a few minutes ago used in the pandemic flu, and ASO2.

You will see here that this column represents the number of subjects protected in each of the groups receiving the different adjuvant formulations. Now control recipients were completely unprotected. That is how the model is set up.

You will see that there was partial protection in subjects receiving RTS,S with ASO4 or ASO3, one out of eight and two out of seven individuals, respectively. But the highest level of protection was observed in individuals receiving the vaccine formulated with the ASO2 adjuvant, and that correlated to about an 86 percent efficacy for the ASO2 formulation.

Now there were additional immunologic evaluations done in these individuals, which included evaluation of antibody responses to the RTS,S protein, and then also some mediated immune responses were

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evaluated, in this case interferon gamma secretion measured by ELISPOT in CD4 and CD8 lymphocytes.

What is interesting to note is that, if you look at the antibody response in subjects receiving the adjuvant systems that contained the oil and water emulsion -- so that is ASO3 and ASO2 -- there was good induction of antibody responses, didn't differ significantly between those two groups. ASO4 induced antigen-specific responses, but at a lower level than the oil and water emulsions. But if you look at the gamma interferon secretion profile, this was different and did differentiate the two oil and water emulsions.

So you can see here, with ASO2 individuals that were protected -- and that is shown by the black bars -- tended to have higher levels of interferon gamma secreting lymphocytes than individuals receiving the other formulation. So there was a good correlation between the cellular response

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induced and protective efficacy.

Now based on these results, a number of efficacy studies were initiated, and this slide summarizes some of the key efficacy data that has been generated in infants and children in Africa.

What you will note is that in separate studies vaccine efficacy against malaria infection, clinical malaria, severe malaria, hospitalized malaria was demonstrated in young children one to four years of age, with long term follow-up showing sustained protection; and efficacy has been evaluated in infants as young as 10 weeks of age in a separate study.

So these results, I think, are very promising, and as a result of these very promising results, a large Phase III program will be initiated in the very near future.

So those were just some selected examples of vaccine efficacy, immunogenicity linked to different adjuvant systems. I would

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now like to spend the last few minutes talking a little bit about safety evaluations and some of the considerations that come from what we learned in our clinical development experience.

So, clearly, the safety evaluations of any new vaccine, including vaccines containing adjuvant systems, must include traditional safety evaluations, and you have heard a lot about these kinds of evaluations this morning: Solicited local and general symptoms, unsolicited symptoms including serious adverse events and, if the vaccine is being used in women of childbearing potential, pregnancy outcomes.

There are additional categories of events which, we believe, need to be considered, depending on the target population for the vaccine and other factors. So adverse events of special interest need to be defined, depending on preclinical data, what information might be available from related

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products, and again the target population.

We also believe it is important to collect information on medically significant adverse events. So these studies are defined of events that prompt physician interactions. These are important, because they generate health care costs but also might be important indicators of important adverse pathology.

Then also new onset chronic diseases with a focus on autoimmune diseases are events that we have tried to routinely capture in our adjuvanted vaccine development programs.

In addition to these traditional evaluations and the additional categories of events of special interest, we think it is important to consider pooled analyses or meta analyses for rare events -- we heard a little bit about that this morning, and I will come back to that in a minute -- and also in some situations, it might be important to use expert review panels to evaluate certain

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events or categories of events, depending on data that emerges in clinical studies.

I would like to emphasize that these considerations apply not only to vaccines that use new adjuvants or adjuvant systems but to any new vaccine, in fact.

So coming back to the example of HPV, this is our largest clinical development program, and I wanted to show you the kind of data that we have collected in our development program, and then show a few examples of clinical data that have come from this development.

So in all of our HPV clinical studies, which go back now to our first study beginning about nine years ago, we tried to collect our safety data using relatively consistent methodology, and we collected traditional safety information, solicited symptoms, usually over a seven-day period post-vaccination. Unsolicited symptoms are typically collected for 30 days after each

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dose of vaccine is administered.

Typically, we have collected serious adverse events and pregnancy outcomes over the entire duration of our studies, and in all of our HPV studies we have also collected information on medically significant events and new onset chronic diseases.

Now the HPV program is a very large development program most driven by the fact that the clinical outcomes to assess efficacy, CIN2+, are infrequent and, as a result, we have had to do very large studies and, in fact, long term follow-up in these studies to generate enough clinical endpoints to evaluate vaccine efficacy.

So this has given us the opportunity to collect a lot of safety data in the course of a development program like this. We have up to 6.4 years of follow-up with an average duration of follow-up in this development program of about two years.

So one of the analyses that was

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done with the HPV program was what I would consider a traditional pooled safety analysis, taking all of the subjects that have participated in this program through the data lock point of this analysis, and looking at a range of adverse events.

This large pooled safety analysis which we have conducted includes about 30,000 females, 16,000 of which have received active vaccine, and the others have received control. This pooled safety database represents a pretty broad age range as well.

Some of the general observations that we have made with this kind of standard pooled safety analysis approach are that the vaccine appears to be generally well tolerated across all age groups. We have not seen any differences in rates of unsolicited adverse events, serious adverse events, medically significant events, autoimmune diseases. I'll come back to that in a minute.

We have seen a comparable safety

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profile in women who had prior exposure to HPV compared to those who were previously uninfected, and overall similar rates of pregnancy outcomes in vaccine and control groups.

So these standard evaluations, I think, can be done using the pooled analysis approach, but there are some events that are infrequent enough that you have to use even broader approaches.

This is an example of a meta analysis which was conducted recently and, in fact, just published in the last month or so. So it is now available as an electronic publication. It should be in print in the next month or two in the journal Vaccine.

In this meta analysis, we have done two things. So first, we have taken all subjects that have been included in the HPV development program and looked specifically at autoimmune diseases.

Now I mentioned that we were

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soliciting physicians, investigators in our studies to report any signs or symptoms that would potentially lead to a diagnosis of an autoimmune condition in the development program, and so there was proactive solicitation.

What you will see here is essentially what I showed you in the pooled analysis. When we look at relative risks of any autoimmune disease for individual categories of events -- this is comparing subjects receiving the HPV vaccine over subjects receiving unadjuvanted controls -- we see relative risks that are all very close to one, confidence intervals that overlap one.

You will notice that there's a large number of events. So in this analysis, which is restricted to the HPV program, we have about 100 autoimmune events in each of the groups. So that might sound like a lot, but that is because we have done long term follow-up with active surveillance and, I

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think, good data capture.

Now I think even more important is this additional analysis which now expands the meta analysis beyond the HPV program to include subjects that have received any ASO4 adjuvanted vaccine in one of any of the three largest ASO3 adjuvanted programs that we have. So this includes subjects receiving HPV vaccine, adjuvanted HSV, general herpes vaccine, and an adjuvanted Hepatitis B vaccine.

What you will notice here is that this analysis includes about 68,000 subjects, 36,000 receiving ASO4 adjuvant, 31,000 receiving control, and the mean duration of follow-up in this study is about 2.1 years. So it is relatively long term follow-up in a very large population of individuals.

If we now look at the relative risks for autoimmune diseases, either any autoimmune disease or individual categories of events, you will see again the relative risks

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are very close to one in all categories. Confidence intervals tend to be relatively narrow, narrower with the broad analysis than with the analysis which includes only the HPV program.

We think this kind of data is very reassuring in terms of looking at risk of induction of autoimmunity over the course of very large development programs.

So in closing, a few lessons that we have learned about safety evaluations coming from these experiences and other experiences with other vaccines that have been through clinical development.

We believe that beyond traditional safety evaluations, it is important to determine events of interest relatively early on in the development program, based on either preclinical data, early clinical data, related products, target population or, in some cases, biological considerations, and use that information to define in advance what you need

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to collect prospectively to make sure that you have good data to do these kinds of analyses.

We think it is also important to define a relevant time period for follow-up, based on biological considerations. So you might think that just collecting more for longer is better. There are, actually, some downsides to having so much data that you might actually have events that occurred, background rates diluting out a potential safety signal.

So trying to define the relevant time period does become important in making sure you don't lose specificity in your detection. Then, of course, make sure that you capture the events of interest.

The other thing that we think is very important is to use consistent data collection methodology, not only across individual studies in programs but across programs using similar adjuvant systems, to allow pooling of data or the conduct of meta

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analyses.

So in conclusion, we think new adjuvant systems offer considerable promise in helping address important unmet medical needs. The selection of the adjuvant system, of course, needs to be appropriate for the specific need, and I gave you a few examples.

The development program should generate data allowing a robust benefit/risk assessment. The studies clearly should demonstrate the value of adjuvant systems but, very importantly, need to include thorough assessment of safety, including appropriate evaluation of events of interest that go beyond what might be considered traditional safety outcomes.

Again, to emphasize, these criteria could apply to any new vaccine, not only vaccines using new adjuvant systems. Thank you.

(Applause.)

MODERATOR SLATER Thank you, Dr.

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Dubin. Actually, I think we are going to hold the questions until the roundtable discussion, because we went a little bit long.

The next speaker is Dr. Greg Glenn, Chief Scientific Officer at Intercell USA, and Dr. Glenn will take us to lunch.

DR. GLENN: Well, thank you very much for this opportunity to speak to this audience, and I am very privileged to be with many friends, and I appreciate this chance to talk about Intercell.

As you may know, I was formerly of IMI, and Intercell recently acquired IMI. So I am now the Chief Scientific Officer of Intercell USA.

I have been interested in listening to some of the previous discussion, specifically about LT and some of the themes of using novel adjuvants and knowing a lot about the adjuvants.

So what I am going to talk to you today about is the LT adjuvant patch, which is

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a potent and safe and, I believe, very flexible adjuvant strategy that can be added to existing vaccines.

By the way, I think I will point to the left, if you want to watch the pointer.

So the LT, as we all know or many of us are very familiar with this adjuvant, has really -- in a way, was the original novel historical adjuvant, and there has been 30 years of tremendous amount of research and understanding about what LT does and how it works.

It comes with baggage, and we had some of that discussed earlier. It has safety issues. However, it is a -- In some ways, it is a very safe adjuvant in the sense that it is not very novel. It is a bacterial product. It is well known. There is extensive human exposure in the sense that it is the key pathogenic factor in enterotoxigenic E. coli with hundreds of millions of cases of exposure.

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So I think it makes for a very interesting discussion to see how one might use a product that is potent, has extensive human exposure, has previous safety issues that could be solved by putting this into a skin patch and providing some of the benefits of immune stimulation at the level of the skin and as well of safety, because it is now a highly sequestered immune stimulation.

I think what I will try to do is walk you through the merits of this and some of the thinking we have done in terms of how to develop a patch.

Just very briefly, as I mentioned, LT is a potent bacterial product. It is normal -- In the natural setting, it is given off by the E. coli, enterotoxigenic E. coli, and it induces massive fluid secretion.

This, by the way, is a profound but transient event. When you look -- This is now looking here at the mucosa. When you look at mucosa post-infectious cholera in ETEC,

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normally the mucosa is not effaced and looks normal. So a profound effect in the natural setting without sequelae.

It is known how it works. It is an avid binder, as Carl Alving mentioned, almost a covalent binding to the GM1 gangliocyte, which is a ubiquitous cell membrane component.

In the case of the enterocyte, it is found in the lipid raft. It binds, forms a structure that is taken into the Golgi through the ER. There's signals that allow it to get into the cytosol. It causes a rise in cyclic AMP and causes fluid secretion.

So I think pathways of how LT is activating in cells have been studied and are pretty well known. In the context of antigen presenting cells, we know that LT induces things that you would hope an adjuvant would do, migration of dendritic cells, of draining lymph nodes which is really a straightforward thing to study in the context of the skin,

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increased antigen presentation, up regulation of co-stimulatory molecules, etcetera.

So this long history and understanding of this adjuvant makes it quite an interesting topic, as far as a potential adjuvant for human use, but it is certainly book-ended by safety issues that have been historically understood as problems that would not allow development by certain routes.

So originally LT was thought to be an ideal adjuvant for oral immunization, but it is hard to find a therapeutic window between adjuvanticity and diarrhea caused by the toxin. The same -- We have discussed earlier, nasal use of LT has caused Bell's Palsy.

So one of the rationales for targeting the skin would be to provide a potent signal in an ideal biological milieu. Now this is a biopsy of human skin. You can see the three layers, the dermis, epidermis, the stratum corneum, and you can see this very

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dense population of antigen presenting cells called Langerhans cells in the skin.

They make, in my view, an ideal target for immune stimulation, but the skin also represents a significant barrier to penetration, and as GM on ganglioside is a ubiquitous cell membrane component, we have LT arriving in the skin and being taken into the body essentially by the antigen presenting cells.

I also would point out that, unlike the nasal passage, the skin, at least in the deltoid, has no vital anatomic structures.

I like this picture. This shows the network barrier of immune cells looking down on it. You can see, the pathogen is passing through that. It would have to encounter antigen presenting cells.

So in a way, by adjuvanting at this level, we are recapitulating the normal immune process where these antigen presenting

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cells are activated.

Now these are Langerhans cells crawling out of the skin. You see these very nice photomicrographs, and we are just really replicating a normal process that happens on possibly a daily basis where there is immune stimulation at the level of skin. The antigens are picked up by these antigen presenting cells where they crawl out of the skin, migrate to the draining lymph node, and elicit immune response.

The we have been working with this concept some, and what I would like to focus on is somewhat of a twist to this, where now we are engaging the skin immune system. We are taking a very potent adjuvant, LT, and we are adding this to an already formulated vaccine -- for example, influenza or pandemic influenza.

Now what we are doing is this has to be done in the same draining lymph node site. The APCs are activated, and they arrive

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at the same draining lymph node, and they have a bystander effect on antigen presentation, immunity, T-cell and antibodies, as I will show you.

I think one of the practical merits of this is that you can avoid -- Formulation is key. You can avoid formulation issues. You can add this to existing formulations, and it makes a very practical way to adjuvant a vaccine.

Now this activation, as I mentioned, is quite regional. Now this is from a mouse where we have immunized it on the back, on the dorsal on the back. The dendritic cells will travel down to the inguinal lymph nodes.

What this shows here is simply that, when you add LT to FITC labeled dendritic cells, you increase the number that arrive at the draining lymph node, and you increase their activation state.

What I wanted to point out is that

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it is also a very regional effect. So it is very hard to detect activated antigen presenting cells anywhere but in the draining lymph nodes of the site at which you have applied the patch.

This manifests itself in terms of the regionality. So this is, again, a mouse patch here. You can see, at immunization -- I believe this was with flu and different doses of LT patches added. So you can see very nice enhancement of the immune response by adding the patch, but when you put it elsewhere, you really get no adjuvant effects. That has been a very key finding for us.

So this is a very potent strategy. I am going to show a little bit of animal data. This is a no-patch. This is tetanus toxoid. We actually used this to some degree to look at the potency of the adjuvant patch, because in one dose we have this very profound enhancement of the immune response by adding the LT patch on top of an injection of tetanus

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toxoid.

So these are serum IgG antibodies on this scale, and you can see the individual mice, a very profound adjuvant by adding the patch at the time of injection.

it also enhances T-cell immunity. I won't go into too much detail. This is a flu study where we see increased IL4, interferon gamma spots by adding the patch after an injection, and similarly with the mucosal responses which is one of the interesting aspects of skin immunization. You can see, these are enhanced mucosal responses based on adding the patch to an immunization.

Then finally,. just to make the point that this adjuvant patch strategy, at least pre-clinically, has been tried in many different antigens, and it is a very effective strategy. Again, this is a trivalent flu. Here is no patch. Here is the patch with the adjuvant, and very big enhancement of the immune response.

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So what has been unique for us as a company to develop a patch strategy is to find a place where we can do all the development work that relates to delivery, coming up with a commercial format, and yet have something that could really be a product.

So we had early success with the delivery of LT as a heat labeled tox with E. coli, and we have done a tremendous number of studies now to focus on this application in terms of optimization.

We have to have something that, when you put a patch on and you immunize with this, is a very reliable system, and certainly as good as pushing the plunger and injecting.

So I would say today after -- this is actually, I think, a little low. It may be something on the order of 37 trials, many trials of optimization where it generated, I think, a very good system for delivery of LT in a reliable manner. I will show you some data, and I think we understand the safety

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profile very well.

So here the extensive data has helped us understand what the issues are, and I think it will validate what I have been saying, that the skin is a safe route to immunization.

Now what is unique here is LT is the key pathogenic factor for traveler's diarrhea or ETEC diarrhea, as I mentioned earlier, and this is actually -- we are just closing the door on the Phase II program and looking to enter Phase III shortly.

So we have a lot of data. We have a formulated LT patch with reliable delivery, and this same formulation, the same system, has then been applied as an adjuvant patch and maybe with some differences in the doses. So today we have this two-step system where we have a pre-treatment, and I will talk about that in a little bit, and then a patch application.

