SOCIETY OF TOXICOLOGY

CONTEMPORARY CONCEPTS IN TOXICOLOGY SECTION

U. S. DEPARTMENT OF HEALTH & HUMAN SERVICES
FOOD & DRUG ADMINISTRATION
OFFICE OF WOMEN'S HEALTH

CENTER FOR BIOLOGICS RESEARCH & REVIEW

WORKSHOP ON:

NON-CLINICAL SAFETY EVALUATION

OF PREVENTIVE VACCINES:

RECENT ADVANCES AND REGULATORY CONSIDERATIONS

VOLUME I

Monday, December 2, 2002

Arlington, Virginia

MILLER REPORTING CO., INC.
735 8th STREET, S.E.
WASHINGTON, D.C. 20003-2802
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[TRANSCRIPT PREPARED FROM A TAPE RECORDING.]

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C O N T E N T S

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* * *

C O N T E N T S [Cont'd.]

P R O C E E D I N G S

1A DR. MIDTHUN: Would everyone please take their seats?

Good morning, and welcome. It gives me great pleasure to welcome all of you to this Workshop on Non-Clinical Safety Evaluation of Preventive Vaccines.

I'd like to start by thanking our co-sponsors--in particular, the Society of Toxicology and the Contemporary Concepts in Toxicology Section--for their help in organizing and contributing to this workshop. And a special thanks to Shawn Lamb, the executive director of the SOT, and her staff, for their extremely wonderful help in putting all of this together.

We'd also like to thank, and very much appreciate the support extended by the FDA Office of Women's Health, and for their significant contribution to the funding for this workshop.

And I'd also like to give a special thanks to the staff of CBER in pulling all of this together.

I'd also like to acknowledge the organizing committee members. A special thanks to Marion Gruber and

Liz Sutkowski, from the Office of Vaccines, for working extremely hard to develop and coordinate the workshop. Marion has been working for several years to develop the Office of Vaccines' policy on preclinical toxicity testing; and in particular, to develop reproductive toxicology testing. And Elizabeth Sutkowski is the chair of the preclinical toxicity testing working group within CBER, and is drafting a guidance for industry on preclinical toxicity testing of preventive vaccines.

We'd also like to thank Mercedes Serabian, from the Office of Therapeutics, and Sally Hargus, from the Office of Vaccines, for their help in organizing this workshop, and for acting as moderators for the roundtable discussions.

We also thank Christine Everett for her help in ushering through the co-sponsorship agreement and the approval of the funding from the Office of Women's Health.

We gratefully acknowledge Francois Verdier, for his help in organizing the workshop; and in particular for his recommending that this first day of the workshop be

dedicated to discuss preclinical safety of testing of vaccines in general.

We also gratefully acknowledge Dr. Kenneth Hastings of the Center for Drugs, for his help in coordinating the workshop; and in particular, for his recommendations for speakers for the workshop.

And we also thank Robert House, of DynPort Vaccine Company, for his help in organizing the workshop; and in particular, for securing the approval of the co-sponsorship for the CCT section of the SOT.

This morning I'd like to discuss the key components of the safety evaluation of biologics; which, of course, include preventive vaccines. I'm going to briefly review approaches to toxicity assessments of preventive vaccines, past and present. I'll touch on current challenges and issues related to non-clinical safety assessment of vaccines. And I'll describe some initiatives addressing non-clinical safety assessments of vaccines, and how the regulatory process is evolving in this area.

Clearly, assuring the safety of biologics is at the forefront of CBER's mission. What are some key

components of the safety assessment? The safety assessment of preventive vaccines is a continuous process that begins with the development of a vaccine candidate at the pre-IND stage. It continues through the various stages of clinical development during the IND phase. And it continues onward after licensure, as well, with post-marketing surveillance, and also with inspections that are ongoing at manufacturing sites.

It includes the characterization of the product by physical, chemical, and biological testing. It includes an adequate control of the manufacturing process, and the development and establishment of adequate lot release tests to assure the safety, purity, and potency of the products. It also includes toxicity assessments in animals, clinical safety assessments and, again, surveillance after licensure, as well.

Over the next couple of days, we'll be discussing the non-clinical safety assessment of a product focusing on animal safety testing prior to introducing the product into the clinic and any further safety evaluation of animals

that may be needed to be performed during and in parallel with clinical development of the product.

How is "safety" defined? Well, in the Code of Federal Regulations it states that "safety" is "relative freedom from harmful effect to persons affected directly or indirectly by a product when prudently administered, taking into consideration the character of the product in relation to the condition of the recipient at the time."

Thus, given the diversity of preventive vaccine products, the safety evaluation needs to consider the character of the product, the methods of manufacture, and the indications. It's critical that early in product development agreement is reached between the Center for Biologics and a vaccine developer, to assure that methods and standards for the preclinical and clinical safety evaluation of a product are adequate.

Historically, the non-clinical safety assessment for preventive vaccines has often not included toxicity studies in animal models. This is because vaccines have not been viewed as inherently toxic, and vaccines are

generally administered in limited dosages over months or even years.

However, progress in the field of biotechnology has accelerated the development of a broad range of novel vaccines, and the composition of vaccine products has evolved from attenuated or inactivated whole-cell organisms, to protein polysaccharide conjugates, peptides, recombinant proteins, DNA vaccines, etcetera.

More recently, there has been a generation of a wide range of complex vaccine products and vaccine technologies that are often combined with novel adjuvants, administered in new delivery systems, and administered by new routes of administration. These advances have resulted in an increased focus on non-clinical safety assessment of these products and whether there is a need for initial phase-one clinical studies to be supported by preclinical toxicity data in animals.

In contrast to most drugs and biological products that are predominantly developed to treat ill patients, vaccines primarily are given to large numbers of healthy people, oftentimes predominantly healthy infants and

children. And this places significant emphasis on their safety.

Also, for several vaccines the incidence of the infectious diseases that they are intended to prevent is quite low. Therefore, a high percentage of vaccinated people will never be exposed to the infectious agent. Thus, the benefit of the vaccine in preventing the infectious disease may be difficult to appreciate at the individual level. Thus, there is low tolerance for significant adverse events associated with vaccines--that is, caused by vaccines.

Given these findings, and the context of novel vaccine development, there is an increased focus on the safety assessment in animal models. If the preclinical safety assessment is deemed to be insufficient, this can lead to a clinical hold for an IND.

The Code of Federal Regulations states that an IND should include data from pharmacologic and toxicologic studies that allow the sponsor to conclude that it is reasonably safe to conduct a proposed clinical investigation.

The main challenge in establishing a predictive non-clinical safety assessment comes from the fact that vaccines act through complex multi-stage mechanisms. Thus, the detection of toxicity for vaccines is likely to be more complex than for conventional chemically-derived drug products, because safety concerns may result from the immune response to the vaccine. Thus, toxicity testing programs recommended for conventional drug products may not always be applicable to vaccine products.

The non-clinical safety assessment of vaccines represents a new and evolving field. And clearly, consensus is needed among industry, academia, and regulatory authorities regarding the most appropriate and scientifically sound approaches to this area.

And there are a number of questions to address: For which products should toxicity testing be performed? What are the criteria for selecting the appropriate route of administration, doses, and schedule? How should the toxicity of adjuvants be evaluated? What animal models should be used? And how should one incorporate alternative methods into non-clinical safety assessments?

Depending on the target population and vaccine indication, it may be necessary to conduct special non-clinical safety assessments. In particular, if a target population for the product includes pregnant women or females of reproductive age, reproductive toxicity studies should be considered. We have dedicated the second day of this workshop to address this important subject.

FDA announced in the Federal Register in September 2000 the availability of a draft document entitled "Guidance for Industry, Considerations for Reproductive Toxicity Studies for Preventive Vaccines for Infectious Disease Indications," providing information to sponsors regarding assessments of the reproductive toxicity potential for preventive vaccines indicated for maternal immunization and females of reproductive age.

Industry has provided comments on this document and, because of the complexity of the issues and the concerns raised, we decided to discuss these in a public forum among experts in the field. Thus, tomorrow we will address technical aspects, experimental design, and animal models for developmental toxicity studies, in order to

reach a consensus on how to best perform developmental toxicity studies for preventive vaccines, and the type of information that can be derived from such studies, to assure that it will be relevant and useful for assessment of human risk.

A number of working groups have been established at CBER, not only to address the non-clinical safety assessment, but a number of other aspects of safety. And I'll just mention these briefly, although they're not the focus today. For example, looking at the safety aspects of DNA vaccines, the cell substrates used to manufacture vaccines, and also keeping abreast of the best ways to test for adventitious agents.

There is a CBER reviewer document, as CBER has been engaged in the process of developing guidance for the preclinical toxicity testing of preventive vaccines. The internal reviewer document is entitled "Preclinical Toxicity Studies for Vaccines To Support Initiation of Clinical Studies." And that's an internal reviewer document. And Dr. Sutkowski will discuss this with you in her presentation.

This document will eventually form the basis for a guidance for industry document for non-clinical safety evaluation of vaccines. The goal is to publish a document that is specifically tailored to preclinical and non-clinical safety assessment of preventive vaccines. And that, besides a discussion of general toxicity assessments, includes special considerations for individual product categories, adjuvantive vaccines and other routes of administration.

Issues pertaining to the guidance document on reproductive toxicity studies will be presented by Dr. Gruber, and will be discussed tomorrow.

How is the regulatory process evolving? Well, toxicity assessments will be a part of the product characterization for certain vaccines. CBER will continue to use a scientifically based, case-by-case approach to toxicity assessment.

In summary, non-clinical toxicity assessment is a key component in the development of preventive vaccines. The challenge in predictive safety assessments for preventive vaccines is due to the fact that vaccines are

not conventional drugs. We need to discuss and reach consensus on scientific and technical approaches for toxicity assessments that are specific for vaccines.

These approaches should be optimized to lead to the generation of interpretable data, the wise use of animal resources; and should facilitate the development of safe products, not delay product development.

It gives me great pleasure to introduce our next speaker. And that's Dr. Liz Sutkowski. She will be presenting the FDA perspective of the non-clinical safety assessment of preventive vaccines.

Dr. Sutkowski is a scientific reviewer in DVRPA--in the Division of Vaccines and Related Products Applications, in the Office of Vaccines--and has chaired the working group on the preclinical safety testing of preventive vaccines. She has a wealth of experience that she brings with her: a background in biochemistry; post-doctoral work in the departments of pharmacology at Georgetown University and the University of Washington. She also had many years of experience in the division of

cytokine biology in the Center for Biologics, before coming to the Office of Vaccines.

So it gives me great pleasure to introduce Dr. Liz Sutkowski.

[Applause.]

FDA PERSPECTIVE:

BY ELIZABETH M. SUTKOWSKI, PH.D.,

OFFICE OF VACCINES RESEARCH & REVIEW, FDA

DR. SUTKOWSKI: Thank you, Dr. Midthun, for giving an overview of the initiatives that are ongoing in our office on the non-clinical safety evaluation programs that we have and our addressing on this evolving field. I'll be giving the FDA perspective today on non-clinical safety assessments of preventive vaccines regulated by CBER.

As Dr. Midthun mentioned, the Office of Vaccines is giving consideration to whether or not, prior to proceeding into phase I clinical trials, there is going to be extra consideration given to whether or not non-clinical safety assessments will need to be supported by toxicity testing in animals.

And so the purpose we have gathered here today is to discuss this evolving field, and to facilitate discussion between regulators and researchers in the fields of immunology and toxicology, and to address specific questions that we have in our minds for generating the guidance that Dr. Midthun mentioned.

The objective of my talk today in introducing this workshop is to just go over the challenges that we are facing in toxicity assessments for preventive vaccines, and to go over how the regulatory process is evolving within CBER, and then to go over the current approach that we are taking to toxicity assessments for preventive vaccines; with the idea that the approach we have is evolving, and is not written in stone, and we are here to seek input from all of you.

Today I just want to focus on preventive vaccines, and say that our office regulates preventive vaccines and therapeutic vaccines for infectious disease indications. So we do not regulate other therapeutic vaccines, such as cancer vaccines.

And I wanted to just make sure that everybody is on the same page, and provide a definition of "preventive vaccine." And I'll just give you a few minutes to read that.

Then another couple of other items I wanted to make sure that we had the same perspective on, in terms of definitions, were the preclinical safety assessments. We feel this includes the product characterization, as related to safety, animal safety testing. And both of those things are required for initiating clinical trials.

And preclinical safety assessment, then, is a subset of non-clinical safety assessment; which would include, in addition to preclinical safety, any further safety assessments that would be required during the various stages of clinical or product development. Such as, if any significant changes are made to the product and/or the formulation, then there may be additional safety studies required; and/or if any safety concerns arise during the phase I or phase two clinical trials, then additional safety studies may be required.

This slide lists the key components in non-clinical studies for preventive vaccines. On the left you see there is product characterization, in terms of characterizing the product by biological, physical, and chemical means. And then the next important aspect is manufacturing and the challenge in developing a manufacturing process that, as the product development proceeds, begins with first having control over the starting materials, and then gaining in process control testing and, as the development proceeds to phase three, to establish validated process procedures and to ensure consistency in manufacture by establishing lot release specifications that ensure product purity and potency, and to fully evaluate the stability.

But for today's purposes, we'll be focusing on the right-hand side of the slide. And this includes safety studies that can be performed either in vitro, or animal studies that would include immunogenicity. And this might be part of establishing the potency of the product. It would also include pyrogenicity testing, which would be

performed as part of the purity analysis of the product. And also, of course, general safety testing.

And for certain types of products, there may need to be some neurovirulence testing performed--for example, for live or attenuated organisms. And then, for possibly attenuated organisms or inactivated toxins, you might look for reversion to virulence for those types of products. And in addition, there may be a need to do some additional safety studies. And this could include a GLP--or "good laboratory practices"--compliant toxicity study in animals.

Okay. Dr. Midthun mentioned that vaccines have been generally thought to be inherently safe products. But there is precedence for CBER requesting toxicity studies for vaccines. For example, when the target population includes pregnant women; when there is either a new route of administration or the product contains a novel adjuvant; and also, as I mentioned, when there are some adverse effects that may be observed in the clinical trials; then the sponsor may be asked to examine potential toxicity of the vaccine in additional safety studies designed to replicate the specific clinical event.

And if you were to look in the currently available guidance for principles on designing toxicity studies, you could look at these available guidance documents. The first one is the CPMP note for guidance--and this is a very comprehensive document--on designing preclinical pharmacological and toxicological testing for vaccines.

And then, there is the ICH S6 document, which focuses on biotechnology-derived pharmaceuticals; and the ICH S5a document, which describes toxicity testing for effects on reproduction.

And CBER has referred to this document in their own. The next one is the draft guidance document that CBER has published in September of 2000. And in the CBER document, CBER elaborated more on the considerations for reproductive toxicity studies.

And finally, there is the EMEA concept paper on the development of the CPMP note for guidance on requirements for the evaluation of new adjuvants in vaccines.

And there are also several published articles on designing toxicity studies for vaccines containing adjuvants; one of which is one published by Doctors Goldenthal, Joy Cavagnaro, Carl Alving, and Fred Vogel. And we at the Office of Vaccines use this article a lot to help design clinical studies, non-clinical safety assessment studies for vaccines containing adjuvants, and for adjuvants.

Given the availability of these guidance documents, we feel that there still are uncertainties regarding the toxicity assessments of vaccines, such as those listed on this slide. For example, there is still uncertainty regarding which of the documents are most applicable for use for developing toxicity studies for preventive vaccines regulated by CBER, and whether or not toxicity testing should always be part of the product development. Is it necessary for every type of product? And if it is required, during what phase of clinical development should the studies be done?

And finally, if a study is needed, should it be designed using the conventional toxicity testing approach

used for drugs; and whether or not that would be applicable for vaccines.

So one of the reasons that it's difficult to write a vaccine-specific document, and probably why it hasn't been done so far, is that vaccines are a very complex, diverse class of biologic products. And it's difficult to come up with an appropriate study design, because vaccines act through a very complex mechanism, whereby the product itself is not the final triggering component; but instead, it's the elements of the immune system that are the effectors.

And so some of the questions that one has to address in designing toxicity studies for preventive vaccines is to try to approach all of these issues at the same time, and to design studies that look for inherent toxicity of the vaccine, as well as toxicity of the impurities and contaminants, as well as any toxicity that may be due to the components and individual antigens and other components interacting, and the toxicity linked to the immune response induced by the antigen.

So in terms of telling you today how the regulatory process is evolving, as Dr. Midthun mentioned, we feel that there is a framework needed for non-clinical safety testing for preventive vaccines. And we plan to use the existing documents as a base to develop the guidance.

And the goals of the working group are to make regulatory recommendations and/or requirements more transparent. And we hope that the guidance document would facilitate discussions between regulatory agencies and sponsors and promote relevant and consistent non-clinical testing and review within CBER.

And so, as Dr. Midthun mentioned, we have formed a preclinical safety testing and preventive vaccines working group. And we have already written the first guidance that we plan to write, which is the CBER reviewer internal document entitled, "Preclinical Toxicity Studies for Vaccines To Support Initiation of Clinical Studies."

Okay. I just wanted to mention that the CBER internal reviewer document is going to form the basis for the next document that we plan to write, which is a stand-alone guidance document for guidance for industry,

entitled, "Non-Clinical Safety Evaluation of Preventive Vaccines."

So we plan to use the CBER reviewer internal document as the basis to describe the general approach to toxicity testing in novel vaccines. And that would be the basis for the document. And then in addition, we would have sections on the individual product categories, such as those listed here, and also combination vaccines, and adjuvanted vaccines, and products given by novel routes. So that is one we are still working on. And one reason why we are here today is to get input on the issues, so we can continue to work on that document.

And now I'd just like to go over what principles we have listed in our CBER reviewer document, so you are aware of our approach so far, although it's not written in stone. This is what it is to date:

We have tried to clarify for what product types preclinical toxicity assessment is needed;

We have tried to clarify the timing, the extent, and the approaches to the design of the

safety studies, to support initiation of clinical trials;

And we have described the extent of preclinical documentation required prior to initiating the clinical trials.

So the principles that we have outlined in the document indicate that there is a need for preclinical toxicity--or that the need will depend on risk-benefit considerations, what the target population is, what the route of administration is. And we will also need to look at the available clinical data from the use of related products. And you'll also need to consider product features, such as novelty. And finally, the availability of animal models, relevant animal models.

And the bottom line is that, in considering all of these different items, one needs to use scientific judgment, and that should be the basis for the decision. And it will be on a case-by-case basis.

And this is just a slide to illustrate sort of the clearer areas where you can decide whether or not a

preclinical toxicity study would be needed for a particular kind of product.

It would likely be needed for a product if it contains a novel adjuvant or a toxic adjuvant for which there is no existing preclinical or clinical data; and if the product is from a novel product class for which we don't have extensive clinical experience; or if it's to be given by a novel route of administration.

And it's likely that you may not need a toxicity study to go into a phase I study if the product category is one from which we have extensive clinical experience, or a product for which there is a great amount of product characterization. And this would usually include already licensed products. And also, combination products, including licensed products, you would likely not need to do a toxicity study again.

In terms of the timing, the CBER reviewer document indicates that if the product is one for which the toxicity study is going to have to be done, then it should be done prior to initiating the phase I clinical trials.

And we recommend in the reviewer document that the sponsors agree with CBER, prior to or during the pre-IND meeting, in terms of the design of the preclinical toxicity study. And this would, of course, require having adequate information from the sponsor on the clinical plan they propose. And we also recommend to reviewers that the sponsors submit the protocols to us for review prior to initiating the animal studies.

And once the toxicity studies have been done and you come in with the original submission, the sponsor should include the toxicity study report, which should include a full tabulation of data and line listings, all organized into well organized tables.

And finally, the additional toxicity studies, in addition to those required to phase I, may be necessary as the product and clinical development continues.

And so the next part of the CBER reviewer document, and of course the meeting today, is to focus on this question: How to design appropriate non-clinical safety evaluation programs for preventive vaccines.

I would like to go over the considerations that we have outlined in our CBER reviewer document, in terms of what things to consider for designing the toxicity study. And the goals of the toxicity study should be adequate to identify and characterize toxic effects. And we understand that no one study design is perfect for all product categories.

But in general, the parameters to be considered in designing the toxicity study should consider animal species and strain; and the clinical plan, in terms of what's the proposed dosage form, dose, and route of exposure, and frequency of exposure, and whether or not the product will be delivered by any particular kind of device; and then of course, the product features, in terms of whether it's novel; and other product features and previous data that may need to be considered in designing the appropriate toxicity study, in terms of what is already known about the product.

And finally, the toxicity study should be designed to try to evaluate potential toxic effects on the

target organs and the immune system. And the reversibility of any observed toxic effects should be evaluated.

So in terms of the approach that we currently have in our CBER reviewer document, the principles are outlined here. But once again, it's not carved in stone.

We would recommend that sponsors could either do a dedicated stand-alone toxicity study, or they could do the toxicity study in combination with other safety, activity, and efficacy studies that they would be planning to do.

We feel it's very important to use the relevant vaccine formulation. For example, if the product will be containing a novel adjuvant, you would need to look at the adjuvant alone and in the formulation that is planned for clinical use.

And in terms of correlating with the clinical study, you also need to use the route of administration and the dose that you plan to use in the clinical study. And the total number of doses should exceed the number of clinically administered doses. And when giving the doses to the animals, it should be done episodically. And the

toxicity study should include control arms, appropriate control arms. And finally, the study should be done in a relevant animal model.

Now, just a few words on selecting a relevant animal model. The animal model should be chosen to evaluate safety in an animal model that mounts an immune response to the vaccine and, if possible, an immune response that's predictive for the human response.

And additional considerations for choosing the animal model might include the age of the animal relative to the clinical study that's planned; for example, whether the study will be done in the elderly, or in the pediatric population. Another consideration for choosing the animal model is whether or not to use naive animals, versus partially immune or immune animals.

So in our CBER reviewer document, this slide sort of outlines the parameters that we recommend be monitored. The study should look for local reactogenic and systemic events and immune mediated events; and should also include in-life parameters, such as clinical observations, body weight, and food consumption; and also, laboratory

parameters, serum chemistries, hematology, and immunogenicity. And full necropsy should be performed, to include evaluation of organ description, weights. And this should also include selected histopathology. And finally, histopathology on immune system target organs should be performed, as well as immunogenicity in the laboratory parameters.

So in summary, I think I have told you that non-clinical safety assessment, we feel in OVRR, is a key component in vaccine development; and that we are developing vaccine-specific guidance for non-clinical safety assessment of vaccines; and that the approaches towards toxicity testing for certain products we have tried to define, but that's still open for discussion.

And so we basically wanted to answer two questions: For which product category type should toxicity testing be performed? And, how to best design appropriate toxicity tests for preventive vaccines.

Just to go over now what we're here for today and what we hope to accomplish, we plan to discuss, and would like to invite you to discuss, the methodologies to

determine potential adverse effects of new vaccines and adjuvants. We plan to discuss toxicity study designs and animal models, relevant animal models.

And if possible, we would like to try to reach a consensus on the most appropriate--that is, the most scientifically sound--yet feasible approach to safety assessment of investigational new vaccine products.

We would like to consider all of these aspects. And we have provided some questions in your packets. And we hope to deal with all of these topics today, and we really are seeking your input.

And then, that pretty much does it for my presentation. If there are any questions regarding clarification of what we are trying to accomplish today, I'll take those questions; but otherwise, I think I'll hold any questions on the topics till the roundtable discussions.

If there are no further questions, then I would--

PARTICIPANT [In Audience]: Liz?

DR. SUTKOWSKI: Yes?

PARTICIPANT [In Audience]: You talked about product class, and I'm not sure what you mean by that.

DR. SUTKOWSKI: I guess I should have said "product category type." You know, is it a DNA vaccine? Is it a live organism? Is it any of those products that I had listed there, product types? Does it have an adjuvant?

PARTICIPANT [In Audience]: There are some product categories that do not [inaudible] safety testing [inaudible].

DR. SUTKOWSKI: Well, the slide where I have where it's likely, no. Those are generally the kind of product categories that it's not required. But that's just a product category type.

Yes?

PARTICIPANT [In Audience]: Do you have a time line for turning your internal document into a formal guidance document?

DR. SUTKOWSKI: We're hoping to do that in the next year.

[No Further Questions.]

DR. SUTKOWSKI: Okay, then. Thank you.

[Applause.]

DR. SUTKOWSKI: Now it gives me great pleasure to introduce the next speaker, Dr. Francois Verdier. Dr. Francois Verdier, PharmD, PhD, is the head of product safety assessment at Aventis Pasteur.

He is in charge of establishing and assessing the non-clinical safety investigations required for new vaccines and adjuvant for clinical trials and marketing submissions. He is also involved in the safety issues for commercialized vaccines.

He worked previously for a contract research organization, first managing toxicology studies, and then advising pharmaceutical companies on the toxicology requirements for pharmaceuticals, and particularly for biotechnology-derived products.

He graduated in pharmacy at the University of Lyons, and received his PhD in immunotoxicology in the University of Paris. And this is still one of his fields of expertise.

Francois Verdier is also a Eurotox-registered toxicologist, and a French national expert for the OECD guidelines.

Francois, thank you for coming today.

INDUSTRY PERSPECTIVE:

BY FRANCOIS VERDIER, PHARM.D., PH.D.,

PRODUCT SAFETY ASSESSMENT, AVENTIS PASTEUR

DR. VERDIER: Thank you, Elizabeth, for this kind introduction.

So thank you, too, also, all FDA and SOT members, for the organization of this meeting. I think it's a great opportunity to discuss vaccine safety and to make progress in vaccine development.

In my presentation, I would like to present the industry perspective. And you will see that there are a lot of overlaps with Elizabeth's presentation. So it means that we have a lot of agreement with the FDA regarding vaccine safety assessment.

Also, I would like to mention that I did not make any survey in the vaccine industry to prepare my presentation, so this is my position. And I hope that

during the discussion session I will have some challenges from my colleagues from other vaccine companies, in order to have a fruitful meeting with fruitful discussion.

First, as an introduction, I would like to mention some trends concerning perceived vaccine safety. It was already touched on by the first speaker, and I would like to reinforce the fact that it's true that vaccines provide undisputed benefits to human health.

But also, vaccine safety becomes a major public concern, particularly in developed countries. It is true that the majority of vaccines are given to healthy children. And it is also true that the risk-benefits ratio is looked at on the individual level. People expect a risk of zero from vaccine, even if it is theoretically impossible. And we know that perception of risk outweighs the perception of benefit in the public. And we can see also an increase in the activity of anti-vaccine groups.

So taking into account these trends, and also perhaps due to some recent public health issues, such as the "Mad Cow" Disease, or the contaminated food product issue, there is an increased responsibility for the

agencies and also for the vaccine manufacturers to work on vaccine safety assessment and to develop new methods in this way.

I would like to illustrate these trends--at least hear some unsubstantiated claims between vaccines and disease. Perhaps for the two first claims there are some scientific hypotheses: Lyme vaccine and autoimmune arthritis, with the possibility of molecular mimicry in terms of vaccine; and Guillain-Barre syndrome.

But for the last four examples, there are no real scientific data explaining these hypotheses: Combined vaccines and autoimmune diabetes; Hep-B and multiple sclerosis, this is mainly a French issue; MMR vaccine and autism, that is mainly a U.K. issue; and recently, aluminum hydroxide on macrophagic myofascitis, again mainly located in France.

So at least from these claims it's obvious that we need to provide good scientific data, good non-clinical safety data, to argue on these claims.

Also, if you are not yet convinced about the usefulness of non-clinical and clinical safety studies, I

have listed here some examples of adverse reactions observed during non-clinical studies or during clinical studies.

And it could be tissue necrosis at the injection site during animal studies. Kidney lesions, also: I observed this kind of lesion in primates after the administration of a cancer vaccine with GM-CSF as an adjuvant. Also, a vaccine antibody binding to animal tissues. And we observed that with polysaccharide Meninges-B vaccine. However, this binding was not associated with adverse reaction.

