Food and Drug Administration (FDA)
Center for Biologics Evaluation and Research (CBER)

Vaccines and Related Biological

Products Advisory Committee

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This transcript appears as received from the commercial transcribing service after inclusion of minor corrections to typographical and factual errors recommended by the DFO.

TABLE OF CONTENTS

P R O C E E D I N G S (8:30 a.m.)

Agenda Item: Opening Remarks: Call to Order, Introduction of Committee

DR. EDWARDS: My name is Dr. Kathy Edwards, I'm from Vanderbilt University, and I will be the chair of the committee today. I'd like to welcome the members first and also the participants, the public, and the audience that's viewing in the webcast. I'd like to start first by going around the table and just having everyone introduce themselves.

Phil, would you like to start?

DR. KRAUSE: Sure. I'm Phil Krause, deputy director of the Office of Vaccines Research and Review at CBER.

DR. NOTARANGELO: Luigi Notarangelo, deputy director of the Laboratory of Host Defenses, NIAID, NIH.

DR. TRIPP: Ralph Tripp, University of Georgia.

DR. MCINNES: Pamela McInnes, deputy director of National Center for Advancing Translational Sciences at the NIH.

DR. SAWYER: I'm Mark Sawyer. I'm a pediatric infectious disease physician at University of California San Diego.

DR. JANES: I'm Holly Janes. I'm a biostatistician at the Fred Hutchinson Cancer Research Center.

DR. WHARTON: Melinda Wharton. I'm director of the Immunization Services Division at the Centers for Disease Control and Prevention in Atlanta.

DR. MONTO: Arnold Monto, Epidemiology, University of Michigan School of Public Health.

DR. LONG: Sarah Long, pediatric infectious disease doctor from Drexel University in Philadelphia and do vaccine policy for the American Academy of Pediatrics.

DR. LYNFIELD: Ruth Lynfield, state epidemiologist and medical director at the Minnesota Department of Health.

DR. PORTNOY: Jay Portnoy, director of Allergy, Asthma, and Immunology at Children's Mercy Hospital in Kansas City, and I serve as the acting consumer representative.

DR. GREENBERG: David Greenberg, pediatric infectious diseases, University of Pittsburgh, and head of medical for Sanofi Pasteur and representing industry today, nonvoting member.

DR. EDWARDS: Thank you. We would like, now, to have Serina Hunter-Thomas read the conflict of interest statement.

Agenda Item: Administrative Announcements, Conflict of Interest Statement

MS. HUNTER-THOMAS: Good morning, everyone. Prior to reading the conflict of interest statement, I'll start with some administrative comments. And again, I'll introduce myself. I am Captain Serina Hunter-Thomas, and I'm the designated federal officer for this committee.

On behalf of the FDA, the Center of Biologics Evaluation and Research and VRBPAC, we would like to welcome you all today to the 146th VRBPAC meeting. Dr. Kathryn Edwards is the chair of VRBPAC. Today's session has one topic that is open to the public in its entirety. The meeting topic is described in the Federal Register Notice of April 24, 2017.

The FDA CBER press media representative is Lyndsay Meyer. If Ms. Meyer could please stand up, Ms. Meyer, so that folks can reach out to you? Okay, we'll seek her out later. The transcriptionist for this meeting is Mr. Chanda Chhay.

When you make your comment, or ask questions, please speak up so that he can record all of the statements today. I would like to remind everyone to please check your pagers and cellphones. Please make sure that they are either turned off or in silent mode. When speaking, please

press the microphones to talk, and when you're done, switch them off.

Please make sure that you speak clearly and loudly into the microphone, as the transcriptionist, members of the public, and those listening via webcast need to hear the discussion. I have also been requested by staff to inform the committee members that if you haven't done so already, you can preorder your lunches for $15 plus tax outside by the kiosk, and we have the pink papers around the table for you.

This price includes coffee, tea, and decaf, as well as donuts which are in the back by that magical door there, and preordering your lunch helps to avoid the lunch hour rush. Members will be joining and gathering for lunch in the adjacent room behind here when we break.

I will now proceed to reading the conflict of interest statement for the meeting into the public record. The Food and Drug Administration is convening today, May 17, 2017, for the 146th meeting of the Vaccines and Related Biological Products Advisory Committee under the authority of the Federal Advisory Committee Act of 1972.

At this meeting in the open session, the committee will discuss considerations for evaluation of respiratory syncytial virus vaccine candidates in seronegative infants. The following information on the

status of this advisory committee's compliance with federal ethics and conflict of interest laws, including but not limited to 18 U.S. Code 208, is being provided to participants at this meeting and to the public.

This conflict of interest statement will be available for public viewing at the registration table. With the exception of the industry representative, all participants of the committee are special government employees or regular federal government employees from other agencies and are subject to the federal conflict of interest laws and regulations.

Related to the discussions at this meeting, all members and consultants of this committee have been screened for potential financial conflicts of interest of their own, as well as those imputed to them, including those of their spouse or minor children, and for the purposes of 18 U.S. Code 208, their employers. These interests may include investments, consulting, expert witness testimony, contracts and grants, CRADAs, teaching, speaking, writing, patents and royalties, and primary employment.

FDA has determined that all members of this advisory committee are in compliance with federal ethics and conflict of interest laws. Under 18 U.S. Code 208, Congress has authorized FDA to grant waivers to special

government employees and regular government employees who have financial conflicts when it is determined that the agency's need for a particular individual service outweighs his or her potential conflict of interest. However, based on today's agenda and all financial interests reported by members and consultants, no conflict of interest waivers were issued under 18 U.S. Code 208.

Dr. David Greenberg is currently serving as the industry representative to this committee. Dr. Greenberg is employed by Sanofi Pasteur U.S. Industry representatives act on behalf of all related industry and bring general industry perspective to the committee. Industry representatives are not special government employees and do not vote and do not participate in the closed sessions.

Dr. Fernando Polack, who is employed by Fundacion INFANT in Buenos Aires, Argentina, is currently serving as guest speaker for this meeting and will make a presentation. Guest speakers are not special government employees. Dr. Polack has acknowledged having financial interests in or professional relationships with some of the affected firms identified for this meeting, namely Janssen, Novavax, and Bavarian Nordic.

Dr. Jay Portnoy is serving as active consumer representative for this meeting. Consumer representatives

are special government employees and therefore are screened for their financial conflict of interests and cleared prior to their participation.

At this meeting, there may be regulated industry speakers and other outside organization speakers making presentations. These speakers may have financial interests associated with their employer and with other regulated firms. The FDA asks, in the interest of fairness, that they address any current or previous financial involvement with any firm whose product they may wish to comment upon. These individuals were not screened by the FDA for conflict of interest.

FDA encourages all other participants to advise the committee of any financial relationships that you may have with any firms, its products, and if known, its direct competitors. We would like to remind members, consultants, and participants that if the discussion involves any other products or firms not already on the agenda for which an FDA participant has a personal or imputed financial interest, the participants need to exclude themselves from such involvement, and their exclusion will be noted for the record.

This concludes my reading of the conflict of interest statement for the public record, and additionally, I would like to provide specific guidance regarding this

particular meeting. Please note that the topic of this meeting, considerations for the evaluation of respiratory syncytial virus vaccine candidates in seronegative infants is determined to be a particular matter of general applicability, and as such does not focus its discussion on any particular product but instead focuses on the classes of products under discussion.

Therefore, VRBPAC's role is to advise and inform the FDA, CBER, OVRR, on the effectiveness of current strategies against RSV virus infection and disease as related to the classes of products being discussed. Presenters and speakers will provide data on clinical trials and various RSV vaccines that serve only as examples for the committee to have a scientific discussion while considering various classes of RSV vaccine related products or clinical trials.

This VRBPAC meeting is not being convened to recommend any action against or approval for any specific RSV vaccine or clinical trial. This VRBPAC meeting is not being convened to make specific recommendations that may potentially impact any specific party, entity, individual, or firm in a unique way and any discussion of individual products will be only to serve as an example of the product class.

This meeting of the VRBPAC will not involve the approval or disapproval, labeling requirements or post-marketing requirements, or related issues regarding the legal status of any specific products.

At this time, I would like to hand over the meeting to Dr. Edwards, and thank you.

DR. EDWARDS: Thank you very much, Serina. I think that was very important to highlight what our charge is today. So as we know, we are going to be considering the clinical evaluation of RSV vaccine candidates in RSV-naive infants. We will begin by having a presentation by Dr. Jeff Roberts, medical officer in the Division of Viral Products in the Office of Vaccine Research and Review. Thank you, Jeff.

Agenda Item: Introduction of Presentation and Questions

DR. ROBERTS: Good morning. I wanted to start by thanking the committee members, our presenters, the manufacturers. We are aware that preparing for a meeting like this is a lot of work. So we really appreciate you joining us today to help us think through some of the issues here as we consider moving forward with the development of these products in RSV-naive infants.

What I hope to do is, what I'm planning, is a really broad overview of the agenda, touching on each item

very briefly from a high level, and I hope that's going to help frame this discussion for today. It really starts with these initial studies with the formalin-inactivated RSV vaccine candidates or FI-RSV.

These candidate vaccines were produced by growing wildtype RSV in cell culture. They are formalin-inactivated and they're adjuvanted with alum. There were several studies. I'm just quoting one of them. In this particular one, infants 2- to 7 months of age were randomized either to FI-RSV or parainfluenza virus candidate vaccine, and among those subjects who were infected with RSV, 80 percent in the FI-RSV arm, compared with 5 percent of the control subjects, required hospitalization for RSV disease.

These findings were really unequivocal. There was clearly a more severe disease in the vaccinated subjects, and there were two deaths in these trials. Obviously this presented a substantial challenge for the development of RSV vaccines, and these trials were done almost exactly 50 years ago, and we still have no licensed RSV vaccine.

In the meantime, RSV epidemiology has been fairly stable, and there is a tremendous burden of disease. Just quoting some topline numbers here of global incidence per year in children less than 5, 34 million hospitalizations

for lower respiratory tract infection, 3.4 million hospitalizations, and somewhere in the neighborhood of up to 200,000 deaths. In the United States, for hospitalizations, around 170,000 and an estimate of 500 deaths in the United States.

So when you think about the comparison to other infectious diseases, it's really striking that once you get outside the neonatal period and you talk about the period from 28 days to one year, RSV is second only to malaria as the leading cause of death worldwide.

There may be some other elements to this burden of disease. As an example, I've put up some data from this New England journal study with the use of palivizumab, which is a monoclonal antibody for prevention of RSV. I'll talk about it a little more in a minute.

In this study, healthy premature infants 33 to 35 weeks were randomized to placebo or palivizumab, and they had good outcomes in terms of preventing RSV, but what they also showed here is a substantial decrease in the events of recurrent wheeze over the first year of life, and a very substantial decrease in number of days with wheeze. This suggests a potential long-term impact on asthma outcomes.

Then there is, of course, the direct health care cost, and there's also sort of an unqualifiable burden parents with babies who suffer, and I think many of us are

intimately familiar with that. In the face of that very substantial burden of disease, I think it's been really encouraging over recent years to see the new developments in this field of RSV vaccine development. And I think one of the really fundamental breakthroughs was the approval of RespiGam in 1996 and Synagis, or palivizumab, in 1998. This showed proof of concept that passively administered antibodies in the form of a polyclonal sera, which is RespiGam, or a monoclonal antibody product could prevent RSV.

In addition to that we've had multiple scientific and technical breakthroughs in producing improved vaccine antigens, like the pre-F protein, and then characterizing them in the vaccine technologies that are being developed to vector some of these proteins and at this point, we've got at this count at least 60 vaccines in development. So it's a pretty dizzying array of different vaccine products, and we'll talk about it a little bit more.

So it wasn't surprising, I guess, that when WHO did this landscape analysis a couple of years ago to think about where to focus their efforts, they considered criteria like the magnitude of the public health burden and the chances of success of the different products in development, and RSV really came to the top in terms of a priority for development.

I have put in this slide just to recognize that this space is really complicated and that there are -- it includes the development of these candidates in many different populations, including in older adults. We have several that have advanced into late phase development. A lot of activity in maternal immunization, including one product in phase III, but the point of this slide is to help us narrow down and focus on the specific population of RSV-naive infants and for active immunization.

What I'm recognizing here is that some of the live-attenuated vaccine products have already been studied in RSV-naive infants, and there is a substantial safety database alleviates to some degree the concern about enhanced respiratory disease. But to our knowledge, no other vaccine candidates have been studied since those initial studies in the 1960s in this specific population.

Okay, so there are many ways to divide up and think about the different vaccine technologies that are going forward, and I have this slide up because one of the things that we want the committee to think about as we move through the day is what the science can tell us potentially about these different approaches to vaccinating and potentially what elements of the scientific data could be filled in to help support the safety going forward with some of these specific technologies.

That's a really brief and broad overview, and we'll get into the details of each of these specific topics. Susan Gerber is going to tell us more detail about the latest on RSV epidemiology. Fernando Polack is going to go back and really dissect some of those initial trials with the FI-RSV and what they can tell us, and talk about the animal modeling of ERD and what each of those animal models can bring to bear.

Sarah Browne is going to give an overview of our evaluation of these data so far, and the two manufacturers here, GSK and Janssen, both have vaccine candidates that they intend to develop in RSV-naive infants. They both have a substantial package of preclinical data to support that and the both have some clinical, some early clinical data. So they've agreed to present those programs as examples for us to help think through the issues.

Okay, what I am going to do is I am going to read these questions verbatim, because I want these to be in the committee's mind over the course of the day as we hear all these presentations, and then we'll go back to them one at a time and put them up on the screen during the committee discussion.

In the meantime, I want you to think about these discussion topics. So number one is please discuss the preclinical data essential to support studies of RSV

vaccines in RSV-naive infants, with regard to the potential risk of vaccine-associated ERD. Please consider the impact of vaccine type, antigen, and/or other relevant factors.

Number two is please discuss the role of clinical data from adults and RSV-experienced infants to support the evaluation of RSV vaccines in the RSV-naive infants.

Number three is please discuss how studies in RSV-naive infants could be designed to mitigate concerns about ERD throughout clinical development, including please consider aspects of initial study design such as eligibility criteria, age de-escalation, and duration of follow-up. Please consider relevant aspects of phase III study design.

That's all I have for now, and I think we can probably go straight into the next presentation.

DR. EDWARDS: Thank you very much, Jeff. Our next presentation on RSV epidemiology will be presented by Dr. Susan Gerber. She is the acting branch chief of the Respiratory Diseases Branch in the Division of Viral Diseases at NCIRD. Susan?

Agenda Item: RSV Epidemiology

DR. GERBER: Thank you very much. I cannot possibly talk about all of RSV epidemiology in the allotted time, but I am going to pick some notable topics to speak about that are somewhat relevant to this meeting. First, a

brief review of clinical manifestations, seasonality and implications for models, approach to pediatric RSV burden, understanding pediatric mortality, special populations, and considerations for future pediatric RSV epidemiology investigations.

Briefly, the clinical characteristics of children under 5, and this is adapted mostly from the NVSN platform and a paper by Hall in 2009. In outpatients and hospitalized patients -- outpatients is the darker bars -- but more lower tract illness in hospitalized patients, but there's also ranges to less severe disease with cough and nasal congestion, so a wide range of symptoms.

A few words about seasonality and how it will factor into some future slides, this is the National Respiratory and Enteric Virus Surveillance System, or what we call NREVSS. It's a laboratory surveillance system. It includes aggregate detections from clinical and public health laboratories, automating public health laboratories into NREVSS. We also have collaboration with our flu program and analyzing seasonality of RSV antigen versus PCR detections.

This is just a sample to look at the RSV season, and just to point out that it's not the same in every region of the United States. And Florida, particularly southern Florida, has a different seasonality, but really

this is something that usually on the average is November to April in the United States, but does vary regionally.

Also, this information has changed over time going from antigen toward more PCR detections. PCR is more sensitive, it's appropriate for all age groups for diagnosis, and interpretation of seasonality data turns out to be different for PCR detections and antigen detections. Antigen is less sensitive, particularly in older adults, culture is expensive and disappearing as a diagnostic assay, but the implications for models of RSV disease burden, it's important to consider the data for seasonality in interpretation of the likelihood of RSV disease.

A couple notes about worldwide RSV estimates, and then I'm going to switch to the United States. For 2005 in children under 5 years, I think Jeff just showed 33.8 million new episodes of RSV-associated acute lower respiratory infection from a paper that Dr. Nair published in 2010. Approximately 3.4 million hospitalizations, and it's an estimation of 66,000 to 199,000 deaths, but it's important to note that 99 percent of deaths are estimated to be in developing countries.

In terms of an overview of U.S. RSV burden in children, it's the most common cause of lower respiratory tract infection among hospitalized young children here.

Approximately 2.1 million children under 5 years with RSV infection require medical attention each year.

In children less than 5 years in the United States, RSV infection results in an estimation of one of 334 hospitalizations, one of 38 visits to an emergency department, and approximately one of 13 visits to a primary care office.

Now, the hallmark syndrome of RSV, which is not limited to RSV, but can be caused by a few other viruses but is a way to measure and understand RSV epidemiology, is the clinical syndrome of bronchiolitis where RSV infects the ciliated epithelium in the upper and lower respiratory tract, and bronchiolar epithelium and type I pneumocytes may become infected. Airway obstruction may occur due to sloughed epithelium and inflammatory cells with mucus and fibrin that get into small airways. So actually, being able to count bronchiolitis during RSV season is one way.

Just a note about how to interpret what is going on with RSV in the United states. There are bronchiolitis guidelines. This is the most recent published by the American Academy of Pediatrics in 2014, which actually notes that there doesn't not need to be routine virologic testing for uncomplicated bronchiolitis cases. In terms of trying to measure what is going on in the United States, if

it isn't tested, it may not be laboratory confirmed for RSV.

First, I am going to start with a model about hospitalizations, published by Dr. Zhou in Clinical Infectious Disease in 2012. These were hospitalizations associated with influenza and RSV in the United States from 1993 to 2008 using the HCUP data, which is Healthcare Cost and Utilization Project data from 13 states at this point.

It was a contribution of influenza and RSV to hospitalizations for respiratory and circulatory disease by using negative binomial regression models. And again, going back to seasonality, using that NREVSS data which I showed you previously, looking at RSV weekly numbers of specimens tested and numbers of positive results by antigen and virus isolation. This was before much PCR testing was received into NREVSS.

Just to point out, not to look at this whole slide, but just to point out that these were rough estimates of children less than 1 year comparing flu and RSV and to other age groups, 1 to 4, 5 to 49, and the largest numbers were in the younger ages.

Another way people have tried to interpret epidemiology of RSV in younger children is looking at another kind of model, and this is basically based on counting bronchiolitis likelihood. This was from a paper

by Stockman et al a few years ago, discharge diagnose codes, at that time ICD-9 codes, looking at all lower respiratory tract illnesses in children less than 5 years.

RSV-associated hospitalizations were all RSV-specific coded hospitalizations year-round, and then approximately 30 percent of wintertime unspecified acute bronchiolitis and about 20 percent of wintertime unspecified pneumonias.

Looking at the average annual hospitalization rates calculated, denominator data was used from the U.S. Census, and stratified by age group, and as you can see, these are ages in months on the x-axis, and hospitalizations per year. You can see overall for less than 5 years of age, hospitalizations were about 172,000, but under 1 year, which is most of the burden, 126,000 RSV-associated hospitalizations or 32 per 1000 infants, and as you can see, looking at months on the x-axis, really the highest number of hospitalizations were in the 0- to 2-month age group.

Now looking at the New Vaccine Surveillance Network data, and this is prospective surveillance data for acute respiratory infections in the United States from 2000 to 2009. In the first iteration of NVSN, there were three locations in Rochester, Nashville, and Cincinnati, looking

at outpatients and inpatients in children less than 5 years.

It's important to know in this prospective active surveillance system, there was a broad case definition and this was diagnosis of acute respiratory infection defined as illness presenting one or more of the following symptoms, not necessarily requiring any of them: fever, cough, earache, nasal congestion, rhinorrhea, sore throat, vomiting after coughing, wheezing, and labored, rapid, or shallow breathing.

There was an exclusion criteria that included respiratory symptoms lasting more than 14 days, neutropenia from chemotherapy, hospitalized elsewhere within 4 days, or newborns who had been hospitalized since birth. It's important to point out that this case definition is very different than the standard influenza-like illness case definition and SARI case definitions, which include a requirement of fever often. And as data that I'm not going to present today, younger infants don't necessarily have a fever when they have RSV infection.

Just to look at a comparison between hospitalized infants, emergency department, and pediatric practice or medically attended RSV infections, again, the highest rates for inpatients are in the 0- to 5-months age group, and we'll look at that a little bit more finely in a minute.

But you can also note that in pediatric practice, there are high rates in the 6- to 11-month age group as well.

Just a little bit about -- this is from the same paper, this is Hall, New England Journal 2009 -- odds ratios for potential risk factors in patients. These are with RSV infections according to the treatment site, but you can see that there were at least simple analysis looking at young age and daycare, house with smoke exposure, more than 1 month old, breastfeeding, high risk conditions, and prematurity, but only a younger age and prematurity were independently associated with RSV illness requiring hospitalizations.

Average -- this is the same data but this is a different paper now, this is 2013 Hall Pediatrics. This is looking at fine slices per month of chronologic age, looking at the average rates of hospitalizations for RSV infection. These are in children less than 2 years of age and as you can see, the highest rates per thousand children on the y-axis are in the youngest infants.

Here is just a table of numbers, but the red box really indicates the highest rate of 25.9, and these are in the 1-month-olds. It does fall off after that, but just to also note there is still substantial burden up until 1 year in some, up until 2 years as well.

Now looking a little bit at average rates of hospitalization for RSV infection for children less than 2 of age, and this is according to weeks of gestational age, the weeks of gestational age are on the x-axis with hospitalization rates on the y, and you can see that there are high rates of premature infants, but these numbers are small.

Most children less than 2 years of age who were either hospitalized or treated as outpatients with RSV infection had no underlying conditions or were perceived as high risk even though very young children are perceived as high risk for severe disease, but most children who are hospitalized in the burden, treated as outpatients or inpatients, have no underlying conditions. Most of the burden of RSV hospitalizations occurs among children overall, healthy children.

A few notes about pediatric mortality. RSV deaths are not nationally notifiable in the United States, and this is different than influenza deaths, which are nationally notifiable. Administrative data estimates depend on laboratory testing, and administrative data excludes community-associated deaths where RSV testing is unlikely to have occurred.

This is a model from Thompson et al, from a 2003 paper, and again looking at influenza deaths and compared

to underlying respiratory and circulatory deaths and all-cause deaths. Basically in these models, looking at excess deaths and looking at the seasonality using the NREVSS data, you can estimate how many excess deaths there are from either flu or RSV on the right-hand side. And again, 124 or the most RSV deaths for children under 1 year of age. I'm not discussing the adult data today in view of time.

Another approach to RSV mortality in children is using two national pediatric databases, either the HCUP KID database, and this is from a paper by Byington et al from a couple of years ago, and this is again the Healthcare Cost Utilization Project Kids inpatient database. Now, since it's years later, state participation increased from 22 in 1997 to 44 in 2009. So more participation.

And also the Public Health Information System she looked at from the Children's Hospital Association, which represents inpatient data from 44 specialty children's hospitals.

Just briefly, looking at the annual deaths, it was less than what people had thought before, and annual deaths, looking at the KID database, 121, and the public health information systems, 56, and children with complex chronic conditions accounted for the majority of deaths.

So it had been estimated previously but not based on -- mostly estimates of 500 deaths perhaps per year, but at least looking at administrative data, it appears to be substantially low, but this is also based on what is available in the administrative datasets.

Again, to look at some value of national health statistics data and state medical records, and this was a study for RSV deaths under 2 years from 2004 to 2007 using death record data, there were 170 RSV coded deaths from 44 states overall. But looking at these four states, California, Georgia, Michigan, and Texas, 32 deaths with matching medical records were evaluated and 26 had RSV positive laboratory results, one with a histopathologic finding suggestive of RSV, three with a clinical diagnosis of RSV, and two with no mention of RSV in hospital records and no autopsy records, and 21, or 81 percent, had a potential high-risk condition.

So seemingly, the death records seem to match the medical records and are at least accurately coded, but may not be inclusive.

A few words about special populations, this is just a map of Alaska and looking at 18 years of respiratory syncytial virus surveillance, and this is in a paper recently published a couple years ago by Bruden et al.

This is some data from the Y-K delta and it has been thought that rates of RSV have roughly at least been threefold higher of this area among young children as compared to the rest of the United States. Interestingly, looking at the RSV rates over time, they seem to have drifted down, but there still seems to be increased risk of RSV-detectable disease and hospitalizations among infants in this area.

This is just, interestingly, the RSV seasons have also changed in this area, and this is from the same data. They've grown shorter as compared to years before and how that impacts the numbers. Still, a very interesting point of investigation.

Just a couple words about increased rates amongst Native American young children, and this is from a paper many years ago in 2002 by Bockova et al. This is a percentage of Navajo and White Mountain Apache children who were hospitalized for RSV infection, had severe disease, but this is from many years ago. Still, consideration of special populations in risk is important.

I'm going to wind up talking about interpretation of all these numbers and approaches to RSV epidemiology in young infants and just try to talk about methods. In prospective active surveillance, using a broad-case definition or requiring fever in a case definition like

some definitions that have been published elsewhere, these make a difference in terms of looking prospectively at hospitalizations or medically-attended RSV infections.

Laboratory-based surveillance can also be helpful, but that would depend on clinician testing practices, but actually might be more inclusive, especially a very severe disease because in acute perspective surveillance, it often is very difficult to enroll very severe, young infants, and to get parents' consent in those types of studies.

Population-based surveillance is also very important, knowing your denominators. The NVSN and some laboratory-based surveillance systems do have population-based data available. Then actually using the models based on administrative data and seasonality data, really understanding the seasonality data in different regions of the United States. I've really concentrated on the United States for most of my talk.

Also taking into account the administrative data, looking at models where there is some matching between common, let's say for hospitalizations, lower tract manifestations of pneumonia and bronchiolitis and chances during RSV season, it is RSV versus another virus such as parainfluenza or hMPV.

