FOOD AND DRUG ADMINISTRATION

THE DEVELOPMENT AND EVALUATION OF NEXT-GENERATION SMALLPOX VACCINES

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Welcome and Overview of Meeting:

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Overview of Orthopoxviruses and the Diseases They Cause:

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Chief, Laboratory of Viral Diseases
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I. Regulatory Challenges for Next-Generation Smallpox Vaccines and the Animal Rule

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Considerations for Licensure of Next-Generation Smallpox Vaccines:

CAPTAIN TIM NELLE, Ph.D.

Live Variola Virus to Support Less Reactogenic Vaccine Development: Continued Evaluation of "Third" Generation Vaccines:

INGER K. DAMON, M.D., Ph.D.
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PARTICIPANTS (CONT'D):

Antibody Responses to Smallpox Vaccines:

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Assessment of the Effectiveness of Smallpox Vaccines: Immunogenicity Assay Considerations:

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Panel Speakers:

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II. Animal Models of Orthopoxvirus Infection and Disease

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a. Non-Human Primate Models

Variola Virus Challenge Models:

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NIAID Sponsored I.V. and Respiratory Models:

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Susceptibility of Marmosets (Callitrix Jacchus) to Monkeypox Virus:

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b. Rabbit/Rodent Models

Development of Aerosol Animal Model for Rabbitpox in New Zealand White Rabbits:

AYSEGUL NALCA, M.D., Ph.D.
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PARTICIPANTS (CONT'D):

Mousepox (Ectromelia Virus) Challenge Model:

R. MARK BULLER, Ph.D.
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Panel Speakers:

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Wrap-up and Recommendations:

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P R O C E E D I N G S

(8:00 a.m.)

MS. KELLEY: Okay, good morning. I know it's an early start. Just a few housekeeping announcements before we get going.

First, of course, I'd like to ask everyone to please put their phones on mute. To note that we are not providing lunch but there's a restaurant in the hotel and others very nearby.

This meeting is being recorded and will be transcribed, so when you speak please come to a microphone and please state your name and affiliation.

As you may have noticed, we have a very packed, tight agenda. We ask that you please allow the speakers to complete their talks. We've allowed for time at the end of each talk for questions, so we would really appreciate your holding your questions until the end of each talk.

That would be the end of the housekeeping announcements, so with that I would like to introduce Dr. Karen Midthun, the Director of the Center for

Biologics Evaluation, and Research.

DR. MIDTHUN: Thank you, Cindy. Well, good morning and welcome to this workshop on the Development and Evaluation of Next-Generation Smallpox Vaccines.

I'd like to start by acknowledging NIAID, who together with us is co-sponsoring this workshop. And in particular I would like to thank all of those who helped to develop and also who agree to participate in this workshop. I know it's been a lot of work and it was really pulled together on a very tight timeframe. So thank you so much for that.

I think we all recognize the development of next-generation smallpox vaccines pose different challenges, in particular with the demonstration of efficacy. This is because the next-generation vaccines that we'll be discussing in this meeting today do not produce a vaccine take which has been the established marker for effectiveness for previously approved vaccinia-based smallpox vaccines.

I think it's also important to note that the

challenges and approaches for developing next-generation smallpox vaccines are distinct from those needed to develop drugs to treat smallpox. And again, the focus here is really on the next-generation smallpox vaccines.

And now I'd just like to briefly set the stage by introducing the goals and objectives of the workshop.

As you can see, the goal is to identify and discuss the key issues related to the development and evaluation of next-generation smallpox vaccines. And the objectives are to discuss challenges and approaches that can inform the license of such vaccines and to identify strengths and weaknesses of various animal models that might be used to predict efficacy of such vaccines. Also, to discuss the most appropriate methods to bridge between the immunogenicity of next-generation smallpox vaccines and licensed smallpox vaccines, and then to discuss how to extrapolate clinical efficacy of next-generation vaccines from immunogenicity and animal

model data.

And so with that now it gives me great pleasure to introduce our next speaker, Dr. Bernard Moss, who obtained his medical degree from New York University, his Ph.D. from MIT, and who is also a member of the National Academy of Sciences. He's been the chief of the Laboratory of Viral Diseases at NIAID for many years and a student of smallpox vaccinia or pox viruses for over 40 years. So please, Dr. Moss, come on up. And thank you so much for your willingness to help participate in this workshop.

DR. MOSS: Thank you for the introduction. So my task is just to present an overview of orthopoxviruses to set the stage for the later speakers.