During clinical studies, I have listed here some adverse reactions. The old but very bad story of the Formalin inactivated RSV vaccine.

[Tape Change.]

1B DR. VERDIER: Also, I noted some fever after the administration of a Japanese encephalitis virus vaccine during a clinical trial. And also--less severe again--swelling after repeated administration of cellular pertussis vaccine.

So in front of these findings there are two potential strategies, two potential positions: either what I call the "ostrich strategy," or the "hunting dog strategy." And you will see at the end of my presentation which is for me the best one.

I would like also to reinforce one of the slides from Elizabeth; the fact that vaccine safety evaluation is not limited to toxicity studies, as for a lot of biotech drugs. It's clear that we have to take into account the data provided by our colleagues from the quality control department. It is very important to know the quality of the raw materials; to know the stability of the product, including the genetic stability for viral construct.

Elizabeth mentioned also some biological assays, such as general safety tests, neurovirulence, replication competency for viral vector. And all these data should be evaluated with the non-clinical safety studies to build the preclinical package for the safety of the product.

Also, it was mentioned this morning that it's quite difficult for toxicologists to work on vaccine,

because there are in fact various kinds of toxic effects which need to be taken into account.

The first one, intrinsic toxicity, is perhaps not the major one with vaccines, because we are giving low quantity of antigens and the frequency of administration is not so high. But it could be applicable to adjuvant or excipient mixed with the antigens.

The other type of toxicity is the toxicity associated with the pharmacodynamic activity of the vaccine, and it's probably more important for vaccine; for example, the cross reactivity between self antigens and vaccine-produced antibodies. It could be also the modification of the TH1/TH2 orientation, and any other potential toxic effect associated with the immune response triggered by the vaccine.

More complex is what I have called the biological toxicity; namely, the adverse responses that are related to the activation of preexisting biological processes. And this is, for example, the exacerbation of preexisting autoimmune diabetes.

Also, we have to keep in mind the potential adverse reaction due to the interaction either between different antigens or between the antigen and the potential adjuvant.

And last but not least, potential toxicity of contaminants and any residual product from the manufacturing process. So you see, the task is hard.

Perhaps this slide seems very basic for a lot of you. But I think it's good to mention the GLP. And there are a lot of new players in the vaccine field which need to take into account this requirement.

GLP is a quality system which is applicable to non-clinical safety. And therefore, non-clinical safety studies must be conducted under GLP. And this includes in vivo toxicity studies--I mean animal studies, either single or repeated dose toxicity studies--but also, all the in vitro tests, all the in vitro toxicity studies performed on vaccines, such as genotoxicity tests for adjuvant, or any new in vitro tests.

However, as is mentioned in the ICH Guideline S6, some part of non-clinical safety studies using very

specialized test systems, such as immunological assays, may not be able to comply with GLP. And in this case, you can clearly mention in your product that one specific test will be performed outside GLP.

So what are the prerequisites for animal toxicity studies to enter in phase I for vaccine candidates? The logic would say that we should start with acute toxicity study. However, it is not always strictly needed, because sometimes you can get this kind of information from your quality control test battery. Plus, you will get data from general safety tests.

And therefore, in a lot of cases we start directly with the pivotal repeated dose study mimicking the human immunization schedule. And this repeated dose will be really a strong support to start the phase I.

It's usually performed in one species, but we will try to add in this repeated dose study a lot of parameters in order to collect the maximum information. We will add immunological investigations, and I will explain that later. And we will also do some local investigation. We will do the histopathological evaluation of the

injection site, in order to get some information on local tolerance; and avoid perhaps to perform another study just for this kind of investigation.

And then, on a case-by-case basis, as it was mentioned this morning, we will add some other parameters, such as safety pharmacology; viral shedding, if we are dealing with a live virus; or biodistribution evaluation, if we are dealing with a genetically modified organism.

So let's speak a little bit about the protocol for this repeated dose study. As I mentioned before, the key rule is to try to mimic to be as close as possible to the human immunization schedule. So we will prefer a sequential treatment, versus a daily administration. For example, I used to give the product every two weeks.

This is true for the vaccine. We will see that for the evaluation of a new adjuvant. We may come back to the classical rule of daily administration for a new drug entity.

We will also try to maximize the exposure. And I usually have one additional injection, as recommended by the FDA, compared to the human design.

If possible, we use the human route of administration. And in the study, I am used to include two necropsy time points: one early, one or two days after the last vaccine administration; and another later time point, two to three weeks after the last administration.

Regarding the number of animals, I mean, we use the rules used for all types of drugs. Usually, we use ten rodents per sex and per time point. And if we are using monkeys, it's two to three monkeys per sex and per time point.

About the selection of the relevant species, I think it's really the essential question. And I hope that we will have a lot of discussion about this point. Brian will present some slides and will discuss the various options and the logic in the selection of the animal species. But I would like here to present some advantages and disadvantages of some species.

It is true that the rat is the preferred species for toxicologists. I mean, we have a lot of background data in this species. It's a middle-sized animal, which allows in a lot of cases to inject one human dose per

animal, and also to collect a sufficient amount of blood sample.

However, we don't have always some immunogenicity data, and we have to acquire this immunogenicity data. And immunologists prefer in fact the mouse. But as far as toxicity, the mouse is, unfortunately, a very small species with limitation regarding samples.

Concerning the rabbit, I know that it is an historical species, and I know that the FDA likes this species. However, it's a very delicate species, and we have few background data in general toxicology for this species.

Regarding the monkey, it's probably the gold standard. We have a lot of information regarding the monkey immune system. And there is a close homology with the human immune system. However, it's an expensive species.

Another question which will probably be discussed today is the number of dose levels: Should we use one dose level or two dose levels? If we use one dose level today we are generally using the highest possible dose level,

using one human dose per animal or, if we can achieve that, we use the maximum feasible volume in the selected species.

However, there is some tendency to think about another dose level. And in this case, the lowest dose level could correspond to the pharmacological dose. I mean a dose triggering an immune response in the selected species.

What about the parameters? And Elizabeth listed some of them. I think for the parameters we should follow the guidelines already existing for classical pharmaceuticals.

In my study I'm used to including body weights, with a weekly evaluation; clinical signs daily; body temperature, particularly in non-human primates, and on several occasions after the first and subsequent treatments; ophthalmological examination; cardiovascular examination, mainly in non-human primates, in order to include safety pharmacology parameters during the repeated dose study; hematology and serum clinical chemistry data; necropsy time points.

Again, we can discuss today about the usefulness to add this type of analysis during the course of the study. Is it sufficient to have data at the end of the study, or do we have to add satellite animals to obtain this type of clinical pathology information?

And then necropsy: We do a full necropsy, with microscopic examination of the tissues and organs. We measure organ weights, and we do a histopathological examination for quite a large list of tissues.

Immunogenicity: I mentioned also that we have to add this type of evaluation for vaccine toxicology studies for several reasons. The first reason is to confirm and to justify the selected species. We need a species which reacts to the vaccine.

It's also a good way to confirm the vaccine administration, as we are not doing pharmacokinetics or toxicokinetics in this study. And also, it's an additional proof of concept of the vaccine in an animal model.

There are two types of responses which can be evaluated: the humoral response, by ELISA or ELISPOT assay, or by other types of tests, such as neutralization

tests. Usually, the humoral response is evaluated in serum samples. But sometimes you can also use nasal or vaginal lavage for the evaluation of the mucosal response.

The cell mediated immune response is more complex to evaluate, particularly because we don't have always the right reagent in animals. And it's also very difficult to collect the cells, and to protect the cells, and to do this assay very rapidly. The methodology: ELISPOT assay, or the intracellular cytokine detection.

What about the timing for this pivotal toxicology study supporting the future of phase I clinical trials? Usually, to design the study protocol we need to know some information about the clinical protocol. So it's sort of a "Catch-22" situation, because we cannot start a toxicology study without information about the next step.

But I think it's clear that the toxicology design will be based on the number of administrations in humans, on the targeted population, etcetera. So we need to obtain this information from your colleagues from the clinical department.

As mentioned this morning, there is a possibility to discuss the protocol with regulatory agencies; and perhaps particularly for non-conventional studies. If we send all study protocols to the FDA, I think they will have a huge amount of work.

Then we can initiate the in-life phase in the study as soon as the product is available. I usually prefer to work on a clinical batch, but it is not strictly necessary. We can work on a dedicated batch, either GMP or GMP-like.

And then, additional tests can be needed. But they can be performed prior to the phase I, or later on during the development of the vaccine before the licensing or before phase II/III trials.

And just to illustrate all these recommendations, I have put here one example of a monkey study, and I have selected one of the most complex designs. You have here a prime boost strategy, with priming with GMO, in fact, with a Canarypox vector expressing the vaccine antigen. And then we really mimicked the human design by repeating this

prime boost strategy. And we have the boost with the antigen alone.

And as you can see, we have reproduced here the human treatment scale with various groups in the study and various frequencies of administration.

As I mentioned before, we have added quite a lot of parameters, classical toxicology parameters, such as ophthalmology, cardiovascular examination, clinical pathology. But we have also added humoral and cell-mediated immunogenicity on-point.

As we are dealing with a live virus, we have added a viral shedding evaluation, to measure the shedding of the virus in the environment. And we have also added at the end of the study biodistribution evaluation by quantitative PCR, as we are dealing with a TMO administration. And this study was sufficient to support a phase I trial in humans.

Sometimes we have to add some specific investigations to this classical toxicology study. And it is really on a case-by-case basis. I have tried to present here some examples. But I think it will be very difficult

in a guideline to list all potential tests which can be required to assess the safety of a vaccine.

I have here just mentioned what we did for meningococcal vaccine. This was a vaccine using the transferrin-binding protein. And one of our questions was: Would antibody against neisserial transferrin receptor cross-react with human transferring receptor?

And therefore, in order to document this question, we did first a literature search, in order to see if there are autoimmune disorders associated with meningococcal disease. And we didn't find any data about this potential link.

Then we worked on computer in order to do sequence analysis, and we compared the sequence analysis, the sequence alignment, between neisserial and human receptor. And we didn't find any sequence homology or similarity.

And then, in addition to this literature search and then to the computer evaluation, we did some in vitro experiments in order to study the potential cross reactivity of antibodies from the vaccine on human tissues

by fluorocytometry. And we didn't observe any cross reactivity.

I have listed here all the examples of specific investigations which can be required either before phase I or later on during the development of the vaccine. It could be the evaluation of antibody dependent enhancement assay for Dengue vaccines. It could be the evaluation of disease exacerbation model for RSV vaccine; viscerotropism evaluation for yellow fever vaccine. And I think the list is long. It really depends; it's really on a case-by-case basis.

But what to do if there is no evident relevant animal model? This could be case, for example, for Dengue vaccine or small pox vaccine.

First, in my opinion--but I will be very happy to share a discussion with all the people in the room--in my opinion, it's very difficult to claim that there is no animal model at all.

Then, perhaps to reduce the number of animals used, but still to do something, you can combine an

immunogenicity study with general toxicity; with viral shedding, if we are dealing with a live virus.

We can also try to reduce the number of animal uses; perhaps to use only one species and to reduce this number to, for example, two-plus-two per group for monkeys; and to mimic exactly the human design, perhaps one single administration, and therefore a very short study.

As was also mentioned before, not all the studies are required before phase I. Some of them can be performed later on. And during the day, we will speak about the evaluation of the risk of autoimmune diseases. And Paul Henri Lambert and Mike Luster will in their presentation present this risk and the methods available.

Also, later during the development of the vaccine, the developmental toxicity studies can be performed. And this will be the subject of tomorrow.

And also, for clinically modified organisms we may have to perform biodistribution evaluations. This is true for GMO, and also for naked DNA vaccine. Brian I think will present a case study on this issue. This evaluation is intended to detect exposure of non-targeted

organs, particularly germ-lined tissues, to exogenous DNA. And the method used is a quantitative PCR on tissue fragments from dedicated studies or dedicated organs. And if tissue or organs are positive by quantitative PCR, an integration evaluation is needed for the remaining positive samples.

Last but not least--and I think that this subject will be also exposed by Natalie Garcon--what are the requirements if we develop a new adjuvant or a new excipient?

And my position is in this case to first define the toxicology profile of the adjuvant or the excipient alone, by doing toxicology studies as we do for new chemical entities. I mean acute toxicity studies in rodents by IV route or IP route; repeated dose with daily administration in two species; pharmacokinetic evaluation; genotoxicity tests; and any other specific tests related to the structure or to the mechanism of action of the adjuvant or the excipient.

And then, when we have the toxicology profile of this product, we have also to combine this product with a

vaccine and to test the combination in a repeated-dose toxicity study. We have also to verify the pharmacokinetic of the adjuvant when it is combined in the vaccine formulation; and also, to perform on this combination the other studies requested for the adjuvanted vaccine.

Okay. So now I would like to conclude on this presentation by saying that a few decades ago vaccines were considered as safe, ipso facto. I think it's clear for all of us that today vaccine safety is thoroughly evaluated as well as all pharmaceuticals.

And I'd like also to put some more emphasis on vaccine safety evaluation. I like this sentence recently published in "Nature," saying that, "Predictions based solely upon epidemiological projections without solid scientific bases are often misleading."

I think it's clear that there are a lot of arguments justifying science-based non-clinical safety evaluation for vaccines. However, there are some remaining gaps between the existing tools, the existing toxicology methods, and an ideal, fully relevant preclinical safety evaluation.

And to finish my talk, I have tried to list here some potential corrective actions. First, by doing a toxicology study we will learn, and learning comes from performing these types of studies.

Second, potential corrective action: Perhaps we should encourage academic groups to make research in this field. I am thinking about the users of juvenile animals and the very interesting research performed in Geneva by Dr. Carol Sieglitz [ph] in this field.

Also, we need also perhaps to encourage to boost collaborative research and validation programs. I am thinking about the ILSI initiatives already done for classical drugs. Perhaps similar initiatives need also to be started for vaccine safety evaluation. Thank you very much.

[Applause.]

DR. VERDIER: Do we have burning questions before the coffee break? Natalie?

[Question Inaudible.]

DR. VERDIER: I think we need a sufficient number of data showing the quality of the preparation of the

product. When you will make your safety study, you need to archive with your raw data the information showing that you have prepared your product according to good manufacturing procedures.

[Question Inaudible.]

DR. VERDIER: For me, GLP is really limited to the safety end points. So you cannot use the word "GLP" for the manufacturing of the product. But it's true that you have to follow the GLP recommendations when you will manufacture your dedicated toxicology batch.

[Question Inaudible.]

DR. VERDIER: I am used to adding in my monkey studies ECG evaluation, plus obviously histopathological evaluation of the herd. In a recent study, we observed some histopathological changes in the herd. And that's why it could be interesting to see if these histopathological changes have consequences on the ECG. We measure ECG and blood pressure.

PARTICIPANT [In Audience]: We find measuring these [inaudible] monkey studies [inaudible] studies to be very unreliable; very difficult to interpret that data, and

often misleading [inaudible]. Changes are difficult [inaudible].

DR. VERDIER: No, I cannot really comment. I mean, I used to do that, and it's performed for classical drugs. So I don't have any argument to say that vaccines are really different. I mean, do you think that a dedicated safety pharmacology study will be better for that?

PARTICIPANT [In Audience]: I don't really want to go down that path. I mean, if you have concerns [inaudible] I might consider that.

DR. VERDIER: I can tell you that in this case we did a dedicated safety pharmacology study.

PARTICIPANT [In Audience]: So if you use that, if you use that approach, what's the point of including [inaudible] study? I mean, the only thing you can see is a very negative effect [inaudible], which I suppose is something. But it would have to be pretty dramatic to be able to see it [inaudible].

DR. VERDIER: You are perhaps right. I'm used to doing it only for primate studies.

Jean Villain [ph]?

[Question Inaudible.]

PARTICIPANT [In Audience]: It sounds like you may have to repeat that question. Or maybe you can go to the microphone. Because I think people in the back may have trouble [inaudible].

PARTICIPANT [In Audience]: My question to Francois was what he means with the cardiovascular importance introduced in the toxicity study, especially in primates. And when I was involved at the RAVM in the vaccine studies on pharmacology and toxicology, we found out that there were important cardiovascular effects on the blood pressure of the classical pertussis vaccines. And I am wondering whether this is a more general feeling in the vaccines, or whether it has been studied even?

DR. VERDIER: Well, I confirm that we do blood pressure and ECT.

PARTICIPANT [In Audience]: Yes. You mentioned in talking about dose, dose appropriate to generated immune response in the specific animal that was being used. However, the position of the FDA is that the dose should be

the human dose; not something less, not dose per kilogram, but the actual human dose. So is this a specific disagreement that you have with the FDA?

DR. VERDIER: No. When I'm using only one dose level, I am using one human dose per animal, or the maximum physical volume. If, for example, I have in mind a mouse study, in the mouse you cannot always achieve this one human dose per animal. So in this case, you will give the maximum volume.

I am not in favor of changing the formulation of the vaccine. Because one way would be to increase the antigen concentration in order to have the one human dose per mouse. But in this case, you change totally your vaccine formulation.

So in rats, in rabbits, in primates, in a lot of these middle-sized species, you can for your highest dose--or perhaps for your unique dose, if you are just using one dose--achieve this one human dose per animal.

My remark was in the case of several dose levels in the same study. In this case, yes, the second dose could be just a pharmacological dose level.

DR. MIDTHUN: If I could say a word, Francois? I think the subject on dose is one that we plan to discuss in the roundtable discussion. And so you may want to defer that discussion until that time, at greater length.

PARTICIPANT [In Audience]: Francois, I've got to--Can I ask you a very nice question? Since you are asking toxicological questions, why one species?

DR. VERDIER: It's a very interesting question. I hope that we will discuss that today.

PARTICIPANT [In Audience]: And he thanks me to ask him.

DR. VERDIER: We say one relevant species, because we think that it's already difficult to select one relevant species. So the second species could be less relevant than the one you have selected.

So you have really an argument to say that one species is more relevant than another one. In this case, why do a second species in a lower model, if you wish? But it's true that if we cannot differentiate the relevance between a monkey species and a rat species, then perhaps you will have to perform a second species; but later on,

not to support your phase one. Perhaps to support your licensing.

PARTICIPANT [In Audience]: In your table of species, the pluses and minuses of using different species, you didn't include guinea pigs. Any reason for that?

DR. VERDIER: It's a good question. I will speak about guinea pigs this afternoon for hypersensitivity reaction. I think for general toxicological studies, it's quite difficult to use guinea pigs, because we don't have-- It's a little bit like the rabbit: We don't have a lot of background data in guinea pigs.

It's a very delicate species. However, with some vaccines--I have in mind, for example, CMV vaccines. There are some publications about CMV vaccine and guinea pigs. So then it's really on a case-by-case. However, I should tell you that I've never used guinea pigs for a general toxicology study, until now.

PARTICIPANT [In Audience]: Have you given a look at the liver; as there are old studies on BCG and the effects on Hexobarbital duration, effects on Hexobarbital

duration, sleeping time. Is there anyone that has included this type of evaluation in their vaccines?

DR. VERDIER: I didn't get all the words. You mentioned the liver?

PARTICIPANT [In Audience]: The liver, the liver metabolism, and Hexobarbital sleeping time is affected by the [inaudible] vaccine, and maybe also by pertussis vaccine.

DR. VERDIER: No, we didn't do this type of specific assays. We have, obviously, the liver as part of the organs for the histopathological examination. We have also some liver enzymes as part of the clinical chemistry parameters. But we don't do any functional assays on the liver.

We focus on hypertoxicity for some vaccines. I have in mind a yellow fever vaccine and [inaudible] vaccine using the yellow fever virus. In this case, we do some investigation on the liver, but it's mainly in vitro assays, rather than additional parameters in animal studies.

I think if there are no more questions, it's time for coffee break. Thank you very much.

[Applause.]

[Morning Recess.]

DR.Sutkowski: I think we'd like to get going again. I do have a couple of announcements.

I just wanted to point out, I neglected to say at the beginning of the meeting that this whole meeting is being transcribed. So by that I mean, obviously, not videotaped, but audiocassette recorded, and then it will be transcribed. And we hope to make the transcription available, along with the speakers' slides, available to you all possibly on some website that either the SOT or CBER would set up, to make the transcription summary or the actual transcription available and the speakers' slides available to you all.

I also would like to remind you that this is not a regulatory meeting. It is instead a scientific workshop. And we sincerely hope you have come here to help us work on refining our approach to non-clinical safety assessment of new vaccines and adjuvants.

We would like to get your views and, if possible, try to reach some sort of consensus on the most appropriate and most feasible methodologies that can be used to

determine the potential adverse effects of new vaccines and adjuvants, appropriate animal models for these evaluations, and the utility of these data for the design and conduct of clinical trials.

And once again, regarding the transcription, we would like to ask, if you wouldn't mind, to please state the question and then possibly the moderator will repeat the question. And if you would like to give your name and affiliation, that might be helpful, as well.

Also, in terms of how we envision these sessions to run, each session will begin with the chairperson giving a brief presentation to introduce and provide a general overview of the specific topic. And then following that, the chairperson may choose to present a case study, to go over aspects of a particular product for the purposes of providing an example of a product type to be discussed from a fundamental point of view.

Then the topic will be opened up for an interactive discussion. And during that time, we invite you to discuss the topic.

We would like to try to keep the discussion focused on the fundamental questions; rather than discussing the particular nuances of individual product categories.

You may either present your questions over the microphone or, if you prefer, you could turn in index cards to some of the SOT staff members. Let's see, what else?

We do know that there is going to be overlap in the issues discussed in the various sessions; but where possible, we ask that you try to stick to the topic at hand. And if the moderator thinks that the question may be more appropriate for another session, we may ask you to defer the question. For example, in general, I would like to suggest that we not discuss the various questions with respect to adjuvants until we get to the adjuvant session.

And if there are no other questions in terms of clarification, then let's begin with topic one.

I'd like to call on Sally Hargus, the regulatory toxicologist within our Office of Vaccines Research and Review in CBER at the FDA, who is going to be the moderator for session one.

ROUNDTABLE DISCUSSION:

SCIENTIFIC AND TECHNICAL CHALLENGES IN

PRECLINICAL SAFETY TESTING OF VACCINES

DR. HARGUS: Hello, everyone. I would like to welcome today Dr. Brian Ledwith. Brian comes to us from Biologic Safety Assessment at Merck, where he is the director. Brian has a B.S. in chemistry from William and Mary. He got his Ph.D. in biochemistry from the Medical College of Virginia, and an MBA recently from the Wharton School, U. of Penn. He also did post-doctoral work at Merck in safety assessment, under Matt Bradley [ph] and Warren Nichols [ph].

Brian, thank you for agreeing to make this presentation on relevance of animal studies for non-clinical safety evaluation of vaccines.

THE RELEVANCE OF ANIMAL STUDIES

FOR NON-CLINICAL SAFETY EVALUATION OF VACCINES

PRESENTER: BRIAN LEDWITH, DIRECTOR,

BIOLOGIC SAFETY ASSESSMENT, MERCK

DR. LEDWITH: Thanks, Sally.

I'd first like to just give a very general overview into some of the concepts in choosing an animal model for carrying out these studies, touching on some of the points that Francois touched on earlier; but then use our studies of our adenovirus-vectored HIV vaccines as a case study, not to provide you so much particular data to those studies, but really use it as a tool to demonstrate our criteria for selecting animal models and other study design factors in developing these preclinical safety studies.

So of course, it's first very important when deciding on the animal models to really determine: What are the objectives in our preclinical safety studies? And of course, the fundamental objective is to provide

compelling evidence to support the introduction of a vaccine into human subjects.

And it's important that we realize that in the phase I population it's generally healthy individuals, and so our animal models are generally healthy animal model systems. And we have an understanding that many rare toxicities, idiosyncratic effects, or potential effects on certain sub-populations in the human population are generally often only addressable in humans. So by and large, animal toxicity studies focus on generally healthy animal models.

It's also important to realize in the design of these studies that we're trying to maximize the benefit-to-risk ratio of developing a vaccine; which means we want to minimize the risk by rigorous safety studies, but we also want to proceed with timely development of important vaccines that can affect human health.

Of course, one of the fundamental things we need to do is determine a safe dose for the phase I trials. And this is basically a no-effect level for toxicity, with an acceptable safety margin for humans. But here it's

important to realize, by "no-effect level" we're talking about significant toxicity, and not the desired immune response.

And in designing the studies, it's important to realize, again, we want very broad measures, because most toxicities are unpredictable. But in certain cases there will be a key theoretical concern for a certain vaccine type. And in those cases, it may be warned to include specific assays that may be more sensitive, or at least additional approaches for addressing those key theoretical concerns.

So for considerations for choosing an animal model, as we've discussed already, a major focus is on the relevance of the animal model with respect to immunogenicity; that it demonstrates the expected immune response that you're looking for in people.

This could be a humoral response, or a cell-mediated response, or both. And when choosing the animal model, it may be important to understand that for not all species--particularly rabbits for cell immune responses--reagents may be limited.

We want to have a sensitive animal model. Hopefully, that way we've demonstrated that it does respond to immune-mediated effects, or has been shown to be susceptible to the intrinsic toxicity of the test article.

It's also important to realize that the intrinsic toxicity can be separated from immune-mediated toxicity. A classical example, of course, is pertussis toxin vaccines, where we use an activated pertussis toxin to remove the intrinsic toxicity. And this is tested in certain release tests. And hopefully, the only effects you'll see are the immune-mediated effects.

But two of the most important criteria from a safety assessment perspective are using models where you have experience, where you have large historical control data bases, so that you can interpret sporadic findings to determine whether they're just sporadic changes in a control incidence, or really a treatment-related effect.

And it's also important to be consistent, because we want to develop correlations with our preclinical animal models with respect to the clinical safety of those products when they reach the clinic.

Some additional considerations that have already been raised are whether it's important to do single or multiple species; whether we should use inbred or outbred strains when we're using rodents. At Merck we tend to use outbred strains because we feel it's preferable to have that diversity and heterogeneity in the test animals. So for mice we generally use CD-1 mice; for rats, Spraig [ph] Valley rats. And of course, we are concerned about species- or strain-specific sensitivity. And that's when possibly a second model may be of value.

As Francois alluded to, there are certain advantages between using large animal models versus the small animals. We tend to be able to use a larger number of animals per group when we're dealing with rodents. However, the disadvantage there is that we may need separate groups of animals for separate end points; whereas in a large animal model, all end points could be carried out in the same animal.

Again, the issue of dose comes when there is a desire to inject a full human dose. Then you're almost exclusively restricted to a larger animal model. But the

disadvantage there, because of limitations in the amount of test article, etcetera, is that you generally have limited body weight margins, safety margins based on body weight. And you can actually have much more exaggerated body weight margins in the rodents, which I'll show you in the case study.

And of course, additional models may be needed on a case-by-case basis, as Francois alluded to. An example of this would be a cancer vaccine contained in the self-antigen, where you may want to test a self-antigen in animals, meaning using the animal homologue of that test animal antigen.

[Tape Change.]

2A DR. HARGUS: Any other comments?

[No Response.]

DR. HARGUS: Okay.

MR. BARKER [In Audience]: Lee Barker [ph], Sequella [ph] Foundation. The second speaker said it's hard to make a case for there not being a relevant species. And I'm wondering how important, in considering whether a species is relevant, the panel would consider natural

disease produced by the microbe of interest simulating human disease. Because that can make it relatively difficult to find a relevant animal.

And another very specific question that I think was touched on towards the end by the first speaker, Dr. Sutkowski, and that is a great many vaccines are given to either newborns or very close to newborn humans. So I'm interested in hearing some discussion about whether the relevant age is newborn or suckling animals. And I'm not sure how commonly that's practiced, but I'd like to hear some discussion of that. Thank you.