Also, the use of controls, we have seen, looking at comparisons between cases and controls, a small amount of RSV disease, or RSV positivity I should say, in controls, but then again, that could also add value in certain populations. Laboratory assays, PCR versus antigen-based assays, PCR are more sensitive and actually can possibly give us better indications of seasonality. Understanding mortality, and again, mortality in the community versus the hospital is a very important, especially when using the administrative data such as the studies in the United States, may miss community deaths because of infants that are not tested. It's unknown if they have RSV.

For influenza, having influenza be nationally notifiable has added potentially more follow-up testing of children who have died and then were subsequently discovered positive for influenza. Also, evaluation of pediatric deaths and utilization of administrative data will be really very important to follow depending on whatever intervention happens.

Looking at other databases, sudden infant death syndrome databases and other adverse effects will be important.

Also, surveillance for other respiratory viruses, the impact for human metapneumovirus, paraflus 1 through 4,

adenoviruses, rhinoviruses and enteroviruses, other human common coronaviruses, looking at their impact and the actual likelihood during different times of the year for them to actually cause similar syndromes of illness and actually being able to follow that out after interventions will be important.

Lastly, priorities for pediatric RSV surveillance. We have felt that utilizing strengths of multiple surveillance systems will help us to understand burden, risk factors, and mortality. Burden by age in different settings is important for hospital and then medically-attended RSV infection, in the emergency department, and outpatient clinic.

Different surveillance systems can serve as inputs into economic models because I showed you a lot of different numbers, but it was not so much as comparing the numbers but comparing the methods and I think that all of this can add value in really trying to understand this population and risk of RSV infection.

This is just lastly a point; finding opportunities within these surveillance systems to integrate laboratory study, to integrate RSV sequencing information and immunologic studies, and this is something that when we talk about all of these types of studies, in the back of our minds we look for opportunities to kill two

birds with one stone and figure out ways to add value through laboratory investigations. Thank you.

DR. EDWARDS: Thank you very much. I think we have an opportunity for some questions.

Perhaps I could start. What does it take to get RSV mandated to be reported to the CDC like influenza? Is that a difficult task?

DR. GERBER: Yes.

(Laughter.)

Maybe Ruth would like to comment from the CSTE perspective.

DR. LYNFIELD: So the Council of State and Territorial Epidemiologists work with CDC to determine which diseases are nationally notifiable. When there is a disease that has a large burden, one needs to figure out what you want reported. I will tell you that, for example, another large burden disease, Lyme disease in those geographic areas where Lyme is endemic, people do not have the resources to report every single case and investigate every single case so that there are different approaches in those states where the burden is high versus others.

So I would put the question back and say, what is it that we want to know about RSV? Is it just administrative data? Is it assessing that there are virological results? Is it all age groups? Is it

pediatric deaths? So really figuring out what needs to be notifiable and then keeping in mind that public health resources are very limited and ensuring that the data are accurate.

So the question is, does one want to know rates and does one want to know the baseline and to be able to follow? Because some years, clearly there are more severe RSV seasons than other years, and so I think one way that the United States and CDC have gone about trying to look at burden and impact of interventions is using population-based surveillance systems and then extrapolating to the United States as a whole.

So right now, there is the NVSN system, but also CDC's working with the Emerging Infection Program sites to look at population-based surveillance in adults and there's now discussion in expanding that to pediatrics. That provides the opportunity to really ensure that there are tight, crisp case definitions and having population base, you can extrapolate.

A big challenge that Dr. Gerber alluded to is that testing biases are going to be a problem. I mean, if you're doing an NVSN study, then certainly all these children have a respiratory swab if they're enrolled. If you're looking at general practice and there are AAP

guidelines that cases of bronchiolitis don't need virological testing, that will impact your results.

DR. EDWARDS: Thank you. It would seem enumerating deaths would be helpful.

Other comments? David?

DR. GREENBERG: I apologize if I missed it. Is it understood why the Native American populations are at higher risk?

DR. GERBER: Actually, I'm going to go to my extra slides. I knew I had them for a reason. If you look at, and this is a slide at the end of the deck which I didn't show, this is looking at village level factors associated with lower respiratory tract infection, RSV hospitalization rates, in children under one year in 49 villages in that Y-K delta that I mentioned, and this is from the same paper from Bruden, et al.

You can actually see P values associated with maybe crowding, lack of plumbing, and actually, this paper did look at risk factors including things, wood as a heat source, as I said, number of people in the household, location, but really, crowding, lack of plumbing for RSV infections, but also for lower respiratory tract infections were important.

So I think that some of these types of risk factors, this does help further our understanding, but it may contribute.

DR. GREENBERG: Just to carry that one step further, I think some of these are associated with higher risk or burden in the general population, right?

DR. GERBER: Yes, they have been shown to be like numbers of households and young siblings, yeah.

DR. NOTARANGELO: Thank you for providing a very comprehensive overview. My question is about variability of seasonality which of course is very important in monitoring of immunized infants in a prospective trial.

You showed a couple of slides. One of them showed that south Florida was different than any other regions in the country across a couple of years, and in another slide you showed significant variability of seasonality in Alaska across multiple years. My question is what do we know about variability of seasonality in other regions of the country, not just across two years but in a longer period of time?

DR. GERBER: That's a great question. We do actually have several decades of experience, but right now, one notable change that has been occurring, because we rely on clinical and public health laboratories to report

aggregate data to us, so we look at the numbers of tests performed and the numbers of positive detections.

This is actually for RSV and for other viral pathogens. However, the mode of testing is changing and we have done some evaluations and investigations looking at differences and testing of labs that report to NREVSS. What's happening is that less labs are actually reporting to us antigen and more are reporting PCR and that this does reflect the practices in the laboratories.

So this does affect NREVSS data in our interpretation and one thing that, I only had a few minutes to talk today, but I have many, many slides of the differences in interpretation of PCR data versus antigen data, looking at percent positivity and looking at ways to capture and looking at normal curves, the season. So it is very different and this is something that we're doing right now, is investigating differences between our PCR data and antigen data over several years' time.

DR. LYNFIELD: I just wanted to follow up with a comment on community deaths. That is also extremely challenging because the highest death rate is in these young infants. That's also a period of time where you have SIDS deaths, and in Minnesota, we actually have an unexplained death and critical illness project where we work with medical examiners who do swab deaths at home that

they are involved in and the problem is interpreting, is it true, true, or unrelated?

I guess the question, these children may have symptoms of a respiratory infection or they may not, and then you have an upper respiratory swab that's positive for RSV or potentially other viruses, and the challenge is to be able to understand what the component may be, that the attribution may be. So one thing to be mindful of is how long these children can carry RSV, but it is going to be challenging even if one makes it notifiable. In many places, that is not done and then, again, figuring out what the attribution is.

DR. LONG: Since the licensure and use of palivizumab, most of us in children's hospitals do have some information about the seasonality and the tightness of that seasonality and at the Committee on Infectious Diseases at the American Academy of Pediatrics, we all talk about this around the table.

And it is rather predictable. It's about 17 weeks for most areas of the country and they do vary by a couple of months. So I think that for most areas, it could be predictable if one was doing a surveillance following immunization.

The other thing is, I was interested, Dr. Gerber, on the slide that shows the National Respiratory and

Enteric Virus Surveillance System, the NREVSS system, why the antigen detection seems to be higher and earlier than PCR is a little interesting. We know that as people are beginning to think about RSV, they do lots of antigen detection, which is in some systems available for emergency departments and outpatients, and PCR is available for inpatients.

So you get different data, but many of those RSV antigen detections, when it's really not on season, which is 10 percent of your samples have to be positive for two consecutive weeks, they're false positives. So there are a lot of moving targets.

DR. EDWARDS: Good comment.

Final question. Jay?

DR. PORTNOY: First of all, obviously the best way to avoid getting RSV is to be born in May so we should all strive for that.

(Laughter.)

My question is about genetic predispositions. I remember a long time ago there were studies done, I think Welliver was one of the authors, looking at the development of IgE to RSV and they actually developed specific IgE antibodies to RSV when they had infection. My question is, is there a genetic predisposition to having more severe disease? Are infants who develop RSV and have a more

severe disease from families that have atopy or asthma as an underlying condition, and is it possible that those who have more severe disease tend to make a Th2 type of response and develop IgE as opposed to those who don't?

DR. EDWARDS: Susan, would you like to answer that or should we wait perhaps after Fernando has talked about that to answer that and come back with that?

DR. GERBER: That is probably beyond my purview to discuss. I mean, I couldn't include everything, but I started with a children at high risk discussion and certainly those who are recommended to receive palivizumab is at least in the latest iteration of the recommendations, and children that we know, as we saw, who are at high risk for severe disease. Certainly underlying lung disease in premature infants is a high-risk group. I think that I really can't comment on further than that right now.

DR. EDWARDS: Arnold, did you have a final question?

DR. MONTO: I just wanted to extend the discussion from special populations in the United States to those in the rest of the world because what kind of information do we have about the relative importance of RSV there since what we decide here is often reflected in vaccines that become available for the rest of the world?

DR. GERBER: I think that is a great comment. I mean, only in the interest of time to talk, I think it's a whole other huge topic to talk about worldwide RSV because we know comparatively so much less, but I think that from country to country in some of our collaborations, we have recognized a lot of difference.

The case definition with SARI surveillance and WHO's case definition, I just, to interpret with caution RSV burden in young infants.

DR. EDWARDS: We are going to have to end the discussion soon.

Karen, would you like to introduce yourself and then also ask your question? Then that will be the last question before Fernando.

DR. KOTLOFF: Sure. It's just a quick comment. I am Karen Kotloff. I'm a pediatric infectious disease specialist at University of Maryland Center for Vaccine Development.

There is a study that was just completed that was funded by the Gates Foundation called PERCH and it was a study of WHO-defined severe and very severe pneumonia in children under 5 in seven developing countries, and used fast-track multiplex PCR to look at the etiology of pneumonia in those children. It's unpublished, but RSV was looked at very carefully and found to be very important as

a cause of disease, but not as important as a cause of mortality.

DR. EDWARDS: Thank you. Thank you very much, Susan.

The next discussion is going to be by Fernando Polack from the scientific director of Fundacion INFANT and he is going to talk on the history of vaccine-associated enhanced respiratory syncytial virus disease and characterization of the animal models designed to mitigate risk in future vaccine studies.

Fernando, we're very pleased to have you with us.

Agenda Item: History of Vaccine-Associated Enhanced Respiratory Syncytial Virus Disease and Characterization of Animal Models Designed to Mitigate Risk in Future Vaccine Studies

DR. POLACK: Thank you. Thank you for inviting me. These are my conflicts, and I have a brief disclaimer. I started working on this in the 1990s and the consensus at the time that nothing but live attenuated vaccines would ever be used in seronegative infants against RSV. Therefore, for all of us working in the field, characterization of these enhanced disease phenotypes was essentially an academic exercise.

So the consensus was that these vaccines were never going to be tested for enhanced disease, but not

these vaccines. The only thing that was going to be used in infants was live attenuated vaccines. So characterizing the enhanced disease phenotypes was essentially an exercise, an academic exercise.

That's why almost every possible immune cell and a number of cytokines were described as endpoints for this loose entity that could present in different forms in different papers. For this very reason, these endpoints were never clearly established and every single finding was contradicted, was refuted by another paper.

So I'm saying that because I guess I'll get questions about a single paper here and there that's saying something opposite to what I'll be saying, and that's something that is going to happen. Everything is going to be at least having an alternative explanation.

So I guess when Jeff asked me to talk about enhanced disease, one of the issues was that enhanced RSV disease has a twin illness and that is atypical measles because measles was also, many years ago, one of the targets for these formalin-inactivated vaccines. My talk will go through both diseases, trying to build sort of a process to understand the pathogenesis of enhanced RSV disease. Then I'll specifically talk about different arms of the immune system, talking of what we know, what are the caveats of what we know.

I'll try to open a little bit the understanding of enhanced disease, comparing it to other diseases that have a similar paradigm that we often overlook. Then I'll try to go over the animal models and if you have questions, I'll try to answer them.

In 1963, there were two vaccines against measles licensed on the same day, a live attenuated vaccine and an inactivated product. This was a formalin-inactivated measles vaccine. It was used until 1967 and it was used actually in several hundred thousand subjects in the United States. Initially, in the first few years, it looked as if it would protect against disease. Then around 1965, during a measles outbreak, people started developing this odd atypical manifestation of measles.

They had very high fevers, they had a petechial rash that was affecting the upper and lower extremities, and they had bibasal pneumonia, essentially pneumonia of the lower lobes of the lungs, and they were quite ill. Some of the differential diagnoses for these presentations were for meningococcemia, severe sepsis; these patients were often admitted to the hospital.

As the live attenuated vaccines were successful, the first vaccine actually caused some side effects like fever and rash but eventually was more attenuated, leading to Moraten, which is the vaccine we use today in the United

States. The formalin activated vaccine was discarded and nobody gave it much thought about what had happened with this product for many years.

At one point in Sweden, Erling Norrby started studying the antibodies, at least he did by this formalin-inactivated measles vaccine, and what he showed is if you looked at the sera of individuals that had been immunized with the formalin-inactivated measles vaccine, this sera, antibodies with fusion-inhibiting activity.

Now, we're talking about many years ago and the techniques were essentially indirect assumptions at the point of the anti-fusion activity of these antibodies, but Erling Norrby postulated that a deficiency in fusion inhibition was responsible for priming the subject to develop atypical measles.

Interestingly enough, he was not only working with a formalin-inactivated vaccine, but he was also working with a tween-ether inactivated vaccine, which had been developed in Europe at the same time and had very similar manifestations and particularly immune manifestations as the formalin-inactivated product, showing that not only formalin but other things can do the same thing.

In 1962 and 1963, there was a brief sort of trial. There were no controls, but 54 subjects were

vaccinated with the formalin-inactivated RSV vaccine. Twenty-one of those were infected with RSV and 48 percent had severe disease and required hospitalization.

In 1966, four trials were initiated in the United States, and you can see them here in this slide. Many of the subjects, the proportions are different in each of the studies, but a substantial number of the kids developed very severe disease. The main characterization of the disease in all these studies was children presenting with pneumonia and wheezing. If you look at one of the studies in particular, the one led in the report by Kim, 80 percent of subjects who had been infected with RSV ended up hospitalized. This was actually mentioned by Jeff a little earlier.

Two of these kids, a 14-month-old boy and a 16-month-old boy, died as a consequence of RSV disease and both of them had RSV recovered from their lungs postmortem. When you look in depth at these two children, these were a 14-month-old and a 16-month-old, immunized between 2 and 7 months of age. One received a vaccine at 2 months, the other received a vaccine at 5 months, and I think they received three doses of vaccine each.

There are some interesting observations in their past medical history before they presented at the final event. One of them had bronchiolitis at 3 months of age.

The other had croup at 11 months of age, and had 14 days of persistent symptoms that worsened about 48 hours before dying and that's when he required admission to the hospital.

They were both quite febrile. One had almost 40 degrees of maximum temperature. The other had almost 39. They had bronchopneumonia and in postmortem isolates, they had gram negative rods, which has generated some discussion over time in the field. It's true that these are postmortem specimens. These children were in the ICU. They were quite sick and it's hard to know what these gram-negative rods were doing there.

This is actually, I think, very, very strong image of how the lungs of these kids look. This is sort of a close-up of one of those bronchioles that you see there. What you can notice is that there's no epithelium. It has been sloughed.

There's some very, very impressive mucus plug. This kid is impossible to ventilate. There's everything in the lungs and that was the reason precisely, I guess, why this child died. In the report, this was a report in the American Journal of Epidemiology in 1969, all that was said about the histopathology of the disease was that there was a peribronchiolar monocytic infiltrate with some excess in

eosinophils, and this sentence, some excess in eosinophils, has shaped the field for 50 years.

So I'm going to try to start building, for lack of a better word, a paradigm of how this was thought of at the time, and then evolving to what we know now. So as I showed you before, Erling Norrby's work had suggested that in the measles field, atypical measles resulted from failure of the formalin-inactivated vaccine to elicit fusion-inhibiting antibody so the fusion element of the virus was gone.

In the RSV field, Brian Murphy and Mark Connors published a study, which I think is one of the most important papers in this area, in Journal of Virology in 1992 where they essentially depleted CD4 T cells from BALB/c mice that had received the formalin-inactivated RSV vaccine, and the mice had very, very limited pathology compared to control mice that received a control antibody.

The next year, Barney Graham essentially for the first time looks at the Th2 profile of this vaccine. So what you see in this slide and if you look at the second line, you'll see that there is IL-4 production with use of the killed vaccine in BALB/c mice. If you look below, there's limited interferon gamma production.

So here is Brian's work showing that CD4 T cells are critical for the manifestations of enhanced disease in

mice, and Barney's work showing that there's a Th2 bias when you use these inactivated products. When you put these two things together, Brian Murphy's group, and Mark Connor's, conclude that the F protein, the one responsible for the eliciting anti-fusion antibodies, the response was being primed by the formalin-inactivated vaccine to polarize to Th2.

So they say, well, if the F protein is not present, what is driving the response is the other neutralizing antigen on the surface of RSV and that's the G attachment protein. So they started working with a vaccinia vector that encodes for the attachment of protein virus vvG.

A couple of years later, Peter Openshaw's group and Tom Braciale's group almost simultaneously tried to address why are these vaccines biasing the response to Th2? And they essentially emphasized that this G protein in RSV lacks the capacity to elicit cytotoxic T lymphocytes against the virus. If you are able to produce CTLs against the virus, the Th2 profile is gone.

So essentially, what comes out as the conclusion at the time is that enhanced disease is a response elicited by this RSV G because F had been destroyed during the process of formalin-inactivation. We're going to see that that is not correct, but this was the thinking at the time.

So this G response does not elicit CTLs and the vaccine does not elicit anti-fusion antibodies, and all this leads to a Th2 bias with eosinophilia.

I'm going to try to show you how the RSV and the measles models inform themselves, how they talk to each other in antibodies, T lymphocytes, and PMNs, and really see what we just discussed through this next set of slides.

So I was a fellow at the time and I was working in Diane Griffin's lab and Diane Griffin had a monkey model of measles that actually was a rhesus macaque that emerged from challenging these monkeys with a Bilthoven strain of measles virus which was a present from Ab Osterhaus to Diane.

What we did in the lab was immunize monkeys with a formalin-inactivated measles vaccine. Some of these monkeys had been immunized as long as 14 years earlier by I think Arwind Diwan in Hawaii, and some monkeys were immunized at Hopkins around 1996 or so.

A few years later, we challenged these monkeys with Bilthoven measles virus and what you see on the left side, the monkey that has the angry rash, well, that's exactly the same rash that was present in the humans. I showed you a picture of one of these subjects before. Here, you see a macaque and that's the abdomen of the macaque. I haven't shown these slides for many years.

People used to ask me if this was the biceps or what it was, this is the abdomen of the macaque.

You can see, macaques stand like I'm standing now to speak on the microphone. So they're a little bit bent forward so that the disease locates right there in the thighs. On the right side here, what you see is classic measles. You could catch this rash if you touch the monkey. So we had a model of atypical measles with the same rash, and I don't know how it projects there, but the same pneumonia that was experienced by individuals in the 1960s.

Now, interestingly enough, we also had systemic eosinophilia in these macaques. So at the time, we thought that this was essentially putting all the picture together. We had a very similar illness that was observed in enhanced RSV disease and was described by the papers I just told you before. The way we were advancing our thoughts was essentially also learning from the RSV field, but in the 1970s, Joe Bellanti published some work associated with atypical measles and I'll explain this a little bit.

One of the concerns when people had been immunized with formalin-inactivated measles vaccine and had not contracted measles was what would happen to them if they would be exposed to measles in the coming years? So the decision was to immunize these subjects with the live

attenuated measles vaccine that had become available, and that way protect them, and that actually worked quite nicely and I'm going to come back to that in a little bit talking about RSV.

That protected them from subsequent measles exposures, but the other thing that happened when these individuals received a live attenuated measles vaccine is that if you biopsied their skin, you could see immune complex deposition on the vessels. I guess you say on the vessels, not in the vessels? These things, after like 30 years living the states, are still confusing to me.

(Laughter.)

IgG, C3, and also measles antigen, which was actually quite surprising because it was different from the perception that everybody has had of the disease being this delayed type hypersensitivity disease, a lot of inflammatory cells, and the eosinophils we have seen before.

But interestingly enough, when we biopsied the skin of the subjects, the monkeys who had developed atypical measles, there they were. Here, you can see the IgG in the monkeys with atypical measles by immunofluorescence and C3. So these monkeys have immune complexes. We performed bronchoalveolar lavages of these monkeys, serial lavages, or over and over, and you can

clearly see that the immune complexes were also present in the macaques in their lungs associated with the pneumonia.

So the conclusion was at the time that production of atypical measles was associated with this immune complex formation with eosinophils and interestingly enough, the other thing we did is we had more sophisticated dose to look at fusion-inhibition and anti-F antibodies, and they were there, and they were clearly present. So essentially, the idea that atypical measles associated with the disruption of fusion-inhibiting activity was incorrect.

Now we knew that this was incorrect and Barney Graham had shown that polarization to Th2 in BALB/c mice could be elicited with the F protein, so you didn't need the G protein, so this was an incorrect assumption that meant this was incorrect.

I'm going very fast; I hope you follow me. I've been doing the same thing forever, but enhanced disease then is not a response elicited by vaccinia vector G and has nothing to do with the anti-fusion antibody specifically, but we like CTLs because this inactivated product that's not processed through MHC class 1 pathways so it doesn't elicit CTLs. We have these findings from, at the time already, many other groups too showing that it polarized to Th2 and in BALB/c mice had eosinophilia.

The vvG observation is not truly all, because there are hundreds and hundreds of papers discussing enhanced RSV disease as the result of G immunization followed by RSV challenge in mice. While that is something that is probably not a good idea, and if you look at the histopathology of the mice and how they lose weight and how they look, you wouldn't want to be a subject in one of those studies, I don't think that has anything to do with enhanced disease. So that is an artefact, a laboratory experiment that is actually quite interesting, but does not reflected enhanced RSV disease.

The other thing we know, and this we knew from the beginning, is that no protective antibody was involved. Now we knew that there were immune complexes in the skin and lung of the monkeys with atypical measles, so I thought it would be a good idea to look at immune complexes in enhanced RSV disease.

I wasn't quite original, actually. If you look at the last or almost the last sentence of the original paper by Kim and Dr. Chanock and Dr. Parrot, what they say is possibly antigen-antibody complexes at the respiratory epithelial surface initiate a sequence of events involving complement fixation, chemotaxis and leukocyte damage which leads to the bronchiolar pathology seen in serious RS disease. So this is the first report, the one reporting

the first two deaths, and people were already considering the possibility that immune complexes could be involved in severe RSV disease.

The other thing that the first report notes is that if you look at complement fixing antibodies with no correlate for protection and you compare them to neutralizing antibodies, the ratio of complement fixing antibodies to neutralizing antibodies is substantially higher, meaning you have a lot more non-protective antibodies when you use these formalin vaccines than when you have a live infection with RSV.

That is actually further shown by Brian Murphy and Mark Connors very early too and you can see that here. I printed this from the internet, it's not that great, but these ratios were very high compared to live infections. I don't know if you can really see them there, but you have to trust me. What bothered me at the time is he tested passive transfer of sera and he passively infused sera from mice that had received the formalin-inactivated vaccine into other mice, challenged them with RSV, and nothing happened, which makes a lot of sense.

So pneumonia is not driven by these antibodies. In fact, it's only probably biased in its profile by these antibodies, but some other features of the disease are. One of those features is bronchial hyperreactivity. When

we started working on this, coming from the macaque model of measles which was so florid, so good at showing disease, we were quite determined to find correlates of illness that would have clinical meaning or could be translated to humans. So that ruffled fur or decreased activity or hunched back, it's very hard to translate that to bronchiolitis. So let's find something that has a direct correlation to bronchiolitis.

One of them was airways hyperreactivity and we started working on these with a scientist at the time at Hopkins and then at NIH, Steve Kleeberger, and what you can see here and you see it in the first bar, far left, is the formalin vaccinated mice receiving RSV and having significantly more airways resistance than those that contracted RSV or were immunized with control vaccines.

So we seemed to have a model. The second thing is we had a model of pneumonia and you can see there, formalin vaccine, RSV challenge, top left corner, peribronchial, perivascular infiltrates. There's some alveolitis, it's hard to see from there. The other groups don't have it except from the last group, which was also formalin-inactivated vaccine generated from an RSV virus that lacked protein G, which was actually generated by Peter Collins's lab who has given me a million things to work with over the years.

So you can see immune complex deposition up there very clearly, C3, IgG, you can see both of them in, you can confocal up and down. So neither G has anything to do with this specifically and these immune complexes seem to be playing a role.

In fact, they do play to an extent a role probably through T cells. You can see here, again, airways resistance. You have a mouse that has no complement, complement C3, or you have a mouse without mature B cells and you see exactly the same type of profile. In other words, these things play a role in airways resistance in the disease.

Now, when we were at this stage, the question was how can we translate this into something meaningful in terms of humans? We were lucky because Greg Prince generously shared with us slides from the kids who had died from enhanced disease, these two children in 1967.

We stained those lungs for evidence of immune complex activation and deposition in the peribronchial areas. So you can see there that CD4 covalently linked to the lung and showing that immune complexes are indeed stuck to the lung of the kids who died of enhanced disease.

This was actually done at the time by the only group that had the antibody. Now it is commercially

available; everybody has it. This was a long time ago. This was, I believe, University of Vienna.

So we said, well, there seems to be a role for immune complexes in enhanced disease, so now we know a little bit more and the question is, what are the immune complexes doing there? Why are the pathogenic immune complexes present in atypical measles and in enhanced disease? I think this is an important concept. It's because the antibody response generated during vaccination using these vaccines can recognize the antigen, can recognize the virus and can bind, but fails to protect. Seems simple, but I think there is a lot in that sentence.

Why do they fail to protect? Well, in this case, there is a particular specific explanation. I'm going to try to go through it. It's probably not the only explanation, but I think it's an interesting one.

This is a very schematic, simple slide and it's very old, so forget the simplicity, but when a new antigen enters the body, generate antibodies, you see them in green, will be held by follicular dendritic cells in regional lymph fluids and will present the antigen.

B cells, with the help of T cells, will start undergoing affinity maturation as they start changing their binding site for the antigen. They're trying to take away, in simple terms, the antigen from the follicular

dendritic cell. Most of these random somatic hypermutation events yield antibodies that are weaker than the original green antibody so they can't take away the antigen and they die.