So what is important for skin

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delivery? The skin is a formidable barrier. Normally, the stratum corneum, the outer dead layer of skin, is very difficult for things to get through, for compounds, molecules, especially large molecules like LT. But if you do some modest disruption -- and we have published this, by the way. This represents -- - This step represented about 25 percent removal of the stratum corneum with a medical grade sandpaper.

You can see, in terms of immune response -- this is anti-LT IgG now -- if you don't pre-treat, you see very little response. If you pre-treat, you have a very nice antibody response.

So we knew that early on. We took that into a design engineering setting, and now what we have is -- This is a strip. On the other side is a small piece of medical grade sandpaper. On the other side of this push button is a little aperture. So this thing slides across the aperture as you push

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the button down. It is a highly controlled process. It is very easy to use, and I will show you some data from that, and from the patient standpoint, it is really a non-event.

Then we have also then, in concert, developed this patch. What we have tried to do is make the matrix of the patch minimal. It has dry stabilizing incipient formulation. It is a very thin little layer, and essentially it dissolves in contact with water, and I will show you some data on that in just a second.

The merits of the dry patch -- it allows you to provide a very stable formulation. I won't go into details, but these are thermal cycling studies where you expose the patches to harsh conditions, and we have a great deal of data.

The dry patch is a very good format for stabilizing it, but how do you make the patch work? You have to add water. We rely on what is called transepidermal water

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loss. So all of us here have some level of transepidermal water loss going through the stratum corneum. When we disrupt the skin, this is greatly enhanced. In fact, it allowed us to optimize the pre-treatment system.

Once that happens, the patch becomes very quickly hydrated, and that allows the LT to diffuse passively into the skin where it is then take up by the antigen presenting cells.

So this is quite a convenient factor. Many dry vaccine preparations require some logistics for adding water. I just wanted to show you very quickly. This is a dissolution profile form the patch.

This is done in the lab. So this is put into buffer, and we simply can't measure how quickly the patch fully dissolves. The LT is fully able to dissolve in our assays in vitro.

There is another advantage to the dry patch. It provides an enhanced delivery,

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and that is because, as you hydrate this patch, you have a high concentration of the antigen forming, super-saturated in a way, and that forces delivery.

We have evaluated that. This is a wet, which is simply pipe-headed onto the gauze matrix versus the dry patch, and you can see enhanced antibody responses to LT in that setting.

So as I was showing you, we have used the anti-LT IgG in the serum as a way of a marker for delivery. It has helped us optimize the traveler's diarrhea patch, and you can see here, this is now a study, a recent study using the patch system in various permutations.

We were entertaining a self-administration format for the traveler's diarrhea, and we have various patches either put on the arm, the arm and the thigh as a prime and boost regimen, put on by clinicians or put on by self.

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All I wanted to make the point is, even through there are various conditions in various anatomies, the end result, the antibody response is very, very tight between these four groups, and I think it represents an indication that the delivery system is really robust and solved.

So now just turning back to how we have tried to show that the adjuvant patch is useful, again we are injecting the vaccine. We are putting the patch over the same draining -- essentially over the site. It is somewhat like adding a Band-Aid. You do the pretreatment step. You do the injection. This pretreatment step leaves some marks here which allow you to register the patch, and you put the patch on instead of a Band-Aid.

This is one of the early studies we did. It was a proof of principle of influenza in the elderly. Here we vaccinated 56 subjects per group, either with young, elderly or elderly who had a patch. Even in

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this unpowered study, we are able to see the effects of the adjuvant patch in this setting.

Recently, we have been in collaboration with Solvay Biologicals, who makes a H5N1 egg-based vaccine candidate, and under an HHS contract we have been evaluating the adjuvant patch as a strategy for enhancing the immune response to the H5N1 vaccine.

So I am going to briefly show you some results from the fairly large trial. This is 500 subjects. It was quite complicated. We did different doses of flu. We did different applications of the patch, and basically we were looking at one versus two doses of the LT patch.

Again, a fairly complicated slide here, but I think that the highlights are that we saw our best effects at the higher doses of flu, and they were quite profound, as I will detail in just a second, and you can see very high responses in the groups receiving two adjuvant patches to the H5N1 vaccine.

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What was most interesting about this data was that the single dose data was at Day 21. First of all, we could measure significant adjuvant effects. What you are looking at here is the percent of subjects achieving seroconversion, which is a fourfold rise, and you can see, we had significant adjuvant effects.

At the high dose adjuvant group, we had a very nice adjuvant effect, which plays into a high level of seroprotection. I would note that our assays -- when they did the assays, the subjects were almost entirely naive at Day Zero, and by Day 21 we had a 73 percent seroprotection rate which, if we had confidence intervals to expand that, as mentioned earlier, would be a license-able vaccine.

So it is a very attractive concept that you could take a single dose pandemic vaccine into a pandemic and decrease the logistics. If you could achieve high levels

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of seroprotection, and maybe -- Also, I would point out that we have a very high rate of priming in these subjects as well.

So the adjuvanted patch seems to allow us to move in the direction of a single dose, and I just throw these pictures up to note how important I think it would be to have a single dose in a pandemic situation.

So just a few words on safety. We have done a lot of work here -- I think north of 35 trials. We have been -- It has been important to us to do randomized, double blind, placebo controlled trials.

I should mention, most of this work is done with the LT patch for traveler's diarrhea, and we recognize that in the adjuvant patch we are early in the dataset, but I think we have a very characteristic picture.

First of all, we don't see systemic signals, as you might expect. The patch is placed on the skin. The adjuvant is

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taken in by the antigen presenting cells, and so you would expect to see no significant differences between the systemic AEs and placebos in vaccinees.

We do see generally mild local site reactions, including rash, pruritus and some post-inflammatory hyperpigmentation.

So we are moving ahead with the evaluation of the adjuvant patch with pandemic influenza, trying to improve on the results that we saw. But we are also interested as a company to have a single dose, Japanese encephalitis virus vaccine, also to add this to some of the important vaccines that are used in the context of the elderly and possibly for HPV compliance and multi-dose pediatric vaccines.

So just to end, I think that LT is a very interesting adjuvant. It has a unique safety profile, and there is extensive human exposure. But it is also a potent activator of the immune system that we can use in a safe

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manner and a flexible manner in a patch.

For example, for H5N1 pandemic vaccine, this patch can be made well in advance, and if the strain of flue comes through and it is not the same as the vaccine strain, we will not have to remanufacture the patch. So it is a flexible strategy for that setting.

I think we are at a place now where the patch has got a good proof of concept. We have a mature product, because of the traveler's diarrhea program, and I think it certainly has borne out the hypothesis that the skin immune system is worth targeting for immune stimulation.

So with that, I will end and take questions. Thank you very much.

(Applause.)

MODERATOR SLATER: So let's go ahead and break for lunch. We will come back in one hour at 20 after one.

One final little housekeeping

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issue. Many of you are going to be leaving for the airports later this afternoon. You can certainly arrange your own taxis or cars on your own, but if you wish, the good people at the registration desk will help you coordinate that.

So if you want to go over there during the lunch break and talk to them, they might be able to help you.

We will see you at 1:20.

(Whereupon, the foregoing matter went off the record at 12:23 p.m.)

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A F T E R N O O N S E S S I O N

(1:22 p.m.)

MODERATOR SLATER: Welcome back. Please take your seats. We are resuming Session 4. The next speaker is Dr. Martine Denis, Senior Director of Clinical Development at Sanofi Pasteur. Dr. Denis, welcome.

DR. DENIS: Thank you very much. So this is really a very long and ambitious titer for a 20 minute presentation, but what I will try to do this afternoon is just illustrate to you a number of questions that we have faced at Sanofi Pasteur in the course of evaluating adjuvanted vaccines.

So my presentation will be divided into three parts. The first one will deal with general considerations in terms of clinical development of adjuvanted vaccines and study design. Then I will move on to some examples to illustrate how we can evaluate safety, and then efficacy immunogenicity.

So as I myself based in France, I

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guess it was very logical to use as an introduction a few words about the clinical aspects of the EMEA guideline on adjuvants. So there is a guideline that came into force in the middle of 2005, and so that is relevant to the work we perform in Europe.

This guideline is not very different as compared to what we discussed so far, and the general principles or general objective that is described in terms of clinical development, we will again refer to that balance we want to have in terms of improving the immune response with the adjuvanted vaccine while avoiding unacceptable increase in local or systemic reactions.

So interestingly, this guideline identifies two different scenarios where the recommendations would apply, the first one being the situation of a novel vaccine.

So that would be novel adjuvanted vaccines corresponding to a disease for which there was no product existing today, as it

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would be the case for HIV, CMV vaccines, for instance, or a second situation where we would somehow modify a license or established vaccine, and this could consist either of the addition of an adjuvant or vaccine, removal of an adjuvant or other changes to the composition.

Interestingly now, this guideline classifies clinical studies in two different ways. So the first type of studies consists of preliminary studies; second part consists of confirmatory studies. So there is no very detailed specific indication as to what a Phase I, II, II or IV trial should consist of.

It is more, I think, logical, general guidance provided. So in terms of preliminary studies, you would be expected there to just have defined what should be your vaccine composition.

So one aspect would be to demonstrate the effect of the adjuvant on the immune response, and that could be done in

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healthy adults. There is no indication in the guideline on what should be exactly the assay to be used. This will depend on the nature of the antigen that you work with, but the guideline, interestingly, mentions the importance of evaluating functional antibodies and also describes cell mediated immunity, and I will come back to that later on.

Also, this preliminary phase of development will include those dose-finding studies, and so snot only for evaluating the amount of antigen in the vaccine but also the amount of adjuvant.

Now the second step of the development will consist of these confirmatory studies. So this would be normally randomized, double blind controlled trials performed in the final population, final target population for the vaccine.

While, interestingly, in this part of the development these new adjuvanted vaccines will be considered just as any other

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new vaccine, and so the same kind of principle for development would have to apply.

Then having said this, I guess you understood that one key characteristic while developing an adjuvanted vaccine is that we have multiple objectives at the early stage of development of the vaccine. So we mentioned we want to justify the need for adjuvant, the dose, select the antigen dose. We also want to establish the long term effects of the vaccine, so multiple endpoints.

I think having these multiple endpoints doesn't necessarily mean that we could compromise on the statistical considerations, and I here would like to illustrate the way we organize and manage a Phase I trial, a recent Phase I trial at Sanofi Pasteur.

So that was a trial of an H5N1 vaccine combined to another oil and water adjuvant. So that trial was organized in the population that may look surprisingly big for

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you, so a total of 265 subjects.

The reason why we could do that was that we organized this trial in a staggered fashion. So we started with a small group of subjects receiving the vaccine, waiting until the two doses of the vaccine had been administered, conducted a safety evaluation at that time before enrolling the rest of the cohorts.

Of course, the reason why we were able to do that was that, while maybe this was the first administration to man of this type of adjuvant, but at least the antigen was not novel, as this consisted of H5N1 split and activated antigen. But anyway, this design helped us generate very meaningful, useful data to the rest of the development of the vaccine. Even very low dose was as low as 1.9 microgram of antigen were sufficient for inducing the type of response that we needed.

Also interestingly, so this trial had a long duration, and so had to generate

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the results that we needed. Finally, I would have to say that we needed to organize a second study to evaluate the dose ranging of the adjuvant itself.

Then another topic I would like to cover relates to the way we control our clinical trials when dealing with an adjuvanted vaccine.

Of course, we may like to use saline as a control. That is a well known type of control for evaluating baseline reactivity, and it is used regularly in Phase I trials. But personally, I have to say I believe that this type of control has serious limitations.

In particularly, we saw in that trial I was just referring to before that this may actually compromise the study blind, especially in this type of situation where the adjuvanted vaccine increases a high level of reactogenicity, so high level of pain which would not be the case, of course, with the

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saline control.

Another type of control we may se is commercially available vaccine. That would be, of course, very useful especially for benchmarking of safety and reactogenicity, and especially at late stage of development. However, this one is not always practical and, therefore, cannot apply to all types of programs that we have.

A further option -- actually, this is one that was mentioned earlier while we were discussing the preclinical evaluation of adjuvanted vaccines -- is the use of an adjuvant-only control.

I have to mention here that this type of control is not recommended by the EMEA guidelines or not recommended for use in clinical studies.

Well, you may say that, anyway, this type of control will not induce an immune response and, therefore, if your hypothesis is that the immune response actually is part of

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the explanation to the type of reactogenicity that you will measure, maybe this type of control doesn't make a lot of sense and would just result in data that are difficult to interpret.

I would just like to say that this is not necessarily the case, and here I am illustrating that point with a result we obtained sometime ago with an adjuvanted HIV vaccine at Sanofi Pasteur where we actually observed that the type of reactogenicity -- and here I am only showing the results in terms of incidence of pain. So the type of reactogenicity we had with the adjuvant in the adjuvant-only group was as high as that that we had measured in the adjuvant plus antigen group.

Then a fourth option in terms of control is, of course, the unadjuvanted antigen. And as you understood before, this is the most useful control to use, especially in early trials where we try to evaluate the

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impact of adding the adjuvant to the safety, reactogenicity and immunogenicity of the vaccine. But before I conclude on that part, I would just like to remind you that, when we develop -- when we perform clinical trials of a product, we always have to take into account very practical and logistical aspects.

To illustrate, this is just a picture of what an adjuvanted vaccine and for this milky emulsion that you heard of -- one of these milky emulsions that you heard of before. So what this may look like as compared to a non-adjuvanted control. Obviously, in this type of situation, the feasibility of a double-blind design may not be -- may be compromised.

So this being said, I can now turn on to a couple of points related to the administration of safety, and I will start here, obviously, with a short description of the type of results we may obtain when assessing the safety of an adjuvanted vaccine.

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So this is just the type of classical evaluation that anybody would perform while evaluating this type of vaccine. So the results presented here again correspond to the situation of this H5N1 vaccine where we monitored both injection site reactions and systemic reactions after our first and second vaccination.

Obviously, in this example we had a very high rate of pain induced, especially after the first vaccination. Well, the question we may ask now is to what extent this is relevant to the true vaccine safety. So I think we mentioned before that it is very important to make sure people do not mix up what is reactogenicity compared to the safety of the vaccine.

I don't think that with this type of profile, especially taking into consideration the fact that this pain was mild, of short duration and resolved spontaneously, so this was naturally a real

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source of concern. So overall, I think our interpretations should, in general, remain very cautious and take into account also all the data that may be available such as nonclinical safety, for instance.

So this brings me to another topic that we considered before, and that related to the type of adverse events that we may be interested in while developing adjuvanted vaccines. So what kind of safety issue can we foresee at the beginning of such a program?

So we discussed a lot yesterday, the type of in vitro data that are available today on the mode of action of adjuvants. I am sure it is very reassuring to all of us to see all the progress that has been made over the last years in terms of understanding better how our adjuvants function. However, I may sound provocative here, but I think that there is still a huge gap between what kind of information we obtain and what understanding we have gained, and to what extent this can

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impact in practice the organization or the design of our clinical trials, as all of this information that has been generated so far doesn't necessarily result in any specific event that we may like to address or like to evaluate in our clinical trials.

I think at the moment, we are still left with this general assumption that probably, as these adjuvants help improve the immune response, probably we have to pay attention to autoimmune diseases.

Of course, there are other types of data that may be taken into account, like nonclinical safety data, also signals that may have been obtained from other clinical trials, even from other vaccines, as one of these examples occurred this year.

So with this in mind, probably we have to significantly revise this contention that a sample size of several thousand is probably enough to allow detection of adverse events in the course of developing a novel

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adjuvanted vaccine, so probably this very simplistic view.

You heard before that we have a number of additional limits to take into account. Of course, it is important to think of the amount of information we will obtain from randomized controlled trials. So that is to ensure the quality of the data.

It is also important to take into account the fact that we may have predefined hypotheses when calculating -- well, to synthesize the safety trial. So you have seen that, when considering the increase in the baseline frequency of a specific event, then instead of just looking at your occurrence of an event in a population, so we will end up with a number of subjects much higher than was the case before.

Also, of course, we have to take into account the fact that supportive data may be available, and also ask questions whether or not all of the data need to be made

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available before registration of the vaccine.

Overall, I would think that in any case we will need a case by case evaluation of the needs for a novel adjuvanted vaccine. However, I think an important area to take into account in terms of safety is everything related to the addition investigations that we have the possibility to initiate.

I think anyone who would start today a new program, including an adjuvant containing squalene, would be aware of the association that has been proposed between anti-squalene antibodies and the Gulf War Syndrome. So I guess in every case we would be interested in evaluating the induction of such antibodies in our clinical development.