So the characteristics of pox viruses, they're large double stranded DNA viruses. They're unusual because they replicate in the cytoplasm and they encode more than 200 proteins involved in gene expression, assembly, and host defenses.

So I'm showing this slide not for you to

look at the details but to understand that it's a very complex virus. After entering into the cell it expresses an early set of genes, replicates its DNA, then expresses an intermediate and then a late set of genes, assembles virus particles, and these then transit through the cytoplasm and exit the cell and infect the next cell.

The 200 genes, again to show the complexity of the viruses, dealing with this is a transcription map of the 200 genes. About half of them are expressed early in infection, a quarter of them intermediate, and about a quarter of them at late times.

The pox virus family is divided into vertebrate and invertebrate subfamilies. The vertebrate viruses are divided into eight genera. Today, we're interested in the orthopox viruses, which is the best characterized genus and also the ones which include the viruses we're interested in today. Variola virus is the smallpox virus. Monkeypox virus produces a clinical disease that's quite similar to

smallpox. It's endemic at this time. I'll say a few more words about that in a moment. Cowpox virus infects rodents mostly. It can be spread to cats and other animals and can infect humans as zoonoses. Vaccinia virus, which is the model of the pox virus family is the smallpox vaccine, and ectromelia virus is important because it's frequently used as a model to study immunity.

The two orthopox viruses that cause lethal disease are smallpox and human monkeypox virus. You can see that they produce very similar lesions. They both belong to the orthopox virus genus. Smallpox has, for an unimmunized population it can be as high as 30 to 40 percent, whereas human monkeypox virus is only around 1 percent. And it has a lower human-to-human transmission rate than smallpox virus. Smallpox, of course, has been eradicated from nature whereas monkeypox, it's thought that there are a few hundred human infections each year.

Now, the clinical manifestations of smallpox, this slide was taken from a paper by Breman

and Henderson and it shows an important feature that there is a silent or prodromal period which can last about two weeks before the overt signs of smallpox occur. And this is important in vaccination strategies because there could be spread of the virus before it's recognized. The spread usually occurs mostly at the time of lesion formation and afterwards as shown in this slide.

Now, the first vaccine against smallpox was variola virus itself. And that was used in China and in India. It was based on the fact that reoccurrences of smallpox were very rare and because of that, practitioners of variolation as it was called, inoculated a small amount of the live variola virus into people and usually it produced only a small lesion and it provided protection. Obviously, this is not an ideal vaccine. In a significant number of people it did cause a severe infection and it could actually be the start of a focus of infections.

The next important step was the observation that inoculation with cowpox virus also was able to

prevent smallpox. And this cartoon shows the events that milkmaids were infected with cowpox virus from milking cows. The cows probably got the infection from rodents, and it was noted that milkmaids had a low incidence of smallpox, probably contributing to the English poets' description of the fair skin of milkmaids because they were not pockmarked.

Edward Jenner was one of several people who observed this, noted this, and he carried out experiments in which children were inoculated with the smallpox virus. And again, this was not unethical at the time because variolation was practiced and essentially what he did was to variolate several boys and then inoculated them with smallpox virus -- I'm sorry, I said it the wrong way. He inoculated them with cowpox virus and then he challenged them by variolation. And the variolation did not produce a lesion and therefore, he concluded that cowpox virus would also be able to prevent a natural case of smallpox. And in fact, history has born that out.

Some advantages of cowpox and vaccinia

virus, a related virus that was substituted for cowpox virus at some time, we're not quite sure, and it could be given as a skin scratch as shown here and it could be given in mass vaccination as shown here. The gentleman doing the surgery here, the cow has been inoculated with vaccinia virus and he's taking some virus out of the lesions and without cold chain or packaging it's going into the arms of all the people waiting. So this is a very easy vaccine, inexpensive, and simple to administrate -- administer.

So how serious was smallpox? Some people consider it the most devastating of all diseases. It's thought that there were hundreds of millions of cases during the last century. And in one year, the World Health Organization estimated that there were over 10 million cases of smallpox and they extrapolated that to say that the death toll may have been as high as 2 million in 1967, which is not so long ago.

However, in 1967, the World Health Organization launched an intensified smallpox

eradication program. Then, in 1975, it evolved from a mass vaccination strategy to a search and containment in which when a smallpox case was discovered, a team would go out and vaccinate the surrounding population. This was very successful and in 1977, the last endemic case of smallpox occurred and three years later the World Health Organization officially declared smallpox eradicated.