But some of these events succeed and the antibody now is stronger and can take away the antigen and these B cells become memory cells and plasma cells. So this is the principle why people have antibodies to measles 50 years after receiving immunization or Jim Crowe detected antibodies against H1N1 pandemic in 85-year-olds that had been exposed to the virus in 1918.

The trick is that these formalin-inactivated vaccines cannot do it. So what you're seeing there in the red circle, panel H, is white squares, open squares, reflecting affinity maturation in monkeys that had been immunized with a formalin-inactivated vaccine. It never gets any better. What you get is what you have, or what you have is what you get. What you see with the black squares is actually affinity maturation with a live attenuated vaccine, far better, and I think built into what we're talking about.

What you see up there, which is still improving but there's limited room now to improve, I'm showing you the end of the tale, is serial vector H vaccinations. H is the hemagglutinin of measles. So all these antigens

presented in the cytoplasm undergo affinity maturation in these processes and get better and better and better. The formalin-inactivated vaccine cannot do it.

The problem is, and you see with the arrow, that affinity maturation for measles and for RSV has a good correlation with protection. You can see there and we did this, this is 2003, we did it for RSV a few years later, lack of affinity maturation leads to enhanced RSV disease. But it leads to this antibody that cannot protect in this case and that was associated with enhanced RSV disease.

So now we have a better picture of enhanced paramyxovirus diseases. Non-protective antibody responses, a primed T helper response biased to Th2, and no CTLs. So let's get a little deeper into non-protective antibody responses.

The first question is is affinity maturation critical for every vaccine candidate? Is this something that must be present in every vaccine? Well, the answer is only if high affinity responses are required to elicit protection as is the case with the inactivated and subunit products we are familiar with.

What am I saying? Well, I'm saying that let's say you have a formalin-inactivated product and formalin has disrupted all the antigens. So if you get affinity maturation for bad antigens, you're going to get bad

antibodies. That is not necessarily true. It's not magic that affinity is going to make it better. You need to have a good antigen.

Or, alternatively, here's a paper by Barney's group a year ago, and what he's showing, if you look at the second, the third, and the fourth pair of squares, for lack of a better word, what you see is that the formalin-inactivated vaccine does not elicit antibodies against the pre-fusion F. That's what Barney is suggesting, that the pre-F epitopes are disrupted, essentially, that's what he's saying.

So if the pre-F epitopes are good enough and you then need affinity maturation, well, then maybe you don't need it there, but we don't know. This is not something that we know at all. What we know is that antibody has to protect. If it doesn't protect, it's not going to work.

The second question then is how do we know if antibody protects? So are neutralizing antibodies a sensitive and specific assay for protection? I think neutralization is as good as your cell substrate, your animal species. It may sound stupid, but actually is quite important.

For several years in our monkey models, the response that we were measuring, the neutralizing response elicited by the vaccine against measles was protective. We

thought, how on earth are these monkeys getting ill if we're getting these good neutralizing titers with Vero cells?

Well, because we were using Vero cells. Vero cells lack CD150 which is the live, the receptor for Bilthoven, the measles virus strain that we had, to elicit disease.

So once we knew to be identified monkey cells and CD150 was present, the sera was not protective so we were actually sort of misunderstanding the nature of our assay. We do not have a great neutralizing assay. I think this is not new. Animal models are often, we'll use a different point, are often falsely reassuring if you challenge too soon after vaccination because you have a lot of antibody.

So this is typical. People challenge their models two weeks after they've done the initial inoculation and then they find protection or they're not fully understanding why on earth they see some symptoms but it looks like the neutralizing antibody assay is not enough to show any protection. Well, you need to wait, basically.

Can disease reoccur? Can enhanced disease happen twice? Well, when we were almost done with the immune complex story, I went to LID, to NIH, and Peter Collins introduced me to Dr. Chanock, and I sat briefly with Peter and Dr. Chanock for a little bit. He was sort of recalling

this story of enhanced disease and one of the things he said, I was actually quite shocked, was that one of the greatest concerns was that the kids that had developed severe RSV disease, enhanced RSV disease, during the winter of 1967 were going to be re-exposed to RSV in the winter of 1968. They didn't know whether things were going to get worse or were going to get better or nothing was going to happen. It wasn't clear.

The answer is no, it should not reoccur. It shouldn't reoccur because once you get live RSV infection, when you're infected with RSV, affinity maturation will correct the antibody mismatch. It will generate antibodies that are protective. So disease should not reoccur.

In fact, that is the principle why live attenuated measles vaccine protected those that had received the formalin-inactivated vaccine in the first place and had not contracted measles. So this is potentially an interesting strategy if you're in the middle of a problem. I'm not advocating for doing any of these, but if you were to protect a child with palivizumab and let him contract upper tract disease, if it's an emergency, maybe that may let him slide through the danger, and then be fine the next season.

Alternatively, if you would, I don't know, I mean, this is for virologists, but if you would inoculate

RSV in the arm, could you alter the profile? This is something that's never been looked at in animals and we probably should.

But this is another important implication and it's that even that the best antibody wins during affinity maturation, if you have a subject that already has a good, strong, long-lived memory B cells that respond to RSV, you're not going to catch enhanced disease. That means that seropositive individuals will not help you make any type of a conclusion when you're testing enhanced disease, when you're looking at vaccines for enhanced disease. You should not rely on these subjects.

T cells and enhanced disease, well, this is the field I'm going to try to talk about. This is an area that people have done a lot of good work and we needn't do much.

So this is one of the original studies I showed you. This is Tom Braciale's group in Journal of Experimental Medicine in 1997. What he's looking at is vaccinia vector G and what he's showing is that if you have no cytotoxic T cells, which is what you have in the VG/22K that you're seeing on this slide, you make a lot of eosinophils.

So the CTLs have been tied to eosinophilia since those papers and another paper in the European Journal of Immunology by Peter Openshaw's group the next year. What

we know comes from the vaccinia vector G literature. So we are essentially assuming the rest which is probably the same, but we are essentially sort of taking one data from there and extrapolating to the formalin-inactivated story.

In this paradigm, CTLs may contribute to sway the T helper response away from T helper type 2 biases which is what they do when you use the vaccinia constructs and may also help by diminishing the antigen loads stimulating primed T helpers. So as you think about it, the more antigen, probably the more exuberant the memory response, which is what you do not want, so CTLs are probably a good thing to have.

I think in general we agree CTLs are a good thing to have for most things you catch. It's good to be able to clear your infected cells. But the statement is not absolute. I'll get back to that.

This is probably the hallmark of enhanced disease and its primed T helper responses biased to Th2. There are two phenotypes that have led this field for years or have been the preferred outcome for many studies for years. One is eosinophils and the other is Th2 cytokines, and I'll talk about that in a minute.

This is I think a very important paper for eosinophils. This is work by Steve Varga's group. What

you see here is that if you have mice that do not make eosinophils, you still get enhanced RSV disease in mice.

So the eosinophils are there but are not in the causal pathway. They seem to be doing something else. So to me, the best analogy is coming home at night and seeing an ambulance at your door. This is not good news, right? But the doctor is not causing the problem. So that's sort of how I think of eosinophils.

(Laughter.)

But they are there. So this is a slide that we never published. It's actually perhaps our best picture ever and we never published it, but what you're seeing on the lower panel are eosinophils in the two kids who died of enhanced disease and no eosinophils in a younger child who died of wildtype RSV disease.

What you can see is anti-myeloperoxidase antibodies, and this was sustained by Jamie Lee from Mayo Clinic, and very clearly shows that eosinophils are indeed present and abundant in the lungs of kids who died from enhanced RSV disease. But they're not present in the blood. This is the CVC of the two children who died of enhanced diseases in 1967 and one had no eosinophils and the other had 5 percent eosinophils.

This has implications for vaccine testing because don't look for them as a sign of relief. If you find them,

you should worry, but if you don't see them, it's not telling you much.

In another of the reports, this is a report by Chin in 1969, 50 percent of subjects with enhanced disease compared to 10 percent of controls had eosinophils, more than 250 eosinophils. The other two studies didn't look at eosinophils, the four studies that were originally published and the only ones that we can go back to to learn about this. So the eosinophils are, I think, a warning sign that should be carefully considered, but it's not absolute. It's a good biomarker to discard a candidate, but that's about it.

It works in BALB/c mice, it's not present in cotton rats. You see it in some bovine models, not in others. That's about as useful as it is. It is absent in many models as I said. Absence does not guarantee safety.

The other trick is that it's important to know your model and select an appropriate vaccine for your control group. So the vaccinia G vaccines generate more eosinophilia than the formalin-inactivated RSV vaccine. So if you have a control that makes a tremendous amount of eosinophils as your positive control, that doesn't even reflect the disease you're trying to test, and you go back and test your vaccine and you see a few eosinophils and you say, this looks pretty good, look at the other one.

Well, the other one is wrong. So this is very important. Your controls are essentially the main drivers of your animal models.

The other thing is if you challenge too quickly, you'll think the vaccine protects. This is still adherence again, and you may miss them.

Last part. How about the Th2 bias? In 1992, as I showed you before, Brian Murphy depletes CD4 T lymphocytes from enhanced disease. He shows the disease is nearly abrogated in terms of the histopathology in BALB/c mice. Barney later does this work with BALB/c mice showing the IL-4, and then there is a suggestion of a combined effect of Th2 cytokines coming from studies of Mark Connors and Brian Murphy depleting different cytokines in mice.

I think that the most definitive work may be Steve Varga's work with STAT-6 deficient mice significantly decreased the ERD phenotype, but let me show you something from the early studies where we inferred that Th2 cytokines were critical for enhanced disease. What you see there in the first red bar is the effect of the regular control, formalin-inactivated RSV vaccine, and what I can see from here with my eyes, it's about 27 percent of the bronchioles having a pathologic score, which goes down to 23 percent when you use anti-IL-4; you deplete IL-4. If you deplete interferon gamma, it goes to 20 percent.

So this is the story of the Th2 bias and I think this is an important contribution I can make. We need standards. We need to know what we're talking about.

Here is, again, the same story. This is the vvG paper. This is Tom Braciale's work and you can see there, vvG, high IL-4, vvF, supposedly a protective control, low IL-4.

I think I missed the green square I had underneath, but if you look directly underneath, you'll see that if we were to graph this based on interferon gamma and we cut our graph at 200,000 picograms per ml, I could show you the same thing. Do you follow what I'm saying? So yeah, there's a lot of IL-4.

So what do we call enhanced disease? This is the battle tower. Low interferon gamma, IgG1, IgG2a ratios, IL-13 levels, IL-10 levels, IL-10 levels have been blamed as the Th2 marker for a long time. Now we think of them as regulatory cytokines. The eosinophils, the eotaxins, combined cytokines, interferon gamma, whatever, you can find everything, absolutely everything, and not necessarily everything aligned, not necessarily everything going the same way.

So I think the critical thing we need is a consensus to the finding. What are we talking about when we say Th2 bias for preclinical evaluation? This I think

is paramount to any safety determination of a vaccine for RSV.

So some form of Th2 bias has been reported in every model of enhanced RSV disease with the caveats that I just showed.

A few conclusions. I think it's important to see the lung, you need some sort of histopathology correlate, so bronchopneumonia and mucus production are important. You know when you are seeing these reports, sometimes alveolitis is present in 11 percent, 3 percent of the lung. So you need to look at the whole lung because otherwise you're going to see a terrible bronchiole and the rest of the lung is clean.

This is a semipermissive virus in most of these animals. I mean, the animals are semipermissive for the virus, I'm sorry. Increased airways resistance is a useful phenotype. I'll talk about alveolitis later.

So in principle, one of the few things that I think everybody probably agrees is they should not elicit eosinophils. These vaccines should not bias the response to Th2 compared to controls.

They should promote the CTL response, they should elicit a long-lived protective antibody response, but we need to get people's minds together and figure out what are we talking about exactly for all of these things? Because

I think it'd be a mistake to leave every single determination at the discretion of individual investigators. Not because of bad faith, but I may understand this one way and you may understand this differently, and I think for safety testing, it's a completely different ballgame. So I think this is very important.

So I know I had a lot of time. I don't know if I've already talked all my time, but I figure, well, this is a good chance for me to expound a little bit. I thought one of the problems with enhanced disease is also that we keep looking, waiting for this miracle factor that's going to explain it and there's no miracle factor. This is actually quite simple from my perspective because, well, atypical measles and enhanced RSV, these are not unique. There are probably many diseases that obey the same mechanism of illness that we see with atypical measles and enhanced disease.

So I'm going to take a detour, a little bit, to show you some examples of what I think are similar types of mechanisms of illness. This is a slide from the New England Journal of Medicine paper from Mexico when the pandemic influenza, when the pandemic influenza first emerged in Mexico early on. What was always striking to me

from the very beginning was that the middle-aged individuals were so severely hurt.

I was in Turkey, actually, got a call from a very close friend of mine who was the president of the Central Bank in Mexico and he was saying that his secretary had died and they were telling everybody to go home and the immediate assumption was, well, if a 30-year-old healthy woman is dying, this is going to kill all the children and all the elderly.

But it didn't. It goes for the middle-aged population. So these guys have seen something that kids haven't seen. We've done some work on that and we identified, actually, immune complexes here again in the lungs of people who died during the pandemic, in Argentina in this case.

We also identified them in the lungs of people who died in the pandemic in Tennessee in 1957, which was also archaeovirology, I guess, by John Williams, who is a super talented virologist who was able to rescue the 1957 virus from the lungs that had been at Vandy for like, I don't know, maybe 70 years.

Interestingly enough, if your first exposure is to a strain that not only elicits antibody but elicits cytotoxic T cells, your cytotoxic T cells are also going to be misguided. So what you see here is a CTL response, an

exuberant CTL response, against pandemic influenza. It's a memory response from the seasonal virus, but it's not working. You still recover virus from the lungs of these subjects. In fact, you see this in other papers that seem to indicate that this can be at least to an extent playing a role in pandemic influenza. Here are studies looking at CD55 which is a molecule responsible for modulating the immune complex response, and when it doesn't work, you get worse pandemic disease.

Here are four studies in Canada where immediately prior to the pandemic immunizing with the regular seasonal flu vaccine seemed to enhance the chance of developing severe pandemic disease. What people tend to think is that this may be a problem with antibodies directed to the stem of the hemagglutinin influence, which are binding but not neutralizing. So if you get these antibodies that target the wrong side, there seem to be recognition but you get into trouble.

At least, I'm not talking about the flu vaccines at all; I'm talking about flu disease. The same thing may apply to dengue hemorrhagic fever. Dengue hemorrhagic fever is the hallmark of getting one certain type of dengue and then coming and getting another one and getting into trouble.

This is one of my favorite papers, this paper by Gavin Screaton in England, and what you're seeing in that blue square is the abrupt cytotoxic T cell response, and he's done a lot of work, this is a Nature Medicine paper just showing you a little bit, where he shows these are low affinity CTLs primed for by the original dengue exposure of his subjects that are going after the secondary -- the secondaries are going after the secondary virus and associating with more severe disease.

We actually developed a mouse model. We've recently published dengue 1 and dengue 2. What you see in the blue are hematopoietic centers in the liver. The arrow in the spleen shows that megakaryocytes and all these show that indeed the CD8 T cells are critical when you prime for the wrong CD8 T cells to develop subsequently an enhanced form of the disease elicited by your secondary challenge.

So this is nothing but original antigenic sin. I mean, there's nothing new. It's every time the wrong sequence of events, which may hide under other diseases that we don't know.

So if you think conceptually under the paradigm of original antigenic sin, non-protective antibodies may emerge because you require affinity maturation. RSV, measles, you have a high affinity interaction with your

receptor, you need a good antibody to protect. Flu doesn't have that problem.

Antigens modified by treatment processes, you destroy your antigen in the vaccine. You use formaldehyde. Or you have a cross-reactive strain of live virus like flu or you have the wrong serotype sequence like dengue. So under cytotoxic T cells, they're absent after immunization in the context of formalin-inactivated RSV vaccine and measles. They may be mismatched in the other examples.

The T helper response, only in the context of inactivated and subunit RSV vaccines, and considering our definitions, we can say that they were biased to Th2.

That's not the case for flu or dengue or anything, so it is conceivable that these problems could emerge if you make the same mistake and you have another composition of cells playing, which I can think of, but at least it's there. So as I said, it may vary in other conditions.

Eosinophils just in general are bad if present. The stakes are too high. I would not sleep at night if I see an eosinophil in one of these things. Then I'll talk about alveolitis which is my last point.

About these animal models, we've been, I think the mouse, we've beaten the mouse to death. So typically we use 4- to 6-week-old females. These mice are

semipermissive for RSV. I think the BALB/c emerge because it's easy to use, it's a friendly mouse, doesn't bite you like Black-6, and female mice are particularly kinder than male mice, and they polarize to Th2 which is also useful, and they make eosinophils. If you do Black-6 mice, you're not going to see eosinophils, so that's why people prefer these BALB/cs.

You need to consider the vendor. I never had this experience because we always had the same vendor, but there are other investigators that are worth talking to, like Steve Varga, who used different vendors and some of the profiles that we're used to and familiar with may not repeat themselves if you change your vendor. Of course, part of the reason we are using mice is the level of sophistication that you can analyze things with.

Well, how about the cotton rat, which is every few years a big subject for discussion? I think this was the paper that led the discussions with cotton rats because I think what happened is Greg Prince recognized that we needed a model to discuss these things and to test vaccines with.

We were in the mouse field, basic pathogenesis, we weren't thinking these may need to test vaccines and give a yes or no and have a specific answer, and what Greg and subsequently Jorge Blanco found is that if you look at

alveolitis, and the orange line is comparing day four alveolitis in formalin-inactivated RSV recipients, these are cotton rats versus formalin-inactivated mock so that's cell lysate, you see a significant difference when you're scoring at the time.

So that alveolitis was present, it was present in mice, it was present in the original studies, so they seem like a good surrogate marker of enhanced disease in Greg's opinion, I guess, and that's how this got started.

So alveolitis had been there for a long time. This is one of those unreadable slides I put here because it's sort of blurry, but what you can see is that 11 percent of the alveolus surface in mice that receive the formalin vaccine has alveolitis. This is Brian Murphy's group, 1992. But if you look at RSV or you look at formalin-inactivated parainfluenza vaccine, it's not dramatically different. Live RSV infection will give you 6 or 7 percent. So it may be very sensitive, we'll go about it, but it's clearly not specific. And there's a trick, cotton rats have no eosinophilia so that's not going to help you.

This is a manuscript I particularly like a lot. I think it's a smart manuscript. It is work by the Novartis group. This is Shaw's paper in Vaccines, 2013, and the reason I like it is because this is something that

every one of us who worked in these models saw before. You just say, well, let's clean it and keep going, but they took the time to really nicely show an effect that should be paid attention to.

What you're seeing here, in fact, this is a Coomassie stain and what you're seeing, look at the numbers, different vaccines that will be inoculated into cotton rats. So formalin-inactivated cell lysate, formalin-inactivated RSV lysate, RSV, live RSV, and purified RSV or semi-purified RSV using a sucrose gradient. It's interesting, the amount of protein in micrograms that is present in all these preparations but the last one. So you have 35 micrograms of protein in the mock and of that, about a fifth, 5 of a 7th, 5 micrograms are albumin. You have a little bit less in the formalin RSV, but you have a lot. So you're going to be giving a lot of other things to these rats in addition to your vaccine.

That's going to have consequences. If you look at mice and rats that receive this formalin-inactivated cell lysate, they do have alveolitis regardless of challenging them with mock, again, or with RSV. If you use the RSV vaccine and you come back with mock, you're going to get significant alveolitis, moderate by these standards, which is going to get a little worse with RSV. RSV is going to essentially act almost as an adjuvant to the sort

of nonspecific protein response. I guess RSV has all these pattern recognition receptor agonists that will push the response or something like that.

Why am I saying that? Well, number one, because if you purify RSV, it gets a little better, or quite much better, and the vaccine substrate was never purified. They didn't do formalin-inactivated purified RSV vaccination, which I think would have been interesting.

What you see in this box is that the main driver -- well, this is hard to say, you shouldn't call it a driver, but the main cytokine associating with the enhancement of the pathology in the lung of these rats is interferon gamma production driven by the albumin. You look at the second column, where it says RSV, go all the way up, and you'll see that what we're saying as the worst possible alveolitis, the worst possible situation, comes from interferon gamma.

Conversely, if you use purified F protein and you do the same assay, you seem to have no alveolitis, and if F protein stops protecting the denature of the protein, you still have no alveolitis. But if you mix those things with cell substrate, you mix them with mock, you're going to get into trouble. Again, you're going to see alveolitis.

I think beyond the issue of is alveolitis any good for us to discriminate, I think there is a secondary

issue and it's that if you have a bad control, you're going to underestimate or overestimate your vaccine over and over. So a non-purified virus will lead you to think that there's an incredible amount of alveolitis in the formalin-inactivated RSV recipients and so I shouldn't worry because I'm seeing a little bit on this other side, or if your live RSV product generates enough, which it will because of course it's cell-derived and you're taking it from cell culture, then your vaccine may look like a negative control, but it's not a negative control. There's a problem with the assay.

Conclusions, I think I've said them all. There are a lot of nonviral products in vaccines and challenge virus. You need to clean it. No vaccine and mock or RSV challenge will generate alveolitis. FIRSV followed by mock or crude RSV generates the worst alveolitis. This alveolitis seems to particularly associate with production of interferon gamma driven by albumin.

If the inoculum is clean, F or denatured F do not generate alveolitis regardless of protection. This is important, the last point, because I want to go to the last paper which is a paper that was recently published looking at a very similar thing, alveolitis in cotton rats. I just want to make a few points. What I thought would be most

useful was comparing these two papers and showing you again how difficult it is to compare studies in the literature.

Everybody in the field talks about this paper and everybody in the field three years ago was talking about the other paper. It's interesting because what you see is that F in this case does generate alveolitis and mock, which are the orange arrows, I got ahead of myself, does not. So mock did not elicit virtually any alveolitis in this study, which was the main finding in the previous paper. The F protein, pre and post, elicited alveolitis, opposite of Shaw. Shaw was the first author of the previous paper.

This was actually quite interesting to me. I found this very, very informative. This, from my perspective, looks like a pretty attractive antibody response elicited by these adjuvants with the F proteins. It looks very much, and that's I think a good example, like the live attenuated measles vaccine compared to the inactivated product that you see below. Remember that inactivated is getting three doses, so it goes up, goes down, goes up, goes down, but it should go down after inactivation, after the first dose of inactivation.

So I thought, wow, this looks like a very good response, and even though it looks like a very good response, there are all these problems. The issue is, is

this truly showing us a problem, or is alveolitis enough for us to consider that we're looking at enhanced disease?

A similar phenomenon occurs with dengue. The dengue community has translated dengue hemorrhagic fever to antibody-dependent enhancement for years, and antibody-dependent enhancement does not explain the full picture of dengue hemorrhagic fever, so you get into trouble if you try to extrapolate. So I think alveolitis is clearly at least not enough. Well, this is indifferent.

Large animal models, well, I think the lambs and the cynomolgus macaques, are pretty attractive models. The reason I'm saying that is there are some studies in lambs who still need to clean your vaccine. There's formalin-inactivated herpes vaccines that elicit enhanced disease in lambs. So you need to be very careful about what you inoculate. The fact that the animal is like 70 pounds doesn't mean it's not going to get a response to your substrate.

The cynos also did, when they used formalin-inactivated measles vaccine, they got some degree of disease, but Rik de Swart in the Netherlands had a study that was actually quite attractive because the monkeys went on to develop full-blown enhanced RSV disease and died from enhanced RSV disease. That I thought was a pretty interesting model that was never further explored. I think

there was a caveat there that the lung pathology wasn't as clear or something like that, but I think they are definitely attractive.

The cows are really what people are thinking would be the most attractive thing for large animals. The problem with the cows is they can look great or they can not tell you much.

I remember the first time I ever went to an RSV meeting. Ruth Karron took me to Florida in 1999, and Laurel Gershwin presented a study -- I think it was 1999 -- where calves who were developing enhanced disease had no eosinophils. None.

I was so excited. I said, well, that shows us eosinophils are not important. Back to back, a Dutch group showed calves full of eosinophils. So you see all these things. So I think we really lack definitive models.

A few tips and I'll finish. Wait long enough to challenge your animal model or you'll be fooled by steric hindrance. If you do it too early, you're going to think your vaccine is better than it is. Clean your positive control, we said that, otherwise you will be testing dirt-mediated enhanced disease.

You need to think mechanistically and your threshold should be very low. This is, of course, I don't need to say that, but think mechanistically because there's

no magic. This seems to be a very, very logical pathway. It should have an explanation.

Do not rely on a single animal model to feel safe. Consider more, I don't know, I think size of animals makes everybody feel better. When I was doing monkeys, it was so easy to publish these papers, and then you move to mice, and people say, well, they're mice. So I guess if you get to a bigger animal, it carries more weight.

Beware of your positive control. It should be formalin and your negative control should be right if you're going to draw any conclusion. Otherwise, you're going to be fooled. Certain subjects could fare worse and you know, I was actually quite interested to notice that one of the children who died had bronchiolitis symptomatic disease at age 3 months and then went on to develop fatal disease. I have no idea what it means. It's an n of 1, but it was there.

Enhanced disease, atypical measles, DHF, do not require pandemic flu, of course. If it changes, you get a new disease. Do not be falsely reassured by your human seropositive studies. There is only one test. So this I think is one of the critical questions and I'm glad I'm not invited here to provide an answer, but I think, can you rely on a mouse to test a seronegative child? I think this is part of the elephant in the room, or lamb, or what

degree of information should you have to be confident that that is fair?

I think the other thing is standards. I think that the RSV community, we owe this to the vaccine community to be able to provide more clear guidelines on what we mean when we say bi-antibody or we say alveolitis or we say Th2. Thank you.

DR. EDWARDS: Thank you, Fernando. That was a tour de force. Thank you.

Questions? Luigi?

DR. NOTARANGELO: That was fascinating. I have a question and a comment. In the BALB/c mouse studies, if I saw correctly your slide, there were two routes of immunization that were used. One was IM, the other was IN, and there was a clear difference.

Both of them indeed result in a strong IL-4 production, but the IN, as opposed to the IM, was also associated with an interferon gamma production, so the question is, how important do you think is the route of administration of the vaccine in all of these?