So that is just an example. I think, in general, we may have other types of reasons to consider such additional investigations. So that may come either from clinical or preclinical data, and may have been generated on the product we have in

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development or any other related product. Also, the question may be related either to the pathogen, antigen itself or to the adjuvant.

So I will just illustrate here two examples or two examples of the investigations that were conducted at Sanofi Pasteur. So the first one relates to a cytomegalovirus where we had obtained information that an adenovirus-gB recombinant was able to induce autoantibodies in certain mice strains.

So this triggered an investigation in the context of development of a vaccine, and here I am referring to a clinical trial. So the gB vaccine produced at Sanofi and combined with MF59 from Novartis was used in that clinical trial.

So as illustrated here, so we performed a number of investigations to evaluate, actually, the induction of autoantibodies in humans. As you can see, the results were quite reassuring.

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Another example is a more recent one and occurred in the context of development of an H5N1 vaccine where, at least hypothetical risk of disease exacerbation had been raised and linked to this observation of the Sixties of an RSV vaccine, so forming inactivated RSV vaccine, so inducing such exacerbation of disease in children.

So part of our investigations to respond to that kind of concern included an animal model, and so data were generated in a monkey challenge model of H5N1, but we also conducted some investigations in our clinical trials, and I am here showing the results we obtained in terms of Th1, Th2 balance, also cytokine response after vaccination of infants.

So these children received either adjuvanted or unadjuvanted vaccines, and we looked at both the induction of interferon gamma, IL-5 and a number of other cytokines. So with this type of data, we are able to

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identify the evidence of the similar bias of the response in subjects that received the adjuvanted vaccine as compared to non-adjuvanted control, and again felt that this information was very reassuring.

So this brings me to the last part of the presentation. So what about efficacy? Now I don't think I need to go very much into the details of what an adjuvant can bring in terms of improvement of the immune response, and I think you have seen over these two days already a number of examples where the adjuvant was able to improve significantly the profile of the vaccine.

Obviously, in terms of antibodies, a number of parameters can be identified, so whether in terms of magnitude of the response, cross-reactivity of the response, also persistence of immunity.

I would just like to stop on the last example here, so related to some mediated immune responses. I think that in a number of

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vaccine, adjuvanted vaccines in development now, we are facing a situation where the disease relates to a situation where we expect some mediated immunity to play a significant role in the protection against the disease and, therefore, these are all situations where generating some mediated immune data is very important.

Of course, we have all the tools needed to generate these results today. So science has made significant progress over the last years, and all of these methods allow generation of, certainly, very useful, interesting data. But the question today is, I think, to what extent we can really benefit from these results in the course of developing a vaccine.

I think, when looking at the package inserts of all registered vaccines today, we never find any indication in the evidence of CMI data proving essential to registration of the vaccine.

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Well, obviously, there are a number of challenges to overcome to make this feasible. So for instance, sample management is much more complex when dealing with some mediated immunity as compared to antibodies. Also, there is need for RSV validation that is not as easy to reach as compared to antibody assays.

Also, in many situations we know that there is increased, let's say, variability with this type of assay as compared to serology. But while these are all challenges, they can be overcome, and at least there is significant progress being made in just considering the efforts made in terms of HIV or cancer, CMI assay.

So in terms of standardization, I think we can be quite positive in terms of what we can expect. So this is just an illustration of the type of CMI data that we have generated so far at Sanofi Pasteur. So in different areas, HIV vaccine, H5N1 vaccine

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or metastatic carcinoma vaccines are all situations where we were able to detect a significant improvement with the adjuvanted version of the vaccine as compared to nonadjuvanted.

So this brings me to a conclusion. Here, I actually thought back to a paper, a title of a paper I had read sometime ago. When looking at this, I am sure that we all want adjuvants to remain our friends in the future.

I think for this to be feasible in the future, it will be very important that we pay specific attention to the way we design and analyze our clinical trials. I am sure all the knowledge that we exchange over these two days will contribute to that. Thank you.

(Applause.)

MODERATOR SLATER: Thank you very much, Dr. Denis. I think we will hold the questions until the roundtable discussion, please.

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Our next speaker is Dr. Ofer Levy, and we are going to have a little platform change. It will take about 30 seconds to do that. Let me just introduce this section.

When we were planning out this session, there was interest among many of us in having some discussion of age related issues, and it was correctly pointed out that we actually could have planned a two-day workshop addressing only age related issues.

Nonetheless, we felt it was important to at least introduce this in some way, and Dr. Ofer Levy from Boston Children's Hospital is going to address some of the issues regarding the neonatal immune response, to start off this last section of Session 4 before the roundtable discussion.

DR. LEVY: All right. Thank you for the opportunity to speak. So the title of my talk today is Distinct Innate Immunity of Human Newborns, Implications for Development of Neonatal and Infant Vaccine Adjuvants.

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This is work carried out in my laboratory at Childrens Hospital, Boston, in the Enders Building.

Just by way of introduction, newborns and young infants have an increased risk of invasive microbial infection, and a statistic that brings that very clearly into focus is that, according to the World Health Organization last year, globally more than 2 million infectious disease deaths in those less than six month of age.

Common bacterial pathogens include gram-positive bacteria such as Group B Streptococcus. Streptococcus pneumoniae is still responsible for nearly 1 million deaths globally per year. It is worth noting that the Prevnar and other vaccines in the pipeline have been a big win in the West, but they don't cover a lot of the serotypes that are prevalent in other countries.

Gram-negative pathogens in this age group include Haemophilus and E. coli, but

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also Bordetella pertussis, the causative agent of whopping cough. Viral infections in this age group include Herpes simplex virus. Respiratory syncytial virus is the leading cause of infant hospitalization in the United States, and diarrheal diseases are still a prominent player. Rotavirus alone is responsible for several hundred thousand deaths per year in infants and newborns.

So, clearly, there is an unmet medical need for prevention of microbial infection early in life.

So our lab has been trying to understand the roles of the fetal and neonatal immune system, and particularly the innate immune system, with the underlying hypothesis that, if we understand it better, we might be able to manipulate it to come up with better vaccine adjuvants.

So as with any immune system, the role of the fetal or neonatal immune system is to protect against infection, but it is also

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to avoid potentially harmful pro-inflammatory or Th1 polarizing reactions.

It is well known that pregnancies that end up in spontaneous abortion or pre-term delivery are characterized by high peripheral blood concentrations of interferon gamma and other Th1 polarizing cytokines in maternal blood.

The fetal and neonatal immune system also mediate the transition from a normally sterile intrauterine environment to a foreign antigen-rich outside world. If you think about it, the first few days of life are quite remarkable.

It is the initial colonization of the skin with fluorides, the initial colonization of the intestinal tract with bacteria, and early host-microbe interactions affect the risk of the newborn for infection, and we will remember that pre-term newborns are particularly susceptible to infection, but even full term newborns after a normal birth

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have a pretty high susceptibility to infection.

Then also, of course, immune system polarization is affected, and we are all aware of the hygiene hypothesis that, in a nutshell, exposure to infection and infectious agents early in life is correlated with less autoimmunity and less auto-inflammatory disease.

So in the past 10 years there has been tremendous progress in defining the pathways by which the innate immune system recognizes danger signals, both endogenous danger signals and also microbial products.

This kind projects weird here. Some of the molecules look radioactive, but nevertheless, this is supposed to represent lipopolysaccharide or endotoxin, which is found on the outer leaflet of the gram negative bacterial outer membrane, and as we know, that signals through toll-like receptor four.

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This is supposed to the surface of a monocyte, macrophage or antigen presenting cell and bacterial lipo-peptides derived from gram positive and gram negative bacteria activate through toll-like receptor 2, and there are signaling cascades that are activated that culminate in NF-kappa B activation.

We know these pathways are important in humans, not just in mice, because human patients who are defective in this interleukin receptor associated kinase-4 or IRAK-4 -- these children present to our clinics with recurrent staphylococcal and streptococcal infections.

I follow a child with recurrent staphylococcal meningitis, and when we sequence the IRAK-4 gene, it is a deficient IRAK-4. This observation was initially made by Jean-Laurent Casanova in Paris. He is now at the Rockefeller.

So we know these pathways are

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relevant in humans, number one. Number two, we know that children with IRAK-4 deficiency grow out of their immunodeficiency. So if they make it to their teen years and beyond, their susceptibility to infection drops, and that indicates that these pathways are particularly important in newborns, infants and young children.

There was recently a paper in the journal Science about MyD88, the adaptor molecule in the Toll pathway, and certain alleles of Myd88 and certain hypomorphic alleles.

You end up with recurrent Streptococcal infections, and once again in that paper by Luke O'Neill and many other co-authors, the children grow out of this susceptibility, so once again indicating this pathway is important in humans, and it is particularly important early in life.

So part 1 of my talk is characterizing the mechanism for polarized

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neonatal monocyte responses. There is a vast literature about neonatal immunity, and it mostly says that neonatal leukocytes don't function as well as adult leukocytes when you test them in vitro. That sums up about 1,000 papers, and it is most of what those papers will say.

Then the question is -- and I don't want to be too dismissive, but I am trying to quickly give you the background. But we decided to take a whole blood screen comparing neonatal cord blood and adult peripheral blood, probing TLR agonists, because when we started this project a few years ago, the pure agonist for various TLRs were just being described.

We measured TLR induced production of tumor necrosis factor alpha, which is pro-inflammatory but, as you know also, Th1 polarizing, and interleukin-6. It is underappreciated fact that interleukin-6 has anti-inflammatory properties. It actually

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inhibits neutrophil migration. It also is Th2 polarizing, unlike TNF, and is regulated very differently.

So cutting to the chase, if you take human adult peripheral blood, and if you take human newborn cord blood, and you incubate them in vitro with Toll agonists and then you measure in the extracellular phase TNF and IL-6 by ELISA, and you plot TNF on the Y axis against IL-6 on the X axis, you find that the adults and the newborns segregate to two completely different groups.

The adults make a lot of TNF and very little IL-6. The newborns make a lot of IL-6 and very little TNF. This is something we published in Journal of Immunology a couple of years ago.

It turns out, as we tried to break apart the mechanism, that human neonatal blood plasma reduces TNF alpha production in response to agonists of Toll-like receptors 1 through 7.

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What we did was we took human neonatal cells, and we spun them down, and we washed extensively with a pyrogen-free buffer, and then we resuspended the neonatal cells in adult plasma, and we did, conversely, adult cells in neonatal plasma, a mix and match experiment, if you will, and then stimulate with different Toll agonists and look at whether TNF production is enhanced or inhibited.

These big black bars shooting up indicate that, if you take human newborn cells and culture them in adult plasma, you dramatically enhance the amount of this Th1 polarizing cytokine, that you make TNF. Conversely, if you take adult cells and put them in newborn plasma, you inhibit production of TNF.

There was an exception to this rule, and we will talk about that exception a little later. But we tried to target here: Let's understand why Toll-like receptor-1

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agonists, these bacterial lipo-peptides, are so inhibited by neonatal plasma.

We did a large study that was published in JI, but to cut to the chase, we found that there is a soluble low molecular weight factor in human newborn cord blood that turned out to be adenosine.

Adenosine is an endogenous purine metabolite made by all the cells in our body, that acts through cognate adenosine receptors. It is an anti-inflammatory factor. It is a counter-regulatory factor that is elevated by hypoxia and stress.

If anybody has been present at the birth of a baby, you see how the baby comes out blue and purple until it takes its first breaths, and we were able to show that adenosine is at very high levels by HPLC measurement in human neonatal plasma, and it acts through seven transmembrane adenosine receptors.

If you block those receptors

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pharmacologically, you can dramatically enhance TNF production, here on the Y axis, in response to a Toll-2 agonist, with no effect on adult TNF production and no effect on IL-6 production. So this adenosine factor selectively inhibits TNF production in newborns.

We think the mechanism is through inducing cyclic AMP. So there is ATP, and under the aegis of the enzyme adenylate cyclase, ATP gets converted to cyclic AMP. That is the key second messenger that earned Dr. Sutherland his Nobel prize and is induced by ligands via 7-trans-membrane receptors like epinephrine, norepinephrine, etcetera, and these are G-coupled.

It is very important to know that in PubMed, if you look at the literature, study after study shows that cells that have a lot of cyclic AMP in their cytosol are unable to produce Th1 polarizing cytokines, but they preserve production of IL-6 and other

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cytokines.

So we took neonatal cord blood mononuclear cells, and we lysed them, and we measured cyclic AMP by competitive immuno-assay, and it turns out that at birth the mononuclear cells in the cord blood of newborns have more than 20-fold more cyclic AMP per cell than adult peripheral blood mononuclear cells.

So the physiology of the neonatal leukocytes is profoundly different from that of adults, and serum confers this. If you culture the cells in newborn serum, you detect cyclic AMP, but if you culture them in adult plasma or serum, you don't detect cyclic AMP. Conversely, neonatal serum when placed on adult cells, will induce cyclic AMP.

So this low molecular weight adenosine factor induces cyclic AMP in these cells. And now we hypothesize that cyclic AMP may be a general regulator of neonatal cytokine production, and we hypothesize that,

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because it is known that newborns don't make TNF alpha very well or interferon alpha very well or interferon gamma or IL-12 or IL-1.

Guess what? The world literature suggests that cyclic AMP inhibits all of these. Conversely, newborns make IL-6. They make the anti-inflammatory counter-regulatory IL-10 well, and they make IL-23 well. In all three of these cases, cyclic AMP either enhances or does not inhibit.

So that is a hypothesis I have put forward recently in a review article I wrote for Nature Review's Immunology, and this is also from that review article, looking at mechanisms that polarize the cytokine responses of human neonatal antigen presenting cells.

There is the extra cellular adenosine binding its adenosine receptor, inducing cyclic AMP production, which through protein kinase-A dependent and independent manner inhibits production of TNF and other

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Th1 polarizing cytokines.

Now part 2 of my talk is the discovery of a Toll-like receptor pathway that is refractory to this inhibition, and here I very much have to acknowledge the work of Eugene Suter and Victoria Philbin in my lab, and we believe that the preservation of this pathway suggests novel neonatal vaccine adjuvants.

I want to emphasize again that vaccines at birth, it has been argued, could be a key to global health. We talked about greater than 2 million deaths per year due to infection in those less than six months.

According to World Health Organization, birth is the most reliable point of health care contact in resource poor settings. If anybody -- if a child is going to see a health care provider at all during their life, it is on the day they are born, whether it is a midwife or a nurse or a doctor.

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So early life immunization, such as with BCG vaccine, is associated with higher vaccine coverage. It is a practical point. However, vaccines effective in adults and infants may be poorly protective at birth, and that includes the conjugate vaccines.

Impaired neonatal AMP responses have been described to most adjuvants. There is, therefore, an unmet medical need for vaccine adjuvants that are effective at birth.

This is the general diagram of the antigen presenting cell, expressing Toll-like receptors and other pattern recognition receptors, and that activation through these receptors can enhance the second signal needed to enhance APC function and lead to long lasting immunity.

This is a figure that was recently made by Victoria Philbin in our lab for a review article that will be coming out soon in the journal Pediatric Research, and it reviews vaccine adjuvants and how they engage innate

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immune pathways.

Incomplete Freund's adjuvant, it turns out, activates through these nucleotide oligomerization domain proteins that act through MF-kappa B. Hemophilus influenza Type B vaccine, the one that is conjugated to OB-C, actually is a TLR-2 agonist because of the OB-C portion, and that was shown by Schreiber and Eike Latz at U. Mass.

BCG or Bacillus Calmette-Guerin expresses Toll-like receptor 2 and Toll-like receptor 4 agonists. Attenuated live viruses engage the RIG pathway that culminates in production of Type-1 interferons, important for cross-presentation.

Then alum, which is the most commonly used vaccine, as you know, through work from Fabio Re and other groups, Gabriel Nunez, engages the inflammasome. So this adjuvant that we have been using a very long time with limited understand -- now we understand the pathways involved, and this

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will trigger IL-1 production through caspace activation.

I am going to tell you a bit about the imidazoquinolines, a family of compounds that activate through Toll-7 and 8, but also engage the inflammasome.

So viz a viz imidazoquinolines, they are synthetic, low molecular weight immune response modifiers developed by Dr. Richard Miller at 3M Pharmaceuticals. This is adenosine, by the way, and you could see the resemblance there.

This is a first FDA approved stand-alone TLR agonist, imiquimod or a Toll-7 agonist. As you know, it is FDA approved as a topical therapy that will induce antiviral interferon in the context of human papilloma virus or warts, and it is safe and efficacious for that indication. Turns out to be a Toll-7 agonist.

Of course, there are a variety of congeners. R-848 which has these ethoxyl and

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hydroxyl groups is more polar, more soluble and also engages Toll-like receptor 8.