DR. HARGUS: Thank you. Who would like to take that? Francois?

DR. VERDIER: I think you touched here on a very important question: Do we have to use juvenile animals for a pediatric vaccine? You know probably that there are new guidelines for pediatric drugs. I think today we need to get more information about the immune system of juvenile animal models. We are not yet ready to use these juvenile animals in toxicology. And that's why I was mentioning at

the end of my presentation that fundamental research should be performed on juvenile animals.

The problem also is the feasibility of this model. I mean, if you give the drug by oral route, that's fine. Or by, perhaps, IV; that's fine. You can use juvenile animals. But if you give the vaccine by IM, I don't see how we can treat juvenile animals.

To give you also another example, for a vaccine intended for elderly people we used aged mice. So we altered mice at four weeks, and then we kept these mice for nearly six months in order to start a study on six-month-aged mice.

MR. : Yes, I just want to comment on what Francois brought up about. There is a draft guidance on juvenile animal studies to support clinical trials with drugs. That guidance I think is probably going to be published pretty soon.

But in there there is no statement that you should routinely do juvenile animal studies. You do it on a case-by-case basis. And I don't know, that's probably what's going to wind up with vaccines.

DR. HARGUS: Yes, another comment?

DR. VERDIER: Yes, I would like to have another comment on this topic, since we are very much involved in this type of work using very young animals. And one important point if we deal with vaccine is to consider the immune status and the development of the immune system soon after birth.

It's clear that a neonatal mouse has nothing to do with a neonatal infant. On the other hand, what we tend to see now is that a one-week-old mouse is much closer to the human infant, in terms of development of the structure of the lymphoid organs, the appearance of follicular and [inaudible] cells, possibly of developing an immune response. In fact, you find quite a lot of the deficiencies which can be seen in the newborn are seen also in a one-week-old mouse.

To what extent this can be used for toxicology and to assess the potential risk that we have there, I think that there is a whole bunch of work to be done there. And we know that for some adjuvants it's probably important to look at young animals as well, because we see different

types of reactions. But the knowledge is still quite limited.

MR. : I was actually going to touch on this in my talk, too, about small molecule programs. And I think we're still approaching the subject of whether it's feasible, whether it's relevant, whether the model is relevant, whether it's feasible to do the dosing.

With small molecule programs you certainly can make the argument about differences in metabolism versus--you know, young versus old animals. Here we talk about differences in immune response, young versus old animals.

It's not clear to me that we are ready to jump off that and try to do those studies now with vaccines. How we get to a point where we might be able to address that question is open for debate.

DR. GRUBER: Well, I just wanted to add a point. I really think we would agree that we are not there yet, asking for toxicity studies to include juvenile animal models to assess the safety of a vaccine that is indicated for an infant population. And not to say that there shouldn't be research encouraged in that field, but the

approach that is usually taken, besides doing a toxicity study, is to then do your first clinical trial in adults, and stepping down to toddlers, and then to infants, to gather more on the safety data in older humans before you then go and do your studies in infants.

And I think that's the approach that is currently taken, and is something that is probably going to be employed for a while before we are at the point that we can entertain the idea of using juvenile animal models for assessing the safety of a product for the purpose of moving into a phase I clinical trial.

DR. HARGUS: Shall we move on to the next question? Go ahead, Stu.

MR. SHAPIRO [In Audience]: Yes, Stuart Shapiro [ph], from the Division of AIDS at National Institute of Allergy and Infectious Diseases.

I'd just like the panel to address the differences in safety testing between therapeutic and preventive vaccines. I notice that the guidance that's being developed is specifically for preventive vaccines. However, we increasingly see people--and it's not the large

drug companies, but it's mostly academic investigators--who have this idea that if they come in first with a therapeutic vaccine, first of all, it won't be reviewed by the same people--which I keep telling them this is not true. It's a vaccine for an infectious disease; it's going to get to the Office of Vaccines.

But secondly, they have the feeling that if they start off testing their vaccine as a therapeutic vaccine, the requirements for tox testing will not be as great; and then once they've had it in ten or 20 humans, they can turn around and say, "Oh, look, it's safe, it's got a safety profile."

But we know, those of us who have some experience with it, that they don't get the level of data that you get from doing a thorough preclinical tox study where you can necropsy the animals, sacrifice them at the end of the study and do thorough necropsies. And just the level of information you get is not the same.

So I would hope that you could first shed some light on the FDA's thinking about this; and secondly, that

when you're writing this guidance document, you take that into account, that this issue really needs to be addressed.

DR. HARGUS: Okay, I'll give this a shot. I can tell you that we don't get that many therapeutic vaccine INDs. By and large, we get--Right. Right. I mean, this is an emerging area.

And typically, the approach that we've taken is to be consistent in requesting a definitive preclinical GLP safety tox study for everything now; not across the board, but for a novel preventive vaccine, for a novel therapeutic vaccine.

And we would expect that the sponsors would provide us with an adequately designed preclinical safety study prior to going into phase I, which would typically be in an adult healthy population. And then after phase I, you would go into maybe a very small group of your target population. And then you would take it from there. But at this point, we are consistently requesting the up-front definitive safety study in a preclinical model.

MR. SHAPIRO [In Audience]: Then your guidance document should probably be for preventive/therapeutic vaccines.

DR. HARGUS: Well, I think we'd have to clarify what kind of therapeutic vaccine. And we'll certainly take that under advisement. Thanks.

Oh, Liz has something to say.

DR. SUTKOWSKI: I think perhaps if Mercedes has anything to add, that she should feel free to do so. But our program, in terms of how it's evolving, we have consulted all along with Dr. Dave Green's group in the Office of Therapeutic Vaccines, and we are striving to put this program in writing for vaccines. But we've consulted with them all along, and we are trying to be consistent.

And if Mercedes has something to add--?

DR. SERABIAN [In Audience]: Yes. This is Mercedes Serabian. I was with the Office of Therapeutics. I'm now with the Office of Cell Tissue and Gene Therapy.

When you say "therapeutic vaccines," I guess the first thing I think of is for cancer, because that's generally the indications that come in to us; not for

infectious diseases, if you will. Again, you have to consider risk-benefit. Many of these--obviously, cancer, a life-threatening disease. It depends on what's required preclinically.

At times there may not be a relevant animal model. It may be just in vitro studies that are done. It depends on your product and your--

[Question Inaudible.]

DR. SERABIAN [In Audience]: For AIDS? Well, I guess it would--You tell me, Karen. It would go to your group?

DR. MIDTHUN: Yes.

DR. SERABIAN [In Audience]: Yes. So I mean, I honestly can't respond, except through OTR experience. But the ultimate call would be with the OVRR group. You're correct on that, yes.

DR. HARGUS: Thank you, everyone. Let's move on to the next--

PARTICIPANT [In Audience]: Actually, I--Is it true? Do you think that you would deal with a therapeutic

vaccine for an infectious agent differently than as a preventative?

DR. MIDTHUN: Yes. This is Karen Midthun. I guess, just to say a few words, yes, it's correct. Office of Vaccines would have vaccines against infectious diseases that are for therapeutic indications, also.

And our approach would be to view those vaccines with the same safety considerations as we would view vaccines for the prevention. So that I think the same issues and considerations would go into that, also.

DR. HARGUS: Okay.

MS. CHRISTIAN [In Audience]: Mildred Christian, Argus [ph] Research.

Well, we'll be spending a day tomorrow looking at reproductive considerations. I think that the speakers this morning also lead into the conditions in which one must consider that many of these vaccines will be given to potentially pregnant women, and also to pregnant women, and to pediatric populations, as ultimate populations that are to be treated.

And I'd like to follow the rationale for species selection a little bit. Because last year at a similar meeting on selection of animal species and testing for developmental immunotoxicity, we decided that it was not appropriate to use Balb/c mice most of the immunotox models that are standard will use for developmental tox studies, based on there not being sufficient historical data.

What I noticed was that developmental tox, when requested--And it will be requested more frequently, because pregnant women will be the test population. When tested, there is a tendency to go to the rabbit, which is fine. But in that case, one is attempting to potentiate the immune response to the maximum amount, either by giving boosters, or testing pre-pregnancy.

And I wondered if the speakers would address the rationale and say whether they conducted sub-chronic or other tests to show when the maximum amount of immune response occurred in an alternative species; since usually the primate is not practical for these tests, and a different mouse strain would be what they had as

information in their companies for developmental tox background.

MR. : I can say something. So in contrast to the other representatives from industry, we are doing most of our tox testing in rabbits.

To specifically address your question--And again, I was going to talk about this in my talk. But you know, we don't do traditional pushing to MTD and those kinds of things; or even daily dosing, which may be an issue in terms of designing a developmental tox study. But we focused on the rabbit. There are issues with historical database, but we do get a complete tox package in rabbits. So we're going down that path.

DR. LEDWITH: In our approach we haven't actually carried out a DART study yet, but we are planning to in the next year or so. And our approach would be to use our validated model that's in-house, which would be the rat, and demonstrate the relevant immune response in the rat.

So rather than trying to adapt the DART testing to an immunogenicity model, validate the DART testing model

that we have established in-house by demonstrating the relevant immune response.

DR. HARGUS: Okay, thank you. Let's move on. Sir?

MR. GREENBLAT [In Audience]: Jay Greenblat [ph], National Cancer Institute.

The case study presented, you had no virus-vectored vaccine. And a significant portion of the human population already has an immune response that had no virus. I was wondering if you thought it would be beneficial to include a group of animals that were pre-immunized who had no virus, or a similar vector?

DR. LEDWITH: We haven't done that in our toxicity studies because, all the evidence we had, that would only diminish the response to the adenovirus vector from a toxicological perspective. As I showed you in my talk, almost all of the findings related to toxicity were most pronounced after the first dose; and many not even observed after subsequent immunizations when the animals have neutralizing antibodies against the vaccine.

The issue raised is certainly an issue in the human efficacy trials. And that's certainly under consideration there.

MR. GREENBLAT [In Audience]: Thank you.

DR. VERDIER: To your question regarding several positive animals, I can give you another example. For RSV vaccine, we did studies with sero-positive and sero-negative animals, in order to study both cases.

MR. : To a certain extent, we've addressed the question, and it may cross over to another question, about a rechallenge experiment, whether we need to put in a rechallenge dose.

In reality, the repeat dose tox study, you might even think of that as a--you know, a challenge in the face of an ongoing immune response, or a preexisting immune response. And perhaps even a more rigorous test would be a rest period and then a rechallenge; which is one of the questions I think we're going to try to talk about, too, about the relevance of that.

But that in my mind sort of gets at that same question: whether the toxicity, in the face of an ongoing

immune response, is different, or a preexisting immune response is different, than in the absence.

MR. : Well, that makes me wonder about a particular issue which Brian had mentioned. And that is, he had conducted tox testing both in animals that you knew were undergoing an active immune response, and hence you had seen changes in lymphoid organs that you might expect: larger lymph nodes, larger spleens--Which, you know, you sort of cast over, saying, "Well, they were minimal." But it's something you'd probably want to see, I think, in an active vaccine.

DR. LEDWITH: Right.

MR. : I don't think it's something to be worried about.

And then, the issue then is, if you're doing standard toxicity studies where you're going into a higher dose of the vaccine to look for unwanted toxicities, would you be also measuring whether there is an immune response occurring at that particular time?

Because I think it would be unlikely, or it's a possibility that if you're in high dose you're going to be

inducing tolerance and you won't be seeing that in your response. So how do you distinguish between the changes you see, whether they're toxic or normal changes, that occur from a vaccine in an animal that's undergoing an immune response, versus those that are not undergoing an immune response? Because they're going to be quite different. I mean, do you monitor that?

DR. LEDWITH: We address it in the sense that the doses that we've used in our animal models are similar to--or are identical to the doses that were carried out in the immunogenicity experiments. So we know that under the dosing regimen we're using for our vaccine, for example, we're not seeing tolerance; that we've already characterized the extent of the immune response, whether we're seeing a boost or whether we're seeing basically a steady duration of the immune response. So those particular concerns really haven't come up in our studies.

MR. : But classically, then, what you're measuring is the toxicity of immune response. And if you're looking at a classical toxicity study where, for whatever reason, you go to a higher dose--most sensitive

individuals you're protecting, or whatever--you're not really addressing that specific question, right?

DR. LEDWITH: Yes, and this gets back to a little bit of what Francois was saying. We're limited by vaccine formulations, compared to small molecules, in being able to push the dose towards what I think you're suggesting, maximum tolerated dose types of things, where we will see overt toxicities.

And basically, what we can do is push to the highest dose that we can in these animal models, and then evaluate the toxicities with respect to whether they're expected, with respect to the immune response, or whether there are really organ-specific toxicities which really are not related to the desired effect of the vaccine.

DR. HARGUS: Okay. I think we'll take one more question, and then we're going to have to move on to the next speaker, in the interest of time. And for those of you who didn't get a chance to ask your question, please write it down on one of the cards and submit it to us. Or, if you want to wait until the next session--I mean the next

discussion period after Garvin, then you can ask your question then.

Go ahead, sir.

MR. SNOW [In Audience]: Yes. My name is Bill Snow [ph], AIDS Vaccine Advocacy Coalition.

And I'd like to go back to the basic question of this panel, which has to do with the animal models. The case study that was presented, presented three animal models: the mouse and the rabbit and the monkey. And each was serving a different purpose.

The question that I have is in the introductory tox, that was not required. I'm wondering under what circumstances--For example, if you had monkey immunology data from a preclinical lot that made the company confident to move forward, when could you cut back from those three? And under what circumstances in an exploratory vaccine program would you be able to get some human data before going to all of the expense and time of doing these tests and the manufacturing at the GMP level?

DR. LEDWITH: I'll take a first stab at that, because it was my talk that prompted the question. First

of all, our choice of using those three different animal models was basically voluntary. It was not required upon us. That was basically our study design, which we did discuss with CBER prior to the initiation of the studies.

And we chose to do two animal models for complete toxicity basically because, as many of you are probably familiar, there was great controversy about the use of adenovirus vectors in a gene therapy trial at that time, and significant safety concerns, some of which we felt could only really be evaluated in monkeys.

So we wanted to have both our more standard, small-molecule mouse toxicity study, combined with a second species, Rhesus monkeys, to adjust really for those particular safety concerns for adenoviral vectors. We don't do both species routinely for all vaccines.

And with respect to having an additional model for local tolerance, again, that was more of a voluntary, in-house procedure, because we've used rabbits for so long. Now, rabbits was not the best model for us for a full toxicity study. It's not validated at Merck for tox studies. But we have used it for intramuscular studies.

So we just chose to use that for local tolerance. But we also evaluate local tolerance in the tox studies. So it's quite possible in a single study to address the local tolerance issues as well as the systemic tox issues.

DR. HARGUS: Ken, do you want to go ahead?

MR. : Yes, just one comment I'd like to make, since you brought it up. One of the things--And I'll just make the comment, and this can be discussed later. I am wondering a little bit about whether the issue of systemic inflammatory response really was dealt with to the depth that I would have expected it to in this preclinical model.

You know, you look at the overall data that was presented, and there's a little glimmer of things going on. And when you're talking about a small number of animals, you want to explore that a little bit more in depth. That's all I want to say about that.

MS. : I just had a more general comment. I thought--And perhaps I'm challenging the panel members and the audience here a little bit. But the session was about the relevance of animal studies and

animal models. And I think what we heard today is that animal studies are critical and necessary to get a feeling about the toxicity profile of a vaccine.

But what I haven't really heard this morning is a discussion about what the relevant animal model really is; and what the relevant immune response really is; and if we really should stick with the note that was made earlier to say we need to work with animal models for which we have a large amount of historical background and experience, so that we can interpret some sporadic adverse events that we otherwise in a non-traditional species would not be able to interpret.

But my question is if we perhaps have to compromise here a little bit and say, okay, we may not have a species that is validated by all means in that we have a large amount of historical data and background data, but that we know it's valid and that the immune response that is induced is somehow relevant to what we are really getting at.

And perhaps we need to discuss it a little bit more; if not today, then we can do it tomorrow. Because

the issues are somewhat similar, I think, and we can perhaps revisit this tomorrow. Because I think that there is a case to be made perhaps to really take a stab at looking at perhaps not the well established animal data in order to arrive at a more relevant animal model. And that's a comment I had to make.

DR. HARGUS: Okay. Thank you. At this point, I'd like to introduce the second speaker for this session, Dr. Garvin Warner. Dr. Warner received his Ph.D. in microbiology and immunology in 1986 from the Albany Medical College, and did a post-doc and was a research assistant professor in David Scott's lab at the University of Rochester Cancer Center.

In 1991, he joined drug safety evaluation in Bristol-Myers Squibb in Syracuse, and expanded the immunotoxicology and exploratory toxicology group there as part of the department of biologics evaluation, and was responsible for the early drug safety and development programs for a number of immunomodulatory, oncology, and therapeutic vaccine programs.

In 1997, he moved to Genetics Institute, Andover, and was responsible for a number of development programs for therapeutic protein. After Genetics Institute was fully incorporated into Wyeth Pharmaceuticals, then American Home Products, he was responsible for development programs in immunology and hemophilia.

He is currently the director of exploratory drug safety, Wyeth Research, Andover, Massachusetts, and is responsible within drug safety and metabolism for the biopharmaceutical, hemophilia, and vaccine programs. Okay.

And Dr. Warner, thank you very much for agreeing to make this presentation on the applicability of traditional drug toxicity study designs for safety evaluation of vaccines.

APPLICABILITY OF TRADITIONAL DRUG TOXICITY STUDY DESIGNS FOR SAFETY EVALUATION OF VACCINES

PRESENTER: GARVIN WARNER, DIRECTOR, EXPLORATORY DRUG SAFETY, WYETH RESEARCH

DR. WARNER: I have to apologize right off the bat because I may confuse people, in the sense that I tend to play devil's advocate. And you may say, "I thought he

said this, and now he's saying that." So bear with me on that. I'll try to generate some discussion here.

Words to live by. I often run into this problem when I'm dealing with--because I do cross over into the discovery groups--of why we're doing toxicity testing when we know that this is okay. This isn't going to be a problem. Why do you push that dose so high? You know, we don't need to do that; that's ridiculous.

But the reality is, we do toxicity testing to look for unexpected effects, not for expected effects. We use scientific judgment and try to think about what we might see, but the reality is we're looking for unexpected effects.

So I just really want to give you a little bit of view of drug safety evaluation from a traditional small-molecule company perspective and touch on: What's the point in a traditional tox study, or tox program; factors that influence the traditional toxicity program; selection of species--some of these topics are going to overlap with what we talked about last time--general flow in our tox

safety programs; and then sort of move on to whether vaccines fit the drug toxicity testing paradigm.

In the concept of maximizing exposure to the test article, does one size--I think we've already answered that question. Does one kind of study fit every kind of program? I think that's clearly "No."

The kinds of study designs: I will present a sort of straw dog study design at the end, really not from a viral--you know, from a sub-unit perspective for a vaccine program.

Issues related to the immunogen versus the adjuvant--we'll talk about adjuvant later--versus immunomodulator. I throw that in. Now people are starting to throw other things in besides an adjuvant; perhaps direct TH1/TH2 responses.

And then I've also put up here--and it's interesting, it's been brought up several times--the test article used in IND enabling studies. What do we need to have for that, in order to do our GLP/IND enabling tox studies?

So in a small-molecule program we establish an MTD. We push the dose to the point where we see toxicity. Indeed, some would argue that you haven't done a toxicity study unless you have toxicity. Otherwise, you've just shown that it's safe at that given dose.

So with most small-molecule programs, we can push the dose to an MTD. And we usually do it in two species-- We always do it in at least two species. And primarily, we're trying to identify target organs of toxicity, to guide clinical research into what to look for in their clinical studies.

We establish a safe starting dose based on the no-toxic-effect level, or the no-effect level in the tox study. And the focus really is on exposure. We maximize exposure. We measure exposure. We do pharmacokinetics. We can talk about exposure relative to the area under the curve of our tox dose versus our pharmacologically active dose; which of course is a little bit of a problem in vaccines.

We can change dose levels. You know, we're non-restricted usually in the formulation; although that can

happen at times. We can increase the number of doses relative to the clinical study. We can change the schedule. We can change the route to maximize; go IV, if we want to look at a maximum systemic exposure.

And the studies are staged with increasingly longer durations of treatment to support the clinical program, and ultimately to support registration.

There are some factors that influence the study design, and perhaps the timing of the studies as we do them. But the reality is it's pretty--I don't want to say it's really straightforward, but it's reasonably straightforward about what we have to do for all of our small-molecule programs.

But there are issues, you know. If we're treating a terminal disease, oncology, that program may look different than treating asthma, for instance. You know, whether it's non-life-threatening disease; and whether there are other existing therapies.

Age of population: We touched on it already. We'll talk about women of child-bearing potential tomorrow

in developmental toxicity, but I also put up here juvenile and pediatric studies.

Now, with small-molecule programs, of course, we would do a juvenile study generally, prior to going into infants, or to less than--whatever, 16 years old.

With vaccines, the history has been that we don't do those studies. And again, as I said before, I'm not sure that they're really relevant in the context of a vaccine program, or whether we have enough information to know that they're relevant.

And of course, the duration of treatment: If we're talking about a drug for an acute indication, a single-dose study, that program will look different than one for chronic lifetime administration.

So we generally pick the most sensitive species in terms of any toxicity noted. I'm really giving my view here. And in terms of rodents, we prefer rats; but occasionally mouse. And certainly, it's often needed for carcinogenicity studies. Our non-rodent, we prefer dog; but sometimes we use non-human primates, or sometimes we do both.

And again--I'll jump down here--often the selection has to do with the relative metabolism in the two primary tox species, and the exposure. So generally, we'll either use the most sensitive species and/or the species that gives us the greatest exposure. So again, everything is exposure based.

I'm going to briefly touch on proteins. You know, historically, everybody says, well, proteins are dealt with differently than small molecules. And certainly, they have different issues. But again, and I bring up some of the issues that cross over into the vaccine area, right? So we need to demonstrate pharmacologic activity in the species selected.

Often, particularly with monoclone antibodies, an MTD can't be established. Okay? So in contrast to the small-molecule program, we can't reach an MTD. And the question always comes up: When do you stop dosing? When do you stop escalating your dose?

Often we end up with, as has been mentioned here before, dosing based on maximum feasible dose, based on the formulation that can be provided to us. And the toxicities

seen here are often associated with the pharmacology; like the vaccine programs where we're often worried about the response and not so much--well, at least historically, anyway--the intrinsic toxicity of the molecules themselves.

But we're able to measure exposure here. And generally, the greater the exposure, the more rigorous the test. And the question is: When do you stop dosing? When do you stop pushing that dose?

Immunogenicity: I'll bring it up. Here we've talked about tolerance already. Early on, immunogenicity was a big concern, "Oh, I can't dose. If I get antibodies in my animal model, I can't dose any more." Well, you know, that turns out to be maybe not the case. You can either dose through the immune response; maybe you induce some tolerance; maybe--I don't like that word; I'm an old "tolerance" guy. But "clonal exhaustion," whatever you want to cal it. But there's an example where, pushing the dose, clearly you can get over issues of immunogenicity. It's relevant to vaccines in the sense that pushing the dose in a vaccine may be the exact opposite way of where you really want to go.

So the general flow here, we do exploratory, non-GLP studies, often single-dose MTDs, with toxicokinetics, range finders, repeat dose. Sometimes these are GLP. And exploratory metabolism.

In phase zero, our IND enabling GLP tox studies, including the full battery of gene tox, generally a 28-day tox study with TK.

Some safety pharmacology. We've touched on that. We do dedicated safety pharmacology, cardiovascular safety pharmacology studies.

And the reproductive toxicity studies to support women of child-bearing potential. And of course, the timing of these is depending on the clinical population.

And that basically is phases I through III. We're just keeping up with the clinic, trying to extend the duration of our studies to cover the duration in the clinical studies; and then start adding in things, depending on clinical plan: juvenile studies, chronic studies, reproductive toxicity studies, and ultimately carcinogenicity studies.

So do vaccines fit the drug toxicity testing paradigm? Does the concept of maximizing exposure to the test article, does it apply to vaccines? Now, we've got to separate out adjuvants. And are we really trying to do two things with our studies? Are we trying to not only assess the intrinsic toxicity, but we're also trying to assess the--I don't know, the pharmacodynamic toxicity, the toxicity of the pharmacodynamic response to the response [sic].

So the question to me is: What is the test article? Is it the adjuvant? I would argue that it's both; it's the adjuvant, and it's the combination. We look for it in the intended immunologic consequence. That is either anti-immunogen antibodies; the cell-mediated response, as several of the speakers have already talked about.

The question of: Will maximizing the exposure to the immunogen actually hinder the intended immunologic response? So Francois talked about using a dose--and we do this, also--a dose that is intended to maximize the intended response, and a dose that we do several-fold over.

And often, it is based on the formulation, the maximum feasible dose.

And of course, I should say in these animals that we're talking about mgs-per-K--or mgs-per-meter-squared, or however you want to gauge it--we typically dose on a per-dose basis, using the equivalent human dose if we can, and then some multiple of that. But built into that is a safety margin based on mg-per-K, as we've already mentioned, particularly in mice.

Vaccines are a complex and diverse class of products. And immunogens--I mean, we've had sub-unit vaccines; purified; recombinant DNA derived; live attenuated viruses, which have their own issues; CD&A vaccines; vector vaccines; and chemically synthesized vaccines and adjuvants. So clearly, for this gamut of things it isn't a "one size fits all" kind of a toxicity study that we can use for everything.

Dose selection? Again, we've already mentioned that a couple of times. I bring up the issue of a special tox formulation. That's come up several times in our discussions. We know what the clinical formulation is, but

if we want to push the dose, should we come up with a special tox formulation where we've increased the concentration of the immunogen?

Schedule: Maximize exposure again, like in a small-molecule program. Honestly, I would never think of going daily dosing in a vaccine study, even with an adjuvant. And we can talk about that later.

And then the question is: Do we trade off--And again, we're doing two things with the study. We trade off an optimum mean response for an optimized exposure to the test article.

Route has come up. Intended clinical route only? I would say, yes. Other routes of administration to maximize systemic exposure: At times, is that a relevant thing to do? I mentioned before, sometimes we'll go IV, even with the small-molecule program, to maximize systemic exposure. Is there a place in a vaccine program to do that, also?

And then, the duration: Generally, as has been brought up several times--We call it the "N-plus-1" convention, the number of clinical doses, plus one. What

also has come up is the rechallenge issue, whether we should build into studies a rest period and then a rechallenge. Because often these vaccines will be used perhaps more than one sequence of immunizations during a lifetime.

Immunogen, versus adjuvant, versus immunomodulator: Do we need to assess individual toxicity in each one of these things? In general, I'll just give you a paradigm for our sub-unit vaccines. In our tox studies we include an adjuvant alone, the immunogen alone, and then combinations of the immunogen and the adjuvant.

Usually, when we do the immunogen alone, it's a top dose, in the combination arm of the study. And if we're doing two dose levels of the adjuvant, it's at the top dose of the adjuvant. And we'll discuss the novel adjuvants and the adjuvant issues later.

So finally, I just want to touch on this issue of test article used in the IND enabling studies. This is less of a problem for the small-molecule program people because by the time we get our stuff, they're talking about their first 50-kilogram batch and things like that.

But there are questions regarding the contaminant profile. It's come up in a couple of other talks, too. So we're not only assessing the activity or the toxicity of the active ingredients, but also of the contaminant profile. So this needs to be representative of the clinical material.

It should have a similar activity profile. It may not be GMP; it may not be fully GMP compliant. But what data should we have to say that it's representative of the clinical material? Especially given that often at the time that you run into tox studies, you don't have the clinical material yet.

A similar biochemical, biophysical profile, is that sufficient? And of course, the question that always comes up in dealing with protein programs, too, is: When is a change a change? So scale, ten-fold scale, a new peak on size occlusion chromatography, those kinds of issues.