So what is the basis for smallpox eradication then? How is that accomplished whereas other viral diseases, such as measles is having such a hard time? First of all, there's no animal reservoir of variola virus. It probably could not have been eradicated if, like monkeypox virus, it was endemic in animals. Smallpox was easily recognized in humans and that allowed this search and containment strategy of vaccination. Cowpox virus and vaccinia virus are greater than 90 percent identical to variola virus, and, therefore, induce a strong cross-reactive immunological response. The vaccine was cheap and easily administered by skin scratch and the

vaccination confers complete protective immunity in over 95 percent of people for at least 5 to 10 years, and partial immunity for greater than 50 years.

The present concern regarding smallpox. There are two registered repositories of variola virus in the U.S. and in Russia, but the presence of undeclared virus elsewhere is possible. General vaccination ceased in the 1970s so that a large proportion of the population is now susceptible. Currently licensed smallpox vaccines can cause severe disease in immunocompromised individuals and that is one of the important reasons why efforts are being made to find a safer vaccine. Many people could be denied current vaccines for medical reasons and no therapeutic for smallpox has been licensed.

The complications associated with the smallpox vaccine is shown in one study in the United States for the year 1968. There are several severe complications listed here. Post-vaccinial encephalitis; progressive vaccinia in which the virus keeps spreading from the site of inoculation; people

with eczema can have a spreading infection as well; generalized vaccinia virus and accidental infection, either in other sites on the same individual or for other individuals. And you can see that the number of incidents is significant but it's low numbers compared to the number of people who are vaccinated. More recently, myopericarditis has also been associated with a minority of vaccinees.

Now, the CDC has put out a recommendation for who should not get smallpox vaccine in a non-emergency situation. And this includes anyone who is allergic to the vaccine or any of its components; women who are pregnant or planning to become so; women who are breastfeeding; anyone under a year of age; people who have eczema and atopic dermatitis; people who have weakened immune systems, such as drugs and transplantation, HIV, et cetera; and people who have been diagnosed as having a heart condition with three or more known major cardiac risk factors. So if you add up the numbers of all of these people and the family members of them, it comes out to quite a high

percentage of the population at large.

The consideration for developing new types of vaccines, humoral and cell mediated immunity are desirable. There are two infectious forms of vaccinia virus with different exposed envelope proteins known as the MV and the EV. The antibodies produced by live virus infection neutralizes both forms and passively protects animals. So it would be desirable to have antibodies to both of these.

Antibodies produced with inactivated mature virions, MVs, does not neutralize EVs and does not completely protect animals. Passive transfer of monoclonal antibodies to either MV or EV proteins partially protects and the combination is most effective in animal models. And this brings up an important topic in this meeting. We need good animal models and correlates of immunity.

The two major forms of infectious virus are illustrated here. The core of the virus is in the center. There's a membrane around the core and this is essentially the MV. And then there's another

membrane around the MV which is shown in red. There are more than 20 proteins associated with the membrane of the MV and a smaller number, about 6, associated with the membrane of the EV.

And we know that there are at least two targets of immunity in the EV membrane, A33 and B5. And there are at least six proteins in the MV membrane that are targets of antibodies. Stu Isaacs will undoubtedly elaborate on this.

Now, T-cells are also important in clearing infections, and because of the large size of pox viruses there are hundreds of CD8+ and CD4+ T-cell epitopes have been identified in the vaccinia virus and a high percentage of them are predicted to be present in variola virus. In general, the majority of CD8 T-cells target early proteins and the CD4+ T-cells target the intermediate or late post-replicative proteins.

There are several types of new vaccines that are being studied. One is the more attenuated strains of vaccinia virus and some of these are being --

undergoing Phase 1 and Phase 2 testing. In animals, recombinant vaccinia or variola virus proteins have been used. Also in animals DNA encoding vaccinia or variola virus proteins are being tested.

Now, I only bring up therapeutics which is not the subject of the meeting because if we had very good licensed therapeutics they could be useful for treating vaccine complications as well as smallpox. And the ideal targets of therapeutics are the highly conserved essential proteins involved in entry, gene expression, DNA replication, virion assembly, and spread. And there are two drugs that are being tested in humans and one, Cidofovir, inhibits DNA replication and ST-246 inhibits spread.