The comment is about complementing T cells. So I think there is now clear evidence that anaphylatoxins can induce T cell activation, and so I wonder whether this has been evaluated in the context of ERD.

DR. POLACK: I'll answer the last one first. We did in fact, years ago, publish a paper looking at C3 and C5 and C3 and C5a, their role in enhanced disease. Of course, in the context of a mouse model, but C3a promoted the disease and C5 actually sort of inhibited the disease. So not having them was actually affecting them in opposite ways. Which that went in parallel to us, you say, findings by Marsha Karp and others about C3 and their role with Th2 and even Th17 profiles.

As for route of inoculation, I don't have the answer. I don't know. I don't know why, I mean, I looked at that -- quite frankly, the first time I paid attention to that was when I pasted it in my slides. So someone may know, I don't know.

DR. KOTLOFF: I am thinking about a vaccine for young infants and there are two things I'm wondering about. One is I know that there are age related changes in Th1 to Th2 bias in response to antigens. Two is that during, in this age group, there are concomitant vaccinations that are being used that could also drive that response. I'm just wondering what your thoughts are about concerns we might have related to those two aspects in vaccinating young infants.

DR. POLACK: Well, let me be very honest. I don't think I can provide any more insight than you can. I'd be

guessing. What I would say is that these two kids and most of these kids were immunized quite young, 2 to 7 months. That was one of the explanations for the age at which they contracted RSV and these two died at 14 and 16 months, so late compared to the typical epidemiology of RSV. I think that's a big warning sign. Children don't die of RSV disease at 14 and 16 months. So that's pretty bad.

But I don't think I'm the person to say -- I understand what you're saying and I think it makes sense, the Th2 bias certainly, but I don't think I'd be qualified to predict that, quite frankly. I agree, but I don't know what to add to your comment.

DR. TRIPP: That was a good talk. I would like to talk a little bit about original antigenic sin and RSV versus flu, because you know people are repeatedly infected with the same and different strains with RSV, and the courses are very different in RSV than the flu. Can you give me some interpretation of that?

DR. POLACK: Well, the only thing I can tell you is that the first thing we worry about -- when we did RSV, the way we conceptualize the different with flu, first from the vaccine standpoint, is that you're going to need the same type of affinity maturation to elicit protective responses for the interaction of flu with the cells.

Of course, we don't know it, but there's a specific receptor somewhere there for RSV or CD150 for measles that you need to interrupt that interaction. So that's the first difference why you don't see it with vaccination.

I guess the other is -- so there is an area that's a little bit conflicting about, but what I would say is when flu is not neutralizable but recognizable is when you get into these problems with flu. RSV is always at least partially neutralizable, I guess, but here that's all I can say.

DR. LONG: A question about your cautious inference that maybe a live attenuated vaccine could rescue if there was enhanced disease related to a new RSV as it did with measles. That would only potentially work if in fact the first vaccine did not enhance neutralizing or did not produce high neutralizing antibodies. Is that a correct assumption, and it also -- go ahead.

DR. POLACK: So, let me answer this in two ways. If you were to use -- this is something that has never been even validated in animal models. So I'm talking completely out of the blue. But if you were to think of something like this in the context of a monoclonal antibody, let's say palivizumab, you would never achieve protective titers of palivizumab in your nose. So you would be covered, say,

conceptually up to here, and of course I would be very scared, but what I'm thinking is if you get an RSV infection in the community even, and you have palivizumab, not only would you be protected from the problem, but you would reset your response from the upper tract. That's what I'm thinking. So if you were to allow a site where you're not going to get into the lung and have problems, that would be the idea. So that's one part of the answer.

As for live vaccines, well, we never faced it, but I would infer from these that from a formalin-inactivated standpoint, it wouldn't be a problem. That vaccine never worked. So you could do it.

A better vaccine. Yeah, you would face perhaps the type of situation that is faced with measles when you have antivirus floating around. It's very difficult to immunize.

DR. PORTNOY: I am intrigued by the importance of the time between when the vaccine is given and when the actual RSV infection takes place, and my question is if you study a vaccine, these vaccines obviously have to be given very early right after birth or within the first month in order to be protective at the time when the infants are most vulnerable. Is there a difference in the response if it's given early and then the infection occurs within a few weeks of the vaccine versus if it's three or four months

later, and has that been studied? How important is it the difference between when the vaccine occurs and when the infection takes place?

DR. POLACK: It may be important. It is clearly important in the animal models, because you have enough antibody that's not protected and but will cover the sites and will lead you to think that you're protecting. So you will be fooled into thinking you have a protective response if you do have a transient protective response. Measles did. So for a while, people didn't contract measles, and then when the response waned, started developing atypical measles. So yes, timing is important.

DR. MCINNES: Fernando, thank you very much. Terrific talk. As I recall, the vaccine from the late 1960s study was hyper-alum adjuvanted. By many fold, compared to what we look at now with alum adjuvanted vaccines, and we know from other alum precipitated products that it -- they in and of themselves bias to a Th2 response. So I think this characterization of the product remains an enigma with this story, and a lot of it is hearsay and people who have known people who were involved in the time, and I think the sort of provenance of that is still a little hazy.

You bring up a really interesting point about characterization of the animal models, and I think this

speaks to the entire effort now around rigor and reproducibility, and you mention BALB/c sexes and I think so many companies that are providing animals are not evaluating genetic background of these animals. They are not being genotyped. They are -- we don't know about them, and I think when you look at what is at stake here, every aspect of this is going to need to be dissected apart again, and there have been efforts.

I mean, I remember 20 years ago, we tried to have standard pools of challenge virus made, you know, all these efforts were made, but there were missing pieces as well, and so I'm wondering what you think would be helpful in trying to bring rigor and reproducibility to this next effort, which has got to take place. Otherwise we'll be another 50 years. I won't be here, but you know, some other people will be talking about it.

DR. POLACK: I won't be here, either.

(Laughter.)

So, to answer the first part of your question, I agree. I think many of these studies I showed had no alum, but I think it's fair to say that if you look at the lungs of the kids who died of enhanced disease, the main infiltrating cell are neutrophils. So it's an exuberant neutrophilic response.

In fact, until we did the staining, I had the autopsy reports which Greg shared with me, and neutrophils, neutrophils, neutrophils. There's almost nothing about eosinophils, and I was really surprised when Jamie stained the lungs and found these. So the problem with neutrophils is neutrophils are common with wildtype RSV disease, too. So what you can tell when you see them? Can you really discriminate anything? So that's why I think they are a little bit useless as a biomarker in this case.

But they are there. So I have no idea to what extent alum contributed. I think you can get this problem without alum. I'm sure, you know, there's been baculo-F, baculo-G, subunit, purified, all sorts of colors of things that have been used in the past, distant past, Brian's group particularly did a lot of work on that, and you still had similar problems. So yeah, there's a lot of noise.

The second, I agree. I think we need particularly some standard definitions, which are complicated. There may be knowledge outside the RSV field that may be necessary in many cases on how -- you know, it's clear that if we use a product we don't clean well, we see things that resemble what we see with our mouse or we get into trouble.

It's clear that if we challenge too early, overinterpret our vaccine, and if we challenge late the --

well, you know. There's a million of these things. So I think a concerted effort to look at each of these questions and formulate how to solve them would be very -- I particularly think it would be easy also for vaccine companies, because there's so much at stake that you -- so that's entirely one issue, and the other issue is if all this is going to make enough information at the time and that's another decision, to see how to make a decision on these vaccines, which is actually -- it's going to be essentially from an animal model to a 2-month-old baby. That's what's going to be.

DR. GREENBERG: Given that you spoke of a number of different animal models, is it your recommendation then that because each model has its own uniqueness, limitations, immunologic characteristics, that multiple models should be used?

DR. POLACK: Yes, I think in part the confusion comes from what I said at the beginning. I think we need to know what the models can inform us about and what they cannot, and see to what extent this information is enough. So today I wouldn't say any model is a bona fide model you can test and you're safe, use your vaccine. That's another situation.

So the context is the cleanest possible information with the most possible variables. I don't know if that's achievable, but that's what I think.

DR. GREENBERG: You spoke also a lot about the controls. In a sense, does a live attenuated RSV become an essential control, or is it immaterial?

DR. POLACK: Well, the live infection I think is approved something that's not going to generate enhanced disease. So that is a good -- so you have a live RSV inoculation. I think we have a lot of experience in intranasal inoculations in children. There's even intramuscular trials at some point. We don't see enhanced disease. So this is informative, but you know, then again, in the context of having the purified virus that you can use, and I think there's context to every control, but you do need your negative control. You do.

DR. EDWARDS: Thank you, Fernando. That was amazing.

Okay, we will have a break until 11. So drink your coffee quickly, and then we will be back at 11.

(Brief recess.)

Agenda Item: FDA Presentation

DR. EDWARDS: If people could take their seats, we will begin the next presentation. This is by Sarah Browne, a medical officer at FDA/CBER and Office of the Vaccines

Research and Review. Sarah will talk about the development of vaccines for the prevention of RSV disease in RSV-naive infants.

Sarah?

DR. BROWNE: Thank you. So I am going to speak about the development of vaccines for prevention of RSV disease in RSV-naive infants and try to outline the FDA perspective on these issues. So the goals of my presentation are to provide an overview of RSV disease in prevention, a brief overview, review different RSV vaccine approaches, and summarize immunologic mechanisms proposed to underlie vaccine-associated enhanced disease, to review recent workshops that addressed RSV vaccine development, and to discuss types of supportive preclinical and human data and the potential design of initial studies in RSV-naive infants.

So as Dr. Gerber has pointed out and Dr. Roberts, we know RSV infection has a great impact on the health of infants and young children. Treatment is largely supportive. There have been passive immunization approaches that have been shown to confer protection against RSV disease, namely RSV Ig preparations, RespiGam, and subsequently the humanized anti-RSV F monoclonal antibody, palivizumab, which is licensed for the prevention

of serious lower respiratory tract disease caused by RSV in children at high risk of RSV disease.

So those observations that passive transfer of antibody can confer protection really provided supported for RSV vaccine development and accordingly, there are many products that are currently under evaluation. Really for three main target populations, adults 60 years of age and older, pregnant women, and infants, which is the topic of this advisory committee meeting.

And really with a focus on these observations of enhanced disease. So clearly described by Dr. Polack as we heard, these studies occurred with the administration of a formalin-inactivated vaccine in infants back in the 1960s, and there was not only an increase in proportion but there was also an increase in the severity of severe RSV disease in infants who were previously immunized with the formalin-inactivated vaccine compared with those immunized with the control vaccine.

These observations largely redirected vaccine development at that point towards understanding the etiologic mechanisms underlying these observations of enhanced disease, which led to the potential mechanisms being described as Th2-dominant cytokine responses and absence of RSV-specific CD8 positive cytotoxic T lymphocytes, immune complex deposition in the lungs, and a

low-affinity antibody response with minimal neutralizing activity.

So in the context of evaluating vaccines in animal models, a number of different protein targets have been considered. The surface proteins G and F and then internal proteins SH, P, N, and M2. Some of these antigens have been shown to confer protection in mice, at least partial protection. F and G have shown complete protection in mouse models, N and M2 perhaps partial protection, and then some other factors that may be protective against enhanced disease such as a neutralizing antibody response and production of CD8 positive RSV-specific cytotoxic T lymphocytes have also been looked at.

The antigens of course have to be considered in the context of the vaccine approach being utilized, and here on this table we can see all the way to the left, the FI-RSV vaccine, which was shown to clearly cause enhanced respiratory disease in infants and toddlers who received that vaccine in the 1960s, and then at the left side of the table, what we see are the live attenuated products, which have already been tested in seronegative infants, and there's been no evidence so far of enhanced respiratory disease in those subjects.

Then in the middle, it's a little bit blurrier. There's the protein and the peptide subunit products.

There are the gene-based and vectored products. There are many of these different approaches. In the case of the protein and peptide subunits, some have shown enhanced disease in animal models. In the case of the gene-based and vectored approaches, many have not shown enhanced disease in animal models.

But I think it's important to recognize that there are many different approaches within this middle area of the table in the context of many different antigens being delivered, and so each of these different approaches likely needs to be evaluated on a case-by-case basis.

So we already saw more of this figure than I'm showing by Dr. Polack. But there are a couple things I wanted to emphasize and point out. First you can see the evidence for alveolitis as a marker for enhanced disease in cotton rats after immunization and challenge with RSV, is similar to the FI-RSV vaccine here. You can see it over here as well.

This is with decreasing antigen dose. So the things that I want to point out is that it's regardless of the adjuvant that is being administered here, whether it's Th1 or Th2 biasing, and then what you can see down here where there's minimal alveolitis at the very lowest antigen dose, there also didn't appear to be much vaccine take.

In another part of this paper, there is a figure showing that higher doses, a tenfold higher dose of these vaccines administered did confer protection and didn't show alveolitis, suggesting that these vaccines really need to be titrated to find the place where you can identify enhanced disease.

This figure may also look familiar. This is a paper suggesting that these evaluation of these animal models is complicated and that cellular components alone can induce alveolitis in the cotton model. So if we quickly walk through it again, interestingly in this study, the F protein alone, compared with the previous study, did not induce alveolitis compared with no vaccine and subsequent RSV challenge, and then you can see irrespective of whether you formalin-inactivate the cell supernatant or you don't formalin-inactivate it or you include the antigen or you don't include the antigen, you can see a signal for alveolitis in the cotton rat.

So moving on to conferences that have recently occurred to discuss issues specific to RSV vaccine development, there was the WHO conference in March of 2015 and what came out of that was a draft -- one of the things that came out of that was a draft document that provided the following perspectives, namely safety data in adults and RSV-experienced children 12 months to 5 years of age,

should proceed evaluation of RSV-naive infants, that studies in RSV-naive infants should extend over two seasons for efficacy, cross-protection, and durability of immune response.

And they also propose case definitions as clinical endpoints for field efficacy trials for both severe and very severe RSV lower respiratory tract infection, and those endpoints used RT-PCR testing, SpO2, pulse oximetry, and clinical signs of respiratory distress.

The FDA and NIH a couple of months later cosponsored another conference that delved more deeply into the science of enhanced respiratory disease, and some of the key concepts that emerged from that discussion included no single animal model demonstrates all features of FI-RSV-associated enhanced disease in infants, that a Th2 biased immune response after challenge of immunized animals is consistently associated with enhanced disease, that a high magnitude of antibody response with poor neutralizing activity may be causally related to enhanced respiratory disease, that RSV-specific CD8 positive T cells mediate viral clearance, but they can also mediate immunopathogenesis in the setting of high viral loads and low neutralizing activity of antibodies, and finally although lung eosinophilia is probably not causal, as has

been mentioned before, it's probably a marker for a Th2 dominant cytokine response.

So here we are. We have animal models. We have expert opinion, and we have new vaccine technologies. So what I would like to do is walk through use of these preclinical models to move into human models, ultimately to support introduction of vaccine candidates into RSV-naive infants.

So the idea is that extrapolation from animal models that have been developed to understand enhanced respiratory disease might be used to inform the risk of ERD in humans. But there are a number of unique considerations when evaluating these preclinical data, including the specific animal model that is being used, the vaccine dose. As we discussed on an earlier slide, that titration of the vaccine may be critical to identifying a signal for enhanced disease in those models. The timing of the RSV challenge relative to immunization, and then fundamentally establishing criteria for enhanced disease in those animal models.

Moving into clinical studies in adults, they can assess the general safety and reactogenicity of the vaccine candidate, but probably cannot assess a risk for enhanced disease. Human challenge models also might have utility in downselecting for promising vaccine candidates and also to

help identify correlates of protection, and finally, it's worth mentioning that immune responses in adults represent a boosting of preexisting immunity and therefore may not predict a protective immune response in RSV-naive infants.

Next to discuss RSV-experienced infants. Like with adults, you can assess the reactogenicity and general safety of a vaccine, but the usefulness in assessing risk of ERD is less certain. Furthermore, persistence of maternal antibody may inaccurately imply RSV experience, although this is less likely after 6 months of age, due to waning of maternal antibody in the newborn.

So using those data to support the potential for moving into RSV-naive infants, we have some considerations around this and some potential approaches to address those considerations.

First, the risk of ERD might be higher at younger ages. So studies in RSV-naive infants greater than 6 months of age might help to predict the risk of ERD in younger infants. We know that enhanced disease was observed in infants immunized at older than 6 months of age in those initial studies conducted in the 1960s, suggesting that if it is a phenomenon, we will be able to see it in that older less vulnerable population.

Secondly, in this older age group, the RSV serology is more likely to be reflective of true RSV

experience, whereas in younger infants the serology could be confounded by presence of maternal antibody.

Next, there's the consideration that vaccine-associated enhanced disease may not be clinically discernible from naturally-occurring severe RSV disease. So one approach to addressing this would be to simply evaluate the relative risk of severe disease between the vaccine arms and the control vaccine recipients.

Finally, the risk of ERD may increase as immunity wanes, and one approach to this issue would be to follow subjects past one RSV season or at least until their first documented RSV infection. We know that RSV, that enhanced respiratory disease can be seen in toddlers.

So in summary, prevention of RSV disease in infants is an important public health need worldwide. Observations of the FI-RSV vaccine-associated enhanced disease in RSV-naive infants have presented a challenge to the development of safe and effective vaccines for infants.

The proposed immunological mechanisms is a dominant Th2 response, immune complex deposition in the lungs, low affinity antibodies, and a lack of RSV-specific cytotoxic T lymphocytes.

Animal models that can mimic some features of ERD are being used to assess risk of ERD in vaccine candidates. Studies in adults and RSV-experienced children might

provide support for those initial studies in RSV-naive infants using an age-de-escalation approach. Finally, the types of supportive data and study design may be product specific depending on the parameters of that and the theoretical risk of enhanced disease carried by that approach.

Thank you.

DR. EDWARDS: I will ask the first one. I think you brought up the point of the presence of maternal antibody, and we know repeatedly that maternal antibody has a big impact on infant responses. The complexities of perhaps immunizing pregnant women with RSV and then studying their -- and then immunizing babies with the vaccine does seem to present some challenges. Have you thought about that or what concerns might there be?

DR. BROWNE: I think that there certainly could be concerns around those competing approaches potentially. I think we know there's increasing evidence that the risk period for severe disease and the morbidity associated with RSV may extend past, well past the time when maternal antibody is thought to be present at high enough levels to be protective.

So in the end, they may turn out to be complementary approaches. I think that under six months of age, because of persistence of maternal antibody,

irrespective of prior vaccination or not, using an IgG sero-status in that population is not going to be helpful to assess risk.

DR. MONTO: Just taking it a bit further, I can see a scenario where maternal immunization might come in before we have a vaccine for children, and given the fact that much of the severe disease occurs before 2 months of age, a maternal immunization strategy would seem to be a very good one to follow. But we are going to have a situation where you're going to have some children who have received the -- whose mothers will have received maternal immunization and others who haven't, and this may be not only an immunologic but a policy dilemma that we are in.

What should be done? Should they be different rules or different vaccine schedules for those who have been maternally immunized, whose mothers have been immunized, and some who have not? So I think we are going to have to think about that right now as we start moving forward.

DR. LONG: Another consideration I think is what's the primary goal? Is the primary goal to prevent the relatively small number of deaths, and if that's the case, that happens so early in otherwise healthy children that it's hard to imagine any immunization schedule will protect those children. So it may have to be a little bit more

like pertussis. Prevent deaths, maternal, prevent burden of disease, would be a later vaccine, but they may be two different strategies depending on the goal.

DR. GREENBERG: I am just trying to put together a couple of the comments that have been made today. One is you remarked that seronegative infants over 6 months of age might be an intermediate step in terms of evaluation, but yet the oldest child who developed severe enhanced disease was 18 months of age, and I don't know what age that child was immunized with the formalin-inactivated product, but can you just help us understand a little bit; is 6 months magical? Again, does it matter when they received formalin-inactivated versus when they were exposed?

DR. BROWNE: Sure. So in those four studies, I believe three of them, the youngest age of immunization was at 6 months of age, and infants in those studies went on to develop severe RSV disease after infection in an imbalanced fashion, suggesting that enhanced disease can be seen in older seronegative infants. Albeit the most, the strongest imbalance, was in the study where the infants were between 2 and 7 months of age.

So the rates could conceivably be higher at the lower end of the spectrum. I mean, I'm handwaving now, but there's the immunologic immaturity of those infants, there are the smaller airways, all of these things that may

contribute to a higher either risk or rate or severity of enhanced disease as you get down into lower infants.

So the 6-month cutoff, I think to some degree is somewhat arbitrary and maybe programmatic. It's discussed as a time when we can be confident that the serological status of the infant is really reflective of RSV experience so we're not going to be misclassifying infants in that population, and they may be at slightly less vulnerable to the negative consequences of a vaccine the older that they get.

DR. PORTNOY: I'll preface this by saying I am not an infectious disease expert. I'm an immunologist. But I don't know where the reservoir for RSV the virus itself actually is, but we have been discussing the goal of trying to protect these young infants from having severe RSV disease; has there been a consideration to perhaps trying to create herd immunity by widespread vaccination, perhaps in adults, in other individuals, kind of like the way we did with haemophilus influenzae, where it protected the infants, but ultimately the carriage rate became so low that the kids just weren't exposed to the infection to begin with. Has there been a consideration of perhaps taking that approach with RSV?

DR. BROWNE: I think that would be challenging, because RSV infection occurs, reoccurs frequently

throughout life and adulthood and childhood. So vaccination -- you know, that middle age of the population from RSV-experienced children to young, younger adults, are really not a target population for immunization because they have already been infected with the sort of best, repeatedly infected with the best potential vaccine, which is the RSV infection itself over and over again, and they still get sick. So I think that induction of herd immunity may not be the best approach in that regard.

DR. NOTARANGELO: Well, I fully share the comment about maternal immunization as being the best strategy to prevent early death due to RSV, but we need to consider also the seasonality of these infections. So month at delivery will matter, and this is further complicating the issue.

DR. BROWNE: Can I just point out that maternal immunization at this point is certainly a very encouraging or exciting possibility, but it is still yet unproven, and I recall from one of Dr. Gerber's slides that there still is considerable impact on health of children beyond the timepoint, although the most severe disease we saw was at zero to 2 months of age, that there still is a significant public health impact in older infants as well when maternal antibody may not be as protective.

DR. EDWARDS: The Hall paper basically said that if you were a baby it wasn't a good idea to be born in October or November. So I don't know whether we can have some public health policy to sort of --

DR. MONTO: Which we said in the first paper we wrote about RSV from our Tecumseh study.

(Laughter.)

DR. EDWARDS: Thank you very much.

Our next presentation will be from GlaxoSmithKline by Ilse Dieussaert, who is the director and lead vaccine development of maternal immunization with GSK.

Agenda Item: GlaxoSmithKline Presentation

DR. DIEUSSAERT: So good morning, everybody. My name is Ilse Dieussaert, and I'm leading the RSV development for both maternal and pediatric RSV vaccines at GSK. I would like to thank the FDA and the members of the advisory committee for giving us the opportunity to present to you today the GSK's pediatric RSV program.

So what I would like to cover in the next 20, 25 minutes is show you some data that we have generated as part of our pediatric program, and discuss some of the challenges that are ahead of us and how we can potentially mitigate those.

So before we dive into the challenges, I would like to give a topline overview of our candidate product

profile. So the global intent is the active immunization of infants for the prevention of lower respiratory tract infection and illness caused by RSV. For that, we are planning to vaccinate infants as early as possible in life, so from 2 months onwards, with two doses of our vaccine. This in coadministration with routine pediatric vaccines.

As vaccine composition, we have selected a recombinant adenovector that is coding for three antigens, the F, the N, and the M2-1. At stage of development, we have completed our phase I first time in human clinical trial in healthy adults, and we have launched a dose-escalation study in seropositive infants. So that study is currently ongoing.

Now there are many challenges that are ahead of us, and most of them are linked to the development of pediatric vaccines, new developments I would say in the pediatric space. One of them is definitely the early burden of disease that requires vaccination very early in life, and I think we heard a little bit about this earlier this morning.

We have an immature immune system, presence of maternal antibodies, and there is this crowded pediatric schedule that can lead to implementation hurdles and potential interference either on the routine pediatric vaccines or also on the RSV components. So all of this

will need to be evaluated very carefully during the development.

Now, there is one challenge that is really unique to the development of RSV pediatric vaccines, that that is the history of the enhanced disease that we heard about this morning. Now the history of enhanced disease has impacted the overall development at many different stages, and what I would like to do with the rest of the presentation is select three of those and go in a little bit more detail. So I would like to talk about the vaccine candidate selection, the preclinical assessment, and the clinical development.

Now the first stage that is heavily impacted by enhanced respiratory disease is the selection of the candidate vaccine, and this happens very early in the development. Now GSK has selected a chimpanzee adenovirus 155 to mitigate the risk of enhanced respiratory disease. So for the rest of the presentation, I'll refer to our lead candidate as ChAd155. So the ChAd155 vector is a non-replicative adenovector that is coding for three antigens, the F, the N, and the M2-1.

We believe the adenovector to be the right toolbox to develop in this target population, as it can induce the appropriate immune response. We have this intracellular expression of the RSV antigens, as is with

live RSV, and we have an induction of a more Th1 induced immune response or at least a more balanced Th2/Th1 response. We believe that the vector is capable of controlling viral replication by inducing neutralizing antibodies. That is triggered mainly by the F antigen in the vector, and by induction of CD8 T cells to clear infected cells, driven by the three antigens, and mainly also by the N and the M2-1, who are internal antigens.

Now the second stage that is really impacted is the preclinical assessment, and we heard quite a bit about it already this morning. Now GSK has generated a large comprehensive dataset in small and large animals, because there is not one single animal that can adequately predict the risk of vaccine-related enhanced respiratory disease in humans.

Now as you can see from the table, we have evaluated different animal models. So the mouse model, the cotton rat, and calf, and they all have their advantages and disadvantages, but they all bring complementary information, and it's really bringing the results together from all these different animal models, and when the results are consistently pointing in the same direction that the vaccine does not show any sign of induction of enhanced respiratory disease, that you can be confident and

reassured to move forward in the next stage of your program.

So what I would like to do is go through each model and show you some data that we have generated as part of the program.