And this is what was also touched on a little bit, too: Sometimes the clinic is used to decide which gets brought forward and how much preclinical work is necessary to do those human immunogenicity studies. I

mean, I think HIV is a particular example of that, where we're bringing a lot of things forward, in hopes that our human studies will tell us what works best, or what is likely to work best. And the question is, in a life-threatening disease like that, is there some minimal amount of work that we can get into a phase I study?

So here's my straw dog of a tox study. And maybe we could just leave this up here and, again, perhaps open the floor to questions. So I'm talking about several questions.

So actually, up to here is a four-dose study. You know, typically, N-plus-1. We're going to do four doses. The question: Interval; how long between doses? I've heard two weeks mentioned. We've done four weeks, because that's what the most aggressive schedule in the clinical is going to be. What should that decision be based on? What should the decision on dose be based on?

Early necropsy? How long after that last dose should we do the necropsy? Should we do two necropsies? Should we look at one--I would argue that perhaps

conducting a necropsy at an optimum point, when the immune response is expected, is the appropriate place to do it.

I certainly haven't listed all the clinical end points here, and there were other ones here. But I'm really lumping everything together because, in honesty, the tox studies that we do--which I've already mentioned, we often do do rabbits--it's a full tox panel that we would do for a small-molecule program. A full histo; we add in measuring immunologic parameters associated with the pharmacology. But it's a full tox path assessment.

So I don't know, questions from the floor? Do you want to open up the questions again? Really a continuation from the last questions.

DR. HARGUS: Let's thank Garvin.

[Applause.]

[Tape Change.]

2B DR. HARGUS: --discuss the topic of animal models along with the topic of study design considerations. Let's open the floor to questions addressing those two broad issues.

MR. VAN DER LAAN [In Audience]: I have first a question on the--Jan-Willem van der Laan. I am a member of the safety work part of the CPMP.

With respect to the dose and the dose level and the number of dosages, I'm not sure--and maybe the immunologists on the panel can answer--whether the immune response is dose dependent. I expect at least a bit.

And if Dr. Verdier is indicating that one dose is sufficient, how to be sure if you have to do yet the phase I, how to be sure what is the human dose. And I think that our questions which came up during this lecture of Dr. Garvin [sic].

DR. WARNER: I think that is a good point. And Francois mentioned trying to get the clinical plan before designing the tox studies, which is sort of related. But you're right, that top clinical dose is a guess, honestly. I mean, in the sense that we're talking animal models, and the reality is that we don't know that it's going to be relevant to the human dose, the needed human dose.

So, you know, it's sort of a development question for the people in development. Because talking to the

research people, they're saying, "Ah, this will work." And that may be true.

But we do try to--I try to influence people to build in some safety margin there: the highest anticipated clinical dose, and then some multiple of that. So that when we get into the clinic, we have some room to move; and that the tox study supports dosing at higher levels than based on the mouse immunogenicity--or in our case, we do rabbit immunogenicity.

PARTICIPANT [In Audience]: Garvin, does it matter whether or not you use body surface area or body weight?

DR. WARNER: So, okay, if you use mgs-per-meter-squared, all you're doing is building in an extra factor.

PARTICIPANT [In Audience]: Yes.

DR. WARNER: If you want to do that, say you've got to, instead of using tenfold based on mgs-per-kg, just say use 30. The basis for mgs-per-meter-squared for small-molecules is based on metabolism and clearance. At least, that's the argument.

We don't know here whether that's relevant or not. If you want to build in additional safety margin, just build it in based on mgs-per-kg. That's my opinion.

DR. VERDIER: Jan-Willem, I agree with you that there is not a clear dose relationship for the immune response. And that's why perhaps one human dose is perhaps sufficient, because with high dose or low dose you will trigger the same humoral and cellular mediated response.

There are some cases where you have to test two doses. If your vaccine is mixed with an adjuvant, one human dose per animal could be too high, and you may have to test lower doses in order to avoid a very severe inflammatory reaction due to your adjuvant composition.

And also, I think I would like to emphasize the fact that I'm not really in favor of changing the vaccine formulation to test higher concentration, even if this is a way for new chemicals.

DR. GRUBER: If I can comment on that, too, indeed, when you have a formulation, and especially when you have the antigen--and I am more talking about recombinant antigen--and you have a specific formulation,

the interactions between each component defines your formulation. If you start going by mg-per-meter-square, or by weight, it doesn't mean anything any more in your formulation.

You change completely the system, and you may see responses that are not relevant to what you would see for the human response. So from our perspective, it's better to stay with one human dose, which in a rat or a rabbit is already in vast excess of what you would have in a human.

DR. WARNER: I agree. I'm not advocating it. I'll play devil's advocate, though: It depends. It depends on the adjuvant that you're using. It depends on whether you can get stability at a higher concentration.

I was really thinking in my mind when I thought this through of maintaining a constant adjuvant level, and then changing the concentration of immunogen, and perhaps doing that in exploratory studies. So we could even define the immunogenicity profile, and see whether it's relevant. Again, I'm not advocating it. I'm just playing devil's advocate.

MS. NOVIKI [In Audience]: Hi. I'm Deborah Noviki [ph], from Chyron [ph] Corporation.

A lot of the comments that I was going to raise just got raised as the discussion was occurring. But I wanted to sort of just raise a point, and it was covered a little bit with the last comment. And exploratory studies are not really exploratory for Chyron, but the immunogenicity studies that actually justify the utilization of an adjuvant.

I depend greatly on the preliminary pharmacology and immunogenicity studies that my colleagues are running. And that actually helps drive a great amount of the way I design a study to do the toxicology.

I, too, use rabbits extensively, because primates and mice tend to be the species that our pharmacology and immunogenicity studies are done with. And what I've tried to do is work very closely with colleagues in immunogenicity studies and actually incorporate some safety end points into especially primate studies; not very much in mice at all.

But I think that working closely with those groups, utilizing long-term studies that toxicologists would almost never run--I mean, we'll run primate studies for a year or more sometimes. And those are important opportunities to garner data, even if it's not done under GLP and even if it's done with research formulation. So I think that's a really important thing to think about.

MR. : I agree completely. And not just for vaccine programs, either; other programs, too. There's a lot of potential safety information that can be gotten out of well designed pharmacology or, in this case, immunogenicity studies.

DR. HARGUS: Okay. Go ahead.

MR. GILMER [In Audience]: Yes. My name is Ian Gilmer [ph], from the EPA.

I was interested in the "N-plus-one" concept. Where I work we're interested also in cumulative and aggregate risk. Kids get anywhere from a dozen to 16 injections of different vaccines over a two-year kind of span.

So I'm just wondering about the kind of cumulative effect of the different vaccines. And perhaps this is more relevant to the adjuvant, mainly aluminum hydroxide. But you know, there seems to be more of an exposure than what's being tested, if you look at what's going on in real life.

MS. : So if I understand your question, it's more in the light of pediatric schedule vaccination, considering all the vaccines they get. What you're suggesting is that maybe toxicity studies should be done in light of those vaccines, and you should somehow prime the animal with those vaccines before testing your new vaccine, or co-administer?

MR. GILMER [In Audience]: Well, I'm interested in how the FDA--or looking at this just as an overall exposure in this two-year window; not just a single component. So, yes, that was my question.

MR. : I'm embarrassed to say that I don't know what has been done, in terms of aluminum toxicity.

DR. MIDTHUN: You know, I think in large part that's why we're here. It's really to say, you know: How should we really be looking at vaccines, all their different constituents, and really be collecting information in a very organized fashion from beginning to end?

You know, I think that clearly, once one gets into the clinical trials, that often times if a vaccine is to be administered on a particular schedule, it will usually be administered in the context of the other vaccines that are already part of the infant immunization schedule.

And you know, it's very important to have good clinical data and controlled clinical trials. But I think we also recognize that there are a number of things that we really need to learn more about, and certainly aluminum is one of them.

I think that we're increasingly moving toward the approach that you really should demonstrate that you need certain adjuvants, that certain excipients are really important to your product. And so I think that if you've

had recent experience with us, we will be asking you, if you come in and say, "We need this aluminum adjuvant of vaccine," we'll be asking you to demonstrate that you really do need that.

And so I think these are all issues that we're all very interested in learning more about. And I think that really ties into part of why we're here, examining some of these issues.

DR. VERDIER: I will not answer totally to your very interesting question. I will just perhaps justify why we need to perform 28-day or 14-day repeated-dose daily studies for new excipient or new adjuvant. It's to answer to your question. I mean, if an adjuvant is given several times in several vaccines, what are the potential toxic effects? And by doing classical toxicological profiles for new excipient or new adjuvant, we will partially respond to your question.

MR. : But part of the problem are some of the old vaccine components. I mean, I don't want to start a riot here or anything, but we did nominate aerosol

to the national toxicology program, and they are doing a workup of that.

DR. HARGUS: Okay. Go ahead.

MR. BAYARDI [In Audience]: Yes. My name is Marc Bayardi [ph], from [inaudible] agency and the University of Paris.

Can you comment a little bit on the immunotox end point you are planning to include in your protocol? There is a question mark, so I'm asking the question.

DR. WARNER: Well, I think we're going to talk about that later; aren't we? Way to pass the buck.

[Laughter.]

DR. WARNER: That is a true question mark, because we have not to date included anything that I would call immunotox end point. Histopath, draining lymph nodes, and things like that have been the only thing that we've really looked at to this point.

MR. BAYARDI [In Audience]: Okay. I've got a second question. It's a question for Elizabeth Sutkowski. It's about when you were talking about which kind of product we should include in a tox program. You described

the combination of products. You said it's not required for well-known products. I agree. And you said it's maybe not required for combination of products. And I just want to know why.

DR. SUTKOWSKI: Yes. I think that was on the slide--

MR. BAYARDI [In Audience]: Yes.

DR. SUTKOWSKI: --on this side, where it was likely not necessary for most commonly approved vaccines when they're in combination and approved individually, and possibly even for an investigational vaccine if there is enough data available preclinically and clinically; in terms of whether or not there is any concern about synergism or added reactogenicity when you combine them. But I think that's sort of a difficult question, and would have to be answered on a case-by-case basis for individual components.

MR. BAYARDI [In Audience]: Okay. So I mean, it is still open that if you're planning to develop a combination of products, that you should do tox studies? It's on a case-by-case basis? You're not excluding right

away from the tox program? I don't know if my question is clear.

DR. SUTKOWSKI: Yes. I think it would have to be considered on a case-by-case basis. I don't know if anybody else would like to make a comment.

DR. GRUBER: Well, I just wanted to add something to that point that Liz just made. I mean, we had long discussions of even to show the slide, likely yes or likely no. Because we knew it would really put products into certain categories where they may or may not belong.

And combination vaccine is really one example. I mean, it really depends. And if you have concern that combining two already licensed products into one product and you have concerns that it somehow may raise the toxicity profile or cause some adverse events, so go ahead and do a tox study. Knowing this in advance is probably something that you don't, right? So that's why it really is something that cannot be answered in this forum. You would have to look at the actual vaccine formulation and the component that you're talking about in order to make a decision if you need a tox study in that case or not.

And the reason why we put up the slide is that we would attempt to categorize in order to decide what is the concern for having to do or to support a phase I clinical study with a tox study. And that's why this rough categorization helps. But it really is not cast in stone. And you almost could say that combination vaccines could fit nicely under both--likely yes, and likely no--categories, depending on the circumstances.

MR. BAYARDI [In Audience]: Okay. Thank you.

DR. SUTKOWSKI: Also, one more point. Due to the evolving nature of the vaccine technologies and safety issues, we highly recommend that sponsors come in for a pre-IND meeting. That's when you get together your basic product characterization profile, preliminary exploratory safety and immunogenicity, and at least an outline of the clinical study that you want to do. And then basically you get our input and our advice before you submit your IND. And you have time then to design and get concurrence with your plan.

MR. BAYARDI [In Audience]: Yes, I agree. My question was really to [inaudible] because since you are

going to have guidelines on this, if there is going to be a category of products in these guidelines, it's going to be difficult. Because in the FDA you're going to go do a pre-IND meeting. You are not going to do likely a pre-IND meeting in Europe, especially in France or other countries. So you will not likely have the contact with the sponsor and the agency before going to phase I, if the sponsor is not asking for this kind of meeting.

So it's important to not maybe try to set up categories in the guidelines. Because that was my feeling of the talk, it's maybe in the guidelines there will be categories of likely yes or likely not. And after it's difficult to manage, these kinds of categories, when you don't have this pre-IND meeting.

DR. SUTKOWSKI: I think in CBER we really do work on a case-by-case basis, and it's hard to generalize. I mean, there are certain things that we can generalize and we can make general recommendations. And I think that's what guidelines are for. And beyond that, if a sponsor wants specific advice on their specific product and their

specific clinical protocol, they need to communicate with us.

You know, you can always submit your IND, but then you risk having made investments that we may not necessarily agree were worthwhile.

MR. BAYARDI [In Audience]: [Statement Inaudible.]

DR. SUTKOWSKI: Yes. I understand. I understand. But I think that it's not cut and dried here.

DR. GRUBER: I'd like to add to this. I think the problem is--and it doesn't really matter if it's Europe or the United States or the U.S. FDA--the only formal forum we have to discuss a toxicity study is at the pre-IND stage. And that is for many companies already much too late to get good advice on a toxicity study, because many times the preclinical development starts ahead of the time. Then you come in for a pre-IND meeting, at which time you should have some sort of idea about whether your vaccine is reasonably safe.

And we have actually struggled with this at the agency. And right now we have to admit we don't have

another forum than the pre-IND meeting. And it has been a problem. We have accepted--And actually, I shouldn't say this here, but we have accepted informal, you know, fax submissions to just look at a toxicity protocol. But what you get is a very informal, non-binding advice, which is usually really not--Since we have many other deadlines to meet, it's not on the top of our priorities.

So the point is that also is not a forum that works very well to get early input on a toxicity study. And sometimes I think, if this is so critical to really decide early on is it a vaccine candidate that is promising or not, we may have to look at some other mechanism of getting an earlier feedback, if this is something that you want.

I've been hearing this comment and concern over and over again, that the pre-IND meeting is fine, but at this time you usually have your toxicity study well underway or conducted. And then, if it was wrong, that could put you way back. And I think that's a problem here. In that regard, I don't think it is the issue in Europe. It's so different than here in the U.S.

DR. HARGUS: Okay. Go ahead.

PARTICIPANT [In Audience]: Yes. I wanted to ask a question on the intersection of your tox studies versus your potency studies. I think inherent in some of the tox discussions you're having here is that whatever animal model you use, there is some sort of immunological outcome that is reflective of the ability of the antigen to do something.

I found it interesting, though, that in the Adno [ph] case study those immuno outcomes were not used as a component of potency. In fact, the potency description sounded like more in vitro or more structural chemical. I'm just wondering if you could comment on that.

And the other part of the question is, this inherent connection between the two would work well if, like the Adno, you can get an immune response in a model, like a mouse or something. But if you use some sort of other vaccine structure that doesn't permit that kind of flexibility, where can you go?

DR. LEDWITH: With regard to your first comment about the potency assay, the in vitro types of assays you

were talking about are our release assays. And the reason is that for a release assay you need to have something that's very quantitative, with a low standard deviation. And you generally won't have that in an animal study. So for a lot release, that's why these types of in vitro potency studies are done.

However, on comparable lots of material, immunogenicity studies in animals are carried out where we do have a dose response, so we know the expected dose response through a very wide range of doses. And there are also other types of characterization studies done on each lot that aren't necessarily the release tests, such as in vitro gene expression, or even some animal immunogenicity experiments. But because they're not as quantitative, they're not the so-called potency assay for release.

PARTICIPANT [In Audience]: So you don't use the immunogenicity as a release test?

DR. LEDWITH: No, not as a release test. It's more of a characterization study. It's not a quantitative release test.

PARTICIPANT [In Audience]: So for the rest of the panel, where you can use that, it seems a viable opportunity. But when you're using a vaccine construct where immunogenicity in a mouse or something is impossible--Because I remember one of the key components is that the toxicity is somewhat reflective of what could happen in people. What do you do if your structure or your vaccine--you know, animal model, doesn't really reflect that? What do you do then?

[No Response.]

PARTICIPANT [In Audience]: Do you understand the question?

DR. LEDWITH: I think you're asking if you don't have an animal model that elicits the immune response you're hoping to get in people? Is that--

PARTICIPANT [In Audience]: Yes.

DR. LEDWITH: That's basically the question?

PARTICIPANT [In Audience]: Yes, exactly, yes.

DR. LEDWITH: Yes, I personally haven't run into that problem. So maybe Francois or somebody might have--

DR. WARNER: We're talking about potency assay for release?

PARTICIPANT [In Audience]: No, sir. I was trying to--Basically, the question is, can you dissociate toxicity from immunopotency? Because it sounds like to do a tox study where immunogenicity of the vaccine is a key component, those two are inherently connected. But if you have a vaccine where the animal model isn't completely predictive of what will happen in a person--i.e., you won't generate the kind of immune response you hope to get in a person--what do you do then?

DR. WARNER: Well, Francois made the comment that it's very difficult to make that argument. And I don't know whether you want to address it. But I don't know whether you're going down the path of using homologues or animal models of different antigens.

But I agree with you. I have some examples where it has been very, I would say, impossible to use an animal model to mimic a human response. However, at that point, I suppose in a certain sense you sort of--I don't know, I don't want to say this trivially, but you throw in the

towel. And then you're stuck with doing a toxicity study, though, that does address intrinsic toxicity, contaminants.

I don't think that gets you out of doing a tox study, is my point. Because there are other issues. There are issues of contaminants. There are issues of intrinsic toxicity. So you're right, you may not have addressed the issue of the pharmacodynamic--or the toxicities associated with the pharmacodynamic response. But I would argue that that doesn't get you out of doing something to look at the other possible sources of toxicity.

DR. LEDWITH: Yes, I think another good example for that is the cancer vaccine I just touched on briefly, where in humans you will be possibly vaccinating with a self-human antigen. So again, we're concerned about immunological effects, autoimmune effects. But we're also worried about intrinsic toxicity.

So if we had a cancer vaccine program, I would want to test the actual human vaccine, cancer vaccine, in the animal models, to address the intrinsic toxicity concerns. But I'd also want to carry out a study with, for example, the Rhesus homologue of the self gene in Rhesus

monkeys, to address those other kinds of peculiar types of toxicity that could arise from long-term expression or autoimmunity, or things like that.

MR. : Which brings me to the next point, which was in those cases where you have a homologue, it brings us to the question of the use of transgenic animals for these kinds of studies.

DR. LEDWITH: Right.

MR. : And, you know, the agency's position on the use of such animals, and whether they're worth it, or not.

MR. : I would personally evaluate the transgenic model versus a homologous gene in an animal model very closely. Obviously, basically, the gene expression of that self-antigen in the two different models. Does the transgenic model really reflect the tissue distribution pattern of gene expression in humans? Or does the animal model using an homologous gene better reflect that? That might guide your choice there.

DR. WARNER: I wouldn't advocate using the animal homologue, though. The issue is related to whether it has

any relevance, in the quality of the material--There's a lot of issues there. Whether it's relevant at all. But I already have problems with most of my animal models, anyway, using the murine analogue or transgenic mouse.

The problem with doing tox studies sometimes is that it gives you false confidence, also. So I don't know, I have some issues with that, too.

MR. : Yes, I think it would be done in the context of an ancillary extra study, in addition to testing the actual test material.

DR. HARGUS: Go ahead.

MR. COPLIN [In Audience]: Paul Coplin [ph], from Merck.

To address the issue that was raised by the gentleman from the EPA as to how the cumulative exposure to alum has been assessed in the pediatric regimen, one of the ways that that has been looked at is in post-licensure studies of pediatric vaccines and adult vaccines.

Recently at Merck we did an evaluation of three vaccines that contained alum. One was a pediatric vaccine given at two, four, and 12 months. The other one was a

combination vaccine given at two, four, 12 to 15 months. And the third one was an adolescent-adult vaccine. And each of these studies contained 27,000, 42,000, and something like 60,000 doses.

And in each of these studies, we compared the duration following vaccination with a period of time before vaccination, and with historical controls who were vaccinated with a pediatric regimen without that added vaccine; to evaluate whether there was any increased rate of adverse events in the people who got the additional doses of alum-containing vaccines, compared to the period before they got any vaccines or historical controls.

And in all of those three cases, we found there was no increased risk of adverse events. And this report was submitted to CBER as part of a justification for using alum in a new vaccine. So that's one of the ways it has been looked at.

My question to the panel is how much the experience with existing vaccines, in terms of adjuvants, how much that safety experience from post-licensure studies would inform new vaccines going forward.

[No Response.]

MR. COPLIN [In Audience]: In terms of doing tox studies, whether the experience with adjuvants from existing vaccines and post-licensure safety studies would affect the level of toxicity studies that would have to be done for pre-phase I for new vaccines.

DR. GRUBER: Well, as far as adjuvant is concerned, there is only one which is licensed worldwide. It's aluminum. Otherwise, you have caron [ph] emulsion, which is licensed for flu vaccine in Italy. And you have the virasum [ph] intra-nasal flu, which has been withdrawn in Switzerland. And you still have a flu vaccine based on virasum. That's the only ones that are licensed. So post-licensing evaluation is kind of difficult on that standpoint.

DR. LEDWITH: But I think clinical data from--whatever--new adjuvants does influence our--

DR. GRUBER: Sure. But I mean, it's not millions of doses that are administered.

DR. LEDWITH: No, absolutely.

DR. GRUBER: So it's a process that has to be built on. But the studies that--Even if you have an adjuvant that has been used already a vaccine being developed, you use another antigen with the same adjuvant. You redo the tox package. I mean, it's first time in line for new antigen with a given adjuvant.

MR. COPLIN [In Audience]: Thanks.

MS. BURKE [In Audience]: This is Rae Lynn Burke [ph], from SRI International.

I wanted to ask a question about choice of animal model. I heard several people say when you were selecting animal models for systemic toxicity that you might not use rabbits because they were not sufficiently well characterized. I wanted to ask, what would be involved in having a "validated" animal model. And I also wanted to ask whether that choice would be different for biodistribution, versus systemic toxicity evaluations.

DR. WARNER: Well, we're using rabbits. We're generating historical database. We had some. There are issues, in my experience, with rabbit supplies and quality rabbits. You know, we so far haven't had an issue using

rabbits. We are actually using them with some other things, too. So all of a sudden we're getting down this rabbit path.

You know, I think it's a nice mesh with the issues of the reproductive toxicity because--I don't want to steal anybody's thunder, but placentation in rabbits is more similar to humans than the other species that we talked about. So we've sort of made a concerted effort to sort of stick with rabbits. We do our immunogenicity studies often in rabbits, if possible. And so if we do do that, we stay with rabbits.

But you talked about validation. We wouldn't validate an animal species for tox testing. We have validated methods and we have, you know--We do everything in a validated sense, but selection of tox species--I've got to admit, nobody was crazy about it when we started off down this path but, you know--

DR. LEDWITH: Yes, I think I meant a similar approach. By "validation," I meant we would want to generate sufficient historical control data before we embarked on a GLP safety study of a test compound.

And so we would probably want to do several different studies with control animals first, just to find out what the normal variation is and the hematological, serum biochemistry parameters, as well as organ weight variations, histological lesions, what's the incidence of them in control animals. So that we have an idea going into the study what would be an appropriate group size to use to control for those types of variations.

DR. WARNER: I mean, I think we are taking risks, honestly, because we don't have a big historical database within our own shop. But we're building our historical database.

MR. : But you do have a fair amount--I mean, from the repro-tox studies with rabbits. I mean, you do dose range finding studies for that. I mean, actually, rabbits--We know a lot more about rabbits than you might think from the discussion here.

DR. WARNER: That's true. At our main tox facility we do repro-tox in-house in rabbits. So everybody said, "Ah, you know, rabbits. Okay." Although that's not a full tox assessment typically.

MR. : I'd just like to add to what Garvin said. You shouldn't think that rabbits are precluded from tox studies. Most places that do any sort of tox testing at all will use rabbits. It's a matter of how much background information you have.

The other thing to consider about rabbits is, it's not quite as simple as doing a rodent study. They are larger. They're more expensive, from the standpoint of increased labor. Housing situations are much more expensive and complex. And so it becomes a matter of what the individual laboratory feels most comfortable with.

But again, echoing what Dr. Hastings says, we do have a lot of information on rabbits. Perhaps the only thing that would argue against them is a limited amount of immunological reagents compared with, for example, rodents.

MS. BURKE [In Audience]: And how about the question of biodistribution? What would you all generally choose as the best species?

DR. VERDIER: I can perhaps answer to this question. And Brian, feel free to answer, also.

Regarding biodistribution, you really collect biodistribution in two species. In the primate species, because if we are dealing with a GMO and a live virus I am used to doing at least one non-human primate study, and therefore I will get some biodistribution data from this study. And I also do, as I mentioned, a general biodistribution study in a small animal species, in order to have several necropsy time points, a large number of animals, and therefore a kinetic of the biodistribution. So at the end, I have rodent and primate data for biodistribution.

Brian, do you want to comment?

DR. LEDWITH: Yes. I think a very similar approach. We typically used mice, which was also one of our tox models. When we moved to doing Rhesus monkey toxicity studies, though, in the first several studies we did biodistribution there, because I think it's very important to have the biodistribution data in your toxicity model for at least a given vector class.

And for example, if we move to developing the rabbit as a tox model at Merck, I would do a

biodistribution study in rabbits, just so I'll be able to correlate it with any organ-specific toxicities.

DR. HARGUS: Okay, one last question, then we're going to break for lunch.

MS. BOYLE [In Audience]: Yes, this is Rosanne Boyle [ph]. I'm with the International AIDS Vaccine Initiative.

Continuing on the topic of biodistribution studies, earlier Dr. Verdier mentioned in his presentation an approach of characterizing the biodistribution and the integration of a vector without transgenes as a strategy, a primary strategy, and then following up with toxicity studies of the actual vaccine construct.

My question is, I'd like to see some comment from the regulators on the panel on this strategy. And I'd also like you to consider, and perhaps discuss, how we can come up with an algorithm or criteria for targeting potential integrants in vectors, since we are all faced with biodistribution and integration studies at this point in time. Thank you.

DR. MIDTHUN: Is Dr. Dennis Kleinman [ph] in the audience? Because he's our expert in DNA vaccines. If he'd please comment, I'd really appreciate it.

DR. KLEINMAN: Your question dealt with viral vectors, or with plasmid DNA vaccines?

MS. BOYLE [In Audience]: Either. Or address both, please.

DR. KLEINMAN: I have some expertise only in the one. I think one of the difficulties that this meeting has had is that we're trying to deal with a plethora of possible types of vaccines and lump them together. So I think that how we deal with the toxicity of a live viral vaccine, versus an attenuated live, versus a DNA vaccine, versus a protein sub-unit vaccine, perforce must be different.

I can tell you that the FDA approach to how we determine what types of toxicity and biodistribution and integration studies need to be done with DNA vaccines has evolved considerably since the first clinical trials were done back in 1995.

Originally, we felt that every new vaccine candidate needed thorough toxicity and biodistribution studies. We no longer believe that. We believe that if the backbone of the plasmid has been well characterized, that simply making moderate modifications--ones which would be unlikely to affect its biological activity--that we would no longer require additional such toxicity, biodistribution, and integration studies.

The same may be true for some of the live viral vectors. It's simply a matter of whether the agency has enough information, has accumulated a background, to make them appear to be well characterized. Until we have that background, I think that for the purposes of safety, we need to require that studies be done on each vector. But once we have that background--perhaps multiple examples from the same sponsor, or single examples from different sponsors--I think that we're far more willing to entertain a less rigorous toxicology study.

MS. BOYLE [In Audience]: Could you comment on the issue of targeting in the backbone, or characterizing the backbone, as it relates to potential integrants?