Now, unfortunately, there are many difficulties in developing new smallpox vaccines and therapeutics. In the United States, work with smallpox virus can only be carried out at the CDC under stringent containment according to terms of the WHO agreement. And much of the work needs to be carried out with surrogate orthopox viruses. And the

proteins are highly conserved but there are differences in host-range and the diseases they cause. Since smallpox has been eradicated, reliable animal models are needed both for surrogate orthopox viruses and desirably for the smallpox virus itself.

So with that I'll conclude my introduction. And I'm eager to hear the talks of the other speakers. (Applause)

CAPTAIN NELLE: Are there any questions for Dr. Moss?

Well, good morning. My name is Timothy Darrell Nelle and I'm a team leader in the Regulatory Review Branch in the Division of Vaccines and Product Applications within the Office of Vaccines at CBER.

Today I'm going to give an introductory -- a second introductory presentation and try to frame the regulatory considerations that are involved with these next-generation smallpox vaccines. The intent of my presentation is not necessarily to cover all the facets that are involved in all these issues but rather broach them so we'll set the framework for the

future presentations that will follow both this morning and in the afternoon.

Okay. So here's a brief overview of my plan today. For the purpose of today's workshop I'll define what we're considering a next-generation smallpox vaccine. I'll also provide some of the associated regulatory background for those vaccines. Since smallpox disease has been eradicated, it's not hard to imagine that licensure of these vaccines will involve the Animal Rule, so I'll review the tenets of this regulation and some of the associated challenges that are specific to these vaccines. Last, I will cover a few approaches to licensure under this regulation.

And so with that we'll go ahead and get started.

So what is a next-generation smallpox vaccine? To define this we have to first define what are the previous generation vaccines? The first generation vaccines are, of course, no longer manufactured. These were the ones that were actually

used during the eradication campaign. However, the manufacturing of these products were crude by today's standards and were associated with rare, serious adverse reactions. Commercial manufacturing of most of these vaccines ceased after the elimination of the disease in the '70s.

Following the events of 2001, we realized that the use of biological weapons was a possibility, and independently the CDC and Russian scientists identified smallpox as one of the greatest bioterrorism threats. To prepare for such an event it was clear that the national thought required a new source of vaccine and there was also a desire to have one which was produced by more modern technologies. This led to the creation and licensure of the so-called second generation vaccine right now which is ACAM2000. ACAM2000 is a clonal isolate that was derived from the Dryvax and is manufactured using modern cell culture technologies and is designed to comply with good manufacturing practices.

Both the first and second generation

vaccines produce a cutaneous reaction otherwise referred to as take. And both appear to have similar safety concerns. Production of this take is an important thing that's lacking in some of the vaccines that are following, so that's, unfortunately, a big issue that we're going to have to overcome. ACAM2000 was licensed in 2007 by demonstration of non-inferiority to Dryvax as opposed to, of course, a traditional efficacy field trial which is no longer possible. The license for Wyeth's Dryvax was revoked in 2009 at their request, so that vaccine is no longer available. So the need for safer smallpox vaccines, as Dr. Moss has said, has led to the development of newer vaccines that we call next-generation.

The ones that we're mainly focused today are what we're calling third generation vaccines for the purpose of this workshop. They are generally based on attenuated vaccinia virus, and usually most of them are in advanced development. They vary in their capacity to produce a take and it's an important consideration as I've just mentioned as a take is

generally accepted as a surrogate marker for protection by the ACIP and WHO. Because these vaccines are based on an attenuated strain, they're expected to have a better safety profile. And so that's the reason why we're looking at them so closely.

For the purposes of today's discussion, it's important to note that we're not necessarily going to discuss fourth generation vaccines in detail because these vaccines are generally in early development, but it's not hard to imagine that today's discussions will also be applicable to those as well in certain facets.

So now that we've laid the groundwork on who the players are on these vaccines, let's talk about some of the regulatory considerations for these next-generation vaccines. As we've already stated, you know, efficacy trials are not possible and licensure is likely to be based on the Animal Rule. It is also important to note that the use of the actual pathogen, in this case variola, as Dr. Moss has mentioned, is problematic and it would be very difficult to conduct.

And perhaps it's not necessary.

To further complicate matters, a licensed vaccine, in this case I'm referring to ACAM2000, is available. This leads to the question of what role should it play in the evaluation of these next-generation vaccines, if any? Regardless of the role, it's not difficult to imagine that some comparative data is desirable to aid us in understanding of the efficacy potential of these new vaccines. I'll discuss this possibility in more detail near the end of my talk.

Okay. So next I'll briefly cover the Animal Rule. Okay.