So the first model is the rodent model. So the mouse and the cotton rat. So these are semipermissive models and they require high challenge dosages. They do not reproduce clinical signs of lower respiratory tract disease but are commonly used for the evaluation of enhanced pathology by the use of surrogate markers. So for mouse and cotton rats, you can look at viral reduction post-challenge in the lungs. You can look at Th2/Th1 balance in the mouse model, in the mouse model lung histopathology looking at goblet cells or eosinophil infiltration in the lungs. For cotton rat, you would typically look at the alveolitis scores.

Now the animals are vaccinated with two doses of our vaccine, three or four weeks apart depending on the animal model, and then we challenge them. It's a homologous challenge with human RSV either two or three weeks after the last vaccine dose.

The groups we have evaluated in these experiments obviously is our candidate vaccine, ChAd155, the FI-RSV group, which is considered to be the benchmark for enhanced

pathology, live RSV, which is considered to be protective and not to induce enhanced pathology, and a placebo group.

So in the first set of results I show you coming from the mouse model, prior to the challenge -- so you can look at some immunological parameters such as T cell responses and functional antibodies that are induced by the vaccine, in panel A I'm showing you the vaccine induced CD8 T cell responses circulating in the blood. So you can clearly see that the ChAd155 vector -- and this is the first group in panel A, is inducing an M2-1 specific CD8 T cell response after challenge, while the other groups that are tested do not.

In the second panel, panel B, I show you the neutralizing responses. Again, you can see that the ChAd155 vector is able to induce neutralizing responses, which are about in the same range as the neutralizing responses induced by the animals that were vaccinated with live RSV vaccine.

Now the overall level of the neutralizing response, as I like to point out, is low. However, if you challenge the animals, you can see -- and these are the results that I show you in panel C, you can see that the animals in the ChAd155 group and also in the live RSV group have a complete reduction of viral replication in the

lungs, while this is not the case with the FI-RSV vaccinated animals.

So already from this first dataset you can see that the ChAd155 vector is behaving differently from the FI-RSV vaccine. Now that is not enough to exclude that the vaccine could induce enhanced pathology. For that we need to look at other markers.

Now the results I show you here are post-challenge in the lungs. So post-challenge, one of the hallmarks you can use for enhanced pathology is the Th2/Th1 ratio. So we know that in animals that were vaccinated with FI-RSV, upon challenge you have a skewing of the immune response toward a Th2 bias. So it is associated with CD4 Th2 bias. These results are consistent with what I show you in panel A. You can see the FI-RSV group, which is the second group on the graph, has a skewing of the immune response toward Th2 bias, while this is absolutely not the case for the ChAd155 or the live RSV vaccinated animals. You clearly see a Th1 bias then.

Now in addition to the Th1 bias, the ChAd155 vector is also able to induce interferon gamma CD8 T cell responses, and these are the results that are shown in panel B. So in panel B, you can see that the ChAd155 vector as does live RSV is able to induce interferon gamma CD8 T cell responses post-challenge.

Now another hallmark for enhanced pathology is looking at mucus-producing cells or eosinophil infiltration in the lung, and these are the results that are given in panel C. So in the first graph, I show you the mucus-producing cells, and you can see that the ChAd155 vector is producing significantly lower levels of mucus-producing cells than does the animals that are vaccinated with FI-RSV. The same pattern you can see in the eosinophil infiltration in the lung. The ChAd155 vector is having lower, significantly lower, levels of eosinophil infiltration in the lung when you compare into the FI-RSV group.

I forgot to mention that for the Th2/Th1 ratio, we used as Th2 marker the IL-13 and for Th1 interferon gamma.

Now if you put all the results together from the mouse model, at least in this model the vector is not -- our lead candidate is not inducing any sign of enhanced pathology.

Now the next slide I show you some results in cotton rats. So the results here is a dose ranging study that we performed in cotton rats. So we have lowered our dosage of the vector up to the level where we found some viral replication occurring in the lungs post-challenge.

So we started with the highest dose, 5 10^7, and we went down to 1 10^6, and you can see that in the last two groups -- and that is the result that is shown in panel A, that in the last two groups you see some viral replication occurring in the lungs after challenge. Nevertheless, if you vaccinate -- if you challenge the animals, and these are the results I will show you in panel B. When we look at the alveolitis scores, the alveolitis scores for all groups in the ChAd155 vector have significantly lower levels of alveolitis scores than the animals in the FI-RSV group.

So even despite some viral replication occurring in the lungs post challenge for more suboptimal dosages of our vector, it still does not induce any sign of enhanced pathology.

Now the last model I would like to discuss is the calf model. The calf model has proven to be an effective model for the detection of enhanced respiratory disease. It shows high similarities in the epidemiology and the pathogenesis of bovine RSV in calves and human RSV in infants. It has a high level of genetic and antigenic similarity between bovine and human RSV.

Interesting in this model is that it is fully permissive for bovine RSV. So you only need low challenge dosages that are required, and this is contrary to the two

other models where you need these high challenge doses. So you really mimic natural infection and natural progression of disease. This is also a unique disease model where you can directly measure clinical signs, and you do not only depend on surrogate markers as it is in mouse and cotton rats.

So the study design of these experiments, you have young calves of six weeks old, and they are vaccinated with two doses of our vaccine. Here we used a dosage 5 10^10. This is the highest dosage that we use currently in our human trials.

After vaccination, we challenged the animals, and here in the results, I will show you we have used two different challenge timings. So some of the animals were challenged one month after the last vaccine dose and other animals were challenged four months after the last vaccine dose, and we did that because we wanted to evaluate the impact of waning immunity before challenging and see whether the animals were still protected.

Now after the challenge, so after infection, we closely -- we intensively monitor these animals for 12 days post-infection. We daily look for clinical signs. We take nasal swabs and bowel samples for viral load. We look at inflammation, and after the 12 days post-infection, we

sacrifice the animals and collect the lungs and perform gross examination and histology.

So the results I will show you here are the groups evaluated are our lead candidate and a placebo group. So here I show you the clinical signs that we have generated in this model. So I show you fever, general illness, and respiratory rate, which is a benchmark of lower respiratory tract disease.

Now the red lines are the placebo groups. So we have two placebo groups according to the two vaccine challenge regimens I should say, and the black lines -- so there are two black lines -- are the animals that have been challenged according to the two different challenge regimens, either one month or four months post last dose.

You can see that there is a peak occurring around day 7, day 9, in the placebo group, and this is the case for fever which is shown in panel A, for general illness and panel B, are the respiratory rate and panel C. This peak is absent in the animals that were vaccinated with our ChAd155, suggesting that they are protected against the clinical signs and clinical manifestations.

We looked at the viral load, and again, here, the same picture. So the red bars are the placebo groups according to the two different challenge regimens. The black bars are the ChAd155 vaccinated animals, and you can

clearly see that again a peak occurring at day 7, 6, 7, 8, in the placebo groups, and significant reduction of the virus load in both cases, either we look in panel A in the bowel samples or we look at the nasopharyngeal samples. We have significant reduction of the viral load in the animals that were vaccinated with the vector.

If we look at lung histopathology, again the same. We have significant reduction of the consolidated lung area in the ChAd155 vaccinated animals. This is also true for the alveolitis score, a significant reduction in the alveolitis score in the ChAd155 vaccinated animals compared to the placebo group.

Now in this model we have also evaluated neutralizing responses. So this looks a bit complicated because we have challenged all the animals at the same day, but as I said before, we wanted to evaluate the challenge according to two different timepoints, either one month after the last dose or four.

So we have vaccinated the animals at different timepoints prior to the challenge. So the blue arrows are the animals that have been vaccinated four months prior to the challenge, and the green arrows are the animals vaccinated with the two doses one month prior to the challenge.

Now the kinetics of the immune response at least in neutralizing response is the same for the two groups. You can see that the first dose only gives you a marginal increase in the neutralizing responses. It's really the second dose that is boosting the neutralizing responses up to high levels.

Now in the group, as you can see, that has been vaccinated four months prior to the challenge, you do see waning immunity. You do see the neutralizing responses go down. But what is interesting is when we challenge the animals and they all have been challenged at the same day, whatever the group and whatever despite the waning immunity, we see an anamnestic response. So I think it is interesting to address this is that even with a delayed challenge, you do see anamnestic response in the animals despite waning immunity up to the same levels as the group who was only challenged one month after the last dose.

Now what are the key messages? Yes, we have generated a very comprehensive dataset. This was a snapshot of the results that we have generated, and we have used different animal models to do that. We used mouse, cotton rat, and the calves, and again, as I said before, they all have their advantages and disadvantages, but they all bring complementary information, and the power thing of

using different models is when all results point toward the same direction, that you can be reassured to move forward.

I want to point out that we felt that the calf model was of particular interest because it's the only model that can have a direct measurement of clinical manifestations of lower respiratory tract disease. So that was for us an additional comfort to use that model before moving into the clinic.

Now the third stage that has been heavily impacted is the clinical development. So this is a topline overview of GSK's clinical development plan, and as you can see, it contains many steps, starting with the preclinical package we just discussed and then moving into first time in human phase I study in healthy adults.

So we have completed the first two steps of the development and we are currently running our first trial in toddlers older than 12 months old in seropositive infants, and I would like to point out here that infants were screened for seropositivity.

Now once you generate data in seropositives, you can further age deescalate into the 6-month-old seronegative infants to finally reach the target population of 2-months-old RSV-naive infants before launching a phase III, which is a huge undertaking and where you will expose large number of subjects.

Now as we gradually age deescalate from a less vulnerable to a more vulnerable population to the consequences of enhanced respiratory disease, we do increase the confidence and the safety profile of the vaccine with every step we take. Important to note also is that we have set up an independent data monitoring committee or a data safety monitoring board. It's basically the same thing. With members that have committed to stay with us as from the first trial in seropositive infants up to the end of phase III, and we really wanted to establish that in order to have a consistent overview of the safety profile of our vaccine. So that was an important safety parameter that we put in place.

Now what does it take finally to get into those RSV-naive infants? So I said before, you need to have the convincing preclinical dataset. Then move forward into the first time in healthy adults before moving into the seropositive population, and said before, infants are screened in this first seropositive study. Since all subjects in this population have been previously primed, they are considered to be at low risk for enhanced respiratory disease.

So this is the population where we can test the highest dose levels of our vaccine where we can look at safety and tolerability of our vaccine, where we can

potentially deselect some of our vaccine dosages before we move into a more vulnerable population. We can look at immunogenicity, but then I think we all agree that this will not be truly representative of what we may expect when we go into the target population when the truly RSV-naive infants. Nevertheless, it can give you an idea of your vaccine take.

Again, important to point out, there is an independent data monitoring committee that has been set in place and doing the safety oversight independently from the company.

Now once you generate data in a safety data tolerability data in the seropositive population, you can then further age deescalate. We are doing it in two stages in healthy full-term infants, naive infants, first in the 6 months old, which is already closer to the target population, but still less vulnerable to the consequences of enhanced disease. And then later on in the 2 months old, and this is the first time you will give the vaccine in coadministration with routine pediatric vaccines, and also in potentially the presence of maternal antibodies. So again, this is why we have this careful age de-escalation.

Now whatever the study, clinical studies must be conducted with maximum care. It has to be done in settings

with availability of advanced medical care but also with accessibility to that medical care. We have set up active surveillance for RSV infection identification, and we will be closely looking up to the progression of disease in these first studies.

We will document the clinical parameters of RSV disease to see whether we can detect difference in patterns on severity. We keep 1:1 randomization throughout the program. We follow up the infants for two years in phase II, and we have decided to use the WHO case definition that was referred to earlier today.

This is where we will measure the immune response, see how our vaccine is performing in terms of immunogenicity, and so all these steps have to be put in place. So we have to maximize the potential to de-risk enhanced respiratory disease before we launch large phase III studies, and it is this delicate balance between how many subjects, how many volunteers do we need to expose prior to phase III so that we can conclude on the risk of enhanced disease and not overexpose them.

So in conclusions and key messages. So GSK's primary goal is to ensure the maximum safety of the subjects in every single step in the development. It is all about patient safety first. So yes, the risks of enhanced disease are there, but there is potential to

mitigate that risk at different stages in the program, either the risk through the vaccine candidate selection, that should be designed to elicit appropriate immune response, or the preclinical assessment and generating a large dataset in the relevant animal models, and then definitely the clinical development where there is a careful age de-escalation and intensive disease monitoring and also putting in place independent data monitoring committees to do the oversight of safety.

For the GSK asset, the current preclinical package does not show any evidence that the vaccine would induce enhanced respiratory disease, and the approach that we propose is quite conservative in the clinical overview, but it will increase our confidence as we go along.

So together we believe that this approach is supporting the evaluation of our ChAd155 vector in the RSV-naive infants.

Thank you.

DR. EDWARDS: Thank you very much. It's open for questions. Yes, Ruth?

DR. LYNFIELD: I was wondering if you could describe what is involved in the active surveillance of the vaccinated children to be looking for disease.

DR. DIEUSSAERT: Yes, so in these first trials, we will actually take swabs every -- I don't know where the

question came from; oh, sorry -- we will take nasal swabs every month and then we will follow; we will document all clinical parameters, follow up the infants very closely, and see how they or not progress in disease severity. So it is really even identification of RSV infection in these very first trials.

Now this is not something that we will be doing later on, but --

DR. SAWYER: At the risk of asking an immunologically naive question, since the biggest burden of illness is in very young infants, is it clear that we cannot immunize at birth? Why do you -- why have you decided to immunize at 2 months?

DR. DIEUSSAERT: Yes, I think that's a really good question. We have talked about that. For the moment, I think we stick to the 2 months old. I think it is probably possible. I think it would be more wise to have some level of efficacy maybe in older children before really going down to the neonates I would say, but it's not that we close the door for that. It's definitely something that we can evaluate, but maybe not at this very early stage of this program.

DR. LONG: Not knowing anything about the kinetics of the cellular response versus the neutralizing antibody response, et cetera, I was impressed in the calf that there

are no neutralizing antibodies after one month. Is there any concern that within the month after the first dose of this vaccine, maybe also thinking we don't know everything about enhanced disease, that that might be a time in which there might be enhanced disease?

DR. DIEUSSAERT: Between month 1, month 2?

DR. LONG: Zero and 1. When you don't yet have neutralizing antibodies but do have something related to vaccine.

DR. DIEUSSAERT: What I have not shown, because I had to make a selection of data that we have generated, we did do the calf model with the one dose vaccine. So with the one dose schedule, and we saw also that the animals were protected. So despite the fact that the neutralizing response is almost non-existing after the first dose, the animals are protected. But I haven't shown these results, because I had to make some selection, I'd say.

DR. EDWARDS: Two questions. First of all, could you just remind us the structure of the F protein that you are using in the vaccine? Then secondly, whether you could tell us a little bit more about the immune profiling that you are planning on doing in the infants?

DR. DIEUSSAERT: So, the F protein is a post-F, because it has been deleted from -- its transmembrane

region has been deleted, secreted. So it probably will switch into an F, post-F fusion.

The cytokine profiling. So in the earlier trials, we are going to do the best effort we can to characterize the immune response at the level of Th2/Th1 ratio. We do acknowledge that it is a challenge in these very young infants to get the necessary blood volumes, to get samples prepared at the sites. I mean, that is a challenge on its own, and the interpretation of the results might probably also be a challenge. But we will definitely try to do this in these very trials. That is our plan.

DR. MCINNES: Thank you very much. I have a few questions. What is the route of administration, please?

DR. DIEUSSAERT: Intramuscular.

DR. MCINNES: You said you had a lot of data that you had to choose from to show us. So I'm curious, and I really only have the calf data to look at in terms of kinetics of the response, but it looks like a particular dose concentration was selected, and I'm wondering how -- how much higher can you drive this titer with different dose concentration at different regimen? How did you get to this selection?

DR. DIEUSSAERT: To 5 10^10? In calf, I'm looking at, we have not done any dose ranging. So it was -- we had another vector before, which was called the PanAd3, in

which they did dose selection, too. So we actually took -- we looked at the dosages that were tested there and we will be doing a full dose ranging in infants. I think it is really difficult to predict from animal models which dosage should be used in infants. So for safety reasons, we just took the highest dose in the calf model to look at safety parameters, but we will definitely be doing three different dosages in infants, too. So I probably have the answer for you in a couple of years.

DR. MCINNES: So, by dosages you mean dose concentration, not number of doses?

DR. DIEUSSAERT: You mean, you were thinking about one or two doses, or no?

DR. MCINNES: No, I was really interested in how you selected what you did for your calf.

Then my third question is can you talk a little bit about your neutralization assay?

DR. DIEUSSAERT: It is done on Vero cells, and I'm not sure exactly what you --

DR. STEFF: I am Ann-Muriel Steff from GSK. I am working in the preclinical development of RSV pediatric vaccine. So our neutralization assay is a plaque reduction assay using RSV-A and Vero cells and revealed by immunofluorescence with anti-RSV antibodies.

DR. DIEUSSAERT: There is a standardization ongoing in collaboration with PATH that we are participating to as well.

DR. NOTARANGELO: Just a comment about immune profiling of the infants. I understand it is a challenge, given the young age of the infants and the volume of blood you can obtain.

There is some concern about measuring of course interferon gamma and IL-13 production in lymphocytes and circulating peripheral blood lymphocytes may not be representative of the situation in target organs. Perhaps one could give consideration to alternatives for interferon gamma, a much better indicator as shown by a number of studies that have to do with in situ production of interferon gamma in target tissues would be CXCL9 measurement. So targets of interferon gamma and likewise similar things could be done for the Th2 response.

DR. LONG: A question about the specificity of a Th1 response when you're giving concurrent antigen. So we give acellular pertussis vaccines and probably would with this vaccine. Did you consider in -- and it drives a Th2 response, no question about it. So it's a question of is the host set up to give a Th2 response even if you give them a vaccine that wants to make a Th1 response? Did you

in any of your studies try to use concurrent acellular pertussis vaccine when studying the bias of the responses?

DR. DIEUSSAERT: No, we did not do that, no.

DR. WHARTON: Have you looked at the formalin-inactivated vaccine in the calf model to make sure that the enhanced disease is detectable?

DR. DIEUSSAERT: Yes, we did. So as you may know, the preparation of the batches of formalin-inactivated RSV is very variable, and it does not always bring consistently the signals of enhanced respiratory disease. So we did prepare bovine FI-RSV, and we tested that, included that group in an earlier experiment that we did in calves, and the animals that were vaccinated in that group did not show any signs of enhanced respiratory disease.

Now, in that particular experiment, we had another candidate vaccine that we evaluated, and in animals, some of the animals vaccinated with that particular candidate vaccine, we did see signs of enhanced respiratory disease. So it would really suggest that the model is able to pick it up when it is occurring.

DR. JANES: Potentially very naive question that potentially also could be fielded by Dr. Brown as well. So pertinent to the early clinical studies in the seronegative infants, so I understand that challenge studies have been done in adults, and I'm wondering have challenge studies

been considered in the infant population, or is there an obvious reason why they would not be appropriate? I'm wondering if it would provide for more safety monitoring of these infants that they could be monitored more closely for severe disease, and would potentially need to enroll many fewer subjects?

DR. DIEUSSAERT: It is a good point, but we have not considered that to do.

DR. EDWARDS: To Holly's point, also would -- again, challenging is always a problem in little babies, but what about using the live attenuated as a way to look at safety before they would be exposed to the wildtype virus? Again, it would involve collaboration between several companies, which -- but again, is that something that might be an extra measure of safety that could be looked at?

DR. SAWYER: So related to that, how are you planning to handle your naive 2-month-old studies with regard to seasonality? Have you thought of immunizing them first in a non-RSV season to see how they do and then moving to the appropriate season?

DR. DIEUSSAERT: Yes, so in the very first, we were planning to vaccinate before the season and then run them through the season with the intensive monitoring that we set up in place. Now when once we come to phase III and

the risked enhanced disease, probably we will be vaccinating all year round.

DR. EDWARDS: Thank you very much.

So we will now have a lunch break, and we will return at 1:15. Thank you.

(Luncheon recess.)

Afternoon Session

Agenda Item: Open Public Hearing

DR. EDWARDS: We're going to begin with the open public hearing. I would like to read some general overview of the open public hearing announcement, for particular matters of general applicability.

Welcome to the open public hearing session. Please note that both the FDA and the public believe in a transparent process for information gathering and decision-making. To ensure such transparency at the open public hearing section of the advisory committee meeting, FDA believes it's important to understand the context of individuals' presentations.

For this reason the FDA encourages you, the open public hearing speaker, at the beginning of your written or oral statements, to advise the committee of any financial relationship that you have with the sponsor, its product, and if known, its direct competitors. For example, this financial information may include the sponsor's payment of your travel, lodging, or other expenses in connection with the meeting.

Likewise, FDA encourages you at the beginning of your statement to advise the committee if you do not have any such financial relationships. If you choose not to address this issue of financial relationships at the

beginning of your statement, it will not preclude you from speaking.

We have two individuals who have declared that they would like to speak in the open public hearing, and then also if there are any others who would like to speak then please come to the microphone. And then after the open public hearing we will then have the presentation from Janssen.

So the first individual who had registered to speak at the open public hearing is Dr. Megan Polanin, senior fellow at the National Center for Health Research.

DR. POLANIN: Thank you for the opportunity to speak today. My name is Dr. Megan Polanin. I am a senior fellow at the National Center for Health Research, and I previously trained at Johns Hopkins University School of Medicine. Our research center analyzes scientific and medical data and provides objective health information to patients, providers, and policymakers. We do not accept funding from industry. So I have no conflicts of interest.

We fully support the development of new vaccines to prevent RSV disease in seronegative infants. The recommendations this advisory committee makes will likely affect the progression of current and future vaccine candidates, and ultimately the safety of these products for infants in very early stages of development.

We urge the FDA to recommend extreme caution and not test these vaccines in children before there is a reasonable level of certainty regarding the product's safety. The World Health Organization recommended that the safety and efficacy of vaccines must first be determined in healthy adults and then individuals who have experienced RSV, before testing in infants who have never experienced RSV.

The FDA and NIH recommended that in order to test vaccines in seronegative infants, virus replication and reactogenicity profiles must be acceptable. We agree. We strongly encourage this committee to determine the specific profiles that are deemed safe.

Currently the methods for testing RSV vaccines are limited. Preclinical studies using animal models offer preliminary evidence of effectiveness and safety to inform the appropriateness of introducing vaccines in humans, but cannot completely predict either in humans.

Clinical trials in adults can be helpful, however adults have likely previously experienced multiple RSV infections and adults also have the advantage of a more mature immune system. Although clinical trials in seropositive children can provide information about vaccine reactogenicity, such studies are not conclusive regarding the risk of ERD in seronegative infants. Due to the

significant limitations, once vaccines are deemed sufficiently safe to test in seronegative infants, it will also be important to conduct long-term studies of infants in order to determine whether the vaccine protects infants and young children through a critical period of development.

The World Health Organization recommended that follow-up for clinical studies should continue through two RSV seasons in order to gather more data regarding efficacy and endurance of the vaccine. We agree. Such long-term studies are absolutely crucial to ensure that the vaccine has not caused long-term health effects that would otherwise go unrecognized.

In addition, all infants 6 months or younger are recommended to receive at least five other vaccinations. Thus testing of this particular vaccine should consider how it might interact with other routine immunizations.

Like any public health strategy, a vaccine's risks must be weighed against its benefits. Given all the mistrust of vaccines in a substantial minority of Americans it is especially important to determine how effective the vaccine is at preventing RSV and for how long. If the vaccine does not protect infants and young children through a vulnerable period of development or contributes to negative side effects such as ERD, then the risks of this

vaccine are too high. Given that there have been significant issues with past RSV vaccines, the FDA should ensure that they do not recommend this vaccine prematurely, especially for such a vulnerable population.

In summary, we strongly urge this advisory committee to prioritize patient safety and urge the FDA to establish high standards for preclinical and clinical studies.

Thank you.

DR. EDWARDS: Thank you. The second open public hearing registered speaker is Dr. Ruth Karron, professor of international health and director of the center for immunization research at Johns Hopkins Vaccine Initiative at the Bloomberg School of Public Health.

DR. KARRON: Thank you for giving me the opportunity to speak on this topic. I'm Ruth Karron, and I'm a pediatrician virologist, clinical investigator, professor at Johns Hopkins. I've served as both a member and chair of VRBPAC, and I've been conducting clinical trials of RSV vaccines in RSV-naive children for over twenty years. My university receives funding from NIH for clinical trials of live attenuated vaccines developed by the Laboratory of Infectious Diseases, and I've served as a scientific advisory board member for Regeneron.

I'd like to offer a few observations about RSV vaccines to be administered to RSV-naive children. First is that we need these vaccines. Passive protection via maternal immunization or administration of extended half-life monoclonal antibodies may protect infants against RSV during the first few months of life, but there's substantial illness in older infants and young children.

A new IMPRESS estimate of the global burden of RSV shows that worldwide more than 80 percent of RSV acute lower respiratory tract illness and more than half of all RSV in-hospital deaths occur in infants over 6 months of age. So active immunization of RSV-naive infants and children is critical for addressing RSV disease.

Second, despite the substantial burden, there is no low-, middle-, or high-income country where the risk to young infants of severe RSV disease or death is as great as the risk that was posed by the formalin-inactivated RSV vaccine. As you've heard earlier today in the study by Kim and colleagues of FI-RSV administered to 2- to 7-month-old infants, 80 percent of those who received FI-RSV and experienced RSV infection were hospitalized, and 10 percent of the RSV-infected infants died. Again, at 14 and 16 months of age, as long as 11 months after vaccination.

Prior studies of FI-RSV in RSV-experienced older children did not predict this outcome, suggesting that a

stepwise progression in a clinical trial from RSV-experienced to RSV-naive will not diminish the risk.

Third, despite years of research by multiple groups, there is no single animal model, biomarker, or immune response characteristic that can reliably ensure that enhanced RSV disease, ERD, will not occur. So as we look to RSV vaccines of the future, how do we mitigate the risk of ERD?

The first answer is that some types of RSV vaccines are not associated with ERD. Experimental live-attenuated RSV vaccines have been administered to many hundreds of RSV-naive infants, some as young as 1 month of age, with no evidence of ERD.

RSV vaccines based on parainfluenza virus vectored RSV-F protein have also been administered to small numbers of RSV- and paraflu-negative children, naive children, also with no evidence of ERD. There are a number of these live-attenuated RSV- and PIV-vectored vaccines in development, both at the NIH and at pharmaceutical companies.