DR. KLEINMAN: There are some wonderful studies, actually done by the folks at Merck, showing that the likelihood of an integration event being influenced by the insert is really quite low. That is, even if you have homologous sequences, the likelihood of integration does not rise considerably. So solely from the standpoint of integration, if you don't change the backbone considerably, it is unlikely that the insert will dramatically change.

Now, what will change that is if you use a different type of delivery vehicle. If you electroporeate, for example, or introduce some tolyposomes [ph], or if you change the nature of the live viral vector itself so that it's more infectious, that will dramatically change.

So we have to keep an eye on that, which is why I think that members of the panel repeatedly point out that some of these things do need to be handled on a case-by-case basis.

DR. HARGUS: Okay, thank you. Right now we're going to break for lunch. Lunch is in the Potomac Room in the main hotel, on the upper level.

And then we'll be back here when lunch is finished. And I'd like to thank our speakers, our panelists, and all of you, for participating.

[Applause.]

DR. GRUBER: We've got to be back here at 1:15.

[Whereupon, the workshop recessed for lunch, to reconvene at 1:15 p.m., that same day.]

A F T E R N O O N P R O C E E D I N G S

3A DR. HOUSE: We'd like to welcome everyone back to this afternoon's session. One thing that has been suggested that we'd like to do is just to give you an introduction to the folks up here on the panel, because I guess the name tags are a little hard to read. So we'll just go down the line briefly, before starting our session this afternoon.

I'm Robert House, director of preclinical studies at DynPort Vaccine, in Frederick, Maryland.

DR. VERDIER: I'm Francois Verdier, Aventis Pasteur, in charge of non-clinical safety.

DR. GRUBER: My name is Marion Gruber, with the Office of Vaccines, at the U.S. FDA.

DR. MIDTHUN: Karen Midthun, Office of Vaccines, FDA.

DR. SUTKOWSKI: Elizabeth Sutkowski, Office of Vaccines, FDA.

DR. GARCON: Natalie Garcon, GlaxoSmithKline, Biologicals, Vaccine Formulation Technologies.

DR. HARGUS: Sally Hargus, pharm-tox reviewer, Office of Vaccines.

DR. LEDWITH: Brian Ledwith, Director of Biologic Safety Assessment, Merck.

DR. LUSTER: Mike Luster, National Institute for Occupational Safety and Health.

DR. LAMBERT: I'm Paul Lambert, from the University of Geneva.

DR. WARNER: Garvin Warner, Drug Safety Metabolism, Wyeth.

DR. HOUSE: Okay. We'd like to start this afternoon's session continuing the theme of animal models and safety assessment. Our first speaker this afternoon is Dr. Mike Luster.

Mike Luster is currently chief of the Toxicology and Molecular Biology Branch at the National Institute for Occupational Safety and Health at CDC.

Prior to moving there in 1996, he worked for many years at the National Institute of Environmental Health Sciences, where he was head of immunotoxicology and neurotoxicology sections.

He has published extensively in the field of immunotoxicology. And it is a personal pleasure for me to introduce him, because Mike was instrumental in teaching me what little I know about immunotoxicology. Mike?

ANIMAL MODELS APPROPRIATE FOR ASSESSING IMMUNOTOXICOLOGY AND IMMUNOPATHOLOGY

PRESENTER: MICHAEL LUSTER, CHIEF, TOXICOLOGY & MOLECULAR BIOLOGY BRANCH, NIOSH, CDC

DR. LUSTER: Thanks, Robert.

I was asked to provide a brief overview of the immunotoxicology tests that have been either validated or are undergoing validation, or maybe even have been thought about.

Regarding hypersensitivity, some of the old assays, the mouse ear swelling test, which hasn't been used to a large extent--I saw Shane Gadd [ph] in the audience. He's actually the person that first developed that assay. Sorry, Shane.

The guinea pig tests have been used for many years; guinea pig maximization test and the [inaudible] occluded patch test. But more recently, that's been

replaced by the local lymph node assay. And if the drug allows it, or the chemical allows it, patch tests in humans are conducted. That's of course before [inaudible].

The reason why the local lymph node assays gained popularity isn't so much that it's more sensitive than the guinea pig assays, but it involves the three "Rs," in replacement, refinement, and reduction of experimental animals.

And this is some data by the peer review panel from ICVAM at NIHS that was published a couple of years ago, showing the concordance between the guinea pig assay and the local lymph node assay. And as you can see, it's pretty similar, compared to the guinea pig maximization test and the Buehler, or any guinea pig test.

And then when you compared the local lymph node assay to humans, the concordance is also very similar: around 72 percent. It's not the greatest concordance analysis, but it is equivalent to the guinea pig assay, which also shows, as you can see, concordance in the 70-percent range.

This is how the local lymph node assays run, for those of you who are unfamiliar with it. It's very simple. The test material is applied to the ear for three consecutive days. And then on day five, the animals are administered tritiated Thymidine. On day six, the draining lymph node is removed, and a cell suspension is prepared. And Thymidine incorporation is determined as an indicator for lymphocyte proliferation.

Keep in mind, it's a screening test. It's going to measure immune activation for almost anything, so including vaccines; not necessarily a chemical that's hypersensitized.

Regarding autoimmunity, there's been a lot of attempts in trying to develop the appropriate models for assessing the autoimmune disease, since there's so much evidence of drug-induced autoimmune phenomena; without a whole lot of success.

Currently, there is a validation--an inter-laboratory effort going on using the popliteal lymph node assay. And in this process they changed the original protocol. Instead of injecting the test material into the

foot node, they're injecting it subcutaneously into the back. But the assay is about the same. It's allowed to enter the draining lymph node, although lymphocytes enter the draining lymph node, and the draining lymph node is removed and measured. And in this case, it's just simply swelling.

And in a sense, in this case it really measures very much the same thing as the local lymph node assay would do. It's measuring immune activation. Because the cells that are increasing in the lymph node are T cells or B cells, usually.

There's been a little bit more efforts going on with using the popliteal lymph node assay in conjunction with reporter antigens. And I'll describe that in a minute.

And there's been work done with autoantibody quantitation for chemicals; quite often, anti-DNA antibodies. Someone mentioned that earlier. But it hasn't been an assay that seems to be very validated or reproducible for drug-induced autoimmune diseases.

There were some efforts a number of years ago in immunopathology. In this case, what was looked at after a chemical or drug was administered was immune complexes in various organ systems. And this seemed like a very good idea, except that from the pathologist's point of view it was an extensive amount of work; since it wasn't clear in what target organ the autoimmune phenomenon would occur. So they would have to almost isolate all, and examine every organ for immune complexes.

There's been work done with genetically or experimentally induced animal models. So for example, for systemic types of autoimmune diseases, some work has been done with the NZB mouse. And for organ-specific autoimmune disease there is some work going on, some of it at Virginia Commonwealth University, with the Nod [ph] mice which developed Type 2 diabetes.

In this case, what's looked for is whether the drug or chemical is going to enhance the development of the autoimmune disease; either make it more severe in the animals, or decrease the time that the autoimmune phenomenon will occur in those animals.

The problems with autoimmune diseases is that it's not just one disease. As you can see from this table, it almost affects every organ system found. There's a lot of epigenetic and genetic factors involved. It's predominant in females, as well. So it's been very hard to develop a model. In fact, genetic factors play a major role in all immune disease. HLA is very strong.

So not much success in having a validated model for testing whether an agent is going to produce autoimmune phenomenon.

The most interesting work has been done by Albers, in which he's used that popliteal lymph node assay in conjunction with reporter antigens. The list of chemicals here, or drugs here, are all agents which have been shown to induce some form of drug-induced autoimmune disease.

And what's done here is that the drug is injected at the same time as antigen, either TNP-Ficoll as a T-independent antigen, or TNP-Ovalbumin as a T-dependent antigen. And the immune response to the TNP-Ovalbumin or Ficoll is measured.

Now, the antigen is administered in the absence of any adjuvant. So without the adjuvant, it's not going to produce an immune response. What this is really saying is, since you are getting antibodies, particularly if you know with the TNP-Ovalbumin, it is that the material here is acting as an adjuvant.

Regarding immunosuppression, this was what used to be done about eight or nine years ago. It was more of a tier type of testing. The tests in tier one are more simplistic types of tests; and then when you get into tier two, they are more functional tests, where the animals are challenged with antigens or infectious disease models. NTP was doing these, as well as the group in [inaudible] RIVM.

And then about nine years ago, we had a sufficiently large database with those chemicals that went through this extensive tier--we had about 50 chemicals at three dose levels--that we thought we could answer some questions.

And two questions we wanted to ask were whether we needed to run all the tests in that panel, or if we could reduce the number of tests; and also, what the

relationship between those immune changes and host resistance tests were, as well, whether it followed a quantitative relationship, whether it was a linear or threshold relationship.

And this has been shown many times before, so I won't dwell on it. But this simply shows that if you run any of three different types of tests, you can get 100-percent concordance. In other words, you can accurately predict immunotoxicity. And in fact, if you use antibody response as a surface microanalysis, along with several other tests, you get high 90-percent concordance.

So what it says is you only needed to really run a couple of immune tests to be able to look at immunotoxicity. But two of them that were key were antibody response and some surface microanalysis.

And we also tried to look at relationships between immune functions and host resistance, asking the question of whether the relationship was of a linear model or threshold; meaning, was there some reserve in the immune system?

And this is just one set of type of analysis that was conducted, where we set up all of the chemicals. And on the "X" axis we have changes in antibody response; and on the "Y" axis we're showing changes in the Listeria mortality. And each number represents, for example, a dose. And there's 50 chemicals, so it's probably about 150 numbers here. I'm just showing it as an example.

And then we did statistical modeling with that type of analysis. And we showed with the three host resistant assays that were used in many of these tests that many of the relationships between the immune function and the host resistance were of a linear nature, not a threshold relationship nature; although some were still threshold.

So it said that as immune function changes, then disease--It seems kind of obvious; but that disease will also increase in a linear fashion.

Several years ago, the question came up of whether we can do an extended immunopathology and identify whether a chemical is immunotoxic, in the absence of immunizing the animal with an antigen. What we did then

was to gather four pathologists together. One was from my agency at NIOSH; one from NIHS; one from Dow; and the person that really set up the study was Frika Cooper [ph] from RIVM. And the pathologists agreed to measure these 13 or 14 different histological parameters, which would represent the "state of the art" immunopathology, histopathology screen for immune changes.

I would indicate that this is the preliminary data. The analysis still hasn't been completed after about a year. But the way the data looks like, as shown here, we ran 13 different chemicals that were examined. And what is just shown here is the antibody response, either in control animals or animals treated with the agent at a low dose, medium dose, or a high dose; and then compare that to the pathology.

And I just summarized all that pathology, consolidated into just a plus or a minus. Either the immunopathologists would say there were significant defects with that screen to say that there's something going on immunologically, or there wasn't. Then the comparisons are going to be made. Although there's a lot more analysis

that really needs to be conducted, but this is probably the summary of it.

So with the first five chemicals, you see that we've got no changes. These were negative controls. You see there's no change in antibody responses. And the pathology picked it up pretty well as well with no changes.

Oxymetholone, we saw no changes with antibody responses, but a change with pathology. What turned out was that Oxymetholone affected T cell responses, CTL and DHR. So this is a chemical that we would have missed using an antibody response, that immunopathology would pick up.

The other six chemicals were all shown to affect the antibody response, as indicated by "D" for decrease in antibody responses. And of those six, three of them were picked up by an immunopathology screen, and three were not picked up by the immunopathology screen. So that's where that status stands. And I guess you can call that reasonable concordance, that the immunopathology is going to pick up at least half of the causatives.

The reason, probably, for the lack of the pathology picking up those other three chemicals is that we

weren't stimulating the immune response. And that's shown here. That figure on the right-hand side is a follicle with a germinal center. And although those will be present in normal animals that are not injected with anything, they are very sporadic; while after immunization these are very large and easily can be observed.

And I guess the argument would be that if these were being measured by immunopathologists following a constant stimulus, that the pathologist would be able to pick that up if there was immune change occurring.

The data has several implications, as far as testing for immunotoxicity for vaccines, and this is shown here. This is some data that Kimbal White [ph] had given me a while back, in which animals were set up into either two different groups or the same group. And in the different group mouse, the same mice were used to measure antibody responses at NK cell activity and--Excuse me, different groups of mice were used to measure antibody responses at NK cell activity.

In the same group mice, we used the same mice to measure both antibody responses after immunization and NK

cell activity. And as you can see here, there is a decrease in the NK cell response. And what it turns out was that the NK cell activity overall is normal in those animals, but as a result of the lymphocyte redistribution due to the fact that there are large numbers of germinal centers occurring, it's a misread.

So after vaccination, if we use animals in our studies and we take the spleen, which is the common organ used for most immunotoxicity studies, one would expect to see a redistribution. So flow cytometric analysis, or NK cell assays which look at spleen cells for NK activity, will show an altered distribution without really being functionally changed.

The other issue that can affect immunotoxicology studies with vaccination is the old--I'm sure many of you are more familiar with this than I am--is antigenic competition. And that's been around for many, many years.

And the original argument about antigenic competition occurred from vaccines that had multiple serotypes. And what that said, as shown in the first third here, was that if you vaccinate with an antigen that

contains multiple serotypes, the immune response to the three serotypes--shown here as "A" prime, double prime, triple prime--will not be as strong as if you immunized individually.

And there's another issue of antigenic competition--similar, but a little different--in which there are subdominant or cryptic epitopes. And in this case, the argument is that if you immunize an individual with an antigen that has both a dominant and a subdominant epitope, that the subdominant epitope may not be expressed. If you immunize with both at the same time, then both of them would be expressed.

The one that applies mostly to immunotoxicology studies is the interference model, which states that if you are under an active immunization--as in the case of "A" from the vaccine, which is a very good epitope and a very good antigen--that a subsequent immunization with another antigen that's not as strong will give an inferior response.

And the reason for that is not quite clear yet; but seems to be the fact that there are certain limited

numbers of dendritic cells and cytokines within the spleen that will allow for a normal immune response, and that those dendritic cells and cytokines are all being used by the high-affinity, high-avidity antigen from the vaccine that we've shown here.

So for example, this is a typical beginning of a germinal center in the center here. The yellow cell with the squiggles is a dendritic cell. The little red dots in it are the antigen. And what happens is, antigen-specific T cells will interact with the dendritic cells, and then the B cells react with the T cells. And over a period of time, a germinal center forms, gets very large. The better the antigen, the larger that follicle is, the one that I showed you earlier.

And there is only so much of that that can occur at a particular time. So hence, vaccines by nature are going to be temporal immunosuppressants, because they're competing--they're taking over the ability for making antigens, which will complicate your interpretation of any studies.

So just as something that maybe you want to be discussing later: issues regarding immunotoxicity testing of vaccines. I don't think any of the traditional methods that we used for immunotoxicity testing will really apply to vaccines.

The local lymph node assay, which is right now the current key assay for measuring sensitizers. The popliteal lymph node assay which is being used, although not validated, for autoimmune diseases, really measures immune activation, which is what vaccines are going to do anyway.

And regarding immunosuppression, vaccines by nature are temporal immunosuppressants for other antigens. So any time one is undergoing a vaccine, one is presumably going to show some temporal type of immunosuppression.

I think there are some specific questions that might be addressed within that particular framework. So for example, if one is looking at a particular agent within a mixture for an adjuvant, like a preservative, then the local lymph node assay or even the PLNA assay might apply. Bimaerosol [ph] was picked up by these standard assays,

which was used as a preservative for vaccines. But by and large, I don't think one shoe will fit all. So I'm not sure how well these are going to apply. Okay.

[Applause.]

DR. HOUSE: I'd like to open up the floor to questions.

PARTICIPANT [In Audience]: Mike, I was interested in the concept of temporal immunosuppressant. Generally, for immunotoxicology we think of immunosuppression as being synonymous. And from the context of looking at vaccines, I think we realize it's much more complicated.

Based on your concept of temporal immunosuppressant, would you think that this argues for looking for immunosuppression as a consequence of vaccination? Or would you have a different approach?

DR. LUSTER: Yes, by definition that is, I guess, immunotoxicology, right? It's causing immunosuppression, even though it may not be long term, and it is part of that vaccine response.

I guess the question I would have as a toxicologist would be, is that maybe still a research mode? How severe is immunosuppression occurring after a vaccine? How long does it last? Do we have a window where we're more likely to develop infections after a vaccination occurring? And if so, then I think that obviously the health benefits of vaccine are going to outweigh that, but at least that's the type of information I think that would be quite useful to have out in the clinics.

So for example, if I was going into the hospital for a week and was going to be vaccinated, well, a physician may decide that the best thing to do would be to vaccinate me on my way out, rather than on my way in. It may be just be that simple a question.

But I think everyone knows that vaccinations are temporal immunosuppressants, but I don't think anyone has really quantitated or studied it. So I think it's a research mode question.

PARTICIPANT [In Audience]: Is there any epidemiologic data that says that that's an issue in clinic?

DR. LUSTER: Not that I know of. But regarding issues with immunosuppression, the only way to answer that question is to have a pre-designed epidemiological study. So if someone comes out and if there is a higher incidence of influenza by 5 percent in the population after vaccines, that's not going to ever be picked up. So one would have to go off and design that epidemiological study.

So that's why I don't even think it's something that would--I think it would be, again, more of an experimental research issue at this point. And it may be in fact that the effect will last three days, and you'll see a little 10-percent drop in immune tests and you go, "Well, gee, that's really not anything to be concerned about." But on the other hand, you may see a larger effect. I mean, I just don't know.

But I think it's real. As a toxicologist, I think it's something that is in my mind as a question that might need to be addressed.

PARTICIPANT [In Audience]: Maybe I can ask you, and we have had the discussion years ago about the Dipth-3 Pertussis Tetanus, in combination with [inaudible]

Influenza-B vaccine. And there was some type of interference that requested of [inaudible] to the medicine board in The Netherlands to do requests to separate the injections in children with 14 days. It was not practical, because that led to every 14 days visits of the children to the physician. But there was some epidemiological, or some evidence for interference of this type.

But I would ask Mike, what is your recommendation, from your immunotox viewpoint, for the selection of animals in the testing of vaccines? We've had earlier discussions on what is the most relevant model. Should it be the fact that we have a lot of data on the rat or the mouse on immunotoxicity and immune responses to characterize the vaccine response? Or should it be the most relevant, the most important, factor the fact that the animal is sensitive to the disease that the vaccine is focused on?

DR. LUSTER: Well, I guess it's kind of easy, because I wouldn't think that it would be a very good idea to be doing real testing for vaccines for immunotoxicity, from what I can see out there. Because I think that the

results that might come about would be very difficult to interpret. And I think both FDA and the drug companies would be banging their heads, why they did it.

But I think in certain instances--For example, if one wants to test material within an adjuvant or another preservative, and one wants to go off and do hypersensitivity testing or autoimmunity testing, then one can run those straightforward local lymph node assays. And that's been done, is validated in the mouse. So hence, you can continue that in the mouse.

As far as the immunosuppression part is concerned, the functional tests have been validated, both in the mouse and rats. And to answer those specific questions of how much immunosuppression does a vaccine really cause, if the FDA feels that's an important question to ask, then really the only model they could do it in is mice and rats, because those are the only models that are validated and tested, where we now then have that information.

DR. HOUSE: Next question?

MR. BARKER [In Audience]: Lou Barker, Sequella.

There are human diseases where immunopathology plays a fairly prominent role, in diseases for which we use vaccines. And I'm not sure if this area was covered by your immunotoxicology presentation. I'm actually thinking particularly about immunopathology caused by cell-mediated immune responses.

But in any case, to take it a little further, and similar to the last question, I just wonder--and maybe you've already discounted this approach--but whether one would hope to discover these problems in advance with animal test systems which may not very closely mimic the human diseases in terms of immunopathogenesis. Could you comment on that a little bit?

DR. LUSTER: I'm not sure I quite understand the question. But the vaccine itself will induce--Well, I mean, you mentioned it almost like immunopathology, but I think it's an immune response that's occurring.

MR. BARKER [In Audience]: Yes.

DR. LUSTER: And you're asking whether that could be used as a measure?

MR. BARKER [In Audience]: No, the question is really about what happens when a wild type virus, or bacteria or whatever it is, appears. In other words, you'd have to have a challenge in order to see the kind of immunopathology I'm talking about; not immunopathology caused by the vaccine, per se, but caused by the natural infection in a vaccinated individual or animal.

DR. LUSTER: Well, like I said, [inaudible] or influenza virus infection?

MR. BARKER [In Audience]: There are lots of examples.

DR. LUSTER: Yes. I'm not sure how to answer that. But can that be used, can immunopathology be used as a measure for a drug's efficacy against that? And the answer would be, yes, of course. I mean, if that's the question. But I'm not sure I'm answering your question entirely.

DR. GARCON: Yes, is one of the examples you're talking about what happened with the RSV vaccine?

[Response Inaudible.]

DR. GARCON: Yes. So in that case, there is a model that has been developed in [inaudible] rats with the RSV infection, where actually you reproduce in [inaudible] rats the same pathology in the lung. And that model has been used by us and others to see how to develop vaccines, again RSV. So if that's what you're talking about, that can be done, but you need the animal model for that.

DR. GRUBER: We were discussing here among us the difficulties in really being able to address this particular phenomenon of immunosuppression in animal models; that I guess the availability of animal models to look at these questions is probably very scarce.

And when I looked at the questions raised regarding immunopathology, or immunotoxicology, I was thinking actually of a perhaps much more simplistic, or let's say naive, approach. That is, looking at the toxicology study really as a signal-generating mechanism that is--Granted, many times our concerns with regard to immunopathology may be theoretical. So that you build in your toxicity study really a battery of tests, sort of as a first-tier approach to assess immunopathologies by just

looking at the organs such as thymus and spleen and so forth and so on, looking at organ weights, and run really the basic parameters of assays.

Then if you see something, you then do additional studies to look at mechanisms of effects. And then at that point, you may have to look at the feasibility of developing animal models to address the specific question. But I think, as part of your basic toxicology package, looking at the potential for immunopathology may need to be much more basic, so that you don't really chase a wild goose.

Millie [ph], do you have anything to add to that?

PARTICIPANT [In Audience]: Well, it's right on there, though. I had a question, and I wanted Mike to answer it, because I'm confused about this.

Certainly, if we looked at a gross level for organ weights, one of them you would weigh would be the thymus. And yet, during pregnancy in mammals, the thymus involutes. And some people say that at least in mice it remains functional, even though there's maybe up to a 70-percent reduction in size.

And I was wondering if we have to have a special concern because of that change in weight, potentially in function. And I don't think it's really worked out, but I might be behind in the literature on that. Mike, could you address that?

And I was thinking about the involution of the thymus, the smaller size, which would mark it as immunosuppression; and yet, that's normal for pregnancy. And then you give a vaccine, and get additional immunosuppression. And we're not sure what it means in the animal models. But do you have any concern for humans?

DR. LUSTER: Well, actually, no, just as a point of information, I thought during pregnancy the thymus involutes because of estrogen and progesterone increases.

PARTICIPANT [In Audience]: Right. Yes.

DR. LUSTER: And that is thymal suppressive, or whatever.

PARTICIPANT [In Audience]: Yes, it's supposed to.

DR. LUSTER: My argument only is that, rather than doing just immunopathology because we have a

pathologist and we can do immunopathology, I think one needs to understand what the ramifications of that response would be. And I guess I have a hard time understanding--I'm not sure the thymus is going to enlarge because of immune response. It's a primary organ. The secondary organs are the lymph nodes and spleen, which are the ones where the immune response occurs. So those are the ones that are enlarged.

And when that enlarges, that will likely enlarge after the vaccine. And to ask whether the chemical has increased immunostimulatory activity, non-specific immunostimulatory activity that caused the enlargement, or are the germinal standards or the size of the spleen not as large as they should be after a vaccine, and therefore there are some materials in the vaccine formula that are immunosuppressant, I mean, I don't think you can--You can't really address that from a pathology standpoint, I think.

PARTICIPANT [In Audience]: No. What I was really bringing up was that it is normal for a thymus to involute and become smaller. And if they were looking

against non-pregnant controls, it could be mistakenly looked at as an immunosuppressant effect in a tox study.

DR. LUSTER: True.

PARTICIPANT [In Audience]: And, yes, the secondary organs would be those affected. But I wondered if there was any functional--as the result of the thymus involution and the changes you get during pregnancy, if we needed any special concern, or if there was any data that showed that we did?

DR. LUSTER: Yes, the answer is I think, yes, I think there is old data that suggests that during pregnancy that the pregnant dams have a decreased immune response. Whether that's due directly to a decrease in the thymic weights, or the fact that there's estrogens around that are generally immunosuppressive and block immune responses that are occurring in secondary organs, I'm not sure, you know, if anyone has ever really quite looked at that.

PARTICIPANT [In Audience]: I didn't see anything, either.

DR. LUSTER: But I guess the only argument would be that the thymus involutes normally after a certain age

at very young age, anyway. And immune responses look fairly normal for a long period of time, even though that thymus is starting to shrink up, if that helps answer your question.

PARTICIPANT [In Audience]: Don't know.

PARTICIPANT [In Audience]: The other side of the coin, there was an interesting paper a couple of years ago from Bonnie Graham [ph] in Nashville. And they immunized mice with Pertussis, the A-cellular Pertussis vaccine. And they found a high circulating level of IL-4, which wasn't novel. But then when they infected the animals with RSV, they had a much more serious pathology.

And we've also done some studies with Pertussis where we see increased allergic sensitization. So there's one argument saying that you can see immunosuppression, but temporarily you may also see a hypersensitization to other antigens. And I wonder if the committee maybe can consider that, as well?

DR. LAMBERT: Yes, in fact, I would like to challenge this whole business of immunosuppression with vaccination. I think that if we look at the data that we

have from human studies, not the mouse, we have the only evidence of immunosuppression has been with some live virus immunization. And very likely, the measles vaccine is inducing an immunosuppression for a short time. And we know the mechanism, that this is a direct effect on the cells.

But if you look at subunit vaccine, I think that we do not have one evidence that giving subunit vaccine in a child, you know, combining several subunit vaccines--I speak about protein vaccine--and if you give them at different sites, you don't see any effect on the response.

When you see some decrease of a response, as has been mentioned with the HIV vaccine, it was when it was given all together in one site. And there you can have interferences and competition at one lymph node site, probably. But I would not call that general immunosuppression.

And when you speak about changing the charge from TH1 to TH2, or TH2 to TH1, I think that this has not been seen in humans. In mice it's true that you can have such effect. In humans, in fact, you have very little general

effect of vaccines on the type of response which is developed at another site.

PARTICIPANT [In Audience]: Does anybody else have any experience with changes in lymphoid organs? I mean, Brian you mentioned you had changes in--what, liver?

DR. LEDWITH: Well, no, the typical reactions we would see with the vaccine are enlargement of the draining lymph nodes from the injection site, which is totally expected.

PARTICIPANT [In Audience]: Right.

DR. LEDWITH: There's only one study at very high doses that we saw some minor liver lesions.

PARTICIPANT [In Audience]: Yes. I mean, but some of those are expected. I mean, if we're using a strong adjuvant, I would expect non-specific stimulation. You mentioned that.

DR. LEDWITH: Yes. And we do see that in a traditional vaccine. With the Alum-adjuvanted vaccine you see the same type of lymph node enlargement.

PARTICIPANT [In Audience]: Right. But I wouldn't see that as triggering a need to follow up in

terms of immunotox. Even though in the conventional drug program, that might.

DR. LEDWITH: Yes. Absolutely not. Yes. It's the expected immune response.

PARTICIPANT [In Audience]: Right.

DR. LEDWITH: Right.