We found some very interesting effects when we started to explore these compounds. We are calling the Toll-like receptor 7 as expressed on B cells and plasmacytoid DCs, whereas Toll-like receptor 8 is on monocytes and myeloid DCs. So 7 and 8 are both located in endosomes.

So when we tested the ability of human newborns to respond to imiquimod or a Toll-7 agonist, it was very much impaired, much like that of the other Toll agonists we discussed. However, we did find a Toll agonists that was refractory to the inhibitory effect of plasma adenosine, and that turned out to be R-848, which is one of the imidazoquinoline congeners.

There is the structure of it again. This is TNF production on the Y axis, increasing concentration of this R-848 compound. This is in whole blood in vitro

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assay of cord blood and adult peripheral blood.

This is the congener from imiquimod with enhanced solubility. It has been in Phase III human trials as a topical against Herpes simplex virus, and did reduce HSV reactivation and shedding, but it did give a local irritation that was unacceptable as a side effect profile for that indication.

Now TLR-8 agonists turns out can induce up-regulation of CD40 on neonatal myeloid DCs. In the world of newborn immunology, that is a pretty big deal, because turning on neonatal antigen presenting cells has not been an easy thing to do.

Most of the literature, again, in this field is how a variety of stimuli fail to adequately up-regulate co-stimulatory molecules on neonatal cells.

Here, we took human neonatal cord blood and, by flow cytometry, gated on myeloid DCs and measured CD40 up-regulation, and of

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all the Toll agonists -- these are Toll-7 agonists -- the Toll 8 and Toll 7/8 agonists gave stronger CD40 up-regulation, both in adults, in black bars, and newborns, in white bars, that exceeded that by lipopolysaccharide and Toll-2 agonists.

Here, we worked with monocyte-derived dendritic cells, culturing monocytes in vitro, and then differentiating them to dendritic cells, and showing up-regulation of CD80 and CD40 and production of IL-12p70, recalling that it is the p70 form of IL-12 that is Th1 polarizing.

It is a good marker for good vaccine adjuvant activity, and R-848 and the 3M002, which are imidazoquinolines activating through Toll-8 were superior to the Toll-7 in inducing IL-12p70 in newborns.

Now there may be some interest in engaging the Toll-7 pathway, because interferon alpha production is an important feature of some adjuvants and induces a cross-

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presentation. So here we looked at interferon alpha production.

Again, the world literature would suggest that human newborn cells are not very good at making interferon alpha, but when we took the Toll-7 and 7/8 agonists, we were able to induce interferon alpha from human neonatal cord blood and up-regulation of CD40 on plasma cytoid DCs. So a combined 7/8 agonist might afford the advantages of both a 7 and 8 pathway.

Here we have used a bioinformatic approach to look at mRNA production in human neonatal monocytes isolated to purity and cultured in vitro in autologous plasma, and compared it to a Toll-4 endotoxin stimulation, and we plotted the LPS response against the imidazoquinoline Toll-8 response.

The dots that you see above the line of equivalence indicate that the Toll-8 agonist gave a superior induction of mRNA transcript for these cytokines, and at the

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protein level by a multi-analyte B platform, we were able to show that the Toll-8 agonist gave a stronger cytokine induction than the Toll-4 agonist with respect to newborn monocytes.

A question I often get when I present this work is you are showing us a lot of work with cord blood; how about peripheral blood from older infants? So we do have some limited data here.

Here is an infant from the United States who was tested, a healthy infant, at two months of age and 15 months of age, the same child. We stimulate in vitro for TNF alpha production in whole blood in comparison to a Toll-2, Toll-4 or Toll-7 agonist.

It is only the Toll-8 agonist that gives a robust TNF production, both at two months of age and at 15 months of age.

Similarly, through a collaboration with the Medical Research Council in the Gambia with Sarah Burle and Katie Fitzgerald

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there, we have blood from a nine-month-old Gambian infant, and again the 7/8 and 8 agonists give the most robust response.

So we know these effects are not evident just in cord blood, but also peripheral blood of human infants.

By what mechanisms do the Toll-8 agonists activate human monocytes? They lead to superior p38 MAP kinase phosphorylation, which is important for TNF production.

So by flow cytometry we are able to show that, when you add these Toll-8 compounds, you get stronger phosphorylation of p38 MAP kinase with a 8 agonist versus the 7 agonist, also a more profound and prolonged degradation of NF-kappa B. So these correlate with the enhanced efficacy, and we published in the journal Blood a number of years ago.

We also have recently showed -- and this is unpublished information -- that the Toll-8 agonists are relatively refractory to inhibition by cyclic AMP. That is the

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intracellular factor that we posit is polarizing the neonatal response.

Here is increasing amounts of dibutyryl cyclic AMP. This is a cell permeable cyclic AMP analog. It is a pharmacologic manipulation to enhance cyclic AMP in the cytosol. As you increase cyclic AMP, you inhibit TNF production as a percent. That is 100 percent with no enhancement of cyclic AMP, and the Toll-8 agonist is relatively refractory to that inhibition.

If we want to develop an animal model for this compound as a neonatal vaccine adjuvant, as discussed, the Rhesus macaque becomes very important as a primate model for Toll-like receptor 8 studies.

There are the protein alignments for mouse TLR-8, human TLR-8 and the monkey TLR-8, and these are the leucine rich repeats that are characteristics of the extracellular domain of the TLRs and, as you could see just by glancing on it, the human and monkey

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structure, the leucine rich repeats are much more similar to one another, and the mouse is divergent; and as you know, the mice express Toll-8, but they don't respond to all the same Toll-8 agonists that the human does.

Also very important work done by Wille-Reece and Seder who are here at the meeting is a Toll-like receptor 7/8 agonist enhanced vaccine responses in adult rhesus macaques in vivo.

They did studies with HIV GAG protein, and also covalent linkage of Toll-like receptor 7/8 agonists to GAG protein, enhanced both the magnitude of the Th1 response, enhanced both antibody responses and cellular immunity. These are published in PNAS an JX MED a few years ago.

We have looked at cord blood from rhesus macaques and peripheral blood from infant macaques in vitro in collaboration with Keith Mansfield at the New England Primate Research Institute, and we show robust TNF

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alpha production from rhesus macaque blood stimulated in vitro with Toll-like receptor 7/8 or 8 agonists.

What you could see here is that Toll-2 agonist in the cord gives very little TNF, much like our human data, the Toll-7 not much, but the Toll-7/8 and the Toll-8 give superior TNF induction, and that is not just in the cord. It is throughout infancy. This is blood collected every week from the same monkey. We follow them as they mature. So that suggests that the macaque is a realistic model for us.

The Toll-7/8 agonist also induces CD40 up-regulation on infant rhesus macaques. So if we take blood from infant macaques and stimulate and then do flow cytometry in vitro, gating for CD40 expression on myeloid DCs, here are the infant macaques. If anything, you get a stronger response in the adult.

Finally, what we have done most recently is take a photoactivatable agonist

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and link it to a model antigen, CRM197, and we can show that that confers Th1 polarizing activity on newborns.

So here is a compound we worked with courtesy of 3M Pharmaceuticals. There is imidazoquinoline backbone, and there is the aryl azide that has been added that will confer photoactivatable conjugation.

Here we could show that this compound in human newborn blood -- and this is adult's response curve -- as you increase the concentration of the compound, you induce TNF. These are the newborns. These are the adults. The newborns give at least as strong a response as the adults.

There is the chem draw reaction for post-reaction mechanism upon ultraviolet light for the conjugation. This is gel filtration, and the pooled fractions by silver staining. So we have CRM physically conjugated to this imidazoquinoline.

Then when we take the conjugate

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into newborn and adult blood, we get a robust TNF induction in a way that the CRM alone did not induce.

It is also important to note a paper by Peng and co-workers that, of all the Toll pathways, Toll-like receptor 8 agonists reverse the suppressive activity of human T-REG cells. That is a paper in Science in 2004. Synthetic and natural Toll-8 agonists reversed T-REG mediated suppression in vitro and in vivo through a MyD88 pathway, and did an adaptive transfer of Toll-8 agonist stimulated T-regs to tumor bearing mice, enhancing anti-tumor immunity.

So TLR-8 plays a key role in enhancing adaptive immune responses. As you know, T-REG cells are very important, and they are there for a reason, but they can also serve to limit adaptive immune responses, and they are particularly plentiful and suppressive at birth.

So this is our current cartoon on

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how these agonists might work. They act through Toll-8, and we have some SI-RNA data showing that, but they also may act by blocking at the adenosine receptor. I haven't shown you that data.

They act on T-REG cells. This is Peng's work to reverse T-REG mediated suppression, and by all these pathways then, enhancing a Th1 type response.

Of course, safety will be a primary concern in developing these, as with any new compound, but there are some reasons that local and transient engagement of TLR-8 might be safe.

Conjugation might localize the adjuvant effect, as discussed earlier. A TLR-7 adjuvant is apparently safe and efficacious in adult non-human primates, at least in those primates studied in the Wille-Reece papers.

A TLR-7 agonist, imiquimod, is FDA approved for human use, and has been used in pediatric indications such as Mollusca pox.

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A TLR-7/8 and 8 agonist also induced counter-regulatory IL10, and systemic mechanisms that keep Th1 responses in check will remain intact if you have a covalently modified local depot effect, the adenosine mechanism described in t he rest of the T-REG cells on the body.

So conclusions and future directions: Neonatal immune responses to agonists of Tolls-1 through 7 are skewed toward a low TNF to IL6 ratio by the adenosine system. Impaired Th1 responses of newborns to Toll agonists may help avoid allo-immune reactions, but contribute to infection susceptibility and impaired neonatal vaccine responses.

TLR-8 agonists activate robust Th1 polarizing responses from adult APCs, exceeding responses to other TLR agonists, even setting aside the neonatal data, and TLR-8 agonists are refractory to the inhibitory effect of neonatal plasma adenosine, and induced robust adult-like Th1 responses from

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neonatal APCs. They activate through p38, NF-kappa B, and I haven't shown you this, BTK kinase, and they are refractory to the cyclic AMP inhibition.

So our hypothesis is that Toll-8 agonists conjugated vaccines will induce protective neonatal CD4 positive T-Cell and antibody responses, and our approach will be to assess vaccine adjuvant potential of Toll-7/8 agonist in neonatal rhesus macaque model.

That has potentially great public health relevance, and will require appropriate partners and resources, and there is my e-mail for any who are interested in helping us in that journey.

Finally, I have a long list of acknowledgments, but just to go through briefly: Victoria Philbin and Eugenie Suter in my lab spearheaded a lot of the Toll-like receptor work. Dr. Michael Wessels is our Division Chief. Dr. Raife Jehine, immunology, has been a mentor, Dr. Zach Bohane in the

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bioinformatic realm, Dr. Keith Mansfield at the New England Primate Research Center, Dick Miller and Mark Tomai at 3M Pharmaceuticals, and our funding, we should acknowledge, through NIH, RO1, NIAID on the adenosine work, and Dana Human Immunology Award, and we have received funding from XOMA and reagents and support from 3M Pharmaceuticals. Thank you.

(Applause.)

MODERATOR SLATER: Thank you. I think we have another platform change. I'm sure there will be questions for Dr. Levy at the roundtable, which we will be starting quite soon.

I would like to invite Dr. Rino Rappuoli to come to speak. He is the global head of vaccine research for Novartis. Dr. Rappuoli.

DR. RAPPUOLI: Well, while the computer goes up, I want to start. My focus is going to be about using adjuvants, especially MF59, in different age groups and

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somehow practice of the best science that has been just described in the previous talk.

I will be talking mainly about immunogenicity, because the safety has been described in the previous talk by Giovanni della Cioppa. So all the data I am going to talk about the safety, you have already seen.

So I am going to talk about the MF59, and I will talk about basically immunogenicity in children, in adults, in the elderly, how the adjuvant broadened the cross-reactivity across different age groups, and finally a couple of slides on pandemic influenza.

You heard a lot about MF59. I will not go into it. I think the only thing I can add is MF59 was born Chiron, and was developed originally by Gary VanNest, who is sitting over there, and was the only adjuvant other than alum licensed the past century. This century just started. We will see how it goes.

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The great merit so far of this adjuvant is having been in 40 million people. We have very robust confidence on the safety, and we are starting to work a lot on the molecular mechanism of adjuvantation, how it works, and there have been a number of papers published.

I think the best way to describe and summarize the way we believe it works is it basically creates a micro environment which is optimal for antigen presentation by recruiting all the cells, optimal like an artificial lymph node or whatever, where things happen optimally. That is all I wanted to say about it.

What about different age groups? MF59 a few years ago has gone into newborn kids in a trial where, basically, the adjuvant was used with GP-120 in newborn infants from mothers which were infected by HIV. So 72 hours after the birth, people were vaccinated with -- children were vaccinated with MF59,

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and three doses followed.

There was one study to find the schedule. The second one just to look at immunogenicity. It was found that the optimal immunogenicity, one dose at birth was able to induce good response, antibody response, to GP-120, and it was found the safety was fine.

So, basically, this was a small study, under 54 newborns, but basically MF59 has gone safely in three doses into newborns, and for three consecutive doses. So this to say that adjuvants as MF59 can be used even at birth.

The second study I want to talk about in infants is on influenza. This is a number reference that tell you that the influenza vaccines that we have for infants are not optimal or they are pretty lousy, and there is a way to improve them.

So we have been using the licensed vaccines for influenza in six-month-old kids. Basically, we need to use two doses, and you

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still get a lousy -- a pretty bad response. So there is room for improvement.

Here we compare in kids from six months to three years the immunogenicity of a licensed vaccine against an MF59 adjuvanted influenza vaccine, the three cell types. The story is the same, one , two, three. They are basically much better immunogenicity. This is a log scale. So you can see the difference, if you use MF59 in infants.

This is a detail about what happens with the B strain of influenza. In yellow, licensed, non-adjuvanted vaccine. Basically, this is six months. You see increasing with age the adjuvant -- The non-adjuvanted vaccine basically at six months is absolutely not effective, and it goes up with age, and when you get to three years, basically you get seroconversion across 50 percent of the population.

With MF59, you get 100 percent from the very beginning. That gives you an

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idea. It looks like the adjuvant basically accelerates the young immune system to work extremely well from the very beginning, independently of the age.

Basically, this is another slide showing a year later, you can revaccinate those kids, and basically the influenza you still see statistically significant difference when you revaccinate them.

So MF59 can be used and works in newborns, the HIV, works and can be used in infants and children from six months to three years, and induces optimal immune response.

This is for infants and children. I want to move now to people -- categories of people that are at risk, some kind of diseases that basically compromise their response to vaccines. So this is chronic diseases. Again, it is still influenza, and the three vaccine strains. Always, the MF59 is much better in immunogenicity than the control vaccine.

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These are basically HIV patients, similar story. MF59 is always better than the control vaccine. MF59 is in red, and the yellow is the control vaccine, and this is people, transplant recipients, same story. MF59 is much better.

In the elderly, which also have a kind of compromised immune system, there is a need for adjuvanticity. You see a similar story. MF59, much better against the three influenza strains, and this is a story that repeats in many, many trials. You always see these kind of things.

We did a meta analysis to see whether in all the trials that we have done in the elderly the MF59 will induce superior immunogenicity, and again here is the ratio. One will be that they are equal immunogenic. Below one will be the conventional vaccine is more immunogenic. Above one means that the MF59 adjuvanted vaccine is more immunogenic.

So for all the three strains in

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this meta analysis of many studies, the MF59 is always consistently more immunogenic.

So this is about immunogenicity in infants, in people with chronic diseases, and in the elderly. What about coverage of strains which are antigenically equal to these vaccine strains, still in the case of influenza?

We know that, when there is a mismatch between the vaccine strain and the circulating strain of influenza, the vaccine efficacy, which is usually in the 60-80 percent, drops down to 50 or 40 percent. So can MF59 broaden the immune response so that, even with a mismatched strain, you can still cover things?

The first data are in children. Here is pre-vaccination, post-vaccination, and against the mismatched strain. With MF59 you get seroconversion in more than 90 percent. With a conventional vaccine you are in the 50 percent or less, similar for -- This is for

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H1N1.

So in adults at risk, a similar story. Against mismatched strains, MF59 is able to cover seroconversion in most of the people, conventional vaccine much less. Elderly, similar story.

So, basically, the MF59 not only improves the immune response in children and infants, in elderly adults at risk, but also in the same populations. It broadens the immune response so you can cover strains that will not cover without an adjuvant.

Now the last couple of slides are about using MF59 for a pandemic, and here is a study, part of which has been just published in the New England Journal of Medicine as a letter.

Basically, this goes back to -- The first immunization was in 1999 when we immunized people with and without adjuvant with a vaccine with a H5N3 vaccine, which today we call clade zero. This was the 1997

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Hong Kong strain.