It was first announced in the *Federal Register* in 2002 and its official title was "New Drug and Biological Products: Evidence Needed to Demonstrate Effectiveness of New Drugs when Human Efficacy Studies are Not Ethical or Feasible." The title pretty much says it all. It's codified under 21 CFR 601.90 for biologics and 21 CFR 314.600 for drugs.

There are a number of potential

misunderstandings about this regulation. The rule does not apply if a product can be approved on standards prescribed elsewhere in our federal regulations. It is not an accelerated or fast track approval, and I think it's important to know the rule is not a shortcut to approval as many people undoubtedly in the audience have already figured this out. In fact, it's generally expected to take longer. As human studies are still required under the Animal Rule, we'll need to provide safety studies in addition to immunogenicity studies in humans.

Another important thing to remember as far as the Animal Rule is concerned is that the product is being developed for the use in humans; it is not for the use in animals, per se. So animal studies must be designed such that the data generated in those studies are relevant to humans. This really means that the animal studies and the clinical studies need to be developed along the parallel track and that you need to have some human clinical data to know what the response in humans is likely to be so that when you're

developing your animal model you can keep that in mind in trying to mimic the human response in your animal model.

Now I'll cover the four criterions of the Animal Rule. The first is that there is a reasonable, well understood, pathological, physiological mechanism of toxicity of the substance in treating, prevention, and/or substantial reduction of disease by the product. The second is the effect is demonstrated in more than one species that is expected to react with a response predicted for humans unless the effect can be demonstrated in a single species that represents a sufficiently well-characterized model that is predictive for humans. The third is the animal study endpoint is clearly related to the desired benefit in humans. Generally, this is the enhancement of survival or prevention of major morbidity. And finally, perhaps the most difficult of all these criterion is that the data or information on the kinetics and pharmacodynamics of the product or relevant data in animals or humans allows for the

selection of an effective dose in humans. For vaccines, we generally think the vaccine dose given to humans should elicit an immune response that is comparable to the response achieved in animals that were protected by the vaccine.

So this all seems pretty easy and simple; it's only four simple rules to follow. So what's the big deal? Well, there are a number of nuances that make applying the Animal Rule to these vaccines a little more challenging. And as I mentioned earlier, one of these challenges is the use of the actual pathogen and definitive animal studies is difficult if not onerous and difficult to achieve logistically. Some might argue that it's unnecessary as well. This is confounded by the fact that the protective mechanisms and relative roles and contributions of the various types of immune responses are not completely understood. As you may recall two slides ago, the first tenet of the Animal Rule is that we have a reasonable, physical, pathological understanding of how these vaccines work.

So as we proceed today, one of the questions to ask is, and we'll be definitely asking for your input, is what are the gaps in our understanding that we should fill as we proceed with licensure of these vaccines under the Animal Rule? However, we do know these vaccines do elicit both humoral and cellular response and the animal model suggests that both of these are important. More details on this subject will surely be followed in Dr. Isaacs' talk later this morning.

Another challenge that we'll be discussing in the second session of today's workshop is a selection of the animal model. This, of course, is made on a case-by-case basis and I won't go into any further details as this will be covered later this afternoon.

Perhaps one of the most important aspects of licensure under the Animal Rule is meeting the fourth tenet, which is this whole thing about ensuring that the kinetics and pharmacodynamics are relevant between animals and humans and allows you to make a selection

for an effective dose. As I've said before, for vaccines we interpret this to mean that the dose given in humans should elicit an immune response in humans as comparable to that achieved in animals where protection has been demonstrated. In essence, this requires an immunological bridge between the animal and the human studies. Selecting this parameter for bridging is important and something to consider is that you would like to have an assay where the same assay can be used for both humans and animals without significant modification or at least an assay where if you have to use separate assays or a modification you're shown that these two possible ways are basically equivalent in their quantitative abilities.

One such candidate which has been proposed is plaque reduction neutralization test, often abbreviated PRNT. We will also discuss other possibilities as the day goes along. Okay. And finally, one final word about -- this leads to correlates of protection. While there's no requirement to develop a true correlate of protection,

it's certainly understandable that if one is found along the way this will certainly help aid in the development and understanding of the vaccines but it's also easy to see that depending on the virus and the vaccine being used, such correlates may not be possible all the time.