However, for other types of vaccines we do need to think about the possibility of ERD. This is particularly true for subunit vaccines and is also something we need to consider for vectored vaccines and nucleic acid vaccines.

Small animal models of ERD, particularly the cotton rat model, as you heard earlier today, have been used to assess risk for potential vaccine candidates. In that context a recent study mentioned earlier by Schneider, Oram, and colleagues, is sobering and raises some particular concerns. That study showed that alveolitis, one potential marker for ERD in the cotton rat model, occurred regardless of RSV F glycoprotein antigen conformation, with highly purified, presumably dirt-free, high-quality, genetically stabilized prefusion and post-fusion RSV F-antigen, regardless of whether Th1 or Th2 response-promoting adjuvants were used -- GLA-SE or alum -- and in the presence of high titers of neutralizing antibodies.

Alveolitis was only observed when lower doses of RSV-F were used to mimic waning immunity. These results suggest two conclusions. First, that for an RSV subunit vaccine, there is no obvious antigenic conformation, adjuvant, or type of immune response that will mitigate risk, at least as observed in the cotton rat model.

And secondly, that this model itself can produce false negative results, that is, absence of alveolitis, if sufficient dose ranging and time interval between vaccination and challenge are not used. For this reason my

own view is that RSV-F subunit vaccines present unacceptable risks to RSV-naive children.

The committee therefore may want to consider a recommendation that, given the current state of knowledge, RSV subunit vaccines should not be evaluated in RSV-naive children of any age, regardless of preclinical testing results, and regardless of clinical trial results in non-RSV-naive populations.

Given the present state of knowledge, I would also extend this caution to any RSV vaccine that involves preformed protein, such as inactivated virus, subunits, or particles. How can vectored nucleic acid RSV vaccines be evaluated for use in RSV-naive populations? As noted in the briefing document, these types of vaccines may behave more like live attenuated vaccines, because antigens are processed intracellularly, and CD8 responses are induced.

However, a very cautious and comprehensive approach is required, in both the preclinical and early clinical evaluation of these products. Preclinical evaluation should be informed by the experience in the previously mentioned study. A range of doses and of time intervals should be assessed, with deliberate suboptimal dosing and evaluation of the longest possible interval between vaccination and challenge.

Consideration might be given to evaluating each of these vaccines using more than one animal model of ERD, again as we heard earlier today. While stepwise clinical trials, first in adults and then in RSV-experienced children, are important to assess other safety features of these vaccines, it should be understood that these data will not predict ERD.

Clinical evaluation of these vaccines in RSV-naive children might be considered if there are no safety signals from any of these preclinical or clinical evaluations, with initial evaluation in small numbers and follow-up over two RSV seasons, to allow virtually all participants to be exposed to natural RSV infection, and to allow for some of the exposures to occur in the context of waning immunity.

In addition to careful clinical follow-up, primary immune responses to the vaccine and immune responses induced by natural infection in the first and second RSV seasons following immunization should be carefully assessed.

Thanks again for the opportunity to share my thoughts.

DR. EDWARDS: Thank you, Dr. Karron.

Okay. Is there anyone else who would like to comment in the open public hearing?

Okay.

I would like now to introduce our next speakers, Drs. Roland Zahn and Melanie Saville, from Janssen Vaccines who will discuss the development of their RSV vaccine in naive children.

Agenda Item: Janssen Vaccines and Prevention B.V. Presentation

DR. ZAHN: First of all, I would like to thank the FDA to give us the opportunity to present our data here today in this meeting, and to show what we have put together for the development of an RSV vaccine in naive infants. Just to give you some introduction to the vaccine we have under development, it's an Ad26-vectored RSV vaccine, it's based on the replication-incompetent human type 26 adenoviral vector. And it is produced on a human PER.C6 cell line.

We have used this vector in the past in humans --

DR. EDWARDS: Could you get a little closer to the microphone; it's hard to hear.

DR. ZAHN: Okay. We have used this vector in the past in humans and animal studies, and it elicited a very strong Th1 response in this context. And as you have heard this morning already, it's expected to be similar more to a natural infection for RSV or live attenuated vaccines, which do not predispose to ERD in humans.

The vaccine encodes a fusion protein of RSV A2 strain, and we have a prototype vaccine, Ad26.RSV.FA2, which expresses the native RSV FA2 protein F. The optimized version which we are having now as a lead candidate is primarily expressing the prefusion stabilized F protein -- which you can see on the left side of the protein slide, under the antigen -- which exposes more neutralizing epitopes than the post-fusion format. This was achieved by introducing five additional mutations to the original FA2 sequence.

We plan to administer this vaccine at the beginning of immunization of 2 months of age, and then use two to three doses intramuscularly given in these infants. And it's planned to be administered in co-administration with other childhood vaccines.

The Ad25.RSV.PreF vector which we have developed was tested in the cotton rat model for efficacy, which were challenged with RSV A2, which is depicted on this slide. And we have on the left, that's the lung viral load, and the right, the nasal viral load. And we have tested it in comparison with a prototype vaccine, RSV.FA2, and as you can see both vaccines provide full protection at the doses given here, as a prime-only regimen.

However, if you observe in the nasal viral load, we see a higher efficacy of a Pre-F construct than the FA2

construct as the amount of animals which had nasal breakthrough are lower in that at both doses given. Which obviously is a hint that the Pre-F conformation provides a higher amount of neutralization of the vector, which has also been shown in humans to be the case, that the most potent neutralizing antibodies are against Pre-F-specific sites of the protein.

In this context also I would like to mention again that FI-RSV predominantly displays post-F, and also induces post-F antibodies.

The nonclinical data package we want to use to demonstrate Ad26.RSV.PreF is effective as a vaccine and does not predispose to ERD is depicted on this slide. It's a two-stage approach, where we want to provide first a rationale to move into RSV-naive infants and toddlers, and then in a second step to also provide additional data to move into infants of 6 months and lower.

The first part of our preclinical plan was to really show in mice of immunogenicity of product, show neutralization, and a high ratio of pre to post binding antibodies, and also to show that we have a Th1-inducing vaccine.

In the cotton rat model we want to show also, obviously, humoral immune response. In this model, a high virus neutralization to ELISA titer ration to really show

that we have preferential antibody induction, as well as a high lung viral replication inhibition and no disposition to ERD.

In addition the latest studies will provide and also data for the young infants, where we want to show that we also can elicit an immune response in an immature immune system by dosing neonatal mice, and to also show that we would achieve priming of an immune response in the presence of passively transferred RSV-specific antibodies to mimic maternal antibodies, which are likely present in the infants which we plan to dose.

The first data which is presented on this slide shows a Th1 biased immune response of this vaccine, and we have here in blue the animals which were dosed with Ad26; and the pre-F vector either dosed once or twice, and we have similar cells to look at. If you can have an interferon gamma induction on member of the Th1 cytokines - -

DR. EDWARDS: Could you move closer again, it's getting harder to hear again, thank you.

DR. ZAHN: IL-4 and IL-5, as well as IL-10, was used for Th2 cytokines. And we build a ratio of these to show a contrast of RSV, which did not induce a strong interferon gamma response in both prime and prime-only in these neonatal mice, did induce a very strong Th1 bias in

contrast to FI-RSV. We observed a similar Th1 bias in adult mice.

The cotton rat which we have used to develop ERD and were predisposition, ERD by our vaccine, is the only model we have used to investigate ERD, as we think this model can be used to study in depth the vaccine-induced immune response, efficacy, and the predisposition to ERD.

As you have heard as well, FI-RSV is inducing in this model as high ratio of binding antibodies to neutralization. So very little neutralization is elicited. There's only limited protection in this model with using FI-RSV, and multiple histopathological parameters in the lung of infected animals are changed due to FI-RSV. And we study peribronchiolitis, perivasculitis, alveolitis, interstitial pneumonia in this model.

We included multiple control groups in our cotton rat challenge studies to ensure well-controlled conditions. First of all, of course, FI-RSV, which is known to induce ERD in humans. We actually used original material which was used in these vaccine studies in the past, in the 1960s, as a positive control. And then two control groups, one of course buffer control group to show there is no background histopathology due to infection with RSV itself, and then intranasally given live RSV at a lower

dose to mimic previous exposure to RSV which in humans does not predispose to ERD.

For our study to be valid, these controls should be significantly different from each other in the overall histopathology scoring.

To give an example of one of the studies we have performed, here we depict the control groups of that studies. On the left, the alveolitis score, and on the right, a cumulative score of the alveolitis, peribronchiolitis, perivasculitis, and interstitial pneumonia, to give a better overall picture. What you can see is that buffer and RSV A2 given intranasally is significantly lower in histopathology scoring than FI-RSV for both alveolitis and the cumulative score. However, also, the buffer and the RSV A2 intranasally given are significantly different from each other, indicating A2 intranasally potentially also induces some pathology in this model. This we don't see in each study, but it can be possible.

In general we think that a comparison to RSV A2 intranasally given which would mimic a normal situation in humans is most appropriate.

Further, we decided that next to one dose range we have shown on the previous slide, we would also investigate the vaccine over a bigger dose range to mimic

waning immunity on the low end of immune response elicited, and on the high end, which should provide full protection. This should give a good picture of overall likelihood that a vaccine would predispose to ERD, and also to include not only one challenge strain but multiple challenge strains, going from RSV A and RSV B challenge, to investigate also that likelihood, because it will be encountered in infants as well.

Of course detectable RSV lung replication, like it is the case for FI-RSV, where immune response is elicited but no full lung protection is seen may be important to identify animals which are especially at risk for developing ERD. However, as we have heard today as well, depending on vaccine you might see ERD also in the absence of RSV lung replication and relatively high neutralization titers.

The data I'm going to present in the next slide are based on this general study design. We have obviously included the control groups in this study, buffer RSV intranasally given at a lower dose, FI-RSV given at two doses, and the Ad26.RSV.PreF ranging from a low dose, 10^5 virus particle dose, to a high dose, two times 10^10 virus particle dose, given as a single immunization.

The animals were then challenged 49 days after initial prime immunization and sacrificed five days later

for assessment of lung histopathology and efficacy of the vaccine. And in addition we had an immunological outcome parameter as well, at day 49.

We pooled data of three independent studies which we deemed to be valid based on the outcome of individual control groups, to provide a better overview of the data we have obtained in this model system.

The Ad26 Pre-F induces high neutralization titers which are dose-dependent, which you can see on the left in blue. From the low dose, 10^5, to two times 10^10. Whereas FI-RSV we as expected does not provide any neutralization in this model, similar to a buffer control group.

For binding antibody titers which are here determined based on a Post-F coded ELISA, you see a similar dose increase, leading to an increase in the ELISA titer for the Ad26, and is in about the same range of elicited titers as FI-RSV given two times. The ratio of VNA to ELISA titer is roughly the same across all doses for the Ad26 and significantly different from the ratio of FI-RSV in these studies.

The protection provided from this vaccine across these studies is depicted here. You see a dose-dependent inhibition of nasal RSV replication in this model in a nice dose response as well here, whereas the control groups FI-

RSV and mock do not provide any protection from nasal RSV replication.

The lung is also completely protected in most of the animals we have dosed with Ad26.RSV.PreF, except the animals on the lowest dose group; however, these animals, which you can see, which have the same RSV replication as the mock control group, did not show any ELISA or VNA titers measured by -- with humoral immune response. The FI-RSV immunized animals have also some level of protection against RSV replication, however only a few animals were fully protected from replication.

The Ad26.RSV.preF vaccination did not induce ERD in this RSV A2 cotton rat challenge model, as you can see with looking at all the parameters described before, peribronchiolitis, perivasculitis, alveolitis, and interstitial pneumonia. Again the animals for Ad26 are in blue, and at the same scoring on average as the mock or RSV intranasally dosed animals, or even lower, depending on the parameter you look at. And they're significantly from FI-RSV induced histopathology scoring.

If we then have a closer look at the overall induced inflammatory state, using again the cumulative histopathology score, the control groups are significantly different from FI-RSV, as expected, and as I said, Ad26.RSV.preF dosed animals have a histopathology scoring

which is significantly different from FI-RSV, and about the same level as one of mock or RSV intranasal, providing the rationale that at least in this RSV A2 cotton rat challenge model we do not see any predisposition to ERD based on this vaccine.

To summarize the nonclinical data that we have obtained so far, to provide that Ad26.RSV.PreF is an effective vaccine, and does not predispose to ERD. We have achieved in adult mice a higher virus neutralization titer and high pre-F/post-F binding antibody ratio, in data which we haven't shown today, and a good Th1 biased cellular immune response in contrast to FI-RSV.

In A2 challenge models we have shown as well that we see a high efficacy of vaccine-induced immune responses and no ERD predisposition. In addition, we have shown in neonatal mice, dosed at 5 days of age, a high potency and a Th1 biased cellular immune response.

Additional data we will generate in the RSV B challenge model, and to also evaluate if you could achieve a good priming in the presence of passively transferred antibodies in mice.

With this summary of the nonclinical data, I will pass on to my colleague, Melanie Saville, for the clinical development.

DR. SAVILLE: Thank you. So I will move on to talk about the clinical development plan for our vaccine. So first of all, by way of introduction, just a reminder of the target product profile for the vaccine, it is to begin immunization from 2 months of age with a two- to three-dose schedule, co-administered with childhood vaccines, as a routine immunization, not as a seasonal immunization.

So there are the three key elements that need to come together indeed to ensure that we can develop a vaccine for a high unmet medical need. You've heard a lot about the supportive preclinical data that is being generated. We also have supportive platform data from our adenovirus platform in the context of safety and immunogenicity. Together with the clinical development plan, where we aim to safely progress into the vulnerable target population, while ruling out the risk of ERD.

First of all, to look at a summary of our platform data. So we have the AdVac platform-based vaccines where we have a sizable safety data base in adults of the Ad26 vectored vaccines that we have had in development. We are developing a number of vaccines using the platform, and we have ten completed clinical trials with over 700 doses being administered, and 14 ongoing clinical trials, with over 3,000 adult subjects immunized.

In addition to that, in the pediatric population, we have a related serotype, the Ad35 serotype, having been administered to a number of infants, in a tuberculosis vaccine, with over 300 infants having received the vaccine. They've received these vaccines at a number of different dose levels, all within the range of the vaccine dose levels that we anticipate to give in the pediatric program. And both the Ad26 and Ad35 vectors have shown a satisfactory safety profile, with mostly mild and moderate adverse events of early onset and short duration.

We also have human safety already in RSV vaccine. We have completed two phase I clinical trials in adults, with the Ad26, with a prototype vaccine of the FA2 insert, and we have one ongoing clinical trial in the old adult age population, with the candidate vaccine, with the Pre-F transgene.

Again, the dose levels that we anticipate to use in children have been administered, and again the safety profile is similar to that that we saw in the platform data that I showed you previously, and we have had no related adverse events or adverse events that led to discontinuation from the trials.

Looking at the immunogenicity in the adult population, this is again data with the prototype vaccine with the FA2 insert. Looking at both neutralizing antibody

and also the cellular response with ICS. If you look at the left-hand side of the slide you'll see that indeed this is a pre-exposed population where you see VNA titers already at baseline, but you see strong response to a single dose of the vaccine, which is durable over the six months period follow-up.

If you then look at the right-hand side of the slide, looking at the Th1 and Th2 balance, you can see that prior to vaccination there was already a Th1 bias in terms of the background ICS data, and you saw that, indeed, the interferon gamma increases with a single dose of the vaccine, so the Th1 prominence is maintained with vaccination.

We also have preliminary data with the pre-F insert in our old adult study, which shows comparable or even higher immunogenicity data with these parameters.

So then moving on to look at the plan in infants and toddlers moving forward. The key objective is to safely progress into the vulnerable RSV-seronegative populations, while ruling out the risk of ERD, measured by the frequency of severe RSV-associated low respiratory tract infections.

It's important that we assess reactogenicity in adults and seropositive children first. In terms of ERD, we feel that there is limited value in assessing ERD in

seropositive children, as it's considered that they are at minimal risk of ERD. Evaluating the immune response also has some limitations due to the bias that we would see from the pre-exposure antibodies in such a population. So we are proposing an age de-escalation in the seronegative population, from the less- to the more-vulnerable populations. So starting at 12- to 24-months-old before moving on to 6 to 12 months, and then finally to the target population from 2 months of age.

Looking at the evaluation of ERD risk in these pediatric studies, for the seronegative population, in terms of the case definition, as we cannot distinguish ERD from severe RSV-LRTI, our definition is an increase in the frequency of severe RSV-LRTI. It's very important to have a clear case definition, and we have chosen the WHO definition. It's also important to have laboratory confirmation of each of the cases. And the cases will be reviewed by an independent clinical endpoint committee to decide on what is a case.

RSV will be monitored through the seasons, and we will not just be monitoring for severe disease, we will be monitoring for any respiratory tract infection with a predefined symptom score during the influenza seasons. We will also be conducting serological evaluations at the end of each of the seasons to see what we can indeed pick up in

relation to infections by serology. We will be conducting an immunological evaluation, looking at both the VNA and ELISA titer ratios, and the Th1/Th2 balance in the seronegative population.

In terms of duration of follow-up, all children will be followed up for two RSV seasons, with age de-escalation occurring after successful review of one season's data follow-up.

In terms of the ongoing monitoring within the clinical trials, there'll be both active and passive surveillance, with regular reminder calls to the parents to report all the RTIs that their children are suffering from, and the sites would follow up all RTIs regardless of severity until resolution. There will also be routine surveillance of hospitals and pediatric records.

Focusing specifically on the RSV severe disease, we would be looking at virological confirmation of all of those, and as mentioned before, an evaluation of all suspected cases would be conducted by a blinded clinical endpoint committee.

We would also convene a program IDMC that would be monitoring per study and between studies the incidence of severe LRTI with statistical algorithms supported by the sponsor. And indeed if there were any signal of ERD, there would be immediate communication to the sites, regulators,

and ethics committee, pausing of vaccination, and increasing surveillance of the subjects to ensure their safety.

This next slide just demonstrates at a high level the approach that we take to age de-escalation. So as mentioned before, the first study would be a study in 12- to 24-month-olds, which would include some seronegative infants. They would be followed up for one RSV season before commencing a study in seronegative 6- to 12-month-olds. There would be a second season from the first study, and first season from the second study, data being reviewed before moving into the target population of 2- to 6-month-old infants. Then a further follow-up season before moving into larger phase III efficacy trials in the target population of 2 months of age.

What I will focus on, though, today, is really these early trials, and our thoughts in terms of how to assess the ERD risk in these first trials. So looking at the 12- to 24-month-old trial, I showed you already that we have some data with the vaccine in an older adult population. We would add to that a population of healthy adults before moving on to healthy toddlers 12- to 24-month-olds, starting off enrolling seropositive toddlers, and following them up for safety in a subset for seven days

after the first dose of vaccine, before moving in to 12- to 24-month-old seronegative population.

In total, we would be enrolling 72 toddlers, of which 40 will be receiving the vaccine and 24 of those will be seronegative.

In terms of the objectives of the study, I think the important thing to highlight here in this size, is relating to the sample size. This is a descriptive study, but it does give us the opportunity to have a preliminary ERD risk assessment in a small of RSV seronegative 12- to 24-month-olds that will be followed for two RSV seasons. With 24 subjects per arm, this will give us the ability to detect the risk of ERD, which is similar to that observed in the formalin-inactivated trials in the 1960s.

Then moving on to the second trial, which is the trial in seronegative 6- to 12-month-olds. We will build obviously from the data from the previous trial. We would have general safety and reactogenicity, immunogenicity data, and one season of follow-up. Within this study we would start with a lower dose of vaccine in a subset of subjects, follow all of these subjects for 7 days for safety and reactogenicity, before moving into a high dose regimen of another group of 6- to 12-month-olds, so enrolling a total of 108 subjects, of which 72 are seronegative and will have received vaccine.

In addition, this gives us a first opportunity to look at the regimen, whether it's a two-dose or a three-dose regimen, that would be needed to optimize the potential efficacy of the vaccine.

So again, then, looking at a little bit more detail in this study, one of the primary objectives here being monitoring of severe RSV-LRTI, and the assumptions that we use for that are to show that the severe LRTI rate is not increased in the vaccine group compared to the placebo.

So we are looking specifically at a difference -- we're choosing not to look at relative risk, because this is still really quite a small trial, and we do run the risk of not seeing any cases in the placebo group, so a relative risk calculation cannot be performed. With the sample size that we have of 108 subjects, this gives us more than 90 percent power to demonstrate non-inferiority with a non-inferiority margin of 10 percent.

So with the plan that I've showed you so far, this brings data to then move into the target population. So it brings safety data, it brings immunogenicity data in seronegative toddlers and infants. It gives you two seasons of follow-up in a small group of seronegative 12- to 24-month-olds, and a single season follow-up of 6- to 12-month-olds. In addition, preclinical data will support

moving into the 2-month-old population in a study that will mimic vaccination in the context of maternal antibody.

So following thorough review of all of this data, a dose finding, regimen selection, and proof-of-concept study would be initiated in a 2-month-old age category.

So then overall, in conclusions, first of all, thinking about the preclinical data. The preclinical assessment of ERD in the cotton rat model should be sufficient to initiate clinical studies. With this model we can have enough animals to test overall a wide range of vaccine-induced immune responses, and a vaccine can be compared with immune regimens that do not predispose to ERD such as the live RSV pre-exposure, and to those that do predispose to ERD, such as the formalin-inactivated vaccine. And using the cumulative histopathology score, we can compare the vaccine to the control.

And with the data that we have to date with the Ad26.RSV.PreF vaccine, the histological score in the cotton rats has shown to be similar to the control, which is the live RSV pre-exposure, and lower to that of the formalin-inactivated vaccine following RSV challenge.

The preclinical data are supportive, but cannot provide evidence of complete absence of ERD predisposition, and vaccine-associated ERD risks need eventually to be ruled via cautious clinical development.

Thank you.

DR. EDWARDS: Thank you very much. Are there questions?

I have a couple questions. In terms of the repeated doses, does there look like there can be any adeno antibody generate such that subsequent doses of the vectored product will not be as immunogenic? Is there any information about that?

DR. SAVILLE: Yes. We do have some other vaccines in development, where we do give repeat adeno, and you do see an increase in the immune response with subsequent doses of the vaccine. So you can give it repeatedly and see an impact indeed on the response to the transgene.

DR. EDWARDS: I guess the other question is will the vaccine that you'd be giving to the youngest children, will that be pre- or post-fusion?

DR. SAVILLE: We're planning -- indeed, the transgene is a pre-F transgene. So that is the vaccine we would be using in the infants, yes.

DR. NOTARANGELO: If I understand correctly, the two versus three doses, the two-dose would be used in individuals who do have a previous history of exposure to RSV. So I wonder, can you clarify how you will be able to define whether eventually two or three doses will be necessary? That's the first question.

DR. SAVILLE: First of all, to look in the first study, indeed, in the first study there are the two groups. There's the seropositive group, and in that group we only give two doses of vaccine, and then we move to a three-dose regimen in the seronegative group in that study.

We really need to get down into seronegative infants to really address the two- versus three-dose schedule, and actually maybe if I can go back to the slide. Here, this slide here, shows a very first look at do you need two or three doses in the high-dose group, where you have adeno, adeno, adeno, versus adeno, placebo, adeno, versus placebo.

Now we also recognize there are a number of different regimens that we would have to fit into. So this is really just a first look. I would imagine that we would really need to get down to 2 months and do some more work in terms in terms of the regimen selection.

DR. NOTARANGELO: My second question is about the AEs that I've been seeing about the adenoviral vaccine. Can you tell us more about those?

DR. SAVILLE: In term of the general safety profile -- so first of all reactogenicity, so we solicit the common reactogenicity local and systemic events, and we see a profile very similar to what you see in relation to licensed vaccines, so most common events being local

reactions. In terms of systemic reactions, it's headaches, some myalgia, some chills, very standard types of reactions, and we see little in the way of fever.

DR. NOTARANGELO: But no severe reactions following subsequent exposure to adenoviral infection? Have you ever encountered that?

DR. SAVILLE: In terms of subsequent exposure to adenovirus, we do have a number of studies where we give several doses, and what we tend to see is the reaction rates go down.

DR. KOTLOFF: I am just wondering if -- what your thoughts are about addressing the children who are at highest risk for hospitalization and death? So part of that is defined by age. So if you're vaccinating at 2 months of age, you may only be able to protect a small part of that age group. And then the second question is your thoughts on how you would develop an evidence base to make recommendations for children who have an indication for monoclonal antibody. So that presents a very difficult group to do clinical trials in, because they have antibodies present.

DR. SAVILLE: Indeed, very good questions. In the context of the population, if I'm understanding you, are we looking perhaps at risk or healthy -- I think really it's very important to say we're really starting off looking at

very healthy population. I know from the presentation today most of the RSV infection is in healthy individuals, but we would start in healthy individuals. So, term infants, good birth weight, so we would have very careful inclusion/exclusion criteria, just to make sure that they are healthy.

In terms of your second question, indeed that is something that we will need to address, and we will need to address those specific risk groups and indeed those who receive monoclonal antibodies, and I would envisage in a second phase that you would need specific studies to do that.

DR. KOTLOFF: My first question actually also covered whether you have any thoughts or plans of trying to vaccinate younger, children younger than 2 months of age.

DR. SAVILLE: Our thought is the first indication being from 2 months of age, and really based on seeing satisfactory safety profile and efficacy, and obviously with the medical need we would consider studies lower. But we would start with the assumption of 2 months.

DR. EDWARDS: Any other questions?

DR. GREENBERG: I didn't hear you mention concomitant vaccines. Are the plans with these initial studies to be alone, or with concomitant?

DR. SAVILLE: So for these first two studies, which are really the ones which we've fleshed out the most, it would not be with concomitantly. And I think there are the windows of time where you could do those studies without falling, having difficulty with fitting it in with the vaccines that would need to be given.

DR. GREENBERG: I think it is interesting to think about the, quote unquote, healthy children in the sense that, I'm assuming you and others would look at history of any bronchiolitis or any bronchoconstriction episodes, but I also wonder if the genetics would play into that at all, and family history. It's not for you to answer; I was just thinking out loud that some of these other factors might be interesting to look at.