Then a year ago we went back and we boosted the same people with a clade one 2004 Vietnam vaccine. Basically, here is what happens. The first experience was 1999. We vaccinated with a vaccine without an adjuvant, and we basically got no response. This is the protected level.

In the same study we used MF59, and we got basically more than 80 percent protective responses. We published this in the Lancet 2001. In the meantime, I think there have been many, many other papers confirming this data. With no adjuvant, you don't get a response. With adjuvant, you do get a response.

Then as I said, a year ago we went back. We got the same people, and we gave them two doses of H5N1 clade one, and priming had been done with clade zero; and we asked, do we get immune response?

Here is what we got. This is a

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log scale. This is the protective level. Basically, Day Seven after the first dose, seven-eight years later, you get -- Day Seven you get antibody responses that are one, two logs above the protected level.

We are proud, very proud, of these responses here. Look at this, and this is against the strain used for boosting. What about the other one, clade two, clade three, all the other ones. Here they are. Basically, by Day Seven you get incredibly -- I mean two logs, 1.5-2 logs more antibodies, protected level of antibodies, levels of antibodies above the protected level.

This is what you get. That doesn't really matter. Basically, you prime with clade zero. You boost with clade one, and in three days you are covered against any strain. That means that we can prime with an adjuvanted vaccine. Forget the things for a while, and then when there is a danger, come back and one dose. In three days, five days,

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seven days, you will be protected against any strain, independently of this thing you used to prime or to boost.

That will take away all the questions, which strain or H5N1 do we put in the vaccine. You don't care.

So these are the data. Only another slide, which says what is the mechanism. We are trying to investigate the mechanism of what is going on here. So people will be mentioning several responses. What happens? Which are the things beyond antibodies that we can measure?

Well, the only thing that we can measure, really, that makes a difference here is after the dose of priming, what we see is the memory T cells, they go up with the adjuvanted vaccine. Non-adjuvanted, they don't go up.

Basically, so the first thing that the adjuvant does is to generate a pool of memory T cells after the first dose. All the

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action starts here.

What is the consequence of that? The consequence of that is that, when you get down here, you boost. The people that had not been primed have no memory B cells. The people that had been primed have huge numbers of memory B cells, and these memory B cells guaranty long term protection.

So this, I think, is a solution for a pandemic influenza. This is starting to understand the mechanism, how it works, and with that I want to just summarize what I think I tried to tell you, that the adjuvant MF59 works for different age groups, is a solution for pandemic influenza, and is safe, and we start to understand the mechanism of action. Thank you.

(Applause.)

MODERATOR SLATER: Thank you very much. We have time for one or two questions before the break, if there are any.

DR. SUTCLIFFE: Hi. Joyce

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Sutcliffe. Just a clarification. On the last study you told us about, was the boost with H5N1 without adjuvant?

DR. RAPPUOLI: No. The boost was with adjuvant.

DR. SUTCLIFFE: Was also with adjuvant?

DR. RAPPUOLI: Yes.

DR. SUTCLIFFE: Thank you.

\ PARTICIPANT: Along those same lines, do you know that adjuvant was required in the prime? If you gave the prime without adjuvant, would the boost have worked?

DR. RAPPUOLI: We did have a little show for simplicity here. We did have a group which was primed without adjuvant. They also responded when we boosted, but the magnitude was lower, and the cross-protection was lower.

PARTICIPANT: Is there any direct interaction of the adjuvant with the antigen?

DR. RAPPUOLI: Do you ask whether

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there is a direct interaction of the adjuvant to the antigen?

PARTICIPANT: Yes.

DR. RAPPUOLI: Well, in the case MF59, no, we cannot measure that, because MF59 is an emulsion. We can spin it down, and the antigen remains in the supernatant. So it is no measurable interaction that we can see, basically.

MODERATOR SLATER: Thank you very much. We are going to take a 20-minute break, our last coffee break of the meeting. We will regroup at 10 minutes to three.

(Whereupon, the foregoing matter went off the record at 2:29 p.m. and went back on the record at 2:55 p.m.)

MODERATOR SLATER: Welcome back. We are going to begin the roundtable discussion.

First of all, I would like to acknowledge individuals who are participating in both this roundtable and this morning's

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roundtable who actually had never been introduced, because although most of the roundtable discussants are either co-chairs or speakers and all the speakers have been introduced, Dr. Emmanuel Hanon from GSK, Dr. Geert Van den Bossche from the Gates Foundation participated this morning. I would like to thank them.

In addition, Dr. Martin Friede participated this morning, and he is participating this afternoon. Dr. Friede from the World Health Organization actually has the distinction of being the only person to participate in both roundtable discussions today. So thank you very much.

Dr. Thomas Holdich from ATL is joining us now. Dr. Thomas Verstraeten from GSK is joining us as well, and finally through an oversight, Dr. Florian Schodel from Merck is not indicated on your program as a roundtable discussant, although he is, and is sitting two places to my right.

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Dr. Ballou this morning discussed briefly the roundtable 2 questions. I did cut off some people who were interested in asking questions of specific speakers. So if you have specific questions that you would like to raise, by all means, write those down, and at some point where it is appropriate, you can certainly raise those with specific speakers, but I am now going to turn the proceedings over to Dr. Ballou who will conduct our discussion.

DR. BALLOU: Thanks, Jay. The questions that we have posed for the roundtable here were discussed by the organizers of the meeting, and without talking out of school, I think when we developed these questions, one of the first questions was should we design, and this was thought to be too incomplete of an approach.

So we would like to -- We wanted to rephrase these to how can we, because we felt that we actually did need to discuss and

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think about how studies should be designed more to provide better information, more complete information around some of the issues that we have heard presentations on today.

I think that one of the first bullets on here is detecting age specific differences in adjuvant responses. This builds very nicely on the last two presentations.

So I would like to perhaps start with this, and to first of all, invite anybody who had questions of the last two presenters that might be in this area of age specific responses, particularly in neonates, to also please participate.

So is there anyone on the panel that would like to make an opening statement of opinion regarding this issue of design around age specific differences in adjuvant responses?

DR. DAVIS: One easy place to start -- it is not the full answer -- is that

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if you can pick up innate immune activation in immune cells, which is PBMCs or cord blood that you can test from different ages. That is a very quick way to start to see if you get a similar level of activation.

DR. LEVY: Hi. This is Ofer Levy. One thought that came to mind immediately was vis a vis animal models. Believe it or not, if you go to newborn immunology meetings, which aren't that frequent and aren't that large, because it is not that large a community of people doing that work, but when we have those meetings, there is actually discussion about what is a newborn.

In the human medical literature, a newborn is defined as birth to 28 days of age. So if you are searching PubMed, that is more or less how a newborn is going to be defined for humans.

Now when you look at other animals that have a different lifespan and a different rate of maturation of their immune system,

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that number might change and is open to some debate among immunologists and veterinarians, etcetera. So that is an interesting dimension.

I think you have to keep in mind what your goals are. One element that I tended to emphasize in my talk, although it is not the only venue to use those kind of discoveries, would be to vaccinate on the day that a baby is born. I think, from a global health perspective, that is a practical advantage, although it is not the only way to go, and we believe that some of the adjuvant effects we have shown are relevant also later in life throughout infancy.

If that is a goal of a particular vaccine development program, then a lot of the mouse, the newborn mouse, literature will look at mice that are a week old or rats that are one week old.

We saw some impairments in immunity, but it might not be the same level

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of impairment as one sees in the first 24 hours of life, and people tend not to look at the first 24 hours of life, because the mouse is very small and harder to work with, but that is something that we are doing with our murine program; because we believe that some of the adenosine and other effects may be acute and particularly relevant in the first few days of life.

So those are interesting elements and dimensions to consider.

DR. BALLOU: Could I ask you just to elaborate a little bit more on this issue around the timing of this first dose. As you know, although BCG is recommended to be given from the day of birth, in practice probably the majority of children in the developing world do not receive it as a birth dose, because they are not -- most of these births are not attended, and frequently receive it in the first month to two months of life; if they haven't gotten it by their first EPI visit,

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will get it then.

I wonder, it was not clear from the data that you presented whether there really is a fundamental difference in terms of this first two-month window when you can have the impacts that you are seeing on neonatal immune responses, or is it really critical to get in these first few days?

DR. LEVY: I think, to turn it around a bit, we see a severe impairment in the first days of life, and then there is a gradual age-dependent maturation. So if you wanted to choose a pathway to stimulate to give you optimal efficacy, if efficacy is defined as co-stimulatory activity as measured by CD40 up-regulation, production of IL-12-p70, a TNF alpha, etcetera, then the Toll-8 pathway appears in our hands, both in humans and non-human primates, at least within the confines of what we have done, to be the pathway that will give you the most efficacious response from the get-go, from the

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first hours of life.

When you look later on, there is maturation, and the infants start to catch up to the adults in terms of the magnitude of responses. What remains true in our hands is a Toll-7/8 or Toll-8 agonist have superior bioactivity with respect to these endpoints than the other Toll agonists that we evaluated in our assays.

We looked at Toll-2 agonists and LPS, and we looked at a pure Toll-7 agonist, etcetera. So in fact, one element of our work, which we tend not to focus on because we are focused on newborn and infant immunology, but if you just look at our adult data, set aside the pediatric data for a moment, in our hands within the limitations of the assays that we do, the Toll-7/8 and 8 agonists are giving the most robust response as compared to the other agonists we evaluated, even with adult cells.

DR. SCHODEL: The other antigen

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that is generally given at birth in many countries, and that wasn't mentioned, surprisingly, is actually Hepatitis B, which is just a good old alum adjuvanted vaccine.

Could you comment briefly on why that works so well, in spite of it not being on any of these adjuvants?

DR. LEVY: So we have an emerging body of data on the bioactivity of alum in an age-dependent way, and that is something that we are working on now and is not yet ripe for public consumption. But suffice to say that there is some bioactivity of alum at birth, which shouldn't be surprising, because the clinical experiment is there.

You get some responses. One dimension is that, if we build a better adjuvant, will we get a more effective response that would require fewer doses and/or provide a higher level of protection with fewer doses or faster, in which case then you close a window of vulnerability.

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DR. GLENN: I just found your talk fascinating, but I have to say I am skeptical. There is a big gap between the data points in the neonates and adults.

I know, with the GI tract and the mucosal immune system, once there is, say, contamination or there were microflora involved, you get rapid development of pairs, patches, etcetera. I would imagine -- and we talked about this at lunch a little bit -- that maybe also to the skin where you may have underdeveloped immune system.

It seems that this picture from cord blood where a lot of your data, and very good data, was generated really needs to be extended to two weeks later or some time period when there has been significant antigen exposure, so a chance to see how differently oriented the immune system is.

As mentioned, that is a more likely time when infants would be receiving these agents.

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DR. LEVY: I went through the talk very quickly, because I had too many slides. As I told somebody, you know, one person tells another: I wrote you a long letter, because I didn't have time to write you a short one.

The bottom line there is I did quickly go through a slide that showed that, if you take peripheral blood from a U.S. born infant at, I think it was, two months of age and then follow that same infant at 15 months of age, the Toll-7/8 and 8 gave the superior efficacy of TNF alpha at that readout.

Then we do have some limited data from the Gambia of an African infant at nine months of age where the Toll-7/8 agonist gave the expected or hypothesized superior activity.

Now there is a limited dataset in the infants, but we are starting to develop some experience with infant blood as well.

DR. GLENN: But that is precisely the point. I think you need a lot more data

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points to make those conclusions.

DR. CHEN: Bob Chen. I would like to follow up on the questions. So as you noted, the first six months of life is a period of very high mortality rate and very high selection pressure evolutionarily, presumably not only for homo sapiens but for all the other species.

So why is it, do you think, that the immune system is configured the way it is, and are we doing something potentially disruptive there?

DR. LEVY: Right. So, obviously, this is not an accident, and it probably relates to the fact that the system has to be designed so that the maternal immune system and the fetal immune system don't attack one another's tissues. That is why pregnancy is an immunosuppressive state, and that is why we recommend to pregnant women not to eat unpasteurized cheese and end up with intracellular infection with listeria, for

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example. So that is a reason at that level.

Then, of course, after birth one can speculate that it is important in the first few days of life when the newborn is first getting colonized in the skin with their first bacterial flora and getting colonized in the intestinal tract. You can imagine what would happen if the newborn had a very Th1 polarized response to that. There would be severe inflammation.

So what we know not just from our work -- this is a global literature -- that birth initiates an acute phase response, an IL-6 polarized acute response. Time didn't allow me to get into it, but we have data from infants, not just newborn cord blood but from infants, a European study we did with collaborators in Rome, that IL-6 levels rise after birth.

That is suspiciously similar -- and TNF levels stay flat. That is suspiciously similar to the pattern of

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cytokine production I showed you with our cord blood cultures. So we believe that that pattern is relevant not just in vitro but in infants as they are growing up.

What we are proposing to do, though, is in a local environment with a conjugated adjuvant locally apply in a reversible way a Th1 polarization that can locally break tolerance so that you can get an adaptive immune response.

DR. KENNY: Rick Kenny with GSK. I just wondered. You know, you said that the neonatal immune response essentially is designed to be polarized against the Th1 response. What do you see as the long term safety implications of trying to break that right at birth, and how would you go about studying that in a way to be able to get into neonates with novel vaccines?

DR. LEVY: Yes. Well, obviously, that is a major regulatory and safety issue for any new drug development and, of course,

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particularly in pediatric drug development, which has typically lagged behind, and then particularly when you are talking about newborns.

For the doubters, you've got to look at the biomedical and public health significance. You have to look at the fact that there are vaccines we give around the world in newborns, Hepatitis B vaccine, BCG. So there are certain proofs of concept.

Now, of course, just because those are safe doesn't mean a new one is safe, but it does show that certain vaccines can be given at birth and result in some protective effects.

We also use imiquimod, a Toll-7 agonist. It has been used and published in pediatric populations as a topical cream for molluscum contagiosum. So local application of imidizoquinolines has been done as a pediatric experience, and some pediatric literature on that.

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Finally, as with any drug development, there is going to have to be thoughtful safety approach and, obviously, careful endpoints. I would suggest in newborn Rhesus macaques looking not just at efficacy but looking at safety endpoints, and that is where the discussion from this morning becomes relevant.

I think it is interesting and important to follow cytokines, to follow lymphocyte patterns, etcetera, but we all, I think, have to agree up front that we don't know at this point in time with our state of knowledge that a level X of cytokine Y definitely proves that you are going to end up with complication Z.

I think it is valuable and important to gather that information, but how to interpret it will be interesting.

DR. WARREN: Just something to think about: You are highlighting the challenges of the regulatory environment in

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terms of vaccines for neonates. Should we immunize a neonate or the mother?

DR. LEVY: Yes. Well, the question of vaccinating maternal immunization is a whole field and discussion, in and of itself, and there are proofs of concept. You know, influenza vaccination in the mother does result in some protection. There was recently a paper on that The Newborn.

From a medical, legal and regulatory perspective, I think that is an even more complicated area. That doesn't mean it shouldn't be pursued.

DR. PETROVSKY: Nik Petrovsky, Australia. I am a little bit confused by your claim that the TLR 7/8 agonists were the most effective, because you didn't show any dose response curves, I guess, for all the different agonists that you were comparing.

So again, with single doses of different TLR agonists, how do you actually compare relativity where that dose is in the

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dose response?

DR. LEVY: Right. That is a cogent point. So there is a difference, of course, between potency and efficacy, and we will define efficacy as the dose at which we can get a maximal response for any of these biological systems. You max out at some point.

We have three publications in this area, two in Journal of Immunology and one in Blood. In each of those, we satisfied the reviewers. We did full dose response curves, and then in the summary plots I showed we selected the concentration of agonists that led to a maximal response.

DR. BALLOU: I would just like to comment that in my world, efficacy is defined as protection against a clinically, medically important disease. I would hope that we try to use that as a general description of efficacy.

The second bullet point here,

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providing long term safety information -- We have had a proposal from one of the speakers today that, really, the best way to do this is prospective observational studies. I wonder if people either agree with that or have different views on how one should think about attaining long term safety.

DR. SCHODEL: Yes. I would like to make a comment on that and point to an important gap. I think Bob Chen has pointed out the value of the observational studies and, obviously, the efforts of the CDC and the rapid cycle analysis.

All these things are great new tools that help discover signals. One thing that I think is severe missing is when we see relatively rare events, it is not always easy to get a clear answer as to whether a signal is not biased by all kinds of different things.

What we are lacking is the power of the observational long term analysis and

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the computerized follow-up to be meshed with a randomized, blinded design with some appropriate control, which we have been into, but we haven't really gotten there with any of our post-licensure or pre-licensure large studies.