PARTICIPANT [In Audience]: I should also just mention, depending on the strength of the adjuvant--I mentioned liver--we do see changes in the liver, too: hypertrophy, perhaps an acute phase response in response to that strong non-specific stimulus, and changes in fibrinogen and "A" to "G" ratios, and things like that. But again, I think those are anticipated, given the strength of that non-specific immune stimulus.

DR. HOUSE: Next question?

PARTICIPANT [In Audience]: Summarizing this whole discussion, I was wondering, in the new draft guidelines that CBER is working for vaccines, if there is any mention of the tests and assays that Dr. Luster just suggested? Or if it's going to be something similar to the FDA guidelines that just came out for non-biologics?

DR. HARGUS: I guess I can't really say just yet. I guess we had hoped to get feedback today. And perhaps we'll have to discuss it some more. I am not sure what we'll have in the guidelines about that topic just yet.

PARTICIPANT [In Audience]: May I add at this question?

DR. HOUSE: Yes.

PARTICIPANT [In Audience]: As far as we have discussed in Europe, and also in other areas, I think that in measuring the immunotoxicity or the immune response, as given in an overview by Mike, it has more the purpose to characterize the immune response than to decide whether or not there's immune suppression or immune toxicity. I think that's not the approach that I have in mind when applying this type of thinking.

I think that when, from a European point of view, we request for characterizing the immune response in all the detail, as also described in our repeated-dose guidelines on immunotoxicity, it's more related to what's happening after a vaccine. And maybe members of the panel can comment on that.

DR. LUSTER: I mean, that's what I was trying to say, as well as that I think the immunotox assays that have been out there and validated are doing what they're supposed to do. They detect immune changes up and down. And the idea of testing a vaccine and putting that into that is probably pretty dangerous. But it probably does have some applications in certain instances.

So for example, if there is a particular material within a vaccine that you would like to test as a potential sensitizer, I think those immunotox assays would be really appropriate. But to put the whole adjuvant in, I mean, it's guaranteed it's positive if we can get to the draining lymph node. You may have to inject it, but, I mean, it's going to be positive. It's going to be positive in the popliteal lymph node assay, because it's doing what it's supposed to do.

DR. HOUSE: One additional question before the next speaker.

MR. RITCHEY [In Audience]: Hi. This is Tom Ritchey [ph], from the Naval Medical Research Center.

We just came from a meeting which considered in large part the development of malaria vaccines. And there was a report on a phase I study of a long synthetic peptide which was a malarial antigen given either, I guess, in aluminum hydroxide or in mimontinide [ph]. And they had the problem of contralateral arm reaction. When a second or third dose was given in the other arm, there was a reaction in the site of previous inoculation which developed soreness and swelling a couple of days--it was delayed--following a new immunization in the other arm.

And during the discussion, it became clear that this has happened with a number of different malaria vaccines involving peptides. And in some cases, it's been more of an immediate type reaction, with the other arm beginning to hurt within minutes of the new injection. And actually, this has led to the cessation of developing a particular product.

This is obviously of concern. And I'm just wondering, from the point of view of looking at animal models, this idea of having to immunize in a different

location with a second shot in order to pick up the reaction, how can animal models--

[Tape Change.]

3B DR. LUSTER: Well, let's see. So generally-- Let's see, I'm trying to think. Not necessarily every time, but most of the time we do inject in multiple sites; rotate sites. So that if that were to happen and if the animal models--This is due to antigen persistence. It sounds like it probably is.

Again, that should have been picked up. I'm curious to know whether in those non-clinical studies--how they were done, and whether or not they used multiple sites or not.

MR. RITCHEY [In Audience]: Yes, I don't know, unfortunately.

DR. LUSTER: Yes. Because we will score both sites, you know; pay very careful attention to sites. And I don't have an example of where that occurred. I can tell you, when I used to immunize bunnies to make anti-mouse IG with Freund's complete and Freund's incomplete, I used to

get it there, where the other sites, the previous sites, would flare up when I dosed again the next time.

MR. RITCHEY [In Audience]: Thank you.

DR. GRUBER: Yes. In order to generate guidance, we need some answers to some questions. And if we are worried about the potential for immunopathology and immunotoxicology induced by immunization with a vaccine antigen, then I think we need to really spend a couple of minutes discussing how we really incorporate potential assessments or the assessment of potential parameters in our basic toxicity package to somehow address that; not really to specifically search for it, but sort of to build in some basic parameters looking at, if you want [inaudible], collectivization of the immune response.

And what we had discussed at the agency was really looking at basic parameters, such as looking at organs; looking at bone marrow smears; looking at blood cells, lymphocytes, the induction of antibodies; sort of in an attempt to have some signal generating tool.

If this is not sufficient, or if this is totally off line, or if this will never answer the question, I

think we need some input here in order to really generate guidance that is feasible. From what I am hearing here, it is that we have some assays which we could apply if there is reason to believe that a vaccine may induce hypersensitivity reactions beyond what could be considered biological plausibility if you immunize with something with an adjuvant that would induce some sort of reaction that you could explain away with the adjuvant without being concerned about a real immunotoxic response.

But I think we need something, some information, from the panel or from the audience, and to hear your thoughts on that.

DR. VERDIER: I can perhaps bring some data to your question. It's true that during general toxicology studies that we are doing for vaccines we have already some parameters which evaluate the immune system. We have the white blood count; we have the bone marrow; we have lymphoid tissue histopathological examination. And sometimes with these examinations we are able to pick up changes.

Are they relevant or not? We can then further answer to this question by additional studies. But it's true that I have in mind some studies with decreasing white blood cell count. I have also in mind studies with changes in lymphoid organs.

And I think that's the first tier. And we cannot speak about immunosuppression or hypersensibility. We just can say that there is an effect on the immune system of the animal which is not only the immune response triggered by the vaccine. That's something else, or that's something which is associated with immune response which is not directly the immune response.

Then with other vaccines--and Natalie was mentioning the RSV vaccine--I think a potential adverse effect, immunopathological effect, should be addressed by specific tests, for the RSV vaccine, for cell-mediated immunity. Perhaps in this case--it's really on a case-by-case, depending on the history about the disease--you should design another test. But I think we will not be able to list all these potential additional tests in a guideline.

I have in mind the RSV issue. We know also that we have this antibody-dependent enhancement. Could it happen with HIV vaccine? Could it happen with Dengue vaccine? We cannot generalize this question to all types of vaccines.

And regarding hypersensitivity, I will address this issue in the last talk. I think it's mainly an issue perhaps for excipients and adjuvants, and not perhaps for the antigen itself.

DR. LUSTER: And if I can add something, too, I think, trying to address the FDA question, I guess the first question I would ask is, if I'm worried about immunological effects, is it autoimmunity, hypersensitivity, or immunosuppression? And try to direct it that way. So again, as Francois says, it's a case-by-case study.

But if there is an issue, for example, with a vaccine that you fear might induce an autoimmune response, I mean, there are assays out there. There's just nothing that is guaranteed that's going to work, or validated. So for example, if you wanted to go back to the company and

ask the immunopathologists to examine immune complexes on potential target organs, I mean, that could be done. There's just no evidence of how successful you're going to be on that. But it's probably the best that we could probably do at this time.

And I'm not sure there's anything you could do beforehand on that as a standard screening assay for picking up these types of effects of vaccines.

PARTICIPANT [In Audience]: Speaking as someone who works on AIDS vaccine development, where our problem has really been getting things to have the activity or the immune response in humans at all, when they have excellent responses in mice, we would really not like to see the FDA go overboard in asking us to do some studies in a small-animal system, where much greater immunogenicity is shown than we expect to get in humans, just to get into phase I; especially when the speaker even admitted that there is very little evidence from clinical studies that there is any clinical relevance to the immune suppression that he sees in mouse model systems in vaccines that do work.

PARTICIPANT [In Audience]: I don't know if there are any people who work with flow cytometry here. But is there an application for flow cytometry to the various animal studies as part of the tox package, to see whether those cell surface indicators of various subsets would give us some kind of a profile that we might be able to use to sort out more of what is seen in the immunopathology?

DR. GRUBER: I just wanted to clarify that I didn't mean to suggest any special studies right offhand in looking at the potential for immunopathology. My point was that in your basic toxicity package what you pretty much want is, you look for your basic parameters. And then if there is some signal, then you go on and see how to best address it by employing special studies.

I really believe that this is a difficult issue. And I really don't--I mean, personally, I wouldn't want to suggest in a guidance document that you have to run "X-Y-Z" studies to search for the potential for immunopathologies, other than doing--that's what I'm trying to get at--the basic package. What is required? What is feasible? What can we ask for?

You know, so that is going to be addressing our concerns. But it's not going to end up in an undue burden. Okay.

DR. LUSTER: I think we have to remember that the immune system is the target organ here for activity. And so we expect to see changes in the immune system. The question is, you know, I mean, they all have to be put into perspective, in a properly designed study. And we can attribute it to the adjuvant, to the antigen. Or maybe putting them together makes things different. I don't know; I almost used the word "worse." But I didn't mean to use that. But different. And that some of those are expected.

I think in terms of immunopathology associated with immune complex disease, my pathologists will tell you, "Well, if I don't see a lesion, I'm not going looking for immune complex." So in the absence of a lesion, I would say that any immune complex deposition isn't relevant. And I would believe the HNE's [ph] before I would go to screening for immune complexes in a whole tissue set.

So I guess my point is that we expect to see changes in the immune system. And I think we have to use good scientific judgment about how those changes might predict some other negative effects. But the fact that we're going to see changes--I mean, I think we have to assume that we're going to see changes.

PARTICIPANT [In Audience]: [Question Inaudible.]

DR. LUSTER: No. Autoantibody production, to me it's difficult to see how the animal models will predict what might happen [inaudible]. Now we're talking about an animal immune response. We're talking cross-reactivity in an animal model.

If I saw pathology, unexpected pathology, in an organ system, I might investigate it by looking to see whether I had somehow induced an autoantibody to that target organ, perhaps by immune [inaudible] chemistry or whatever. But again, in the absence of pathology in the tox study, I wouldn't look.

But once again, autoantibodies haven't been a good indicator to predict drug-induced autoimmune disease in animal models. It usually comes and humans get it and

they can see it. But when they give it to the animals, it doesn't happen.

One thing that was brought to mind that people have used--again clinically--to diagnose autoimmune disease is cytokine receptors in immune activation markers [inaudible]. But again, I don't think that--I think you're going to have a hard time--I don't know how you could interpret that post-vaccination. I mean, I can't think of anything that can be at this point enough information that you could predict as a screening tool [inaudible].

PARTICIPANT [In Audience]: I'd like to bring up a point. I think this raises a fundamental question on study design considerations once again. And that question is: When do you look? Do you look the day after? Do you look after the first injection? Do you look after a prime boost regimen? Do you look two to three days after the last injection, to look at immediate effects in a prime system? And then, do you look two weeks, four weeks later, to see what happens as the animal recovers?

I think I'd like to hear some discussion amongst the panel and the audience as to when might be the most appropriate timing in terms of looking at immunotox issues.

DR. VERDIER: I think we have a good case study, which is Brian's presentation. We have several times: after the first administration; immediately after the last administration; and two to three weeks after the last administration.

And again, I would like to avoid the words "immunotoxicity end point." At the end of this period we are doing an evaluation of the animal, an evaluation of the immune system of the animal.

PARTICIPANT [In Audience]: Okay. Yes, I recognize that.

PARTICIPANT [In Audience]: Concerning the assessment of the toxicity or the immunotox of the vaccine, I don't know why we should be very different from drugs. In a way [inaudible]--In a way, for example, I agree with your question: When should we look, if the immunotoxicity is going to be after the first injection?

I mean, basically, the question is not very different than from drugs. I mean, we are going to perform a standard toxicology assay. And after, you can include a [inaudible] group for 15 days, for an example. It's a way to address the question.

And also, I don't think we have any evidence that we have to do something more complicated than that. And I don't think we can address the issue by saying, okay, for these ones we are going to say we are going to look at the response a day after, or two days after, or three days, or seven days. I don't know. I think we don't have any evidence that we should be really different from the drug approach.

And concerning for the immunosuppression, what we are doing now for drugs, in Europe there is a guideline that's saying that you need to perform a functional assay, a [inaudible] assay. Because we know that some immunosuppressive drugs are not always picked up by the immunopathology. Okay?

For the vaccine, maybe this hypothesis set up by Mike concerning the temporal immunosuppression is maybe

true. Maybe it's true in animal models. But I agree with Mr. Lambert that maybe in humans it will not always be the same situation. So the question is: Do we need to perform a functional assay to look if the vaccine is inducing immunosuppression by competition? And if it's temporal, what is the consequence for humans?

I think so for immunotoxicity testing, I think if we go for immunopathology, that's fine; meaning that we look at the modification in target human organs. That's fine. Performing a functional assay, for example, [inaudible] assay in rats, to look if there is immunosuppression on this [inaudible] assay due to the vaccine injection, I think this is a very complicated question for something maybe that does not exist right now. Okay. I don't think--We don't have any of that scientific background to address this kind of question very precisely.

And so for vaccine, I think the form is different from drugs now. For drugs we need to perform functional assay, because we know that some drugs are not picking up in the pathology. For vaccine, I don't think we are at the point that we need to do the same assessment as for drugs.

DR. LUSTER: Marc, the only thing I'm a little confused about is, my understanding is that with vaccines and timing for toxicity studies, you might want to be looking at early effects following a typical toxicity study for direct effects or potential for the vaccine formula to induce inflammation. And then you have to also look at the antibody or immune response forming, to see if there are any potential effects, toxic effects, from the immune response. Is that not how you see it?

PARTICIPANT [In Audience]: The way I see it is if you want to address the immunotoxicological end points, you do immunopathology at the end of your toxicological protocol, which I think is standard.

Of course, I'd say I guess you're going to monitor the immune response towards the vaccine during the tox protocol. You can do several--How do you say that? You can draw blood several times during the protocol, to see what the human response is. That's the way to assess the immunogenicity.

But after that, I think, concerning the immunopathology, we need to have a [inaudible] group. And

why should it be different? I am asking the question, why we should be different than drugs concerning this kind of approach.

I'm not talking about the information, which is another end point. I'm not talking about adjuvants, which should be addressed maybe separately.

DR. HOUSE: Speaking of adjuvants, at the risk of interrupting a very productive discussion, I think we should move on to our next speaker.

And our next speaker today is Dr. Natalie Garcon. Dr. Garcon is a Pharm.D., Ph.D., in immunotoxicology and immunopharmacology. She spent the past ten years working on vaccine adjuvants.

Dr. Garcon joined SmithKlineBeecham Biologicals, which is now GlaxoSmithKline, in 1990, where she set up the vaccine formulation technologies group. She is now in charge of the technology area program on vaccine formulations, alternative deliveries, and preclinical operations encompassing vaccine formulation design, development, preclinical testing through formulation, and

animal laboratory sciences and toxicology evaluation. Dr. Garcon.

SAFETY EVALUATION OF ADJUVANTS:

SHOULD THEY BE CONSIDERED SEPARATELY,

OR ONLY IN THE CONTEXT OF THE FINAL VACCINE?

PRESENTER: NATALIE GARCON, GLAXO-SMITH-KLINE

DR. GARCON: Thank you. So I mean, we've seen how it was easy already for the vaccine, so we're going to talk about the adjuvants now.

[Laughter.]

DR. GARCON: There is one thing I would like to say. The presentation is on adjuvants plus recombinant protein. This doesn't concern DNA vaccine or live vectors. And you'll see that makes a difference for some of the points.

And what we call "adjuvant" is basically vehicles and/or immunostimulant. So basically, should we evaluate adjuvants? And if we do evaluate them alone, should that be done like a drug? And that's what has been discussed many times today. Or should we only consider it in the

context of the final vaccine, which means in the presence of the antigen.

So if we look at drugs--and that has been said, also--the drug, this is the final trigger of the effect you're looking for. And basically, what you want to see when you assess the toxicity is to evaluate toxicity, or absence of toxicity, in animals, before the first time in man.

You want to determine the maximum tolerated dose.

You'd like to identify potential target organ toxicity, and its reversibility.

You'd like to have an idea of the safety margin for your molecule.

When you talk about vaccine, again, this is the whole debate of the immunotoxicity somehow. The vaccine is not the only trigger of the effect you're looking for. The immune response is a big part of it.

So what do you want to do? Again, like for the drug, you want to assess the toxicity, or the absence of toxicity, in animals, before the first time in man.

You don't really want to determine the maximum tolerated dose, because somehow the dose is defined by the immune response you want to induce; so looking at the maximum tolerated doesn't make much sense.

You still want to identify potential target organ toxicity, and its reversibility.

You don't really want to look at safety margins, since you are not looking at maximum tolerated dose.

You want to determine what is the local and the systemic reactogenicity.

And you would like to evaluate the toxicity which is linked to the immune response that you induce.

This is a busy slide, but the point of this slide is that when you look at all the testing that is in the regulation for evaluation of safety of drugs or vaccine, you see that basically it's about the same testing.

The difference comes in the way you perform it. Like for the sing-dose acute toxicity for drugs, you do it in two animal species. In the European guidelines it is said that you can do it in one animal species.

Repeated-dose toxicity study, again, you have differences in the way it is performed. In particular for drugs, it's a daily administration; for vaccine, it is recommended to do it every two weeks.

For the reproductive toxicity study, again, you have the same type. You have the three arms of the study: fertility; embryo/fetal in two animal species--here it's only in one species; you have the peri- and post-natal in one animal species--and this is the same for the vaccine. And again, here it's recommended to do a daily administration, while for vaccine you do it so you optimize the immune response in the animal model, so you do see the effect that the immune response would have.

Genotoxicity study is mandatory for drug. It is not recommended specifically for vaccine. The same thing for carcinotoxicity.

Local tolerance study should be evaluated in both. And here it says it can be part of the repeated-dose toxicity.

Toxico/pharmacokinetics is mandatory for drug; it is not required for vaccine.

Safety pharmacology should be performed, especially on circulatory and respiratory systems.

Immunotoxicology, yes, for the drug. And basically, what you want to see is the effect of your drug on the immune system. Whereas, for the vaccine, if you want to look at the immunotoxicology, what you want to see is the effect from the immune system and from the stimulation of the immune system that you induce with your vaccine.

So what are the guidelines for the adjuvants in Europe? So it's the CPMP guidelines. That was the need for guidance that was issued in '95. Well, it is said that for several adjuvants that are not currently a component of a licensed vaccine, appropriate preclinical studies should be developed on a case-by-case basis, again.

And the following points should be considered:

Injection site reaction, so the local reactogenicity, fever, immune mediated events, teratogenicity, genotoxicity;

Additive/antigen should be compared to the adjuvant alone, or the vaccine without the adjuvant;

Toxicity studies should be done on additive alone;

And the evaluation of the adjuvant effect on the immune response should be done when relevant models exist.

So the next question you should ask yourself is: Why would you perform safety evaluation on the adjuvant alone? Since anyhow it is contained in the vaccine, and you will have that when you do your safety evaluation of your vaccine.

Well, you can do that to discriminate the potential effect that you will see in your final vaccine. But the end point of that somehow is it's in the interest of the manufacturer to refine its adjuvant system and modify it in case you do see some toxicity.

Well, you can consider your adjuvant as a new additive to be injected in humans. And then it's like defining a safety data sheet-like system.

Or you would like to establish a safety package on the adjuvant to be used in various human vaccines. And again, it's in the manufacturer's interest somehow when you do a DMF-like system.

Well, the approach we see that fits best the purpose of the adjuvant is to consider them as new additives to be injected in humans, but to do testing that is adapted for the vaccine environment.

And what do we mean by that? Sorry--I'll tell you that after.

So what we mean is that the way to proceed should be, or could be, for an adjuvant that you will bring to humans for the first time, to first do what we call a profiling package that will be performed before the first time in man, where you look at local tolerance, repeated-dose toxicity, safety pharmacology, and genotoxicity if you use components that are unknown or if there is a concern that there could be any effect.

And how should this be completed? Well, after your first profiling study, you can test your adjuvant in combination with the vaccine when you do the toxicity

testing program. And so you have the separate adjuvant group.

And then you complete it by a longer-term repeat-dose study, if you haven't covered that period of time in the first study. You do perform the reproductive toxicity. You do hypersensitivity and autoimmunity, if there is a relevant model. And apparently this is still open for discussion.

And as for the biodistribution, you only do it if you want to understand the mechanism of the potential toxicities that you have seen, and if this is relevant. What we mean by that is you're doing a biodistribution of molecules that you inject in the amount of microgram and for which you will need to develop a method of labeling so that you can follow it. It's not always an easy task.

So how should the testing be done for the adjuvant alone? Should we do it on the single component of the adjuvant mix? It's not really relevant. On the final adjuvant formulation? This is certainly the most relevant method of doing it, since you may have interactions between

your ingredients. That will be part of the safety that you will assess in your study.

Dose level? Well, human dose, or the highest feasible if you are in an animal species that contact 0.501 [inaudible]. And you can look at injecting additional lower-level doses if you see effects in the human dose.

The schedule and the route? Well, it should be intended for human use. However, when we talk about scheduling, that's not something that we have covered. But it will be difficult to do a tox study where you inject zero, one month, and six months later. But you can argue on the relevance of doing a toxicity study where you inject every two weeks.

So as a start for the discussion, I would like to propose that the evaluation of the adjuvant should be done as follows:

No acute toxicity study;

The repeat-dose toxicity study, as defined by vaccine;

Reproductive toxicity study, as defined by drug, but not doing the fertility;

Genotoxicity, as it is done by drug, if it's relevant;

No carcinogenicity study;

A local tolerance study, as defined for the vaccines;

No toxico/pharmacokinetics;

Safety pharmacology study, like defined for the vaccine;

And the immunotoxicology study, like defined by vaccine, but if you have a relevant model that you can use for the purpose.

Thank you.

[Applause.]

DR. HOUSE: Questions for Dr. Garcon?

PARTICIPANT [In Audience]: [Inaudible.] I have two questions, one on the carcinogenicity. Why not carcinogenicity? Because I think in the early '60s there was some concern with some adjuvants that some components were carcinogenic.

DR. GARCON: If we're talking about oil and--In the case of emulsion--

PARTICIPANT [In Audience]: Yes.

DR. GARCON: --and we do have emulsion in some of our adjuvant systems--the components that are used have safety data sheets that are used already for the single component.

PARTICIPANT [In Audience]: Well, I'm asking that for the new formulation like--Well, I just gave an example.

DR. GARCON: Yes.

PARTICIPANT [In Audience]: But there have been some cases before that. There were some issues with the carcinogenicity.

DR. GARCON: Yes, we don't consider that as a relevant testing for adjuvant as a stand-alone.

PARTICIPANT [In Audience]: Okay. The second question is on the immune response to the adjuvant itself. Like Dr. Midthun in the morning mentioned that even FDA is going to ask that if you have an adjuvant, why you need it. Somehow, if you have an adjuvant, you show you need it. But if you have antibody response or strong immune response to the adjuvant, how you are going to deal with that in the long term?

Like the MPLs. We have a lot of antibodies to MPLs. I don't know if there are any long-term studies that are relevant [inaudible].

DR. GARCON: Well, I'm not sure there's a lot of antibodies to MPL, actually, because I mean, I don't--

PARTICIPANT [In Audience]: I don't know. I have a hard time [inaudible].

DR. GARCON: I haven't seen any data showing that. But I think one way to answer the question on the potential injection of antibody against your component of your adjuvant, the consequence of it would be that upon immunization you won't have the efficacy of your adjuvant basically, because you would have an immune response against it. And that's not something we do see in any vaccine we're testing. But we do try to look at antibodies against the component of the adjuvants, yes.

DR. HOUSE: Question over here?

MR. FREES [In Audience]: Yes. Lou Frees [ph], ID Biomedical.

I think before we go down the track of accepting the idea that there ought to be a nice separate tox package

for your adjuvant, we ought to grapple with the fact that there are more than one classes of adjuvant out there now--of which I'm gifted with one--that fundamentally change their physiochemical characteristics when they're mixed with the antigen, an antigen or any one of several classes of antigens; such that their solubility changes, their charge changes, their hydrophobicity changes, their particle size changes.

And I would just ask that we consider the fact that toxicity studies done with those adjuvants--or slash-delivery systems, if you will--in vacuo, without antigen, may not be relevant at all to the performance of those adjuvants when the antigen is present.

You can make pseudo antigen-type adaptations in some of those systems, but sometimes at the cost of adding other components which may have their own toxicities; for example, detergents, or what-have-you.

So I think that we have to consider very carefully before insisting on an adjuvant-alone package and that, where feasibility dictates, that package might have to be some variant on that.

At the extreme, the adjuvant and antigen have to be considered as a unitary unit; or alternatively, if possible, the antigen alone, followed by antigen plus adjuvant, and then assess your adjuvant essentially by subtraction. Or, last but not least, the adjuvant with perhaps multiple different antigens, even antigens that seemingly are well known, as a way of getting a glimpse at the toxicity behavior of the adjuvant.

But I think all of those things have to be considered. Because there are types of adjuvants which simply will not lend themselves to the adjuvant-alone type experimental design.

DR. GARCON: Well, I think as soon as you consider a new molecule that hasn't been into humans, whichever way you do it, you will have to do a tox study.

Now, seeing if you take it from the side of the adjuvant, test the adjuvant alone, and then add the antigen, if you have that adjuvant system with various antigens, is one approach.

Doing it as you suggest, where you take your antigen alone and test your vaccine and you do that for

different antigens, so then it is a different approach. And I can see that that can vary from one system to another, especially if we are talking about systems like what we are concentrating on here, which are more delivery systems, per se, than immunostimulants. I think, again, it's a case-by-case approach.

PARTICIPANT [In Audience]: Yes. I'm wondering about why you wouldn't do an ADME study, if you saw signs of systemic toxicity.

And the second thing is why you wouldn't want to do a fairly complete package to support a DMF, if you were a manufacturer of an adjuvant, so that another sponsor wanting to use that adjuvant couldn't just refer to that DMF in order to do clinical trials?

DR. GARCON: Well, at the end of the day, if you do your adjuvant study like you would do for a drug, that can be part of your DMF, clearly.

For the pharmacokinetics, it's a basic technical issue. I mean, if you can't label or can't follow the molecule you inject, I mean, it's very difficult to do the biodistribution. This is the main reason.

We can go with the example that was given before with MPS. This is not one molecule. This is a mix of 13, 15 molecules, which you can't really label, which you can't really quantify in different organs. So it's not a task that is easily undertaken.

In the case of a molecule like [inaudible] or any system that you can label easily, and you know that that is the molecule you are going follow for all of your biodistribution, I can see you can do it. But in the vast majority of the cases, this is not possible.

PARTICIPANT [In Audience]: May I have--I thank Ken for his point. I think that your "No" circle in the ADME studies has to be given more nuance--

DR. GARCON: Yes.

PARTICIPANT [In Audience]: --as on a case-by-case. And it depends on the adjuvant, I think, yes. And I would ask, not only in case of using an adjuvant, not only for an ADME study on the adjuvant itself, but maybe on the type of biodistribution or supportive evidence; whether or not the adjuvant gives a delay of the release of the--

DR. GARCON: Antigen.

PARTICIPANT [In Audience]: Of the antigens. And so such as the absorption or the use of those orals. And the background of all that adjuvant is in a lot of cases not very clear. It's only empirically, and not supported by a lot of data. That's the difficulty.

DR. GARCON: Actually, the adjuvant, the only one that is licensed for human use, is the one which is the most empirical. I mean, nobody knows how it works, nobody knows the biodistribution. I mean, it has really not much known about this one. It's for the new one for which, I agree, you have to attempt for better characterization. Actually, I believe that if alum was coming now, it won't be accepted.

PARTICIPANT [In Audience]: I have another point. That is the species selection for the adjuvant testing. I think it's very important to have a combination with the vaccine to the antigen itself. That will come back again on the question: What type of animal should be selected for the testing of the antigen then in combination with the adjuvant?