And then, I meant to ask earlier today -- so this isn't specific to your studies -- but I'm really unfamiliar with the histopathologic scoring, and I don't know in my mind how to compare 0, 1, 2, 3 -- can you give us some sense of it?

DR. ZAHN: So, basically this is a non-linear scale, so basically 0 is with no lesions detected, 1 is 5 percent, 2 is 25 percent, and then 3 is 75 percent, and 4 would be 100 percent of severe lesions in the histopathology.

DR. GREENBERG: Percent of the area.

DR. ZAHN: On the slide, of the analyzed area.

DR. GREENBERG: Thank you.

DR. LONG: Was there any indication in your immunization of experienced individuals that pre-existing antibody interfered or blunted response to vaccine?

DR. SAVILLE: Previous RSV, or -- RSV. So we saw a good response in all individuals. There were very few that we didn't see a response to at all.

DR. JANES: Can you expand on the collection analysis of the severe RSV cases? I assume that you would be collecting and analyzing those in real time, in order to do interim monitoring, to detect early on an increased rate of those events ion the vaccine group. And so if you could just briefly comment on that, and to what extent can you actually act on that information? Assuming that by the time, I assume, most of those events would be detected, the vaccination series is complete, and so what you could do on the basis of that evidence?

DR. SAVILLE: Just to be sure I am answering your question properly. Talking in relation to the ERD risk and the capturing of cases? Indeed, the approach that we are taking is -- during the RSV season, that indeed that we would be actively monitoring the children and swabbing them as soon as possible. We would also do a near-patient test, that would become a probable case of RSV infection, and we

would be doing PCR as well to finally have a definitive diagnosis. So as soon as any cases become available, they will be reviewed by the clinical endpoint committee and forwarded to the IDMC so they would be able to do ongoing monitoring.

To your point about children already being vaccinated by the season, indeed, our first study we would want to vaccinate before the season, and I think the important thing is that we are getting the balance right of the individuals in the study, so not to expose too many too soon. Hence the vary staged approach and the careful thought about number of subjects that can be useful to draw some conclusions versus not putting too many of those children at risk.

DR. MCINNES: Dr. Saville, are you able to share whether, is this a U.S. development, your clinical development piece? Are you going internationally, are you splitting it? What are your thoughts in terms of sites and how well your epidemiology is characterized in these places?

DR. SAVILLE: To start off with -- well, obviously we have the possibility of both northern and southern hemisphere -- but we are planning to start off in Europe. The main study is in Europe and the United States, where they have good characterization, and also good medical

care. There are also countries in the southern hemisphere such as Australia who would also be good countries to do that and who generally have quite good epidemiology. Yeah, so we're really going for the countries that have well-characterized epidemiology and good healthcare, for the children taking part in the study.

DR. EDWARDS: Could you comment on what immune studies that you might be doing to assess immune responses in the infants, or have you designed those yet?

DR. SAVILLE: So yes, indeed, we would be looking at both the humoral and cellular immune response, with all of the caveats of the limitations of the volume of samples. So the main focus being on the VNA and looking at binding antibody with an ELISA, but also looking at the cellular response with ELISPOT and ICS. And there are a number of potential exploratory assays that we can look at, but obviously we would have to prioritize those with the limited blood sampling.

DR. NOTARANGELO: A related question. Some of these studies are rather sophisticated, and they might benefit from centralization. On the other hand, you will be dealing with a variety of centers, so how can you handle that problem?

DR. SAVILLE: Yes, these are not studies that can be done in a single center. So what we're trying to do is

limit each study to even a couple of countries, and try and standardize things as much as possible there, across the study. Importantly, in terms of diagnosis, for example, of RSV infection, we would be using a centralized PCR so that we would get the same quality of data out of each of those. But yes, I appreciate that there are some challenges that we need to work on.

DR. EDWARDS: Are there any other questions? Thank you very much.

Agenda Item: Committee Discussion

DR. EDWARDS: We have Dr. Roberts and Dr. Browne at the table now to help us in the discussion, and we will see the questions that are posed to us for committee discussion on the screen. Jeff or Sarah, would you like to read the questions? The first question?

DR. BROWNE: Sure. So, the first question is please discuss the preclinical data essential to support studies of RSV vaccines in RSV-naive infants with regard to the potential risk of vaccine-associated ERD. Please consider the impact of vaccine type, antigen, and/or other relevant factors.

DR. EDWARDS: Let's discuss each question as they come. Anyone would like to start with the first question?

DR. KOTLOFF: I think there were a couple of thoughts that I had. One goes back to the issues of

whether there's an animal model that can be used to really specifically look at the impact of age. So, you know, the target population is going to be 2-month-olds who, my understanding, who have a Th2 bias in their responses. And so I think one of the models that is used is the neonatal mouse model, but I'm not sure if any of the other models really address the immaturity of the immune system that mimic the neonatal human immune system, and I think if we could get some data specifically on that phenomena that it would be very helpful, because we have so little experience in that age group with this type of vaccine.

And the second issue that I was wondering whether animal models could address is co-administration of vaccines. I think that's a big unknown at this point.

And then a third issue that I think we just heard will be addressed to some extent, is the idea of doing heterologous challenges in the animal models, because the quality of antibody that is induced by vaccination against heterologous strains may not be the same, and I think it would be helpful to get more information on that.

DR. EDWARDS: Thank you. Additional questions or concerns?

DR. LONG: I don't know about questions or concerns, but considerations -- I think from what we've heard, I think it would be best to have three animals.

Mice, cotton rat, and calf, rather than relying on one to try to understand both the kinetics and the types of responses. And I think we haven't heard enough about the longevity and the times of challenge in those animal models at different points in the waning response, because we know that disease doesn't protect more than a season the first time around, doesn't even protect for a whole season in nurses who are re-exposed frequently during the RSV season. So I think that that timing is something that is important.

I think we're convinced that any of these vaccines must induce a neutralizing antibody response that's robust and that there be cytotoxic T cell response. Ruth just reminded me about the age and the Th2 biased 2-month-old. I don't know that so well, but we do remember in whole-cell pertussis vaccine days that that was a very good Th1 response induced in 2-month-olds and 6-week-olds with an endotoxin containing, or whatever was in whole-cell vaccine. So I don't know about that.

DR. NOTARANGELO: A couple of comments. First of all, I also agree that we should use three animal models. Personally I have some concern that the neonatal mouse model may not be fully representative of the immaturity of the immune system in newborns, in humans, or in young infants.

And I also share the concern that -- I'm not sure that we should just be looking for Th2 responses, and it's not absolutely clear that infants are that Th2 skewed early in life, so I agree.

DR. TRIPP: I have a question about the vaccine type, and whether or not we have -- what kind of transmission can we block that with some of the vaccines. Is it possible, since we're using infectious (indiscernible) -- is that a possibility to consider that we can actually get blocking antibodies and stuff in transmission?

DR. LONG: If we're continuing in all of these things, very personally, I don't think we know enough to say that we should not -- there should not be pursuit of subunit vaccines, from what we know to date.

DR. EDWARDS: I was intrigued by the primate model, and wonder whether we need to think a little bit more about that. I must say that Tod Merkel's beautiful model of the primates has taught an awful lot about pertussis that we really didn't know before, and so I wonder if some additional assessment or looking at that model might be helpful as sort of that Fernando suggested that it might be. And so that maybe we need to enhance some of the models we have.

DR. PORTNOY: So I agree that three different animal models seems to be the standard, and it seems to provide a good signal for whether the vaccines have an increased risk of ERD. But my concern is that even in humans there are different kinds of responses to RSV.

Some humans have really severe disease, others have very mild disease. In other words, native RSV infection seems to cause ERD in some humans just naturally, perhaps due to genetic variation in the humans, and I'm wondering if additional strains of animals or types of mice or rats that can get RSV might be studied just to see if there was this same genetic variation in development of ERD in animals that maybe wouldn't be identified if we only look at three strains, three different animals.

DR. MONTO: I agree about the variation in humans, and I think we need to reconsider that as we start looking at the human studies. I just wanted to echo -- support use of primate, nonhuman primates. After all, this virus started out life as the chimpanzee coryza agent.

DR. EDWARDS: I am not sure who's going to answer this, but are there well enough established risk factors for severe RSV disease in terms of various cytokine responses that we could, that we should screen for? Populations of individuals that are aberrant in that way? Luigi, perhaps you could answer that best.

DR. NOTARANGELO: I don't think we have enough data to support it. There are obviously categories at risk that you would exclude from the trial, but I don't think you can use cytokine profiling to assess eligibility.

DR. LONG: For me, I am confused between severest RSV disease, which I see in otherwise healthy children in the hospital all the time, and ERD, which I think is very different. I don't think these children who have severe RSV disease that are in our intensive care unit likely have antibody immune deposits in their lung or anywhere else. They're very young, they sometimes have apnea and don't breathe, they sometimes have pulmonary hypertension because they're so premature and they're prone to that. So I think they're different things. Clinical RSV in small children is not the same as ERD.

DR. EDWARDS: Are there any specific issues about antigens, have we heard that there is an antigen that should not be included in the vaccine, or other relevant factors? Have we really heard that today?

DR. MCINNES: I can't answer that question, but I have another thought. I asked several questions about the dose concentrations and the dose calibrations, and I have the sense that the dose calibration that is used in the preclinical models -- in the animal models -- is a really important parameter, because we're going to be potentially

in challenge studies, and in certain types of vaccines in viral replication, and to be sure that we are being able to detect ERD within a range of dose concentrations, not just number of doses.

DR. EDWARDS: And certainly the paper that we saw is a little bit concerning, that the finetuning of those doses may be exceedingly important.

DR. PORTNOY: I am still confused about the F protein, and the fact that the purified F protein in one study showed good response without ERD compared to the formalin-inactivated. Whereas in another study, the F protein, the same purified F protein, showed ERD. It seems like a third study ought to be done, or somebody ought to figure out whether it actually is harmful or not. We need to know that information, because otherwise we don't know what to do with purified F protein as a vaccine material.

DR. JANES: To me, what that brings to mind is Dr. Polack's comment about the need for standardization in these preclinical studies, for understanding the animal models and the stock of the animals and the immunogenicity assays and antigens and so on, and I don't know the mechanism for that, but obviously there's some need for someone to take ownership of standardizing those attributes of the studies.

DR. EDWARDS: Certainly the NIH has done some of those repository and having stock strains and those sorts of initiatives, right, Pam?

DR. MCINNES: There are other organizations that are involved in that now, but certainly for years there have been efforts to try to standardize, but there are so many aspects to standardization. I go back to the genetic background of particular strains. I think it's not being done uniformly.

DR. WHARTON: So, there is a lot in the preclinical data that's been presented and additional work that hasn't been presented that actually suggests that we've learned an awful lot about the likely pathogenesis of the enhanced respiratory disease, and these approaches seem really, really promising to help reassure us that proceeding down a clinical development pathway is likely to not result -- or decrease the risk that we're going to see the enhanced respiratory disease.

But following up on Pam's point about the importance of looking at a full range of doses, there's also the issues about timing between vaccination and exposure to the RSV, which I think is the other half of that dose ranging question, in terms of where you are in the kinetic response. So I think that's also an important

variable that probably needs to be looked at in a number of different ways.

DR. KOTLOFF: I agree with that. And I think some thought needs to go into how far out we should go, because some of the examples of the children who died were vaccinated at 0, 1, and 2, or 0, 1, and 4, and then when they were toddlers they got disease, which is a much longer time than I think any of the preclinical models have used.

DR. EDWARDS: Yes, Jay.

DR. PORTNOY: Again, the fact that I'm not an infectious disease expert will show up, but I'm still kind of curious about how many strains of RSV there are and how much variability there are in those strains. Which of these proteins varies between one strain and the other? Is it the F protein, the G, or some other protein? And if we make a vaccine against a particular version of the protein, will it protect against the other strains, and how important is that?

DR. NOTARANGELO: One other comment I have is about the variability of data that have been obtained in various animal models. In particular, histopathology score. But I've seen across the board some significant variability within the same animal model, in terms of each of the responses. So that calls for a large number of animals that have to be studied, which might be a challenge

with primates, but still I do think that you need to investigate quite a number of animals in order to get a clear picture.

DR. EDWARDS: In term of the question of the heterogeneity of the responses or the neutralization of the different A and B strains, do any of the companies want to comment on those responses in terms of the vaccine? Does it seem to be working, neutralizing both strains?

DR. ZAHN: So, we have performed studies against RSV A and B strains, in vitro at least, and we see good neutralization against multiple strains, with the previous FA2 vaccine, but also the prefusion F vaccine which we presented today. So we see a good cross-neutralization, also protection also challenge studies, which we didn't present today. So the F protein is also more conservative than the G protein, to just come back to your point; the F is relatively highly conserved across strains as well.

DR. STEFF: If I may just add to also, maybe to clarify about heterologous challenge models. In the calf model we also verified that by immunizing with an adenovector carrying an F protein from human RSV, we were also inducing bovine RSV neutralizing antibodies. So not only we have crossreactivity between A and B human RSV strains, but also two bovine RSV.

DR. LYNFIELD: One question that came up is concomitant vaccines. And I'm wondering if people are also looking in the animal studies and other studies at the potential impact of regularly scheduled vaccines on type of response.

DR. LONG: We focused a lot on the enhanced respiratory disease and severe disease. Almost surely this vaccine is not going to be applicable to -- it'll be applicable, but not protect from infant disease that ends up in the intensive care unit.

So it will be very important, I think, to know how much it does impact on non-hospitalized burden of disease, and if palivizumab is an indication, antibody alone probably does not protect against infection and probably not much against clinical disease that's doctor-attended, as it by itself only decreases by 50 percent the likelihood that you'll be hospitalized. So we know that antibody alone is not the answer. So I think we're looking for something with vaccine that's much more than palivizumab can deliver.

DR. EDWARDS: One of the interesting aspects I think is the administration, as you said, of routine RSV vaccine for seasonal illness, which is sort of different than what we've generally been doing with seasonal

respiratory illnesses. And maybe that's because we have to use it every year because the seasons change.

But the evolution of the kinetics, when the virus comes, how old the child will be when they're vaccinated, will be a very complicated model, and I think if there are data from some of the studies that are being done with NVSN or any of the other surveillance populations to allow people to get some sort of burden of illness in various age groups in children longitudinally, I think that will be really helpful. Because the complexities of all of these moving parts are a little bit mind-blowing, as well.

DR. KOTLOFF: So I wanted to also get back to the concept that both adults and children have recurrent RSV disease. And we also know that there are bad RSV seasons, followed by quiet RSV seasons.

And I -- my own ignorance is, what is largely responsible -- I don't know what's largely responsible for that ability to become re-infected, and how much of it is the host's immune response, and how much of it is the virus's ability to change. And I'm just wondering if we have any sense is this like flu, where you have a drifting virus, and that would affect the efficacy of vaccines? Or is this more related to the host's immune response?

DR. EDWARDS: Fernando, do you have any thoughts about that?

DR. POLACK: There are other people that can provide more sophisticated explanation than I can, but clearly it's not an issue of drift, it is essentially waning immunity, and protecting the upper respiratory tract is a lot harder than protecting the lower tract. Adults and the elderly will get these upper tract infections -- you can't hear me?

So what I was saying is there are people in the audience that can give you a more sophisticated response than me, but essentially it is waning immunity happen back and forth, and protecting the upper respiratory tract for RSV is not easy, so you know you will get upper respiratory tract infections over and over through life. That's something that just routinely happens, but it's not that you have shifting RSVs that behave a little bit like flu. That's not the case.

DR. MONTO: In terms of what goes on in other populations, I think we're learning more and more about what goes on in adults, especially older adults. We're now involved in a CDC-sponsored hospital prevention study which started only involving flu and now has expanded to RSV. And we see in a fairly influenza-immunized population that RSV disease causing hospitalization is about 50 percent as frequent as influenza.

I think that as RSV vaccines are developed for other populations, other than very young children, we need to be looking at what protects and what doesn't protect and other factors, to try to inform what we do in terms of design of trials of vaccines for very young children. We also -- there's also a consideration, because of waning immunity, which results in reinfection in adults unrelated to shift and drift, there's discussion about annual vaccination with RSV vaccines for older individuals and then will we have a repeat vaccination issue with RSV vaccines as we now have with influenza vaccines? So just to add to the complexity rather than give us simple answers.

DR. EDWARDS: Are there any other comments that people want to make about the preclinical data? And also from our FDA colleagues, are there any other specific preclinical questions that you'd like us to address that we haven't, for the first discussion topic?

DR. SUN: In listening to the committee -- first of all, Wellington Sun, Division of Vaccines. In talking about preclinical models, we've focused on animals. Are there any ex vivo human preclinical models that can be informative in this instance?

DR. EDWARDS: Certainly not that I would be knowledgeable to discuss. I think that organ cultures and

those sorts of things might be able to be looked at but I'm not quite sure that they're where the animal models are at this point.

Are there others that have knowledge of in vitro models that they would like to mention?

DR. LONG: Not on that, but we don't have listed in there route of administration. We heard a little bit about in some animal models they were using intranasal, but it sounds like for human studies the plan is intramuscular, and it certainly seems like mucosal immunity is important for a vaccine for RSV, so just wanted to mention perhaps that the route of administration and the impact of that needs to be considered as well.

DR. MCINNES: I want to give a shout out for assay, again, and that the assay -- a lot of attention be given to the assay in the preclinical development phase, because it's going to have to carry over into the clinical development phase in order to have confidence between being able to map back to both of them.

DR. EDWARDS: So, I think then that the preclinical data, everyone feels it's very important, and it needs to be more than a single animal, and whether it needs to be more than three, and how the additional primate models need to be developed. I think everyone agrees that we need to have standardized assays, standardized viruses,

and animals that can be compared one with another. And indeed, this is a very important aspect. Any other?

DR. NOTARANGELO: Again, in favor of the studies in primates, there are better tools investigating immune response in primates than in cows. So that's another argument, if you want to characterize carefully, the immune response, that's another point to consider.

DR. KRAUSE: We're very pleased with the breadth of the discussion that we've heard here, and this of course is not a voting topic, and we've certainly heard a lot of suggestions for things that could be done to investigate this issue further. As you've heard there are companies who are moving forward with these ideas and who have described studies at least in seropositives that are ongoing or nearly planned, with the idea of potentially moving forward into RSV-naive infants fairly quickly.

And so what I'm not getting a complete sense of from the discussion here yet, and maybe it's not fair for me to ask this, but nonetheless it's -- hearing the suggestions, one could for instance develop a primate model, and based on my understanding of what's known about that, it might take five years to get there, or more.

And so what I would like to understand is, of these suggestions that we've heard, which of these are things that members of the committee feel strongly really

need to be done in order to support progressing into human infants who are RSV naive? And so perhaps this can be asked, based on the kind of data and the models that have been described so far, is there a sense that there are strong reasons not to proceed and that substantial additional research needs to be done before companies can proceed? Or is it a question of taking the existing models and making sure that the doses, the timing of challenge, inclusions of perhaps an additional model that has some characterization or whatever, that that is adequate.

Sorry to do this, but I would like to hear a little more on the preclinical topic.

DR. NOTARANGELO: So, my two-cents answer, I would favor at least the three animal models that have been discussed, although I am still in favor of the primate. I would say, exploring alternative routes, I think this is important. Multiple doses. And standardization of assays. These are critical points in my mind.

DR. MONTO: Just taking off from the comments, I just wonder how much of our discussion really is focused on the word essential that's in the question. How much of this is essential, and how much of this is something that would be great if it could be done within a relatively limited period of time, given the state of development and

the -- what we've heard about some of the lack of predictability of some of the models?

DR. WHARTON: I think the standardization issues and assay standardization issues are essential. Looking at the dose variation and the interval variation is essential. I think it's essential to look at more than one animal model. I'm not actually sure it's essential to look at all three of the existing models, I don't know that it's essential to look at additional ones, but I do think that making sure that findings are in general consistent across models is probably important given that we're using these models to provide reassurance to move forward to a very vulnerable population.

DR. LONG: I think the core value of what has to be done is to do no harm to young infants. So I think that would sort of help us with essentials, and I think that might differ by the kind of vaccine that it is. We're probably none of us as worried about an attenuated or a vectored as a subunit for causing enhanced respiratory disease. So that's one thing is be sure that we would do no harm when it got to young infants. It would get worked out if it was effective or not, and that can happen in clinical trials.

But the other thing is then to do all the standardization that is essential so that you can translate

what you learn from one thing to another and to try to evaluate the immunologic response so that we could better predict or feel assured that some other kind of vaccine would not cause harm when it got to the naive.

DR. SAWYER: I want just to add in support of the importance of the interval measurement, is if this is going to be proposed as a year-round vaccine, there are some babies who may get it 9 months before they get exposed to RSV, so that really seems essential to me, to look at that interval question.

DR. KOTLOFF: Yes, I was going to mention the interval. And then, this is actually more of a question. So I think the concomitant vaccines is a very important issue, but I don't know whether you can use the animal models to assess that. So I'm wondering if that's feasible to do. I don't think it would be exorbitantly difficult to do if the model would support the information.

DR. EDWARDS: It would seem to me that an approach that separates the standard from the new experimental RSV first would be a prudent one, because I think that that -- giving multiple antigens and with alum and different kinds of substances at the same time may make a vaccine that looks very different given by itself quite different when it's given in a combination. So I think it would be safer to study it by itself rather than combined. In humans.

DR. KOTLOFF: What about studying it first in animals? That was what I was asking.

DR. EDWARDS: Certainly, we've learned a lot, and perhaps should have learned a lot sooner, about some of the mouse data with pertussis. It does seem to be interesting. I mean, obviously mice are not men, but I think that there may indeed be looking at concomitant vaccines, or some of the vaccines, that may be helpful in that regard. In animal models.

I think, Phil, no matter what the preclinical data show, that if we were going to be doing studies in human infants and when they will be done, I think that we will be reassured, but we'll all be wanting to make sure that those are done very cautiously.

DR. MCINNES: And at the highest rigor and quality. A negative answer is reassuring, but we don't understand enough to know that if we picked another variable we might have triggered a positive. So that's -- I feel bad that I can't sort of have some prescient answer for you, but that's what I think you're seeing around the table. Negative is great. But is it expansive enough? Have we looked at enough variables? Had we just happened to pick some, and is the quality and rigor with which the study's done sufficient? And I would insist on that. I think that has -- everything has to be put on the table.

DR. MONTO: I think some of this uncertainty is reflected to what we're going to hearing about our discussion of item two. How much of this is going to be useful in being able to predict what's going to happen in seronegative children? Because this is, in general when we look at animal models, this is just to clear something before we start really learning what goes on in humans, but here it's a bit different.

DR. PORTNOY: That reminded me, there is one difference between the preclinical studies in animals and the results in naive infants in that none of them are going to be antibody negative. All of them are going to have some RSV antibodies from their mother. What effect does having those residual antibodies have on immune responses? And in these animal models, none of them have preformed antibodies already there, and maybe it would be necessary to look at what effect that has on the immune response.

DR. EDWARDS: I think we're ready for question two. Sarah, would you like to, discussion topic, would you like to review that?

DR. BROWNE: Please discuss the role of clinical data from the adults and RSV-experienced infants to support evaluation of RSV vaccines in the RSV-naive infants.

DR. EDWARDS: Well maybe I can start, since no one seems to want -- I think that it's all very nice to know,

it's certainly very nice to know that the vaccines would make a brisk immune response. It's nice to know what kind of cellular and humoral immune responses are given, are induced, when the adults and seropositive children are immunized. And certainly a measure of safety would be helpful. But it really isn't the same as the naive infant. So I think it's all very necessary, but won't provide all that needs to be provided.

DR. LONG: I think I would be interested to see viral titers in I guess the nasal pharynx, in these experienced in adults, just to be sure that at the time they are exposed naturally that there isn't a major rise in titer that was unexpected.

DR. NOTARANGELO: I also think this would provide an opportunity to investigate in greater detail the immune response to the vaccine, given for granted, of course, that we're targeting at a different population than the naive infants, but at least you would have an opportunity to standardize, again, a number of assays that might be relevant when studying the naive infant cohort.

DR. EDWARDS: Would there be any benefit of challenge studies in these populations, of RSV challenge studies? I mean, certainly you could time the challenge, and you wouldn't just have to depend on when RSV circulated, and certainly that has been done with some of

the pharmaceutical, RSV pharmaceutical agents. Would that be helpful?

DR. PORTNOY: I think you almost have to, because how do you know when somebody is infected with RSV? Especially adults. They may just have a cold. Are you going to test them every time they get a runny nose? And a lot of them are getting subclinical infection. You don't even know if they have RSV. So the only way you can really tell what response they have is by challenging them and seeing what they do.

DR. EDWARDS: Arnold, you look like a doubting Thomas.

DR. MONTO: I've always felt that there are enormous limitations with challenge studies. They appear to be a nice experiment, but there are always questions about the potency of the challenge virus, how much this really resembles natural infection. There are limitations in size. These studies generally have IRB issues and often can be interpreted only in terms of if they come up with a negative result in terms of protection, for example.

If you're not protected in these challenge studies, chances are you're not going to be protected against natural infection. I think here, if we're really worried about ERD, I'm not sure what they would contribute. We know how to do a reasonably large scale clinical trials

now. They're not simple. They require good surveillance and good specimen collection, but you learn a lot more for your investment.

DR. KOTLOFF: I think it depends on the question that you're asking. I think if the question is safety, challenge studies in experienced adults are not going to be helpful at all. I think that challenge studies can be very helpful for infections that, for example, aren't endemic in the study population. So if you're looking at cholera or shigella in the United States.

But I think we know multiple examples of vaccines being efficacious in primed individuals, but not efficacious in naive individuals. So I think as Arnold said, maybe if it doesn't work, that's helpful, but if it works, I'm not sure that's going to really predict what will happen in a naive individual.

DR. LONG: There is more and more experience with continual surveillance in families, looking for respiratory viruses over a year's time, and both from Utah and from Wisconsin. The Wisconsin one was very nicely done, where you put saline up the nose at home and have them let it flow into a baggie and then test it for viruses every week. So you can get around how clinical it is or isn't.

I don't think we discussed this, but by necessity these are going to be placebo controlled? We're going to

require placebo controlled? If that was the case, then you could do these kinds of surveillance over a year's time.

DR. EDWARDS: Let me just summarize, that we all would like the clinical data in the adults and the RSV-experienced infants to provide immunogenicity and safety data, and would have to be positive. But in spite of that, going to RSV-naive infants still -- all the questions have not been answered.