I think that would be really sort of getting the two worlds together and give you the best answers for not the very, very rare things, because they are just too infrequent, but for the answerable questions. Obviously, both GSK and I have shown -- and Merck -- have shown it with interception for rotavirus with a specific hypothesis that these things can actually be answered in prospective randomized, controlled studies -- of course, very expensive, and you can't do this for everything.

So what we would need is another public health tool -- and it can't just depend on the companies, I'm afraid, because of the finances involved -- that allow us to mesh the

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power of randomized and blinded groups with computer follow-up in an automated way to some of the things that Bob and others have so nicely built up at the CDC.

DR. VERSTRAETEN: I would like to comment to that as well. I certainly agree that observational studies, Phase IV, have their value and have their place, but there is still the outstanding question of what safety data do you collect in your clinical trials.

I think we have talked a lot about immediate reactogenicity. A lot of the presentations yesterday were about that. I don't think anybody has any doubt about that.

Now there's a lot of debates between industry and the regulators on how much more and how much longer do you have to follow up in your clinical trials. You cannot push everything to Phase IV. I think that merits some discussion.

We, as Gary has shown, have talked to quite a few experts in the field of

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autoimmune diseases to understand what is the risk area that we really should be looking at or, in other words, how long after vaccination do you expect you could see something as an adverse reaction, a true adverse reaction following your vaccine?

When we did that, we usually get the same response in sort of a couple of weeks, a couple of months at most, and very rarely have we had feedback that you should look for five years or 10 years.

So our position has been it is more useful to look at that immediate -- if you can call that immediate -- couple of months after vaccination and make sure you capture as good as possible information, and do a proper comparison of that information than just go on and on and on and collect data from which you really don't know anymore what was the cause of that event.

So I think, even if we go for large Phase IV trials with electronic

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databases, we still have to agree for clinical trials what is really the period at risk.

There is another comment I would like to make. That is that we should distinguish between the risk period and the follow-up period. A lot of people have these long term diseases in mind, like multiple sclerosis. That may take years to develop. That is true, but that doesn't mean that your vaccine can cause these diseases during all these years.

I think what we should agree is what is really the risk period, how many months or years, if you wish, but I think it should be months after vaccination, and then in addition do you want to calculate in your study some additional follow-up time to make sure that you identify those diseases, if they occur on the longer time scale.

So I think it would be good to have some debate on this period.

DR. DELLA CIOPPA: Well, I think

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we should maybe borrow from other areas of development to get some ideas as to what kind of studies we could do to realistically assess long term safety.

One of these areas that kind of goes in the direction of the previous comment is that of the so called large, simple clinical trials, and I am borrowing this from the cardiovascular area where the key word is simple. Can a company do a study in -- I don't know -- 120,000 subjects, randomized clinical trial, pre-license?

In the current setting and with the kind of things that we measure in clinical trials, the answer is, in most cases, no; or even a company like Glaxo or Novartis, you do it once. You cannot do it all the time. However, the reason -- The main reason for this is that we load our clinical trials with too many questions, which is, obviously, understandable.

There is another approach, which

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is a minimalistic approach, and I will give an example. Let's say we are interested in autoimmune disease. A large, simple, randomized clinical trial in autoimmune disease would be as follows. You randomize the subjects to either adjuvanted or non-adjuvanted vaccine, and then two years down the road you ask the one question: Did you get an autoimmune disease or did you get one of these diseases. And the answer is yes/no. The end.

This is a simple, large clinical trial. This is a doable trial. Now people laugh, because why not adding some other information? Why not some immunogenicity? Why not this? Why not that? And then the trial implodes, and then the cost becomes impossible.

So strong recommendation of how I would spend my money as a company to provide long term safety information before approval through these large, simple, randomized

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trials. The word simple has to be such that, when you kind of tell it to people for the first time, they have to laugh.

The second statement, suggestion, recommendation that I would have, speaking with regulators, is to allow to elevate as pivotal evidence of safety the pooled analysis and the meta analysis, which today are not considered as pivotal evidence.

In order to do that, there are two features that are in my mind essential, the first one being pre-definition. You have to, of course, define beforehand what we are going to collect and how. The second is, of course, standardization. But if these two features are met, I don't see why a large, well done, well defined, pre-defined, pooled analysis could not be elevated to pivotal evidence of safety.

Now it is intriguing that in a submission we do have to do the integrated summary of safety, but that as such is not

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used as pivotal evidence like a normal clinical trial.

My third point, I think that not everything can be determined pre-approval. I think, more and more, we should bridge -- This concept between pre-approval and post-approval is a little bit artificial, because it is based on the assumption that we will know the story by the time the vaccine gets out, but in most cases that is not the case.

So I think proper agreements on post-approval commitments is a third way forward. Then, of course, the company or whoever gets the approval is bound to do the study, to do the study according to the predefined rules, and to submit results and to take action in case the results aren't improving the safety signal.

Think of these three methods, large simple studies, a meta analysis, and post-approval commitment which include the prospective observational studies. We can

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move in the right direction in a way that is sustainable.

DR. BALLOU: Thank you for those helpful comments. Let's get some responses from the audience.

DR. CHEN: Let me make two comments. First, I would like to propose a different design for large, simple trials that we need to think about. That is, in this era of almost great transition to electronic medical records in large HMO-type national health services, rather than necessarily -- and, obviously, for the typical set of numbers, you will want to go kind of solicit adverse events. But for these sets, let's just let the regular health care system run the way it is.

Yes, we need to define ahead of time which might be the adverse events we want to analyze, but allow the natural pattern of visits to emerge, similar to how we currently do the post-marketing large linked database

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studies. But we could do it pre-licensure using, more or less, a similar setup.

The second comment relates to a different challenge that we need to face, and that is, as Tom said, that even though we want to get as much answer in the pre-licensure domain, inevitably certain things will need to be addressed in the post-licensure setting, and how can we track these individuals that were exposed pre-licensure as well as folks who are exposed post-licensure in the real world domain where many people may be getting all sorts of different vaccines, different adjuvants, etcetera?

At the end of the day, to me, the only way that could happen is if we track the vaccine exposure with the adequate level of specificity down to the lot level. What would be needed is that each vaccine manufacturer, as they produce that lot, would need to report to a centralized database all the different information about what went into that specific

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lot, be it the adjuvant, be it the other excipients, other adjuvants, etcetera.

Then down the road whenever new issues come up or whatever studies we want to do, we would then be able to link it together. One proposal that has been out there is let's get a smaller bar code. The newer sets of bar codes, they are two-dimensional, can collect all sorts of information.

So as we produce the new vaccines, let's incorporate that and make sure that information is captured, because in analyzing our safety data, a huge part of the problem is that the nurses that have been giving the shots in the clinics have been used to writing DTP for so long, when there is new DTAP, ITV, Hepatitis B. They only have a certain amount of time to write, and so the amount of error in that information is incredible.

So we need to automate all that, and I think perhaps those might be two ideas for the folks to think about.

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PARTICIPANT: For the gentleman that suggested that the survey for such things as autoimmunity ought to be time limited, I would be curious for you to be a little bit more specific about how limited the time should be, because let's keep in mind here, the idea of the vaccine is that you want to induce life-long or near life-long immunity.

If the adverse event that frightens us most is related to the actual mechanism by which you are inducing immunity, then there is no reason to imagine that the risk for the adverse event would be there as long as the actual beneficial effect that you are trying to induce.

Now, obviously, that means that you can't withhold licensure until immunity wanes in all your Phase III trials, and that does bring to the idea of the post-marketing commitments.

I guess the concern with that, and it is a reasonable issue, but in the United

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States we don't have provisional licensure. Right? I mean, once it is licensed, it is not licensed for five years and then you get to take a look at it again.

So, therefore, isn't it true that, in a sense, industry is not as motivated to get that post-marketing data as one would hope, particularly in a country such as the United States that doesn't have national health registries as Europe does?

DR. SCHODEL: You've brought up a number of interesting issues. Let me tell you that we are very motivated because, obviously, there is the perception that vaccines might cause adverse effects of long consequence are very detrimental for the public health usage of the vaccines. So all of us who are in this particular field are very, very nervous about these kinds of allegations.

So I think both -- I can certainly say that from industry, we are certainly very interested in finding out whether such adverse

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events might happen and whether they happen in the long run. But I would just like to point out one very simple complication of this kind of a question, which is that what you are painting is a uni-dimensional world in which the only effect is the vaccine.

Now even the harshest adjuvants that we use are very similar to things that happen during any infection that you have in the meantime. As you go out -- and I think that is what Thomas was saying as well, in a way. As you go out further, you dilute your effect.

It is not so much that it couldn't theoretically happen a lot later, but the likelihood that you would be able to detect it over all the other things that happen becomes increasingly smaller. Besides, in most cases vaccination is actually, from a biological point of view, a very limited exposure to exactly the same thing that happens in a much more dramatic way when you encounter a

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pathogen.

So I think there are practical limitations to this. It is very different from the drug world where we have continuous exposure to the same drug over long periods of time, and we can really look at long term effects. Here, we often have a single shot, sometimes two or three shots, and then you ask us whether there is a consequence five years later.

The antibodies stay around, but the original sin, so to speak, is hardly detectable anymore.

PARTICIPANT: I appreciate that.

DR. DAVIS: I will just follow up on that comment, and I think it really depends on what your putative mechanism of action is for the autoimmunity.

So if you are working with an antigen that you expect to have molecular mimicry of a self-antigen, then yes, a longstanding antibody response is something to

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think about long term, but that is not the case for most of our situations.

So in our case with the CpG, the innate immune activation, which potentially could play a role, is largely over within three days or four days, five at the max. If it is the DNA presence itself, it is gone within a few weeks.

So, really, you have to think about why you are worried about autoimmunity, and then look at it as to what would be a reasonable time from that point of view.

PARTICIPANT: I'm sorry. I agree with you. As a matter of fact, I would even state that in many cases, if you are worried about triggering autoimmunity, my guess would be that you are triggering it in those who are prone to it anyway. But on the other hand, a couple of years without MS is better than a couple of more years with it. But I agree with your point.

DR. VERSTRAETEN: Rip, can I just

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answer that? To answer your first question on that period that we actually established, I'm not the expert in autoimmunity. That is why we went to these folks. They were experts in neurology, rheumatology or autoimmunity in general.

We settled on a period of six months after the last dose, and we agreed with the European regulators that we would integrate that in our Phase IV trials as well.

DR. GRUBER: I had a question or perhaps wanted some clarification on a comment that was made earlier on, and that is the idea of performing large simple trials. I think this is something that the agency is very interested in.

I just wanted to ask you. You had indicated that you could envision a large, simple trial in which you would randomize subjects to either vaccine only or vaccine adjuvanted arms, and then follow them up.

I can see that being a possibility

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where you perhaps have a product that is already licensed and that you now want to combine with an adjuvant, that you can then design these studies. But let's say you have a novel vaccine antigen that you combine with a novel adjuvant. There, I think the idea of randomizing to vaccine only and vaccine adjuvanted arm becomes a little bit more challenging, because you must have a rationale for adding the adjuvant in the first place.

So I think it is the idea of then including study arms where you do the vaccine antigen only may become rather challenging, and perhaps even not that feasible. I would like for you to comment on that.

DR. DELLA CIOPPA: Well, yes, certainly, the choice of a comparator depends very much on the nature of the vaccine you are testing. In some cases, it is doable when you have an equivalent, as you said, like in flu, for instance, that work without the adjuvant.

In other situations where this is

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not viable or actually not interesting, then the large, simple study trial would be made, carried out, comparing the new adjuvanted vaccine with either no treatment or a placebo or an alternative vaccine of a different kind.

I think the value of the large, simple study is still there. Then the nature of the question that you try to answer is slightly different, but the end product is still of value in determining whether the adjuvanted vaccine is detrimental or not.

DR. GRUBER: Thank you.

DR. BALLOU: I'll put on my Gates Foundation hat here. We are talking about studies and concepts here that can probably only be done in settings such as the United States or Europe or other developed countries that have health systems that can detect these kinds of events.

What about the rest of the world where, increasingly, we are seeing even the large manufacturers going for vaccine

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development programs? What do we do about assessing long term safety in populations in diverse places, some of which have zero to little medical infrastructure that could capture --

DR. DELLA CIOPPA: Actually, I would argue the other way around. In my mind -- and maybe I am wrong, of course, but the large simple trial concept is one of the few that actually can be implemented in countries where the medical system is not advanced, because if the -- Again, if the question is simple enough, then most medical systems can answer that question.

If the question is were you hospitalized for -- I don't know -- MS or for lupus, I think you can easily do it in Africa or in Asia. It is the more complicated trials that we typically do that cannot be done in less rich health care systems.

DR. GLENN: I have a question for the panel. If the adjuvant is a natural

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compound like MPL, for example, there is historically a lot of exposure to that, and one can expect that these very short events would be somewhat like an infection. But if it becomes a more exotic adjuvant, more synthetic, less like something you would find in the natural setting, it seems to me that it would change your thinking about the long term follow-up, and maybe you need to know more about that or maybe it doesn't play into it.

I would be interested in other comments.

So if the adjuvant is truly a bacterial product like MPL, for example, it seems to me that historically through years and years or centuries of exposure, we have already sorted out whether there are going to be important long term signals with that. But if it is a new, novel synthetic adjuvant, maybe that is a different track.

DR. SCHODEL: I would say that it probably depends on the pharmacokinetics. We

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should go back -- On these things, we should go back to basic pharmacology.

I was a little surprised, actually. I was glad that Ripley said what he said about the value of the preclinical studies, because that is exactly the way I would see them as well.

We do look at general toxicity of the compounds. We figure out what the maximum doses are that we can actually test, and it does give guidance as to what we want to even try in people. So I think it is extremely valuable work.

What I was saying, the question I was asking from my preclinical colleagues was actually a little different. I was saying, okay, I am quite happy with what you are doing anyway, because that is sort of the prerequisite for doing anything. We have to know whether these things are toxic and so on.

So I would comment on your comment a little bit the same way. I would say you

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just -- If it is a compound that has a very short half-life, very rapid kinetics and it doesn't have any clear -- from all the preclinical work, you haven't any. Of course, you repeat that in your Phase I studies. If it doesn't have any long term consequences, well, then you look mostly on the indirect effects that it might elicit.

If it is something that actually does stick around for a long time, well, you've got to figure out what that does, similar to implants or things that are around or depot solutions or pharmcos that have a very long half-life and that stick around.

I would try to simplify these things, and if it can't be metabolized and it is an inert compound that stays around, well, you got to look at what that does.

DR. MALONE: Thank you. this is exactly the point I wanted to make. It seems like we are reinventing the wheel. All we have to do is turn to our CDER colleagues.

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Aren't we asking the question, what is different about adjuvants from other vaccines? In a way, all vaccines may be associated with some risk associated with autoimmune response. What is unique about adjuvants is the adjuvant component.

How do we assess the duration of risk associated with the adjuvant component? Well, that is a function of metabolism, right? So if we have a CpG that is phosphorothioate that is designed for long life, we should know what that half-life is, its clearance and pK, and that should inform that decision.

It seems to me that this remains -- - This determination remains in the domain of the dialogue between the competent regulatory authority and the sponsor. I can imagine there would be some appropriate guidance, but my sense is that we are inventing complexity that unnecessary right now in this aspect of the focus.

DR. GOLDING: I want to make a

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comment and then to also pose a question. We keep hearing this comment from different members of the panel.

DR. BALLOU: Hana, could you put the microphone toward you? It's a little hard to hear.

DR. GOLDING: Yes, sorry. That after all, many of the novel adjuvants are basically derived from bacteria. We have been exposed to bacteria all our lives. Then, really, what is the difference? Why are we worried about them?

I think this is kind of a little bit of oversimplification. It is true that we are growing with bacteria on our skin, in our GI tract, and maybe in our mouths, but that is not the same as introducing bacteria and bacterial derived product systemically.

Even though most of the vaccines are administered intramuscularly, they clearly have the potential of systemic distribution, and as we know, that is -- If bacteria does

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get into your blood, you are likely to have very serious consequences, including death due to bacteremia.

So I think the sort of concept that we are always living with bacteria, therefore, anything that comes from bacteria is now okay, I think, is a little bit underestimating of the potential.

We have seen it, actually, in the clinic, that bacterial derived product when administered as a vaccine product generated responses that were very strong. I don't want to mention any specific examples, but I am sure most of us know about those examples.

I do want, though, to ask the panel, since there are other people who are involved with clinical trials and Phase I trials, in particular, should we start thinking of some additional type of measurements of immune parameters that we may have not looked at up to now.

The general SS biomarkers, blood

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chemistries that we are doing may overlook certain signals that can be generated in some of our exploratory preclinical studies, not necessarily in the rabbit but in the mice or in the non-human primate.