But it might be that the adjuvant might be combined, and that's something arguing against the DMF; that it might be combined with different antigens, and whereas another antigen can be tested in another species. And that's the difficulty.

DR. GARCON: Well, yes. In our case, you know, we are using rabbits. And there is one main reason for that, which is that when we started evaluating adjuvants we did look at system-like emulsions. And rabbits are very sensitive locally for any type of local reaction, and that's why we went for the rabbit. It's an excellent marker for any local reactogenicity with any adjuvant. Now, you can argue that rat would be better for the evaluation of the vaccine with the antigen, but, yes. Monkey--well--

DR. VERDIER: Natalie, I would like to challenge a little bit your list of tests. If you consider the recent draft guidelines for excipients, they asked for two animal species. I agree that you cannot classify an adjuvant as a new excipient. However, you may have to do at least the same number of evaluations. So I'm a little

bit embarrassed by the fact that you are proposing only one species for the evaluation of your adjuvant.

DR. GARCON: Well, I think it's the same train or so that you had this morning for also proposing only one animal species. Take the best, and why test it in a lesser one?

In our case, the best for the adjuvant system we are testing is certainly the rabbit, so why go for one that will show you less effect?

DR. VERDIER: With an adjuvant, you can deal with a new chemical entity.

DR. GARCON: Yes.

DR. VERDIER: So if you want to select only one species you need a strong argument saying that all other animal species are not relevant. And personally, I think that's the only case when you can justify one species for a new adjuvant or a new excipient.

DR. GARCON: Yes.

DR. VERDIER: If you don't have any argument to reject a rodent and a non-rodent species, it seems to me

that in this case you have to do both rodents and non-rodents.

DR. GARCON: I think there is one thing we have to remember also in the case of, like we said this morning, vaccine or adjuvants. We are talking about micrograms or hundreds of micrograms, or eventually milligrams, of material that you do inject three times in many years. And the effects you're looking for are much more minimal than what you would see with any drugs given daily.

So I see your point that it makes more sense for a drug anyhow to use two species, because you want to increase the potential to see any side effects. But for a system like that, I'm not sure.

PARTICIPANT [In Audience]: I would argue that for an adjuvant, for a novel adjuvant, the important thing here really is acute toxicity; and that perhaps establishing an MTD as in a single-dose study, given our intended clinical use of--whatever, monthly dosing, periodic dosing--might be sufficient to identify target organ toxicity, establish an MTD. And that might be beneficial in a drug master file to understand that;

notwithstanding the fact that some adjuvants may behave differently with or without being loaded with antigens.

But I'm not sure whether your plan was to use the adjuvant alone--And then the vaccine study, when you did it with your combination, then of course you would--not of course; not that everybody is doing it. But you would include an arm where you did adjuvant alone. And then you got your repeat-dose toxicity of adjuvant alone, relative to adjuvant with immunogen. I mean, that seems a reasonable approach to me.

DR. GARCON: Well, I have some difficulty seeing doing an acute study with an adjuvant system. If you take an emulsion, for example, if you have to give that every day, I'm not sure the animal will survive long.

PARTICIPANT [In Audience]: Right. Right.

DR. GARCON: And it doesn't mean anything for the use or the efficacy of your system for your vaccine.

PARTICIPANT [In Audience]: Right. I mean, I little bit agree with Ken. Hate to admit it, but--

[Laughter.]

PARTICIPANT [In Audience]: If you did establish an MTD, that you might want to know metabolism, kinetics, and those kinds of things, certainly with a synthetic adjuvant.

DR. GARCON: Yes.

PARTICIPANT [In Audience]: What is the difference between it and a small molecule?

DR. GARCON: Yes. At least in our case, a lot of the molecules we are using are not scientific, for the time being anyhow. So it's a difficulty.

MS. HELPERIN [In Audience]: Yes, I'm Jane Helperin [ph]. I'm from ID Biomedical.

And maybe I misunderstood you, but it seemed like a lot of the points you had for testing adjuvant alone would be to the manufacturer's advantage, if they were envisioning their adjuvant as a multi-purpose adjuvant for various people. And that's true, and that would be the manufacturer's responsibility.

And I'm wondering the relevance of this to a regulatory guideline where, if a manufacturer had an adjuvant that they weren't interested in doing that, and

they were only interested in an adjuvant for a single purpose, I was wondering what the FDA thought. In a situation like that, would you necessarily be required to test your adjuvant individually? Or would a complete testing on the combination be sufficient?

DR. GRUBER: This is not an FDA perspective, I'm afraid. But I agree with you, Jane. I'm having all along a difficult--It's difficult for me to understand why we need to look at the toxicity of the adjuvant by itself, when what we really are concerned of is what the safety of the final vaccine formulation would be.

And even if you were to conduct these studies to establish things such as the MTD, would it be conceivable that these parameters change if you combine your adjuvant with vaccine antigen? And then you're again left with the question, you know, "Well, now I have my nice package here with the adjuvant alone, but the minute I add the vaccine antigen things change, and I sort of start over."

So in my very minor, perhaps, but personal opinion, I mean, I would actually rather see a toxicity study where the adjuvant is investigated when it's already

combined with the vaccine antigen. And then perhaps include a control arm where you use adjuvant alone in that study. But perhaps I don't see the full benefit of doing a toxicity study with adjuvant alone.

I mean, are there additional parameters that you could look at, that you couldn't establish in a vaccine antigen?

[Tape Change.]

4A PARTICIPANT [In Audience]: --from Biologics Consulting Group.

How do you deal with the issue of dose of adjuvant, or dose of antigen, as in dose/response relationship, if you don't do an adjuvant study by itself? I'm just not hearing a whole lot about dose/response relationships in this whole meeting.

And I'm a little concerned because dose and dose/response relationships are a very basic part of pharm-tox in any other drug, other biological products, cytokines and so forth. And with vaccines, the issue of dose seems to be--It doesn't seem to be emphasized very much.

And I personally think it's a very basic issue with relationship to interpreting pharm-tox data. So I would like to challenge the panel to deal with the issue of dose a little bit more, and maybe think about that when you write this guidance; both the dose of the vaccine and the dose of the adjuvant, and how you interpret tox data, and whether or not the effect is due to the product if you don't have a dose/response relationship.

DR. VERDIER: I think we quickly addressed this point this morning with Jan-Willem, saying that with the antigen we cannot really speak about a direct dose/response relationship.

However, I get your point for adjuvant. And it's true that with a lot of adjuvant we can use this classical way of dose/response relationship. And that's why, perhaps, I am in favor of a study where the adjuvant will be given in milligram-per-kilo, or with the traditional way. Because in this case you will be able to make your dose level relationship, and you will be able perhaps to identify target organs, as we are used to doing with classical drugs.

But Ken, I would like to give you the microphone. You will perhaps develop a little bit more this question. Please, go ahead.

DR. HASTINGS: Yes, well, the example that I would think of is something like this. You have a novel adjuvant, and in your tox studies, let's say, you see elevations in liver enzymes. Okay? Now, the question that might come up, is this secondary to some sort of inflammatory response, or is this because the adjuvant biodistributes to the liver and causes damage to the liver itself. And I think that's the kind of basic question you want to answer in a tox study with an adjuvant.

DR. WARNER: I'll play devil's advocate. Does it matter if you establish the target organ and a dose/response relationship?

DR. HASTINGS: Well, if it turned out that this was an experimental adjuvant, and it turns out that it's a liver toxin, do you want to use that as an adjuvant in your vaccine product?

DR. WARNER: Well, that's a sponsor's question, about whether you want to--

DR. HASTINGS: Well, it is, of course.

DR. WARNER: But the safety aspect of setting about those, or understanding what to look for in a clinical study first in man with a new chemical entity, is it sufficient to have identified target organ toxicity and a dose response to that, relative to your intended clinical dose?

DR. HASTINGS: It might help you understand the adverse effects you're observing in your clinical trials.

DR. HOUSE: Sir, you've been very patient. Please chime in.

MR. FEDER [In Audience]: Okay. Martin Feder [ph] from Apovia [ph].

Natalie, you said something: Very little is known about alum.

DR. GARCON: Yes.

MR. FEDER [In Audience]: And I think this highlights one of the problems of vaccine toxicity. Very little is published. Having had to write a short review of alum toxicity recently, I was horrified to find how few publications there were on this. And I see a problem.

We are all working on vaccines, and most of us here are working on adjuvants, as well. And we're doing horrendous numbers of tox studies. And we're taking all of that information; we're giving it to the FDA. They don't have the time to sift through this and really tabulate who is finding what, with what adjuvant. And the rest of us, we're just sitting on the data. I think if we were to publish a little bit more of our preclinical tox studies, this would help.

Now, at GlaxoSmithKline [inaudible], I can see publishing for them would be not good for the shareholders. It would give the opposition an advantage. Further, looking through the participant list, I see at least half the people here are funded by the government. And the rest of us in small companies, we receive money funded by the government.

So perhaps, to the government agencies, it should be an obligation that our preclinical studies on these adjuvants are published. Because this would provide us with a way to seek possible toxicity, to reduce future

toxicity studies, and maybe to make a new adjuvant. Who knows?

[Applause.]

DR. GARCON: What am I supposed to answer?

[Laughter.]

PARTICIPANT [In Audience]: Chyron has an adjuvant that's approved in Europe. It's MF-59. And I think that this illustrates a lot of questions that people are asking about novel ones. It's an oil and water emulsion, so it's not tremendously imaginative. It's not a molecular adjuvant, or anything like that.

But we kind of made a commitment to that adjuvant as a platform. And I think that's when it makes sense to generate a DMF type safety profile that you can just refer to over and over and over again.

And now when I run a new protein antigen adjuvanted with MF-59, all I do is have an MF-59 group alone, and an MF-59 plus the antigen of interest. I don't run a saline control or any other control except for local reactogenicity now, because we've got this data base on MF-59 that just keeps expanding.

If it's truly a novel one, and you're running your program where you've got a control group which is untreated, or a saline or something like that, adjuvant alone, adjuvant plus antigen, then you are basically covering what you need to do to at least get into first-in-man and determine whether the adjuvant is any good in humans, and then worry later on about what kind of data package you want to put together to have a platform use of that adjuvant once you know it's good. That would be the way that I would think about it early on.

DR. GARCON: Yes, I agree. But not all the adjuvant systems can be considered as one system to which you add an antigen without modifying your adjuvant system. So it doesn't work all the time. But if you can, yes, it's the easiest way.

[Statement in Audience--Inaudible.]

PARTICIPANT [In Audience]: Yes, we usually expect to see a control arm with antigen alone, just to-- You know, maybe it's not from the safety point of view about looking at synergism, but in terms of just demonstrating that there is synergism produced by the

adjuvant. And then of course, to look for any reactogenicity or increased reactogenicity. When you have them both, you compare it to the antigen alone and the adjuvant alone.

DR. GARCON: But is it for the toxicity purpose? Or is it to show the benefit of adding the adjuvant, so that more looking at the immune response than the toxicity?

PARTICIPANT [In Audience]: [Inaudible] having antigen alone?

DR. GARCON: Yes.

DR. HOUSE: In the interest of staying on schedule, I believe I will go ahead and terminate the discussion at this point. I'd like to thank these early afternoon speakers, as well as all the discussion.

We'll have a coffee break. Please be back in the room at 3:40.

[Recess.]

MODERATOR: It's time to get on to the next speaker, please, if everybody would take their seats.

It gives me great pleasure to introduce Dr. Paul-Henri Lambert. And he is a native of Belgium, where he

trained as an M.D., and was boarded in internal medicine. And then his interest for research and immunopathology took him to Scripps Clinic and Research Foundation in LaJolla, California.

From there he joined the University of Geneva Medical School, as head of the research unit, and became professor in the department of medicine. And after that, Dr. Lambert joined the WHO, where he was asked to lead the immunology research and training program of the WHO. And he stayed there for many years doing research, and then became the chief of vaccine research and development at the WHO.

And Dr. Lambert is a professor in the department of pathology at the University of Geneva. He is responsible for the coordination of the European Research Consortium for the Optimization of Early Live Immunization, and is directly associated with the recently established Center of Vaccinology at the University of Geneva.

He is also director of the International Advanced Course of Vaccinology, organized under the auspices of the Fondation Milieu [ph]. And finally, he is author or co-

author of over 400 publications, and is a member of several international scientific boards.

And he'll be speaking today on the non-clinical approaches to assess the risks of vaccine-associated autoimmune disease. Dr. Lambert?

STATUS OF NON-CLINICAL METHODS FOR AUTOIMMUNITY

PRESENTER: PAUL-HENRI LAMBERT, M.D.,

CHIEF, VACCINE RESEARCH & DEVELOPMENT, WHO

DR. LAMBERT: Thank you very much.

First, I would like to tell you that my voice remains somewhere between Paris and Washington, and I apologize for adding this poor voice to my poor Belgian accent.

I promise you that I will not give you the answer to the question of whether vaccine can cause autoimmune disease.

What is the issue? First, we know that we have examples of confirmed vaccine-associated autoimmune diseases. I have listed here three of these examples. One is encephalitis associated with a rabies vaccine, this is the old rabies vaccine, the sheep brain; thrombocytopenia associated with MMR and measles vaccination; and the Guillain-Barre associated with the swine influenza vaccination.

And I attract your attention to the fact that the incidence of these complications is still low. Although it reached a level of 30 per 100,000 for rabies encephalitis, it goes down to 0.8 per 100,000 when we speak about swine influenza.

And this is probably one of the key issues when we speak about autoimmunity and vaccination: that we deal with low incidence of complications, which are extremely difficult to pick up in the clinical trials.

The second point is that there is an increasing incidence of some autoimmune diseases, and this is resulting with an increasing risk of coincidence with vaccination events.

Here I'll just take one example, which is Type I diabetes. And all over the world the incidence is increasing. Here we just have the European picture, where we see that there is an annual increase of incidence of about 6 percent in the group of children between zero and four years of age.

And in this picture of increasing incidence, we know that Type I diabetes is occurring at an age which

starts from six months to 15 years of age, which is when we have all our vaccines given. So it's practically unavoidable that any case of Type I diabetes will occur some time after one vaccination event. And it's always very difficult to disprove an association between the two.

The third point is that several autoimmune diseases appear to be caused or exacerbated by infection. And just a few examples: We know that a number of bacterial infections can be associated with rheumatic heart disease, reactive arthritis, Guillain-Barre syndrome, chronic arthritis, also with a number of viral infections. We have association with ITP, idiopathic thrombocytopenia. And even diabetes has been associated with various infections, but an association which is not very good.

So the question is: What is the potential mechanism by which vaccines might induce autoimmunity and autoimmune disease? And what can we do, in terms of non-clinical assessment, in relation to this mechanism?

First, the question of molecular mimicry. We know that molecular mimicry means that there is a similar B- or T-cell epitope on the vaccine and on host antigen.

If we speak first about the B-cell epitope mimicry, we have to consider first oligosaccharide, oligosaccharide epitopes. And here we have a good example, which is the Guillain-Barre syndrome which can occur after campularvector [ph] [inaudible] infection, and is associated with the development of antigangliocyte [ph] antibodies. This is clearly associated with the mimicry between some epitopes on the LPS of campularvector and neurogangliocytes with the [inaudible] the association of GT-1 with one of the LPS type, in green GM-1, which is the same type, and in rat another LPS with another type of gangliocyte. So this is clearly shown, this correlation between this mimicry and the development, which is frequent, of Guillain-Barre after campularvector infection.

It is clear that in this situation it would be probably extremely risky to base vaccine against campularvector on this type of molecule. And for this reason, we could say that this type of homology involving oligosaccharide epitopes can be sufficient to select out the vaccine antigen.

In this case we have to recognize that the knowledge of an association of infection with autoimmunity is of critical importance to take our decision. And we don't have that for everything.

If we go to the Group B meninges capsular polysaccharide vaccine, again we have an antigen which is very similar to capsular polysaccharide which is expressed in humans developing neural tissue. And this is this poly-alpha neurominocasein [ph]. In this case we have no known association of Men-B antibodies with any autoimmune manifestation. What do we do? It is a question which is still open today. And obviously, it's a situation where we may like to move to animal models.

And what can we get out of animal models? Well, the animal model in this kind of case can be used if the cross-reacting epitopes are conserved. It can be used to test the possibility to induce cross-reacting responses in animals, keeping in mind that we do not always see the antibodies because they can be absorbed on the host tissue. They can be masked in some way. We can also assess the

pathogenicity, but we know that in this particular situation it has been extremely difficult to show anything.

If we move to protein epitopes, there again we know that on a microbial antigen, like on any protein, most B-cell epitopes are exposed on the surface, they are conformational, and they are discontinuous.

If we look at autoantigens and autoantibodies, it's exactly the same thing. B-cell epitopes are seen by autoantibodies, and they are seen as conformational; they are surface exposed; they are discontinuous. And this makes the problem for identification.

Here we have the example of the GAT-65 islet cell antigen of islet cells in the pancreas mapped. And that's here, this area. It represents the B-cell epitopes as they are identified with autoantibodies coming from Type I diabetes patients. So this is clearly not a question of simple sequence. These are antigens which are highly complex, and where the confirmation is essential.

So the question: Can one predict the risk of autoimmune disease comparing these two things? The study can be based first on "in silico" studies, computer

studies; in vitro analysis; and then finally in vivo analysis.

So the first animal experiment I would like to speak about is the experiment with the computer mouse, in silico prediction. Well, here is the first thing which is usually done, is to search for sequence homologies. I would say that the search for extensive sequence homologies is still of importance, because it means something. But short peptide homologies have no significance, or little significance, when we speak about B-cell epitopes, in view of this importance of confirmation.

Then one can move to the identification of B-cell epitopes. And we have a number of algorithms which have been developed for that, looking at areas of low hydrophobicity, high hydrophilicity, high flexibility, sophistication, antigenicity. And these B-cell epitopes which are identified can be compared between the vaccine antigen and the human protein epitopes.

Well, let's be clear. This is feasible, but it's really feasible when we know which are the target proteins.

And if you don't know what to look for, this is practically a nightmare.

If we move to in vitro analysis, here we can search for cross-reactive antigens on tissue secreted protein with specific antibodies which are induced with the vaccine antigen in animals or in humans in initial trials.

We can also move to in vivo studies. And there I would think that the important point is to look in vivo for the possible binding of these antibodies, for their pathogenicity during active or passive immunization experiments. And this obviously can be done if the cross-reacting epitope is present in the animal.

So B-cell epitope mimicry I would say that this is more a concern for oligosaccharide than for protein. And when we speak about protein, this is of particular importance, maybe of importance, for vaccines which are against diseases which are known to be naturally associated with antibody-mediated autoimmune manifestations.

We have also to keep in mind that autoantibodies do not mean autoimmune disease; and that to be pathogenic, autoantibodies must have access to target antigen, they

must have functional or cytopathic effects, have a sufficient ability, or be able to form pathogenic immune complexes.

If we move to T-cell epitopes, here we know that T-cell epitopes are small, linear epitopes--small, linear peptides. The size is different if we speak about CD-4 epitopes or CD-8 epitopes, from 11 to 20 amino acids, to eight to ten. We know that some core amino acids are important and can be recognized and used to identify these epitopes.

We know also that some infection induced T-cells can be associated with an autoimmune disease. And here I list a few examples, which are rheumatic heart disease, chronic Lyme arthritis, or reactive arthritis where T-cells and corresponding epitopes have been identified.

So can one predict the risk of autoimmune response if there is a mimicking T-cell epitope on a vaccine? First, again, we go to the computer and search for sequence homologies with the human protein data bank, looking for small peptides which are homologous, six to nine [inaudible].

And there usually you get in despair. Because what you find is a large number of homologies. Here you just have an example from a study which was done by Joel Tonnard [ph], who gave me this data, where the frequencing of sequence similarities has been studied between tetanus toxin and 15 human proteins. And you can see that at the six [inaudible] level more than 200 human proteins have peptide similarities with tetanus toxin and tetanus toxoid. Even if you go to the eight [inaudible] level with one mismatch, you still have 95 proteins which have similarities. So if this would be important, no one could be immunized today with tetanus toxoid.

Then, the next step is to search for common T-cell epitopes, using all kinds of algorithms for epitope prediction--which we call classical. And the questions which I ask are, first, are these mimicking peptides likely to be appropriately processed by the antigen presenting set? And if we speak about CD-8 epitopes it will be the question of processing at the [inaudible] level. And can this processed peptide bind to the various HLA molecules;

particularly, entering and being captured in the groove of this molecule.

Well, this has limitations. We can again get a number of results, but we know that advanced HLA binding predictions are now visible only for a few HLA alleles. So we are limited in what we do.

In addition, even as this is being done, again, we can find quite a number of similar peptides on unrelated protein, predicted to bind to the same HLA allele. And taking just an example, again, tetanus toxoid, if we look for one TT DRB-1 binding epitope, this can be found on 12 unrelated human proteins. Again, tetanus toxoid would appear very dangerous.

And if we go one step further, then we can search for common T-cell epitopes using epitope prediction based on structural modeling. This is quite fancy in this modeling approach. The question which is being asked is: Is the mimicking peptide likely to be presented with a similar HLA peptide complex structure as a cell peptide?

And here we have an example where this has been done, comparing the binding in the HLA groove of an APC

expressing the B-cell 27 class I antigen. On the one hand, in yellow, cell peptide of B-27, which is considered as a target in reactive arthritis. And here, the chlamydia DNA primae peptide which is shown, in green, to bind very similarly, to have the same structure as the cell peptide.

This is very fascinating. But as you can imagine, it can be done if you know the target protein. And it's taking so much time, in fact, that it's practically hopeless.

So the conclusion regarding this "in silico" prediction of T-cell epitope is that little useful information is likely to come out of random search approaches. And the search is more relevant when we deal with vaccines for infections which are known to be associated with autoimmune manifestation, and particularly if the target antigen, or one target antigen, is being suspected.

We have also the possibility for T-cell to look for in vitro and in vivo approaches. Animal models are not as good. But if the vaccine, again, is for an infection associated with autoimmunity, two questions can be asked.

One is, can the vaccine antigen induce self-reacting T-cells in an appropriate model? And here I take the example of injecting chlamydia into a mouse which is transgenic for HLA B-27, and expresses a nice groove that we have seen before.

The second question is, can identified common epitopes be recognized by the patient T-cell? And again, this has been done in some studies, taking the patient T-cells from the articular fluid and showing that this can react with the same epitope which is being identified.

When moving in that direction, I would say that in this study of chlamydia, for example, starting with more than 80,000 putative potential mimicking epitopes, going down to the stage of reactivity in this in vivo model, this is allowing to restrict to eight or nine epitopes of potential significance.

One point which I think is essential is that the stringency of these different factors involved in T-cell stimulation and their potential role are very different according to what we look for. We know, for example, that

in terms of binding to MHC there is a certain stringency, but not very high. Many things can bind.

In terms of recognition by T-cell, recognition by autoreactive T-cell, again, surprisingly, this is very degenerated. And many T-cells of low affinity can bind to many peptides. This is not selective.

So the real point: The selection, in terms of what comes out of this mimicry, depends on other things. It depends on the presence of co-stimulatory signals, which are provided either by an infectious agent--possibly by a very strong adjuvant. It has also to escape regulatory mechanisms, such as CD-25, CD-4 cells, which appear to be quite efficient normally. And probably, it needs as well a local inflammation in a target organ to get this really to lead to a pathogenic response and recognition.

So all this is so stringent at the end that it is very rare to get this complication. That's probably why every time we are infected we are not developing an autoimmune disease.

If we look at the other mechanisms, bistandard activation, this is different. The question is the

relation to the fact that some infections have been shown not to induce autoimmune disease, but to trigger an underlying silent autoimmune disease. And the question is: Can vaccine do the same?

I just have here an example. We know that infection with the influenza virus in man has been shown to induce exacerbation of relapsing multiple sclerosis in one-third of the patients within the following six weeks. This is quite impressive. Fortunately, if these people are vaccinated, they do not develop this manifestation.

And we understand that now, in the following way; that some viral infections, particularly with IL-12 inducing viruses, such as the influenza virus, or exported to a number of microbial products, activate dendritic cells. And this activation can be strong enough, through [inaudible] receptors, to induce a release of a high level of pro-inflammatory cytokines, such as IL-1, IL-6, and IL-12. This can then lead to what we call a bistandard activation of other T-cells which are primed, which recognize different epitopes.

And the question obviously is raised: Can new vaccines with new adjuvants, such as MPL, the LT toxin, [inaudible], QS-21, CPG, or DNA vaccines, or some live attenuated viruses or viral vectors, particularly if they induce IL-12--Can they do the same thing? Can they induce all this mechanism and really create the same risk? This I think would be much more significant than any mimicry in the world.

And the question is: Can non-antigen-specific effects trigger an underlying silent autoimmune disease? How can we look at that? What kind of non-clinical assessment do we have?

Here, in vitro methods, we don't have much. We could imagine that we could compare the level of induction of cytokines, particularly IL-12, using different adjuvant formulation and using human PBMC or human purified dendritic cells, and look at this data. But the significance I think is still very difficult to define.

Other approaches which might be more relevant, in vivo, is to compare different vaccines, different adjuvants, in animal models of autoimmunity. And here we

can look, for example, at the enhancement of murine lupus, tracking of EAE. So diabetic mice are not very good for that. And I am sure that this could help for the clinical trial planning. I don't think that the decision could be taken from animal models, but I am sure that we could better know what kind of monitoring we have to include in the initial clinical trial.

I just have here an example of spontaneous lupus, a model which is used in our center, and using New Zealand and B and W mice. In such a model, it's a model of systemic antibody-mediated autoimmune disease, although it's very much influenced by T-cells.

And we can test effects on anti-DNA; on the production and level of antiretroviral antigen, DP-17. We can assess the clinical expression. And here we see on the left the cumulative incidence of proteinuria in control New Zealand mice, as compared to mice which received adjuvants which are derivatives of LPS, which clearly do not accelerate the appearance of proteinuria; may have even some protection effect. And on the right, we see the same

curve for cumulative incidence of mortality. And again, we see that the two adjuvants do not increase mortality.

So we can monitor survival. And we have to look at the histopathology to see to what extent the picture can be changed by what is given to these mice.

Another model which I think is very relevant--although like all models we don't know what to do with the reserves at the end--is that this is the model of silent priming for autoimmune experimental encephalitis. There are different possibilities, but the principal is to have mice which are primed.

Again, myelin is in the top part with infection with a thallus virus, or immunization with myelin-based protein in complete foreign adjuvant, or to use genetically predisposed mice which are transgenic for antimyelin T-cell receptor. These mice do not develop any clinical disease unless they are exposed to strong adjuvants, such as complete foreign adjuvant; or to IL-12 inducing viruses. In that case, the murine CMV. And this then leads very rapidly to delineating disease.

This is a kind of model where different adjuvants can be compared, different vaccine formulations, and see to what extent this kind of bistandard effect leads to real pathology.

One thing that I want to say is that I believe that non-antigen-specific effects of live or adjuvanted vaccine, at least the ones we know with the existing vaccine, appear to be time limited. And they are often localized to the regional lymph nodes. They are also very likely influenced negatively by regulatory mechanisms--the CD-4, CD-25 T-cells--and therefore, they are very unlikely to lead to these kind of dramatic results as we see in these models.

And I just take here the example of BCG. BCG is really considered as the strongest TH1 vaccine that we can give today. Well, in the studies in which we have collaborated with a group in The Gambia, we looked at the effect of BCG given to young children at birth, together with other vaccine, to see to what extent it is influencing the response to the other vaccine.

What has been seen is that if BCG is given at the same time--that means at birth or at two months--as the hepatitis-B vaccine, in the same arm, there is a very significant increase of the Interferon-gamma response to the unrelated vaccine, to the hepatitis-B. However, if BCG is given two months before or two months after hepatitis-B vaccine, there is no effect at all. This is really transient. It's like an adjuvant. If BCG is there at the time you get your vaccine, you get the effect.