So the third discussion topic, Sarah?

DR. BROWNE: Please discuss how studies in RSV-naive infants could be designed to mitigate concerns about ERD throughout clinical development. Please consider aspects of initial study design, such as eligibility criteria, age de-escalation, and duration of follow-up. Please consider relevant aspects of phase III study design.

DR. EDWARDS: Very difficult questions. I guess one of the things that goes without saying is that the parents of the children who participate in these studies will have to be very, very carefully educated, will have to understand all the nuances of the questions that are going to be addressed, will have to really fully understand the risks and the benefits in ways that have to be reassured.

And so whether there's testing, or whether there's individual monitors, or how this looks, I think it's going to have to be very, very carefully done, and

people are going to need to understand all of the nuances in a way that is clearly described to the lay parents.

DR. NOTARANGELO: In regard to what we heard from the two companies, one thing I would personally recommend, is a different kind of monitoring. I heard one monitoring was based on monthly nasal swabs. The other one was based on swabs at a time when you have any signs of respiratory tract infection. I think this, in naive infants, calls for a much more active and continuous monitoring, with weekly phone calls and recording of the situation.

DR. EDWARDS: Perhaps weekly home visits, something of that nature.

DR. PORTNOY: This is a study that's really different than most pharmaceutical studies that I participate in. Usually the consent form says that you have a right to withdraw from the study whenever you want to. Once the vaccine is delivered, you no longer have the right to withdraw from the study. You have to stay in the study, and be monitored for the full year-and-a-half, two years, even if you don't want to be in the study anymore, because you can't undo the vaccine.

So it's really a different kind of a study, and I think parents need to be aware of that fact, and that it's really quite different. IRBs might have a difficult time dealing with that concept also. It's not something they

can just withdraw from. I do agree that it needs to be a two-year study, because the ERD can happen months 18 months later, so you have to monitor them for at least two years.

These are naive infants, you're worried about ERD, you have to know when they're vaccinated. I agree, I think maybe weekly swabs need to be happening, plus in addition any time you have respiratory symptoms, you need to do another swab. So it would be more than weekly swabs need to be done. These infants just need to be monitored very closely.

DR. EDWARDS: What about eligibility criteria? Should these children have siblings, or no siblings? Certainly we know that the RSV bearers are generally siblings. Should that be something that would be looked at first, or what other eligibility criteria or conclusions or concerns might you have?

DR. MONTO: Other than lack of underlying conditions, I really think it could introduce unnecessary complexity. It's hard enough to recruit a study like this. I think the key thing is going to be timing, in terms of when during the year you want to be doing this study, whether you want to have a challenge quickly or longer.

Given the natural history, if you want to call it that, of the old inactivated studies, even this doesn't seem to make any difference, because the events occurred

further out, and if anything, I think you'd want to know sooner rather than later, because you might be proceeding down the path given the small numbers. And I think this is going to be very, very tricky because of the numbers involved. When you think of the frequency of this kind of event, how long it will it take to detect it if it does occur?

DR. EDWARDS: I guess it depends on how common the event is, yeah.

DR. KOTLOFF: Along those lines, I think that one of the studies that we saw was excluding a 30 percent incidence of ERD, and I think that's the worst-case scenario, but I actually think the bar should be lower than that.

DR. GREENBERG: A naive question about these naive children: is there any antiviral on the horizon that would be considered as a rescue if something bad were to occur?

DR. EDWARDS: There have been those reported, so that is an interesting question. However, it might not be the virus. It might be the immune response that we're dealing with.

DR. WHARTON: Would we expect an antiviral to be effective, given what the thinking about pathogenesis is?

DR. EDWARDS: What do people think about, instead of -- certainly when we had LAIV in the very beginning, we

used it sort of as a challenge of natural flu, and is that a way that we could conceivably look at the live attenuated RSV vaccine as a model to see whether more disease would occur instead of waiting for the natural disease? I'm just saying is that a safer approach?

DR. MONTO: It all depends. The problem -- the good part is that it's attenuated and is not supposed to be causing disease. The bad part is, is that really what you want in this situation? Again, I think it's like any challenge model; if the results are positive, then you know not to proceed.

If the results are negative, then this might give you assurance, and in this case, this might be valuable, because it's a different question, and with the questions we usually come up with or try to answer in the challenge model.

DR. WHARTON: Just in thinking about your question, is that a question that could be answered in an animal model, about would the live attenuated be less likely to elicit enhanced respiratory disease following -- rather than wildtype virus challenge?

DR. EDWARDS: I guess you could use the formalin-inactivated vaccine and then challenge with the live to see whether -- yeah.

DR. SAWYER: The question was asked earlier about cytokine profiling as a predictor of trouble, and the answer was we didn't think that would work. Is there -- how about simply family history of immunologically-mediated disease? Is there any likelihood that that would predict a more severe reaction, and should that go into the study design, is why I ask?

DR. EDWARDS: Were there any data from the old studies that suggested that these patients were from atopic families or Fernando or anything that --

DR. POLACK: One child had had bronchiolitis at 3 months of age. That's all I know at least.

DR. EDWARDS: So that bronchiolitis wasn't confirmed to be RSV. Because that would have been worrisome if it was, right?

DR. POLACK: You know, all I know is reading the autopsy reports. There is a case of bronchiolitis. One, the other child had croup. So 11 months. But I don't know that there is anything else, and nothing about the parents. I think these kids were actually had no parents at the time.

DR. EDWARDS: It is an interesting question, for sure.

DR. PORTNOY: My understanding, as I recall, the Tucson cohort, there was the respiratory study that

Fernando Martinez did for many years. They looked at infants who were born and did bronchial challenges. Actually they measured hypo-responsiveness using cold air challenges to see which infants had airways hyper-responsiveness. Then they followed them to see which ones got bronchiolitis due to RSV and how severe was it, ended up in the hospital, and they were able to show a correlation between bronchial hyper-responsiveness prior to getting the RSV and the severity of the RSV infection.

So there may be a predictor in that respect. That predicts severity of bronchiolitis infection. It doesn't predict ERD, but I don't know whether that can be useful information on designing a study like this.

DR. JANES: We are following up on the question about the criterion for what constitutes an unacceptable increased risk of ERD in these trials. So I may have misunderstood, but I thought based on the Janssen studies that the criterion that was laid out was an absolute risk of 10 percent or more, which is substantially lower than the increased risk that was seen in the earlier studies. I don't know what the right number is, and obviously that would be a key parameter that would drive the size of these trials with the smaller that margin is, the larger the trial would necessarily be.

DR. KRAUSE: I'll just add we don't necessarily need you guys to tell us exactly what that number is. We understand that the tradeoff is that the lower that number is, the larger the initial studies have to be and the larger the number of children who are theoretically placed at risk if in fact there's a problem. So that's going to be a fine line to walk for sure.

DR. KOTLOFF: Another question for a phase III study is are we aiming to try to prevent all RSV disease or are we aiming to prevent severe RSV disease, and then this is probably even in the post-licensure phase, but apnea is another cause of morbidity and mortality that we see in these kids, and at least at some point during the lifespan of evaluating this vaccine, I think we should look to see if there is an impact of vaccination on apnea if that's possible, or sudden death.

DR. EDWARDS: Which would take large sample sizes. And fortunately sudden infant death is much less common now with positions.

Okay so the eligibility criteria, I think we have talked over a little bit. Some people feel just sort of healthy babies. Others have concern about asthma, reactive airway disease. So that any other eligibility criteria that we -- Sarah?

DR. LONG: I think that the gestational age would be 37 weeks and more. At that point, there are not data that RSV is more likely to lead to hospitalization at least. So I think that would be the cut point, and it also would presume that you would have your mother's antibodies, that a term infant would have or close to a term infant, and anybody under that would be quite different.

DR. EDWARDS: So should there be any screening of maternal antibody? Should the --

DR. LONG: Well, aren't the studies of naive going to only be done in infants who have them not experienced themselves RSV and who are antibody-negative at the start? Or would that -- did I misunderstand that?

DR. EDWARDS: I think that will have to be defined whether you -- by definition they wouldn't have been infected, because they weren't around in a season, but if their mothers had antibody, then they still would be naive, but they would have antibodies. So will that be something that needs to be looked at in the studies as well? Do you want -- or should it, would it be better to have a little bit of maternal antibody for the first patients to be -- or is that irrelevant? Probably needs to be known at least what the maternal antibody is, but whether it's a restriction, I think it --

DR. MONTO: Logistically, how will you do that in terms of --

DR. EDWARDS: You just have to get maternal. I mean, you just, well, you'd have to get the baby's antibody I guess.

DR. MONTO: Yeah, it would be the baby's antibody.

DR. ROBERTS: So, Kathy, are you are suggesting that we maybe ask for cord bloods? Cord bloods are very hard to --

DR. EDWARDS: Well, that would be nice, but you know, it is hard to know -- it's hard to know at that the time of birth that you're going to enroll in this study in 2 months, particularly one this complicated. So that I think would be difficult. I think you just have to measure antibody levels in the baby, which is reflective of the mother's.

DR. LONG: And go ahead with enrollment not knowing the answer and then looking at it after the fact I think would be what it was. I think we know from antibody decay in the clinical onset of hospital-type RSV disease that it goes away really quite fast. I mean, it's why you see that big uptake at the -- in disease and hospitalization and mortality at the end of the first month, and others probably know that decay better.

But it's pretty darn rapid and I guess it would depend on what time of the year you were born and did your mother just get boosted or is she six months away from her last boost, might all be variable, but I think you would need to know these data when you analyzed whether these were naive and antibody-negative or naive and antibody-positive or what they were.

DR. NOTARANGELO: I agree. And in fact I would actually collect serum at the time the vaccine is given and then follow the antibody titers following immunization. That would be the best way to assess the situation. I would personally not consider eligible any infant who has a previous history of bronchiolitis, of course any infant with newborn screening positive for CF, any heart disease, lung disease. Those should not -- and premature babies. Those should not be eligible.

DR. GREENBERG: One thing that strikes me is it seems as though the whatever study is taking place for any vaccine, candidate vaccine, in this, say, the 12- to 24-month-olds or the 6- to 12-month-olds who are seronegative, because if you have a toddler who is seronegative, I don't know what immunologic assays are going to be helpful there, but if that's your last chance to understand whatever you can prior to going to that 2-month-old who is seronegative, who is naive.

So I don't know what that is or what those endpoints, critical endpoints, are, but if there's anything that could be learned from an 18-month-old who is seronegative that would help understand what's going to happen in the very younger infant, obviously that would be reassuring to everyone.

DR. EDWARDS: So, we sort of talked about in a way age de-escalation. I mean, the older that you could have, the older that you could be and be seronegative, those would be the ones you would study first, and then younger in terms of -- okay, Sarah?

DR. LONG: I am just thinking about your comment, David, and you might get at it a little bit about in your enrollment of 18 months old did they, were they born in such a timeframe and did they have a history of any kind of wheezing diseased observed or not observed. Since most of these infections are clinical and they're not colds, they are some kind of wheezing event if you get in the first year of life.

DR. MCINNES: I have a question for the manufacturer who is proposing that study. I think it's a Janssen. What do you anticipate your screening to enrollment ratio to be to identify those seronegs that are 12 to 24?

DR. SAVILLE: That is a very good question. It really also depends a little bit on where we do the study. So for example, we have looked at some countries like Finland and the UK, and from that we believe that we might see some differences, and I think that's largely a little bit different -- the differences are related to the countries and how early kids go to daycare and all of that sort of thing. So it will be variable. So for example, in Finland it's the second year of life they go to daycare.

So the data that's available in Finland suggests you might have a higher rate than the UK. So it is variable, but likely to be more seropositives than negatives. So it might take a while to find those toddlers who are seronegative.

DR. MCINNES: I was thinking, I mean, it may bias towards finding only the 12-month-old. I just wasn't quite sure what your spectrum would be between 12 and 24 months, what your distribution might look like in thinking a little bit about David's question. It sounds awfully civilized to get thrown into daycare at least in your second year of life.

DR. LONG: Are they collecting, Kathy, are they collecting serum before immunization? You're planning on in the toddlers. So you might be able to get to it. I don't know; you probably know if you had it in your first

year, would you potentially be seronegative 12 months later? But at least if you weren't, you would know they were not naive.

DR. EDWARDS: Natural RSV infection isn't always a good immunizer.

DR. MCINNES: Aren't there some T and B cell assays though that -- no, they may be in experimental stages, that can detect natural infection, prior infection, even though your levels are low?

DR. EDWARDS: And certainly we agree with the duration. Yes, Holly?

DR. JANES: Did I understand correctly that the planned studies in the 6- to 12-month-olds would not screen for seropositivity? Is there a sense that there would be some variation in seroprevalence in that population?

DR. SAVILLE: In the 6- to 12-month-old screening and taking the seronegatives specifically, because we really want to get in that population. When you get down to the 2-month-old, though, there's obviously the maternal antibodies. So we would anticipate taking all comers into the study, but measuring the antibody.

DR. EDWARDS: Yes, Luigi?

DR. NOTARANGELO: Just to emphasize the point of RSV. So what I think Pam was talking about is CD8-positive T cells that are stained by RSV-specific tetramers. I

think that would be a much better way to investigate the frequency of RSV-specific CD8-positive T cells in peripheral blood and should probably be encouraged in such a trial.

DR. EDWARDS: Thank you. Duration of follow-up, everyone agrees it needs to be at least two seasons? Okay.

DR. LONG: Kathy, just on that. Two seasons depending on when you were immunized. Two seasons distant from your immunization. Two seasons after you get your third dose if there are three doses. So if you started in October, I don't think the first season would really be your first season, if we're thinking about ERD.

DR. EDWARDS: Maybe just as WHO, two years.

DR. LONG: Well, and then for others, I think two years might be more time than you needed. I don't know how you make those rules, but it sounds like you could figure it out that you could halt some a little sooner than the other, depending on the -- or change your month of enrollment so that everybody would be 4 months old 4 months after their vaccine when they got to their first season. Then I think two seasons would be fine, and you would be ending up with just under 24 months. Am I right?

DR. NOTARANGELO: What about 30 months? Would 30 months actually help there?

DR. MONTO: What kind of surveillance would be necessary during that time?

DR. EDWARDS: Well, going to their house every day for 30 months, they would be ready to shoot you by the time that they --

(Laughter.)

DR. MONTO: That is why I am bringing it up. Are you looking at intermittent collection of bloods? Are you looking at illnesses of all types to detect whether they're getting infected with RSV?

DR. EDWARDS: Probably would need to be respiratory.

DR. KARRON: I was wondering if I might comment on surveillance, because we have done it for many, many years in our studies of live attenuated vaccines, and I think we have learned something about what's feasible. So we followed children from every medically attended febrile or respiratory illness. If you see children for every runny nose, you will be seeing them constantly, all the time.

So during the RSV season and then when children have those events, we then go and examine them and sample them, and that seems like a reasonable balance between burden on families and study personnel and gathering the information you need in terms of ERD.

DR. EDWARDS: Certainly there are certain respiratory assessment tools that have been recently published from your group and also from the Rochester Group in terms of how to assess or stage or qualify those. Have those been helpful, Ruth?

DR. KARRON: We have not used those specifically, and a lot of those -- so the Rochester grading criterion and the one that was from Argentina with Fernando was for hospitalized children and, again, if you are talking about the United States, the rate of hospitalization is still very little. I mean, we have had one or two children hospitalized over years and years, and part of it is we preselect children who haven't wheezed and are otherwise healthy. So we are still in our early stage trials we're selecting very healthy people, but we don't have a good scale for the kind of outpatient medically attended illness that I think we are mostly talking about.

DR. EDWARDS: And you have been -- you sort of excluded children that have had any respiratory issues before or what about family histories of wheezing or anything?

DR. KARRON: We have not excluded children with family histories, but we have excluded children with their own histories.

DR. PORTNOY: I think one thing that we haven't really discussed is how are we going to define ERD in these infants if they get it, because how do you differentiate between ERD and just a really bad episode of bronchiolitis, since ERD is the endpoint of the study and we are assuming that these kids aren't going to necessarily die so that we can do histopathology on their lungs. They're just going to have a really bad episode of bronchiolitis. How are we going to identify who they are and whether that is what they have when we're trying to determine whether the vaccine caused that?

DR. EDWARDS: I think it will be difficult. Probably one might want to look at amount of virus, whether that would be helpful in terms of titer, whether there would be eosinophilia or other kinds of markers which we heard if they're not there, it's not helpful anyway. So I think that's an excellent point.

DR. PORTNOY: But there are host variabilities in production of those things. I mean, could we maybe look at cytokine profiles, draw blood from these infants and look at Th2/Th1 profiles like they did in the animal models as a way of doing that? We could take sputum and look for eosinophilia perhaps. Those types of things could be done, but we really need to think about this, because otherwise kids with severe bronchiolitis are going to be grouped with

ERD. Maybe they are the same thing. I just don't know the difference. I haven't heard anyone provide evidence that they really are a different thing.

DR. NOTARANGELO: I think Fernando showed that actually eosinophilia would not be indicative of ERD. You may find no eosinophilia and yet have ERD. So I don't think eosinophilia would help. Personally, I think there is no real way to do this. That's a challenge. I mean, then I don't think you can easily distinguish, unless you have a lung biopsy, which of course you don't do.

DR. POLACK: I don't know the answer. I would still get blood. I would still look at the differential, because a negative won't say anything, but frequency plus, you know, there's one of the studies, one of the epidemiologic studies by Chin, that does show that you can see some increasing eosinophils.

So a negative, the kids that died didn't have anything but a positive may go a long way, and I think it is also frequency, and they had very high fevers. They had 39-degree fevers. So it's not that anything is going to be the answer, but I think frequency plus all these things may help you recognize the risk.

DR. NOTARANGELO: I agree on collecting blood. However, if you include eosinophilia to define ERD, then one negative is already means something, that you can't --

that patient would have not been diagnosed with ERD, whereas pathology clearly show that it was ERD.

So I would not make it an absolute criterion to define ERD. So yes, that's what I wanted to mean. There is no way to define in an obvious manner that a patient has ERD. You can probably either define a constellation of criteria, but this will be after hoc(?). I don't know that right now we have enough elements that allow us to predict which patients really have ERD versus those that don't.

DR. POLACK: No, I don't think we do.

DR. EDWARDS: So certainly, that's something that has to be defined and really dissected in the outcomes of the study.

Karen?

DR. KOTLOFF: I just wanted to go back to the efficacy endpoint and the idea that you would have to visit the families every day for three years. I think that it's possible just to look for efficacy endpoints during the RSV season, and I think there's also a pretty good model. So the pivotal LAIV studies that we did through the VTU did surveillance every 7 to 10 days during the season.

So then parents were instructed to call for certain other endpoints. So I think there is a feasible model that was able to demonstrate efficacy in a 6- to 23-month age group.

DR. EDWARDS: We actually had drive-by swabbings.

(Laughter.)

DR. MONTO: Actually that's become more and more easy to do, because now we have home swabbing and all sorts of things. What I wanted to comment on was I really think that we need to follow up in terms of defining ERD, because there are going to be vaccine failures, and a simple vaccine failure with a relatively severe disease is going to look superficially a fair amount like ERD.

DR. PORTNOY: I am hoping that perhaps this group can come up with criteria for defining ERD or perhaps some research can be done to do that, but in lieu of that, we may have to just rely on statistical difference between the vaccine and the nonvaccine in terms of severe episodes in order to determine whether the rate of ERD/severe bronchiolitis is greater in one group than the other, because otherwise we're not going to be able to point to any one individual and say they have it, but statistically we can determine whether there's an increased risk among vaccinated versus unvaccinated infants.

DR. EDWARDS: So certainly the placebo group is very important.

DR. PORTNOY: The placebo group has a lower prevalence of severe episodes, we can say that the vaccinated group probably is having some increased signal.

DR. KOTLOFF: I am wondering if there are any proxies that correlate with ERD, for example in the primate model. So if you can look for circulating immune complexes, if there's anything that you can look at in the nose as a proxy for what's going on in the lung, if that has been examined at all. So I'm wondering if there are any noninvasive ways of getting a sense that this is going on.

DR. MCINNES: I am a little confused, because the Janssen group presented a case definition and defined it and then defined how they were going to monitor infection and assess the vaccine, et cetera. So are we saying we don't agree with that case definition, or that they didn't have one? I'm not sure where this is going.

DR. LONG: I think seeing an awful lot of children hospitalized with RSV now that obviously don't have ERD and reading a lot about those and living when the measles business was going on, they are quite different. The RSV, you know, you get sick, you have some fever, you're at your worst three days later. It's not that you're chugging along and then as the ERD seemed to be have high fever, low respiratory tract symptoms, and abnormal chest x-ray that would be in an unusual way for bronchiolitis, but I think clinically they are quite different in the way that they act.

Can you definitively say? No, but I think with some other things that you might look at, maybe nasal eosinophilia, maybe nasal cytokines, nasal immunologic responses, I'm not sure, or virus titer. Because these are going to be high virus titer situations if they have ERD.

DR. EDWARDS: So, clearly, there needs to be a consensus on that definition.

Let's go to the phase III study design as the final aspect, and are there any other -- obviously the numbers will be complicated because the larger the study will be, the greater the risk, but the more information that will be engendered. Certainly we'll need to make sure that there's not a single episode of RSV that's missed. So there will need to be very, very close assessment. I think in that, in the LAIV study that was done in the VTU, I think that during the winter season, the mean time the patients were studied was six or seven times.

So we're going to have to get a lot of samples to make sure that there's not any missed. Would we be comfortable in doing that in a home situation where people are getting their own samples or maybe not the first time, but maybe subsequently we could look at that to make it a little easier.

DR. LONG: It seems like weekly through the RSV season would certainly be enough, and then keep track of

the clinical symptoms of course all that time, but you are certainly still going to get colonization or you are going to find the RSV, the one before, the one after they became symptomatic if they became symptomatic on day 2, 3, 4. The first one is going to be positive or it's going to still be positive a few days later. So I think weekly through the RSV season. So that might be 20 a season. I don't think that's overwhelming.

DR. NOTARANGELO: Something I missed actually when I discussed the exclusion criteria, now the newborn screening for -- it's not really just for severe combined immune deficiency but for T cell lymphopenia. It's widely used in this country, and there are many other causes of T cell lymphopenia, much more common than severe combined immune deficiency. All of those infants that are positive newborn screening, they should be excluded.

DR. EDWARDS: Any other comments about relevant aspects of phase III study design? Phil, do you have any other really hard questions that you'd like us to address?

DR. KRAUSE: This may not be that difficult, but one thing that was mentioned and discussed on some level was actually your idea, Kathy, of doing challenge studies using -- well, initially you suggested even a wildtype virus, but then you thought maybe the live attenuated virus in children who are immunized, and of course that's

something which has some theoretical advantages which you know when the exposure was, you can follow the children very closely if something bad starts happening, you know it immediately. You presumably can minimize the number of children who are exposed to risk.

So there are a lot of things that sound very appealing about that idea, and of course, Melinda pointed out that if one were going to use the live attenuated virus, you would like to know that it actually mimicked what one saw with wildtype virus, but given the potential advantages of that kind of thing, and this is a totally hypothetical and theoretical question, I wouldn't mind hearing more broadly from the group.

Are there people who have concerns about doing something like that? Because in general, we also worry a little bit perhaps about doing challenge studies, especially in infants or vulnerable populations. So are there people -- maybe I'll sort of put it this way. Are there members of the committee who think that the concerns associated with that kind of an approach might outweigh the potential benefits of pursuing that a little further?

DR. KOTLOFF: So, we were talking about the VTU LAIV study. That was done. So there was no H1N1 circulating during this season when the LAIV vaccine was evaluated. So children were challenged with monovalent

LAIV to try to get an assessment of efficacy. So these were 6- to 23-month-olds. It was accepted by the scientific community.

DR. EDWARDS: I think that RSV infection, like income tax, is everybody's going to have to be subjected to it. So I guess that it's probably better to get it -- well, you might say it's better to get it naturally than to get it from someone else like a vaccine.

But I think it's not that everyone wouldn't be exposed. So I think that you're not giving someone something that they won't see. So it would seem to me that it might be a gentler kinder way to give it, if we're looking for the safety of the vaccine. So I guess that would be my thought.

DR. SAWYER: So I think it might pose some challenges to recruitment to your clinical trials. It would delay the ultimate answer, because you're not only asking parents to get this vaccine that might have a negative effect when you're naturally infected and then you're going to let your child get challenged to virus on purpose that might do that. So I am not sure it's worth the decrement in enrollment that might happen.

DR. KOTLOFF: I guess I was thinking that this wouldn't be the efficacy trial. There's enough RSV that the efficacy analysis would be with natural infection. I

guess this is to -- would it be acceptable to answer specific questions in a limited number of people? I think if you're giving a safe agent and for the reasons that Kathy said and because it's been done safely before, that's the setting where I thought it would be okay.

DR. LONG: I agree. I think it is a great idea to answer the first pass of safety, and the logistics are just that these would undoubtedly be two unapproved vaccines made by different people, different companies having to work together to do this in a limited number of children. But I think it would be great to be able to do.

DR. PORTNOY: That would be a great phase II trial looking at safety, but when the question was phase III, that's a study of efficacy. In that case, you do a randomized placebo controlled trial. You enroll as many patients as you need to get statistical power, and then you follow them over time and look at the difference between those, how many of them get RSV and how many of them don't.

My understanding is all of them are going to get it, but some of them will have milder disease than others. So the outcome won't be you get it or you don't get it, like in a lot of vaccines. It is the severity of the infections. So there has to be a severity score with all the way from maybe 6, where it's the most severe, down to 1 where it's the least severe.

Somehow it has to be a scoring system and then you have to look at the difference between the two groups to determine whether there is a statistically significant difference in the severity of the disease in those who are vaccinated versus those who are not. That's the kind of model I would expect to see in a phase III trial.

DR. EDWARDS: Very well said.

Any other comments? Any other questions that people would -- any other comments from the committee? Any other questions that the FDA would like us to address?

DR. KRAUSE: Thank you very much. We appreciate the comments.

DR. EDWARDS: Well, I think then we are at the end of the day. I think that we certainly have had some wonderful presentations and a lively discussion, and I hope that this has been helpful to the agency.

Thank you very much.

(Whereupon, the meeting was adjourned at 3:42 p.m.)