When do you think it will be justified to bring some of these new parameters, biomarkers -- I don't know -- T-cell subsets, cytokine measurements into the clinic, into the Phase I to really follow up and see whether they will give us additional tools to decide whether to move forward to Phase II or even select the right dose, the maximally tolerated dose, etcetera?

DR. BALLOU: My own view is that we are increasingly seeing these kinds of tools being brought into Phase I and early Phase II studies, and trying to make an assessment about whether or not they are actually telling us anything more than we would have known otherwise.

Without a doubt, you have a lot

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more information to look at, but whether it actually allows you to form a different opinion about the way forward or not -- I think that has been, to me, the biggest part of the puzzle, and of course, as we move forward down clinical development, there is, I think, the appropriate attempt to simplify study design so that you focus on the most important endpoints.

A lot of times that is not going to be chasing lots of markers and other ancillary readouts. That is my opinion.

DR. ROTROSEN: I would add to that, that I think within a few years we may be in a position to draw some reasonable conclusions about immune markers being correlates of immunogenicity and efficacy. I think we are probably far, far away still, though, from immune markers being a signal for safety. I think that might be very useful, again, for efficacy and immunogenicity, but to assign a particular profile to the safety

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concern, I think we are still years away from that.

DR. LEVY: Yes. I would like to amplify on those comments. I think that those markers and those cell types, the new lymphocyte types that we are aware of, the T-REG cells, etcetera, can be very powerful from the standpoint of trying to understand mechanism better, to ask certain questions about how the formulation is interacting with the immune system.

I like something that was said earlier this morning, the notion of a safe haven where the information is collected, when possible, when financially feasible, bearing in mind the comments that we load up these studies with so many endpoints, they get very expensive.

So that is another element, but to the extent that they are measured, being up front about the fact that they were exploratory, and maybe the long term goal is

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to find surrogate markers for efficacy and safety, but that we are not there yet, and that they are going to be there to be hypothesis generating or getting better insight onto mechanism, but not to assume that a certain signal there proves either efficacy or safety.

DR. SCHODEL: Ripley, I actually want to turn your question back to you a little bit, because I think most of us would agree that simple trial design and the power of appropriately randomized studies with whatever the controls are in populations which are the users of these vaccines and where we particularly want to deploy them would be extremely helpful, and simple is, of course, good.

One of the problems with simplicity here is -- and just to exemplify that for those who don't maybe think about this all the time -- is that if you run a very large study and you lose a certain part of the

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population to follow-up, you don't know what has happened to them.

That is the biggest problem with these simple designs and populations that you can't necessarily reach, because you don't know whether there is something hidden underneath or, you know, maybe they have died. Maybe something else has happened.

So the question to Ripley is: Then since this is such an important area and not any manufacturer alone could actually really resolve it -- I mean, we are all making some efforts in our own ways, and we all have pretty large studies in developed and in developing countries, but we are struggling with this issue.

Is there a plan from the Gates Foundation and associated consortia to build some sort of a network in which these studies could actually be a practicality, could be done?

DR. BALLOU: Well, there is

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certainly not a plan, but I do think that the Foundation does recognize that the whole issue of pharmacovigilance as it relates to issue around in the developing world is a big, important vacuum right now, and one that we are thinking about heavily.

I don't think that we are -- We don't have a plan, no. But I think it is an issue that needs to be addressed.

I would say, though, that my experience in studies done in the developing world is that we have higher follow-up rates, lower dropout rates in those populations than any of the studies I have done, part-studies, in the developed world, simply because people are just not that noble, and you can usually find somebody who knows where somebody is.

DR. CLEMENS: Ralf Clemens from Novartis. I have a bit of a difficulty with the entire discussion. We are talking since an hour about rare events, very rare events. We didn't talk a single minute about the

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benefits of adjuvants.

We heard from Gary that adjuvants are critically important for some vaccines for malaria, vaccine -- vaccine, for example, not adjuvant. There is the malaria vaccine. We heard from Rino that adjuvants are very powerful to make an effective flu vaccine.

So why do we only look on the one side of the corner, and we don't talk at all about the benefits. How can we quantify these benefits better? I think, if we don't that, we miss an opportunity here.

DR. BALLOU: Well, I think everybody in this room believes that adjuvants are the jewel in the vaccine crown, that they are the thing that is going to make the new vaccines work. So if there is a sense that we are worried about those perceptions or actions that we fail to take to protect this important tool, I think that is reflecting the discussion here, but I think if we didn't believe they were important, we wouldn't be

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here today.

Are there other -- Geert?

DR. VAN DEN BOSSCHE: Geert Van den Bossche from Gates Foundation. I am just all the time asking myself the question, if indeed we would be able to prove that we deliver only locally -- and we have had Greg's presentation, for example, on the intradermal delivery -- would this change in any regards the kind of safety concerns we would have for -- and for example, these type of studies could easily be done in animals, right?

If we really prove we deliver only locally, would this change the whole discussion or would we still be concerned, as mentioned by all the questions we are discussing?

DR. SCHODEL: Well, isn't all immunity local? So in a way, maybe all autoimmunity starts somewhere, too? I think it is an artificial question.

You know, any positive and

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negative immune response has to start somewhere, probably in the lymph node.

DR. VAN DEN BOSSCHE: So you wouldn't distinguish from systemic distribution?

DR. SCHODEL: No, I would, but from a pharmacology point of view. So in other words, if I have an adjuvant or a drug that has strong systemic effects, obviously, I've got to study them. I've got to look at the maximum tolerated doses and all the classic pharmacology. It's very simple, and we all know how to do that.

If I have a much more short acting drug, then I don't have to do as much on that side. So I would agree with you there, but on the other hand, the consequences of a strong local immune response can still be strong consequences, and it is not necessarily because we have circulating interleukins that something bad happens. That is not -- I think it is a juxtaposition that is not quite right.

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DR. BALLOU: I have done a fairly poor job of going through our questions here, but I would like to actually address the third bullet on this first question, because I haven't seen any data presented here on this question, particularly from the large manufacturers that have fairly fixed formulations.

Is there the need, and has there been done but we haven't just seen it, careful dose ranging of adjuvants, the way we typically do for adjuvants -- for antigens? And is this something that -- Is this an opportunity that we are missing to help reassure us on issues around safety?

DR. DENIS: Maybe I can take that question, because I think I at least partly addressed it during my presentation. While indeed we evaluated the dose ranging of the adjuvant in our H5N1 program, I didn't present the data today.

To me, the question remains to

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what extent these results can really help. Of course, we can always use the data to make sure that the balance of immunogenicity and reactogenicity that was obtained with a selected dose is better than for the other doses that were evaluated, but that is, of course, limited information that you get from this.

In our case, so that was additional information obtained from an additional trial. So it required a doubling of the investment as compared to a single trial, but it was done anyway, as it was considered as required.

DR. GITTLESON: I would like to comment as well. So we have done dose ranging work with the adjuvant, which I didn't show today, where we have looked at a number of escalating doses with the adjuvant where the antigen has been held stable.

The differences can be very, very subtle. If you are doing it early on in a

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Phase I study, you actually have to have fairly large patient numbers in each group to be able to tease out the differences. If you take just the standard Phase I study approach with a very small study and small patient numbers, you are going to miss it.

What we have seen is that, when we have a look at a humoral response, that when you use very low doses of, for example, the ISCOMATRIX adjuvant compared to higher doses, you will get a higher immune response as you dose range up, but you will get a flattening.

Where we have found value is specifically for us, because we are looking at T-cell responses. What we see is that the higher one goes with the adjuvant dose, that you get a broader response, and you can induce CD8 responses with the higher adjuvant dose.

Some of that work has been done. When we have a look at safety, in our hands with ISCOMATRIX adjuvant, we have not seen on local reactogenicity a dose response that

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seems to occur with increasing antigen, because we have done the same. We have held the adjuvant dose stable, and then we have dose escalated on the antigen.

There, as you increase antigen, we tend to see greater reactogenicity. Where we have seen a trend toward increased number of AEs is with systemic side effects, such as myalgia and fatigue, if you go to higher adjuvant doses in some patient populations.

Perhaps later on I can comment on some of the work we have done with ages, looking at the elderly, because we have not touched on looking at the elderly and the affected immunosenescence, and not all elderly are the same, and how do you tease that out and look at responses. Perhaps we could talk about that afterwards.

DR. ROTROSEN: Can we comment on whether animal models, mouse or rodent, other rodent species, were useful in predicting those changes in the human response based on

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the adjuvant to antigen ratio?

DR. GITTLESON: So adjuvant dosing has been done as well as antigen in rodent species. We didn't see increase reactogenicity that we were able to detect. There again, it is very much, when you looking at reactogenicity in the mouse, they don't say "Ah." In our hands, we haven't had animals ending up limping and such like as we have dose escalated.

So we needed to go in our Phase I programs and dose escalate with the adjuvant.

DR. BALLOU: Can I ask if there is a comment from either Novartis of GSK in regard to dose ranging in adjuvants?

DR. DUBIN: Yes. So I think that the situation is potentially even a little bit more complex when you are talking about adjuvants that have more than one component or adjuvant systems, as we define them.

The approach that we have generally taken is to do dose ranging of the

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components in different ratios pre-clinically, but once we enter the clinic, we tend to use a fixed ratio of components of dose ranging of the adjuvant system.

The reason for that is that you could argue that, when you change the ratio of the components, you are actually changing the adjuvant system, because in some cases, there are interactions between the components. So that is the approach that we have used, and in clinical trials this is something that is becoming more standard to do dose ranging of the adjuvant system with a fixed ratio.

DR. DELLA CIOPPA: Well, personally, I believe that dose ranging of both components is necessary and essential. However, I believe it is only useful if you manage to do it in the same trial. Here, I am going to suggest that there is a methodological tool that allows for this.

This is so called factorial design where you can kind of identify two or more

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factors, and you put them together so that you can dose range in the same trial the level of adjuvant, assuming it is only one, and the level of antigen in various permutations.

Of course, I realize that you do introduce another confounder, which is the volume, because you will have to do bedside mixing. Then the volume changes, but still you could have very useful information.

I would like to make also a slightly provocative remark. I think we should do much bigger studies in Phase I, and actually much bigger toxicology studies. Either not do them at all or do them big, because the same doubts that people have with very small clinical studies, they have them exact the same when you get three rats, three female, and three male rats. The information you get from that is questionable.

So, yes, you have to do it. Actually, I think the balance between the kind of investment we make in Phase III and the

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kind of investment we make in earlier stages has to be changed a little bit, and the earlier stages have to go toward bigger studies. So some of the studies that were presented, I think, are going in the right direction.

DR. BALLOU: Steve, did you have a comment?

DR. REED: My only comment was that in our approach where we try to keep the emulsion constant. With TLR-4 agonists, both synthetical and natural, it is easy to find doses that are optimal for rodents and for macaques, and so we have used both of those to help us choose the human dose, and it is very apparent that it is quite easy to use too high a concentration of agonist and actually get a poorer response.

So that is something to keep in mind. Certainly, more is not better in our experience, especially in the monkey system.

DR. BALLOU: We have five minutes

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left. Charmaine, I think you raised an important issue, the issue around the adjuvants in the elderly, which is, in fact, one of the populations where we are going to see adjuvants used more heavily in the near term. Could you please comment?

DR. GITTLESON: So one of the things that I thought was of interest, typically when we consider the elderly, the data is often presented for patients who are over 60. One of the things that we did in a study was we took that patient population and broke it up to actually have a look and see whether there are different responses and to what degree do patients have immunosenescence.

We found, interestingly, that if you have a look at patients who are, say, between the ages of 60 and 74 who are ambulatory and community dwelling, or compare that to patients who are 75 and over who are fairly well and community dwelling, and then have a look at patients who are over 60 who

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are in long term care facilities and who have a lot of comorbidities, what we were interested to see was, was there a different response in those patients in terms of the ability of an adjuvant to overcome immunosenescence.

So we compared it to a licensed vaccine, and we did see that there was, certainly, a trend toward patients who were in long term care facilities, so 60 and over, then compared to those patients who were community dwelling, that they actually seemed to benefit more if we had a look at the fold increase in the GNC titers of interferon gamma, looking at their CD4, CD8 responses, or having a look at their humeral responses.

So it was really interesting for us to try and have a look and try and tease out why that might be, and it is a question I would like to put forward just to say that not all elderly are the same.

Which are the elderly patients

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that would benefit more? What are we actually overcoming? Which adjuvants would be better placed to overcome some of the issues of immunosenescence? I don't have the answers, but I think that that needs a lot more teasing out.

One of the challenges that we faced in our program is that again patient numbers were fairly small, and I take the comment that I think there does need to be -- If we are exploring this from a scientific basis and not so much just trying to get the product registered but really trying to tease out the science, that we do need to be looking at larger patient studies.

DR. BALLOU: I know that the elderly population have been looked at fairly extensively by both Novartis and GSK in the development of their pandemic influenza vaccine. So people from either one of those groups want to comment on their views on the elderly?

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DR. RAPPUOLI: Well, I can try to answer some of this. Well, we have done trials where we have done with pandemic influenza in the elderly and the adults, and you do see that, when you go in the elderly, you get lower responses, but the plans are the same. So we have done that study, separating the two populations.

Talking about the elderly, I think, we've got into a fascinating field, because the way you define the elderly, I think, from the immune system point of view may change over time, may change with different populations.

Thirty years ago, 30-35 years ago, basically, the people that were hospitalized in Italy were basically mostly -- in internal medicine were mostly 60-75 years old. Today, the same place, you get 75, 85, 90 years old. So which one of the elderly?

So you mentioned that when you get 75, you do get a different response than you

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get in the Sixties. There are people that are starting to do studies, what happens to the T-cells. Basically, they do find that in today's population, when you get to 70-75 that both CD4s and CD8s, basically, they basically go down dramatically.

So I think we need to do a lot more basic studies about what is the underlying immune system. What are the T-cells, the B-cells, the cytokines. The beautiful things you are doing with the infants need to be done in the elderly, and we need to define the elderly, because maybe the elderly in a population has a life expectancy of 85 years is not the same as the elderly in a population that has a life expectancy of 50 or 60 years that they have in some developing countries.

So I think you really need to define what elderly means for the immune system.

DR. ROTROSEN: Let me just add to

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that. NIAID has a handful of programs looking specifically at special populations, elderly, transplant recipients, and the like, looking at immune responses. We haven't focused on adjuvanted versus non-adjuvanted vaccines, but if there are manufacturers here who are interested in evaluating products through those clinical research networks, we would be happy to talk to you.

DR. DUBIN: And just a quick comment. In the context of pandemic flu, we have seen that different adjuvant systems appear to have different effects on reconstituting immunosenescence or restoring immune responses in the adjuvant system that we are currently using for our pandemic vaccine.

The ASO3 adjuvant system appears to be one that is particularly good at restoring immune responses in the elderly, in particular.

DR. GLENN: May I ask how the AE

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rate looks? Is it different as you get into the older population?

DR. DUBIN: What we have typically seen, comparing the same adjuvant system in younger versus older individuals, we typically see lower AE rates in the elderly. I mean those AEs that are temporally associated with vaccination, reactogenicity, etcetera.

Now why that is, I don't know. I think Rip alluded to this this morning as well, but that has been the general pattern across different adjuvant systems, lower rates in older individuals.

DR. GITTLESON: So if I can comment on that as well, in the program that we looked at, we had within the same trial younger adults together with our older adults. Yes, when you just compare looking at licensed vaccine or looking with the ISCOMATRIX vaccine, your elderly patients had a lower incidence of reactogenicity.

What was really interesting and

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why we wanted to have a younger adult population and need to compare was what we wanted to see was that if we reconstituted an immune system and were able to overcome that immunosenescence, which we did show, that we were able to get immune responses in the elderly at much the same levels as the young adults with just the licensed vaccine.

What we really wanted to see was did you take the AE rate up to the same instance as the reactogenicity seen in the young, and it was reassuring to see that we didn't.

So whilst we boosted the immune response in looking at humeral responses as well as interferon gamma responses, we didn't see an increase in the AE rates to the same extent as what one sees in the young with a licensed vaccine.

DR. PETROVSKY: Maybe just again a word of caution, that we shouldn't say the elderly as an extension of the young, just as

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we can't extrapolate to neonates. Therefore, maybe we need to look for a different adverse event profile in the elderly.

Certainly, I think it is a general observation that the elderly get less local reactogenicity, but maybe other things start to come into play. I guess one of the potential issues is the data in mice that TLR-4 is important in myocardial infarction and, if you actually look at TLR-4 knockout mice, they actually are protected against atherosclerosis and myocardial infarction.

Now, obviously, that is not going to be an issue in a younger population, but in an elderly population in potentially that pathway, if you activated strongly, may result in myocardial infarctions. Again, that is not a typical adverse event of vaccines that are used in younger populations, but maybe we would have to look at that specifically as something unique to an elderly population.