And something which I found myself most surprising is that BCG has been used by an Italian group as an immunomodulator for the treatment of patients with multiple sclerosis. And they found no major adverse effects. And in fact, they even claim there is a beneficial effect of BCG. That means that BCG does not change completely the individual into a super TH1 person.

So in conclusion, I think that we can say that the potential risk of vaccine-associated autoimmune response is generally very low; often difficult to predict on a purely theoretical basis, such as mimicry; that for vaccines against infectious diseases known to be associated

with an autoimmune pathology, we have to consider the theoretical risk of autoimmune response. And I think that a case-by-case approach has to be selected to see if this is a reality or not.

And finally, the potential risk of triggering an underlying autoimmune disease through non-specific bistandard effects, adjuvants, some live vaccine, is probably low, and even very low. But I think it would be a mistake to ignore it completely at this stage. Thank you.

[Applause.]

DR. LAMBERT: Okay. So we have questions?

PARTICIPANT [In Audience]: I have two questions. The first one is the "in silico" analysis. Even if you do it on a longer stretch of amino acids or a longer peptide, you're going to come up with matches. So for example, you're developing a vaccine, and you come up with a match. What do you recommend on the next steps? You have no idea what this could be related to. Do you have any recommendations?

DR. LAMBERT: I think that, basically, if we suspect the potential B-cell epitope, we have to look for

it, but we look for the cross-reactivity. And this may mean more.

At the T-cell level, I think that with the present studies which have been done, and if we have no indication at all that such an epitope, such an antigen, might be involved in an autoimmune manifestation, I think we might as well forget it. I think it's falsely giving the idea that this may be important, and I think that it's not.

PARTICIPANT [In Audience]: Okay.

DR. LAMBERT: You know, the real problem is that when we speak about vaccine and infection, we are used to speak about T-cells which have a relatively high avidity because they are directed against foreign antigens.

When we speak about the self-reacting peptide, and if they are seen by T-cells as they are seen by T-cells which have not been tolerized normally, and therefore they are the left-overs, they are low-avidity T-cells, low-avidity T-cell receptors, and they can bind as well these peptides to [inaudible] identified, but many other

thousands, as was even without any amino acid in common with the first one--So it means nothing.

PARTICIPANT [In Audience]: Okay. So that leads actually into my second question, where you had those beautiful animal models of diseases that could be induced. I just wanted to make sure you're not recommending that we actually start using these animals indiscriminately; that you only use them if you think that there is a suspicion that whatever you found could cause or could induce some sort of autoimmune disease.

DR. LAMBERT: No, my feeling is that this type of model now has only a usefulness to compare different adjuvants, different formulations. For example, if you had the same adjuvant with some variance, or you derived different molecules from the same one, you might compare and see if, with the same level of activity, one is more likely for the question of binding capacity, diffusion, the kind of cell that would be seen--is more likely to induce this kind of bistandard activation than another one.

I think that by itself I would never recommend to do this kind of test just to see if you get the positive results, you know. By itself alone, it does not mean much.

PARTICIPANT [In Audience]: That's interesting. I'm trying to put that into perspective. I agree with you about the diabetic mouse not being a very good model. I'd like to hear why you think that; sort of what the criteria are for what you think are or would make good models; and at the end of the day, how you use the information you get from some of these things.

DR. LAMBERT: From which model do you speak of?

PARTICIPANT [In Audience]: The NOD mouse.

DR. LAMBERT: Yes. Again, I think I would put on the same level all of these kinds of models, spontaneous or induced models of autoimmunity. It's not giving you directly an answer, or the capacity to induce or reduce the disease which is present.

I think if we would have an adjuvant--For example, if in a model of NZB mouse we would inject LPS or [inaudible], we know that this will have a tremendous

effect on the disease, will accelerate considerably the disease.

In this situation, this would tell you that with this kind of molecule, maybe you have to watch out. And when you move to your clinical trial, I would not stop the clinical trial for that. I would move into it, if you can, and then have maybe a special monitoring during your clinical trial for some of the indicators, some of the markers of systemic autoimmunity.

PARTICIPANT [In Audience]: Okay.

STATUS OF NON-CLINICAL METHODS

FOR HYPERSENSITIVITY

PRESENTER: FRANCOIS VERDIER, AVENTIS PASTEUR

DR. VERDIER: Perhaps we can have questions on autoimmunity after just a few slides about hypersensitivity.

Thank you, Paul-Henri, for this superb presentation. I will try just very briefly to cover another aspect, which is hypersensitivity reaction. It was already discussed briefly this afternoon.

And it's true that with vaccines we have some case reports about adverse hypersensitivity reactions; mainly these case reports are presenting anaphylactic reaction, also called "type I" hypersensitivity reaction. However, it's sometimes related to the vaccine excipients, and not to the antigen itself.

There are also some case reports of vasculitis. But it's difficult to classify vasculitis. According to various textbooks or papers, it's either classified as a

type III hypersensitivity reaction, or as an effect in autoimmune disease.

Anyway, for both types of reactions, there are very few, or even no, animal models, except perhaps the guinea pig model which has been published.

[Tape Change.]

4B DR. VERDIER: And then it's followed by an IV challenge. And after this IV challenge, you monitor the clinical signs, and perhaps the death of the animals. You can also take the serum from the animal and do a cutaneous passive anaphylactic reaction, using additional recipient guinea pigs.

This method is partially validated for large molecules, or weight molecules, which are known to trigger in humans this kind of adverse reaction. And this model can detect their potential toxicity.

However, we know that we can obtain false positive reaction with mammalian proteins. So if in your vaccine you have some mammalian proteins, you may get false positive results.

We know also that this kind of model cannot detect haptens, small molecules which need to be combined with the carrier molecule. For example, this model will not detect antibiotic hypersensitivity reaction.

So my interpretation is it could be used sometimes for new excipients, if you are sure that you are fulfilling this limitation, the limitation of the test.

Perhaps the local lymph node assay, which was initially developed to detect type IV hypersensitivity reaction, can used also to detect immediate hypersensibility. I know that the group developing the LLNA is trying to use cytokine assays to see if we are triggering a TH2 or a TH1 orientation. But it's not yet totally validated for this type of acute reaction.

I just would like to share also with you some results obtained recently with this guinea pig model with not sub-acute immunization, but with intranasal immunization. We did a start of a validation protocol using intranasal administration on days zero, two, and nine. And then we did a challenge IV administration on day 23 in these guinea pigs.

We tested positive reference products; namely, ovalbumin and amylase, which is a [inaudible] known in humans to trigger hypersensitivity reaction. And we added also in this study a negative reference product, a drug which is already on the market and which is a bacterial ribosome fraction.

And we had groups of animals receiving the reference product without adjuvants, and also groups receiving a strong TH2 adjuvant--a mixture of cholera toxin and cholera toxin subunit B. And in this protocol we were able to detect the two positive reference products, but only in the groups receiving also the strong TH2 adjuvant. And therefore, it's just a start, but we can perhaps say that this test may be used with some limitations for the evaluation of components of intranasal vaccines.

Regarding vasculitis, we did a literature search, and we didn't find any good animal models to detect this type of adverse reaction.

Regarding another type of hypersensitivity, contact sensitization, it's not something which is really reported for vaccines today; perhaps because all vaccines

today are given by intramuscular or subcutaneous route of administration.

However, in the future it may be required for a vaccine which would be given by patch, because we will have a topical application of the vaccine formulation. And in this case, we may have to use a very well-known test which has been validated for a long time for this type of topical sensitization; namely, the Magnusson-Kligman test in guinea pigs, or the Buehler test.

As a conclusion, I would like just to repeat one sentence that I have put already in my first slide, regarding the status of non-clinical methods for hypersensitivity: very few or no animal models for this type of adverse reactions. So we should be very cautious about the use of the model, and be sure of the limitations of these models. Thank you very much.

[Applause.]

DR. MIDTHUN: I think that these talks are open for some discussion now. But before we do that, I would just like to address a question that we received earlier on. Someone raised the question, or they understood what I

said to mean that we would ask for exactly the same type of testing for a vaccine indicated for therapeutic treatment of an infectious disease, versus one indicated to prevent infectious disease.

And what I meant to say was that the same considerations hold. You need to ask the same kinds of questions regarding: Do you have sufficient safety for this product to enter into the clinic, based on the risk-benefit. But just because you have the same considerations doesn't mean that you come to exactly the same conclusions. So I think, or I hope that that clarifies what I meant to say.

And perhaps we can now proceed to discussing some of the topics presented.

I think everyone is tired.

MR. KENNEY [In Audience]: Rick Kenney [ph], from IMI.

I'd like to raise an issue that was brought up earlier that I think wasn't fully discussed. You know, DNA vaccines and things are certainly out there as being developed. But there are a lot of vaccines, protein

vaccines and things, that follow more of a traditional model. And from my experience, these vaccines often change relatively significantly between the preclinical period and phase III.

And I'm wondering if there is sort of a place for a graduated assessment of toxicity along that path? We've heard a lot of "Rolls Royce" approaches by the "big three," and sometimes that's pretty expensive.

DR. GARCON: Well, I can speak first. I don't know what you call the "Rolls Royce approach." I guess it's doing everything before phase I. This is not the approach we have. In particular, for everything that is with repro-tox study, we only do that prior to phase III when the final process of the vaccine is established. So that you are not in a situation where, if you do have a modification of the process during your evaluation of your product during phase I, IIA, IIB, you don't have to repeat the tox study, the repro-tox study.

So basically, we do the local, the repeat toxicity studies, genotoxicity if necessary, safety pharmaco, prior to phase I. And when we arrive before

phase III, if the process has been modified in such a way that we consider it to be significantly different from the first product that went into humans, we redo the repeat-dose toxicity study, and we do the repro-tox study at that time.

PARTICIPANT [In Audience]: I actually was going to ask a similar question in a different way. I have the same basic answer for what we do prior to phase I, in that the DART studies are later, prior to licensure, unless it's specifically targeted for pregnant women.

But one of my questions is whether there's a minor change in the formulation, you know, just to increase stability and things like that, where you really haven't changed active ingredients. And is there a way where we can demonstrate equivalence or comparability so that we don't need to repeat the toxicity studies? Such as through demonstrating comparable immunogenicity, comparable biodistribution, or something to that effect.

DR. SUTKOWSKI: I guess I'll take a crack at it. You know, this issue is something that's very difficult to say across the board. It's so case-by-case dependent, in

terms of how significant the change is, whether it's a simple formulation change or a more significant change.

And since this whole series of events we're discussing today is evolving, those are the kinds of questions that we have to begin to address more frequently now, if we're going to be requesting toxicity studies more frequently. So it's still an evolving question that we'll need to address as we go along now.

I don't know if any of my FDA colleagues want to add anything to that.

DR. WARNER: It may not be an appropriate example, but for a therapeutic vaccine where we did change the formulation, we were able to bridge based on immunogenicity, at least during the clinical program, showing a comparable quantity and quality of immune response in terms of that immunogen. So at times anyway that seems to have been appropriate.

I also disagree to a certain extent about the "Rolls Royce approach." You've mentioned the "big three." I think there's four pharmaceutical companies up here. I'm not sure where that leaves me--

[Laughter.]

DR. WARNER: --but, you know, I think we take-- Here at Wyeth, we do a package that is scientifically based. We often only include a single species for IND enabling studies. Again, it's scientifically based. We put together with the immunogenicity studies a whole package; address the questions that are involved with that. And I honestly think that--I don't think we're going overboard.

DR. LUSTER: If FDA goes off and decides to do adjuvant testing separately, is there any value in using a standard protein antigen so you can have comparative data across different adjuvants, across different laboratories, that sort of thing? Just a thought, anyway.

DR. GRUBER: I'm not quite sure if I understand the question. So you're asking about some adjuvant standard to compare your different formulations to? Can you--

DR. LUSTER: Yes. Well, it was discussed earlier from the audience that if one is going to start looking at adjuvants separately, that the formulation is going to be

modulated because of whatever protein is part of it. And I'm not sure how else you can look at different toxicities and their efficacies if that's the case, unless you happen to use the same protein all the time so you have that type of comparison.

DR. GRUBER: I think we certainly would have to think about this. But you know, I really think we are not there yet to make a decision whether we really want to recommend or require adjuvant studies by themselves, that this would be a prerequisite in order to actually proceed to a clinical study, in addition to looking at the vaccine adjuvant formulation. I really think that is something that we need to discuss first before we then go and basically answer question "B."

I think we heard lots of comments for doing adjuvant studies by themselves, and against perhaps. And I think we're going to be considering all these comments. There may be situations where somebody would think it's of advantage to do an adjuvant-only study. But there may be situations where it's plausible or it's more feasible or it

would make more sense to just test it combined with the vaccine antigen.

And I think that, you know, any time you write guidance, you try to be flexible. And I think these types of things could also be discussed in the guideline.

And I wanted to add, actually, to something that Liz had said about, we've got these questions, and I'm going to be discussing this tomorrow, for us to please define the type of product changes that would necessitate additional reproductive toxicity studies, and even in this case, of course, additional toxicity studies.

And I think that is really, as Liz said, a decision that needs to be looked at on a case-by-case basis, because it depends on the plausibility, on how likely it would be that doing a minor change in formulation would really increase the adverse event profile of the vaccine, if you will.

And I think it's probably safe to say that if you have data demonstrating the equivalence between your study or your batch that you have done the tox study with, with

your new, improved batch, then I don't think that we would be requesting additional toxicity studies.

But again, I think you would have to justify why an additional toxicity study is not necessary, as much as you would also need to justify why you're going to use only one animal versus two animals, and the type of animal that you're going to be using.

So I think, again, many of these things come back to sound scientific judgment. And that's how we're going to try to address the issues in the guidance document, I hope.

MR. BALDRICH [In Audience]: Hello. I'm Paul Baldrich [ph], CoVance [ph].

In light of us perhaps having a new guideline, wouldn't it be useful if we can clarify a difference between an excipient and an adjuvant? Because obviously, the FDA brought out some draft guidance on excipients. And if we're calling adjuvants different property, then that could have some connotations. Would the panel like to comment on that?

DR. LUSTER: Well, if I understand your question, the draft guidance on excipients, I mean, you know, an adjuvant is not, I don't believe, considered an excipient in that guidance. I mean, an adjuvant is an active component of the vaccine product.

DR. VERDIER: Sometimes it could be different to make a clear wall between an excipient and an adjuvant. If you take, for example, a liposome which will carry the antigen and would present the antigen to present in the cell in a better way, the liposome can be considered an excipient, because it's a carrier. But it can be also considered as an adjuvant, because it's boosting the immune response. So in some cases, the difference between an excipient and an adjuvant can be very difficult to make.

MR. : How would you consider the liposome to be an adjuvant, exactly?

DR. VERDIER: Natalie, do you want to answer to this question?

DR. GARCON: Thank you.

DR. VERDIER: As you are working on liposome more than me.

DR. GARCON: Before that, I think the definition of an excipient is that it's something you add to a drug or a vaccine or whatever, for which the sole purpose is to maintain your product under a certain stage and help for the stability. It doesn't have any activity whatsoever. So it can be [inaudible]. It can be anything.

The liposome, for me, it's a carrier. This is a delivery system. This is a carrier. So the definition we take of adjuvant is that it's an immunostimulant and/or-- Sorry, it's a delivery system and/or an immunostimulant. But it's not an excipient. It does have an effect.

DR. VERDIER: Yes, but if I am right, in the drug guideline from the FDA they give an example. And they say that carrier molecules are excipients.

MR. : Excipients, yes.

DR. VERDIER: So you see that they are overlapping fields.

DR. GARCON: Yes.

DR. VERDIER: If you take the recent draft guidelines, they say excipients can be carrier molecules.

MR. : They can enhance penetration, they cay--

DR. VERDIER: Enhancing penetration.

MR. : Yes.

DR. SUTKOWSKI: Perhaps that's something we'll have to--

DR. GARCON: Then we are stuck.

[Laughter.]

DR. SUTKOWSKI: --define more clearly in our guidance document.

MR. : That was very clear, right?

[Laughter.]

MR. BALDRICH [In Audience]: I have a secondary question on this. Jan-Willem was talking about adjuvants. Juvenile animals, we touched upon that this morning. Obviously, if you're developing a new drug substance with a clinical indication in pediatrics, there's possibly a need to use juvenile animal toxicity testing. And if we're talking about developing a vaccine in largely a pediatric population, should we be doing our animal studies in very

young animals; for example, one-day-old rats and two-week-old dogs?

I'm just talking about it. We don't want a conflict of interests, where we're pushing to do our pediatric animal studies for drugs going into children; whereas for vaccines where, I agree, the immune system of very young animals is not very well known--But the argument could also be used for NC's [ph].

MR. VAN DER LAAN: May I add to this question, Paul? I'm bringing back to the question, the main question of this morning that has not been discussed: What is determining the relevance of an animal model? Is it the immunogenicity, even in a young or an older animal? Or is it a challenge against the organism for which the vaccine is derived? And I think that's the main issue, maybe for the pharmacodynamics of a vaccine. But it's very much related to the toxicology and the safety of the vaccine.

Can anyone give their answer? Marion, I think you would add your questions to this question.

DR. GRUBER: Jan, I wanted to ask you what you suggest that the answer is to this question. What would it be, in your mind?

MR. VAN DER LAAN: We in Europe have made a difficult decision, maybe, to request at least a challenge model in our guidelines; at least to think about a challenge model. And that's what's lacking this morning in the presentation of Elizabeth only talking about immunogenicity as a pharmacodynamic or that type of end point.

And I think that's also related to, if you want to use juvenile animals, can we have a model in juvenile animals resembling the disease of an organism? And we have early this year developed a small pox guideline in which we have introduced also such a challenge model with an organism homologous to the species used.

DR. GRUBER: I don't know if I have a real good answer to this. But my answer would be that you do what is most feasible and practical. If you are in possession of an animal model that is susceptible to the pathogen, to the human pathogen, if you are so lucky, and if you can do a

challenge study, that is of course almost the ideal world. And if you can define your animal model in terms of being susceptible to the disease and being able to mount an immune response whereby we can now discuss what is the relevant immune response, then I think this would be wonderful.

But I think the basic message should be that you do what is practical, and you try to do the best you can. You have to justify your animal model, why you think that is the most "relevant" model to use in a certain situation.

And personally, I think it should not be really driven by, "Do we have a lot of historical background data for this animal model?" It may be that you don't because you have to divert to an alternate species. And then you perhaps have to consider revising your study design, perhaps to include bigger control arms, because you lack historical data.

So I think there is no one answer for this. It really depends on the vaccine product you have, the type of disease that you want to prevent. And you do the best you can there.

I feel that we cannot answer this question. There is in my mind no one definition of what a relevant animal model is. But that's--Yes, Francois.

DR. VERDIER: No, I just want to say just one word about juvenile animals. I think we said this morning that we are not ready to do juvenile animal study for vaccines. So we should not forget this question. We should perhaps do some research on juvenile animals. But I don't feel we can tomorrow do GLP study with vaccines given by subcutaneous route or intramuscular route of administration in, let's say, one-week-old rats or one-week-old mice.

And regarding the relevance of animal models, I fully agree with Marion. I think it's true that the ideal model is an animal model giving a humoral, a cell-mediated response; but also, giving perhaps pathological reaction to a challenge. But it's not always the case. And we have to take the model which shows some relevance, and not perhaps all ideal relevance.

MS. SAGER [In Audience]: Polly Sager [ph], from NIAID.

I just wanted to follow up a little bit on this use of the relevant animal model and the notion of, if you have a challenge model, that that really is the model that should be used. We at NIAID are in the process now of developing a number of vaccines related to biodefense, and are working on right now anthrax vaccines and some small pox vaccines.

For both small pox and anthrax, we have challenge models. In the case of anthrax, it's rhesusercinose [ph], and for small pox it's cinose [ph] with monkey pox challenge.

If I'm hearing you correctly, you're saying that the most relevant model for the toxicology for those vaccines would in fact be rhesusercinose. Is that what I'm hearing? I just wanted to check.

DR. VERDIER: I think, yes, if you have good arguments. If you have arguments saying that, "We have, unfortunately, to use non-human primates, then we have perhaps to do a non-human primate study with a limited number of animals." I know that we should consider the

ethics of using non-human primates, but if it is the only one, we have to do a non-human primate study.

MS. SAGER [In Audience]: Well, that's perhaps what you're saying. I'd like to, I think, hear what the FDA is thinking on this.

DR. SUTKOWSKI: I think you're confusing a safety study with an efficacy study. And for an efficacy study, you would need to justify whatever animal model you choose. And for your safety study, I think we would prefer that you use a well-characterized toxicology animal model. Okay?

MS. SAGER [In Audience]: Thank you.

DR. SUTKOWSKI: Anybody else?

MR. VAN DER LAAN: May I comment? I think that in the European situation we try--And I think also in the ICH-6 document regarding biotechnology. The relevance of the model is defined by the relevance of the efficacy. So that I prefer the difference between toxicology and efficacy. We try to define the relevance of the animal model from an efficacy standpoint. And we have had discussions internally about influenza and a lot of other vaccines.

And with respect to the European situation, there is a statement from the scientific steering committee from the European Commission, which is located in Brussels. And that also explains the use of non-human primates, and maybe even chimpanzees, for vaccines on apes on tuberculosis and that type of vaccine. So even in our part of the world, where the use of animals for developing pharmaceuticals is under high pressure, this is an accepted political standpoint.

PARTICIPANT [In Audience]: If I could, I think the discrepancy is what you mean by "efficacy." And I think for the animal models that we use for toxicity testing, a surrogate marker for efficacy being the immune response, the antibody response, or the CTL response, would be an adequate measure of the efficacy; since we are interested, one, in the intrinsic toxicity of the test article and, two, the toxicity of the new mediated events.

To take that one step further, to demand that the model also respond to the infectious pathogen you're worrying about I think is putting a hurdle that is an undue hurdle and an unnecessary hurdle; because I think different

animal models could be used to tie those surrogate markers of immunogenicity with protection. But for the toxicity studies, I think the surrogate markers, the immunogenicity markers, should be an adequate measure of efficacy.

PARTICIPANT [In Audience]: [Inaudible] from GlaxoSmithKline.

Can the panel comment on co-encoded molecular adjuvants in DNA vaccines, how they'd be viewed? I'm talking about molecular adjuvants within a DNA vaccine, not a separate plasmid.

DR. MIDTHUN: Is Dennis Kleinman here?

[Pause.]

DR. KLEINMAN: I'm sorry I came.

[Laughter.]

DR. MIDTHUN: I'm not.

DR. KLEINMAN: DNA vaccines are getting increasingly complex. By themselves, in small animals, they seem to be both immunogenic and capable of inducing protective responses. But in higher mammals--us--they seem to be less immunogenic, and perhaps therefore less

efficacious. So various sponsors are trying to manipulate those DNA vaccines to improve their immunogenicity.

It is perhaps relevant to Dr. Lambert's presentation that one of the approaches has been to incorporate either cytokines themselves in the formulation with the DNA vaccine, or cytokine-encoding plasmids, including GMCSF, IL-12, IL-6, Interferon-gamma.

This is rather interesting. Because in one sense it means that the self-antigen being encoded by that plasmid is now perhaps being seen as an autoantigen. And that raises a very different issue than the cross-reactivity or molecular mimicry that Dr. Lambert was referring to.

What the agency so far has required is that since agents such as GMCSF, by themselves, can be such strong immunomodulators, we have asked that they be tested independently in the context of vaccine adjuvants, in terms of safety. Moreover, we have gone so far as to request that that be done in the specific animal model. So for example, human GMCSF is not going to be biologically active

in mice. So we have asked that the companies would come up with the mouse homologue to GMCSF, and demonstrate safety.

PARTICIPANT [In Audience]: [Statement Inaudible.]

DR. KLEINMAN: Right. So in our preclinical toxicity studies to date, when using cytokine-encoding plasmids we have requested that the cytokine itself be studied independent of the co-administered DNA vaccine, to evaluate whether by itself--And remember that there is a rationale behind that, which is, as you know, when you co-inject the two different plasmids, they need not be taken up by the same cells, nor traffic in the same way. So the concern would be that even after you mix them, there is the possibility that, for example, the GMCSF-encoding plasmid could go elsewhere or do other things. So it seemed prudent, at least early on, to look at them independently.

Whether this will still be the case in another two or three years--or six months, for that matter--as we accumulate data on the safety of the co-injection of additional plasmids, I can't say. That's constantly under re-review.

PARTICIPANT [In Audience]: [Statement Inaudible.]

DR. KLEINMAN: So the question is: What if you're a very smart company and say, "Okay, we're going to put both plasmids within both encoding regions in a single plasmid"? We have dealt with that. And the fact is, if that is going to be your final product, it would be unreasonable for us to make you break them apart. So in that case, you would simply have to do your safety with the GMCSF plus "X."

The disadvantage to that is that most companies are not interested only in developing a vaccine against a single product, but would like to mix their, for example, GMCSF with multiple other plasmids. And then to facilitate that, they should test them independently.

DR. MIDTHUN: Thank you.

MS. BENNETT [In Audience]: Hi. I'm Jillian Bennett. I come from Australia, where we have a small vaccine manufacturing company there. We interact closely with our agency, Therapeutics Goods Administration.

I guess I just wanted to pick up on a point that Natalie made about the repeat-dose study in a single animal species. The way that we've actually viewed that is that in our repeat-dose studies we have a novel adjuvant that we're mixing with a number of different antigens. And so that's actually our platform technology.

What we're trying to do is build up a database of experience in the rabbit model for repeat-dose studies. And when we present in our clinical trial exemption applications--which equate to an IND in the U.S.--to our agency, we usually present the preclinical data as a data package. So that there's supportive data for all other antigens, plus our adjuvant alone which is used in each study. So that they can actually see whether there's any trends associated with that particular adjuvant, or whether it's associated with the vaccine formulation.

But to build on the repeat-dose single species, usually what we also have done is that, unlike for new chemical entities, we've usually developed a number of animal models where we've tested the dosage regimen that

we're proposing to take into the clinic and that we've tested N-plus-1 in our repeat-dose study.

We've also done dose ranging studies, to give us an idea of what sort of dose we do want to take forward. And we usually do go forward with our maximum proposed dose in the repeat-dose study.

And so I think, in terms of conventional toxicology, although it looks like we're only doing a single species, we actually have a body of data in a number of animal models. And I think that that's actually something that we should remember.

And so I would hope that FDA would consider this in preparing their guidance document for industry, that often there is a lot of other relevant supportive data. We do tell where something hasn't been done to GLP, and explain what the deviation is from GLP. But I think it's still very useful supportive data that helps build the picture to do a good safety assessment of a vaccine prior to taking it into the clinic.

DR. SUTKOWSKI: I think we would agree. That's a very nice comment. Thank you.

DR. MIDTHUN: Okay. I guess one last question. We just wanted to see if anyone had any more questions on the selection of dose. Otherwise, we'll wrap it up. And possibly even schedule a regimen.

DR. GRUBER: I think the question of dose maybe is certainly an issue that we can sort of continue discussing tomorrow, because we'll have sort of the same issues to deal with there, I think. So if you want to think about this this evening and come back tomorrow refreshed and rested, then we can basically discuss it.

All of this, what we didn't accomplish today, we're going to be discussing tomorrow, in addition to discussing reproductive tox assessment. Okay? Great.

DR. GARCON: Actually, I have a quick question. Denise, don't go away, since you're here. Continuing on the DNA vaccine, we have talked about safety assessment of DNA vaccine; we have talked about safety assessment of protein adjuvanted vaccine.

Now, in the case that many people are going, that route of prime boost vaccine going into humans, and if you have already a safety package in your DNA approach, you

have a safety package in your protein, would you have to do again a safety assessment of the combination of both?

[Pause.]

DR. GARCON: Yes. Okay. Thank you.

[Whereupon, the workshop recessed; to reconvene at 8:30 a.m., the following day, December 3, 2002.]

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