So again, it is just this issue of

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should we be looking at the different pharmacology and the different behavior of the elderly when we start saying whether or not they are going to get more or less side effects.

DR. VERSTRAETEN: I absolutely would endorse that. We talked a lot about neonates and potential effect of vaccinating neonates, but when you go to the other end of the spectrum, we are not talking about autoimmune disease anymore. we are really talking about atherosclerotic process or cancers. I think that deserves a special -- not a special design, but special attention when collecting serious adverse events.

DR. BALLOU: Are there other comments from members of the panel, the roundtable, that have not had a chance to voice and opinion or make a comment?

DR. HOLDICH: Yes, I would like to just raise a somewhat different aspect, which is really from the aspect of therapeutic

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vaccines, and one of the issues that we have is the detection of rare or long term side effects, bearing in mind that is general speaking.

The inherent natures of the program are not large in terms of patient numbers. On the other hand, the degree of monitoring and selection of patients is perhaps more intense than with the prophylactic vaccines. Therefore, the issue that we need to deal with is in that context. How can we go about assessing perhaps the longer term or the rare side effects?

DR. FRIEDE: Better put my two pennies' worth on this.

It is to do with risk management, and the fact that we've got many manufacturers using their own proprietary adjuvants, which work across essentially similar mechanisms. So we've got the oil and water emulsions from most manufacturers. We've got several groups using TLR-4 agonists. You've got several

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groups using TLR-9 agonists.

So when one of these groups with their particular vaccine has an adverse event -- Just take an example of the recently publicized Wegener's syndrome. How does this impact the other manufacturers' products that are working on similar mechanism or similar targets?

My feeling is that each vaccine is separate, and we have to view each vaccine as being separate. So we cannot say that an adverse event seen in one manufacturer's study immediately becomes a detraction to other manufacturers' approaches. But the design of the studies subsequent to this, we should perhaps be taking this into account.

So this could give us some ideas of at least factors to be included in future clinical studies to say, if that was a real event that was seen, what parameters could we design into the study of those manufacturers that are working in a similar area.

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This would also then come back to the issues of squalene antibodies. So Novartis has done a lot of work to eliminate those concerns. But I think this risk management issue is of concern to the entire environment.

DR. BALLOU: Would someone from the FDA like to comment on how you approach this question? You're brave.

DR. GOLDING: I think this is actually a very, very important point, and there is always sort of the balance between proprietary information, that really, we are not at freedom to divulge, yet when a similar product comes in our door, how do you address it?

So I think, again, there is no one answer, but clearly, when the problem with the myocardial, pericardial adverse reaction was seen in the case of the smallpox vaccination, that clearly affects the way we started to look at all pox-derived, and our clinical

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reviewers were asking manufacturers that were using either new Dryvax-like or host in a vaccine as well as MPA-like vaccines and so forth, to looking for any type of signals that related to what was found.

So I think there is clearly an influence, and of course, if it is in the public domain, it is much easier to explain to the next manufacturer why it is asked. I really think that this is a very good time to plea to the manufacturers to make these type of adverse reactions, even if they are already part of the public domain, because ultimately it will help the whole field to move forward, and there is nothing wrong with making it public, because rare adverse events are exactly that.

Nobody can be blamed from finding them, because we couldn't pick them up at the earlier studies, but once we have seen them, we should be able to now retrench and at least look for these kind of signals in the other

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trials, because I think ultimately it will benefit the whole field.

MODERATOR SLATER: Just to answer that, problems aside, we have to look at the question from both directions. It has been expressed several times today and yesterday that we need to look at antigen-adjuvant units, that we need to look at safety issues for the whole vaccine, for the whole adjuvanted product.

That actually cuts both ways. If one consequence of this is the view that, well, if we had an adverse reaction with one particular adjuvanted vaccine, we should not carry over those concerns to all other vaccine candidates that use the same adjuvant.

Likewise, there is a limit to how much reassuring data we can accumulate with other adjuvanted vaccines that use the same adjuvant. In other words, if we have an individual product in which we observed an adverse event, there may be limits to how

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reassured we can be that four or five or six other candidates have been studied using the same adjuvant without reporting that particular adverse event.

It is a difficult situation. It is not obvious how you should approach that, but the logic, unfortunately, carries both ways, and we have to be very careful as to how we handle it.

DR. SCHODEL: I think we also have to be very careful as to what we consider as a signal. A single event of anything is not necessarily a signal. It is a single event, as Hana said as well.

That is why I asked earlier from the preclinical colleagues as to whether there is any approach toward mechanistically thinking, because that is what we would do in any other circumstances. We would try to figure, you know, is there any biologically plausible correlation, if you really think this is a signal.

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First of all, of course, you would like to know whether it is a signal, but let's assume it is a signal. Then you would try to find out, is it plausible? Then if you had a model in which you could actually study whether the adjuvant in question elicits such a mechanism or has an influence on it, then you could rule out or rule in whether you have to do more.

That is somewhat where we are stuck, because we see a single case of something, and we regard it as a signal. Then basically, the observation stops right there.

DR. VAN DER LAAN: Yes, I will give some comments from a preclinical point of view. I think you are fully right. Just a single event is only a single event.

Toxicology is done with much smaller groups, and that is not a real problem. Toxicology is not the final answer. Toxicology is just preparing the clinical studies, and toxicology is only raising

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signals or not, and there should be -- There is always the discretion, are the groups big enough in toxicology? Never, but that is not the issue.

You are playing with the dose. You are playing with the mechanism of action, and you try to understand what is the biological relevance rather than the statistical relevance. Of course, you have to look at statistical relevance, but the causal relationship and pharmacology is also very important for the interpretation of your toxicology studies.

That is what toxicology can offer the clinical experience also with respect to adjuvants.

DR. BALLOU: I would like to thank all of my fellow colleagues up here for their willingness to participate in this, and for the very interesting discussion and dialogue we have had with the participants in the audience.

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Jay, do you have any final comments? If not, thank you very much, and there will be a wrap-up session immediately following with Hana and Chuck.

(Applause.)

MODERATOR SLATER: The wrap-up session starts immediately.

DR. HACKETT: Okay. So it comes down to us. So let us do the wrap-up.

What we would like to do, Hana and myself, is to provide a sort of a high level view of some of the points that we pulled out from the earlier sessions, and not go into really details of how things are going to be done, but some of the ideas that we got and some of the things that we wanted to take home, and they can be perhaps titles of future meetings, perhaps ideas for new initiatives ultimately, and new foci of our research.

So that will be what we will be doing. We didn't, obviously have enough time to go over the roundtable 2. So you have to

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kind of treat that as -- You have to digest that yourself, but Hana will start out with the roundtable 1.

DR. GOLDING: So first of all, I think that I really want to thank all of the participants for being here. I think this by itself is sort of a success point of this workshop, that we were able to bring into the same room a significant number of representatives from the manufacturing of vaccines and novel adjuvants, of regulatory and NIAID that is supporting a lot of the sort of discovery agenda in this area, and CDC, etcetera.

The important thing was not really to come up with answers to all the questions that were posed, either in the roundtable 1 or 2, but to agree on the questions, to agree on the gaps, to maybe together -- If any of us went home and then said maybe this is a point that we should start thinking about designing some experiments, either in vitro or in animal

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model or in our next Phase I trial, that maybe we can start to address, I think we already achieved something.

With that, I would like to just sort of summarize what I got out of participating in the first day and the first roundtable.

There was a lot of very specific questions about preclinical studies, but what we actually heard from the panel was that the most important word is flexibility.

We really have to think about product-specific issues. They may include both the studies that are likely to give us meaningful information, may include both novel in vitro studies as well as in vivo studies in animals; and not all of these studies necessarily have to be conducted with a GMP product, which is required for the pivotal preclinical tox studies.

Animal studies may be a progressive process, including post-Phase I,

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to explore mechanism of an unpredicted AERs, and that has already happened.

We need to take into consideration when designing all of these preclinical studies the specie specificity of the adjuvant mode of action, if it is known, of course, and the availability of reagents to fully evaluate biomarkers in a given animal model.

That, I consider actually an important gap in the field right now, because I think, as we are trying to understand better both the efficacy and the potential toxicity of novel adjuvants, once we identify the models that are appropriate, we really have to know that we have all the reagents, and that should be an area where I think some both financial and research be addressed.

Ultimately, studies with both adjuvant alone and adjuvanted vaccine formulation may be informative during early vaccine development, as well as the GMP tox. This issue was debated. Some of you felt

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that, really, the final product that goes into the human arm is the one to test in preclinical, but others felt that it was important to try and understand the mechanism of action as well as the underlying mechanism of toxicity associated with a given adjuvant.

My personal view is that probably there is definitely a place for both types of studies at this point.

I would like to now open the floor to some other comments related to the first day and the first roundtable before we move to the next set of conclusions.

DR. HACKETT: Well, yes, there should be enough time to bring up any other points as we move ahead.

I wanted to sort of briefly give my take, and again this will also be something we can discuss, on some of the research topics that were highlighted in the workshop. I have a few, and I am actually going to flesh out each one of them, and also I can send

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everybody all these slides from our sessions. So you don't have to write anything down.

Let me go one by one through these, because what I did was try to -- as I listened to people talk about the things they did, the things they wanted to do, especially in the basic studies and some of the preclinical and clinical, I tried to pull out some of the ideas that we were hearing and some of the research foci and needs that we should develop.

So under the topic you might call immunological markers of efficacy and toxicity, it seems that one of the really valuable approaches is to have a definition of the relevant immunological profiles according to the adjuvant mechanism.

I have put down TLR and non-TLR receptor targeted and APC uptake activation, because it seems to me that what you can see is you can always do gene expression, and that may be very relevant to analyze pathways if

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you pretty much know the pathway. And that may be true now with the TLR and many of the non-TLR.

In some of the other adjuvants which, for example, that might stimulate effective antigen presenting cell uptake and activation, maybe the genes -- I don't know if we know the genes, but maybe the genes are not what you should be looking at, but some other parameters, maybe such things as ways of measuring expression of peptide MHC complexes on APC surfaces, maybe actually kinetics of ingestion. I don't know, but I am saying, I think that if you look at how your adjuvant should be working, probably the next step is to refine those profiles.

The other thing is the standardization of reagents, analytical approaches and controls. I think in some of the studies I was thinking of what is the control, actually, and some of them are good, and some of them aren't. I think that is

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something that we as a group could standardize reagents in particular and looking across different animal models and so on.

The in vitro/in vivo correlation is actually very telling. I think Bali Pulendran showed in his paper using systems biology that he was getting -- when he looked in vitro at human cells versus his in vivo studies with the yellow fever, that he was getting quite a high percentage of similarity, but it wasn't 100 percent. I don't know if everyone remembers, but it was in the sixties or so percent.

So that is something that is very telling, but we have to know what the actual correlation is.

Human and animal model correspondence: There are probably many areas where the correspondence is excellent. A long time ago in immunology, people used to say, if you are looking at a real fundamental process, it is going to be the same in animals and

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humans, like loading peptides into MHC.

It is probably a certain amount true, but that is sort of -- You have to actually quiz yourself as to whether that is going to be true or not, because when you get to the innate immune system, it was pointed out, well, I think that, no, we don't have the same number of Toll-like receptors even as a mouse.

So there will be some areas where there will be processes, I am sure, that are reasonably well indicated in the animal model, but tying these together is very important.

The profile with and without vaccine antigens, actually, Hana mentioned. I think everyone mentioned that.

What I was wondering about is what happens, really, in interpreting already effective vaccines. So if you wanted to study the yellow fever or polio vaccine, that has its adjuvant and its antigen together already.

You can say, well, we have the

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double-stranded RNA already in the vaccines, but you also have the antigen. So I think it would be very instructive to try to figure out what the antigen actually does, because we often think of the antigen as being only the adaptive immune system and the other one being only the innate, but an emerging idea, which is, I guess, not that emerging, is that there is really an enormous amount of interface between the two, and that is probably right where that happens.

The other point was the adjuvant mechanisms that drive distinct T and B cell subsets. This is probably one of the real joys of having a pipeline of adjuvants, is that you can probably start to think about driving in the different directions of CTL and so on. But exactly how that drives is a lot less evident to me than I thought it would be.

It is not easy to say this is the reason you get a Th2 response with alum, exactly. I think there is a lot of

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contributors and, certainly, some of that has to do with the dendritic cells, the macrophages, the cytokine profiles, co-stimulatory molecule patterns, but also the type of T and B cell responses that you get.

It is particularly important, I think, to understand this cross-protection and the repertoire differences that could be one of the most valuable parts of what we are seeing with adjuvants. So how does that happen? I think -- I believe we don't know.

Tools and resources: Certainly, systems biology computational approaches -- there is a vast amount of things going on in terms of cellular pathways, different cells and so on.

We also heard a lot of talk about clinical samples of interest. That means to me that high and low responders, infants and elderly, serious adverse events -- to have access to samples where you could actually probe and decide what is a normal response,

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which I would like to know.

We saw in some of Bali Pulendran's data that there are people that had a very low CTL response, had a high antibody response, and some had a high of both. I don't know, but I would imagine all those people are protected. They got the vaccine, and they probably are protected.

So I think we need to be able to define those, and that will be by studying people who have been profiled, who have shown that they have different responses.

Then there is development of a database, and I wonder if Hana could just say a couple of words about maybe why that might be valuable to the community.

DR. GOLDING: Actually, this is something that had been presented today by Solvay manufacturer, that I think are really taking the lead. They say we are going to design prospective studies to capture a large amount of follow-up clinical data that

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ultimately would help us as well as the field, the public health, to look back and detect low frequency adverse events that happen and to maybe identify other types of biomarker or clinical endpoints that predicted or can correlate, or both, either with the efficacy of a given vaccine or possibly with unexpected adverse reactions.

It reminds me a little bit of the early days of the gene therapy field. It was very obvious that we are entering a new era, and we need to have some sort of a registry to follow up people that receive gene therapy, so that we can accumulate stepwise, long term safety data to see what happens in five, 10, 20 years from this treatment.

Arguably, our adjuvants are not as novel as earthbreaking, but nevertheless, as we are starting to introduce these adjuvants into larger numbers of people, I think together with the CDC and there may be a partnership between the manufacturer and the

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government, that should allow to build a really good database that eventually should be very helpful in terms of meta analysis and identifying the tendency or trends toward a unique type of adverse reaction.

Actually, I think there will be -- It is important to have everybody at the table, both industry and government, to suggest how best to go forward and build this kind of a database.

DR. HACKETT: And, really, the final thing that I wanted to highlight was really the need for new collaborations. I think the field actually has grown to this level in part because -- from some of the earlier stages.

Biochemists and developmental biologists, Drosophila biologists, and so on were collaborating with immunologists. I think in the future there will be a lot of room for computational biology and model building to make sense of some of the complex

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profiles that we see in immune responses.

Certainly, biophysical chemistry is an area. Biophysics of these compounds and so on, how they interact with cells and tissues probably holds a lot toward more rational design, a lot of potential.

In vivo imaging -- Nobody actually really mentioned that, but it seems like it would make some sense. If you want to know how long is your adjuvant lasting at a certain site, what cells are going there, what are some of the hints that you can get about pathology, that would be something that could be done, could be started now.

Another thing is the sample sparing assay development. Several people mentioned, well, you have to make a choice about what cytokines you want to look at and what markers you want to use and so on. Probably in the future, you can do them all, if it was possible to miniaturize and do things in a very small scale where you could

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get good readouts.

So that would probably be the ultimate. If you just try everything that we know about that the immune system can do, ultimately something like that.

So I think those are areas of new collaborations that we will see developing, and maybe we should also think about if there are some meetings or ways of sort of catalyzing these reactions, we should really think about that.

So that is all I had to say. Certainly, I think we have enough time before the wedding or whatever is supposed to happen in here, to have more input comments. As I say, we can send you our slides, certainly. So you don't have to write down any of these things. Any other feedback, we would certainly -- Feel free.

MODERATOR SLATER: Thank you again. I was asked about three more times today about the slides, and I will just say

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again what is going to happen with the slides.

We are going to ask all of the speakers for their permission to put their slides on the website. Once we have secured that permission, we will do so.

I think you can be fairly sure that all the government originated slides will be made available on the website. I don't think that is going to be an issue, but we do have concerns about individuals from manufacturers and from academia that they may or may not wish to have their slides on our website, and we will respect that.

Give us a few days to sort that out. My suggestion is check back on the website in a week or, better yet, 10 days, and hopefully, we will have a link to all the ones that we will be able to share with you.

Aside from that, if there are no comments, thank you all very much for participating. Thanks again to the organizing committee, and have a safe trip home.

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Thank you.

(Applause.)

(Whereupon, the foregoing matter went off the record at 4:35 p.m.)

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