In the next few slides I'll attempt to put together -- piece this all together into some strategies that can be used under this regulation. The two approaches that I will cover are the anthrax PEP model, or at least a portion of the anthrax PEP model, and one that incorporates a licensed comparator. Certainly, there are many other possibilities and permutations that are possible; however, for the purposes of today's introduction I'm just going to cover these two possibilities.

So in the following slides, which are a courtesy of Dr. Drusilla Burns of CBER, I'll illustrate the approach that's going to be used in collecting the pivotal data that will be used for licensure of anthrax PEP model or post-exposure

prophylaxis. This was discussed in detail at the November 2010 VRBPAC, and for the purposes of this talk I will not be going into any details of exactly what PEP is and the associated background or any real data or even how they plan to tie this pivotal data with the supportive data in the total package for licensure. All I'm really hoping to do is to relay some of the important features of how they're approaching the Animal Rule.

So the first thing that is talked about is estimating the protective antibody levels in animals. That's the first step. And to do this the animals are immunized with different amounts of vaccine to yield a range of antibody titers that are measured at relevant time points, for example, a peak titer or immediately after a challenge. Then the animals are challenged with infectious agent and then survival is assessed. Then it's possible to plot the relationship between the antibody levels and survival. In the case of the anthrax studies that have been so far, you actually see a nice relationship like this that's on the

screen. However, such a curve may not be achievable for each agent and vaccine, so we're not saying that everybody's going to have such a nice curve but we would hope so. So that's the animal side of the rule.

On the human side, what we should do, of course, is determine the antibody distribution titers for the vaccine itself in clinical trials. And to connect this population data so you would extrapolate the protection in animals to human data in such a way by connecting the two with an antibody bridge. In this case you can see the relay of one on top of each other allows these datasets to be linked with this bridge, which is the antibody titers.

In this example in humans, antibodies in the purple region would be expected to have a greater than 90 percent survival in humans. Having antibodies in the blue region would be between 70 and 80 percent of expected survival and so on. It's important when you make this extrapolation that the antibodies are assessed at relevant and comparable time points in both animals and humans. For example, you might

compare peak antibody titer response in animals to the peak antibody response in humans. In doing this it's possible to assess whether the vaccine is likely to provide clinical benefit.

Okay. So this sounds pretty reasonable and understandable. So let's try to make it a little more -- well, I guess one thing you might say and this is -- we're talking about smallpox today and not necessarily anthrax. And, of course, there are many differences between anthrax and smallpox that makes this approach a little more challenging. Of course, for anthrax, you can easily use the infectious agent in challenge studies, which is not the case for smallpox. The mechanism of anthrax is well known as antibodies to anthrax protective antigen are sufficient for protection. In contrast, the mechanism of protection for smallpox is less clear. Plus, there is a newly approved smallpox vaccine, ACAM2000, which also complicates the landscape.

So this leads to the question of whether demonstration of non-inferiority to ACAM2000 in

animals and/or clinical trials is required for licensure. Obviously, any comparative data would be supportive at the very minimum, but what we're really asking is whether non-inferiority to ACAM2000 is an appropriate endpoint for licensure. If so, can it be incorporated into the Animal Rule approach? Of course, it would definitely mean that an additional study group would have to be added to the animal study portion of the licensure approach. In this case you would have to add at least one group of animals who would receive ACAM2000, which would be separate from those receiving the vaccine candidate.

Each of these studies may include endpoints such as survival from challenge and PRNT or another immunological marker that could serve as a bridge to the human data. In this scenario where PRNT is used, the choice of virus for the neutralizing assay becomes important, especially when the vaccine candidate is significantly different than that of ACAM2000. In such cases, it would be important to select a virus for neutralization that could be equally neutralized

and would not show any artificial bias towards one vaccine or the other.

Although it's not necessary for licensure, one also could conduct a non-inferiority study in humans as well. Obviously, even if such a study is not considered pivotal, as we said before it arguably would be informative. However, since ACAM2000 is only recommended in the military and laboratory workers, these studies cannot be conducted in a general clinical trial in the general population. So that adds another layer of complexity. And finally, it's important to state that regardless of whether ACAM2000 is included in these studies, human safety data is still a requirement for licensure.

So, and of course, you know, it goes without saying that the ideal situation, since ACAM2000 was licensed based on non-inferiority to Dryvax, it would be better for these next-generation vaccines if we could do the same thing and use the same comparator. Unfortunately, Dryvax is no longer available. However, we believe that ACAM2000 will be equally

effective against the disease as it also induces high neutralizing titers and also produces a vaccine take. Unfortunately, the matters are a little more complicated here because the level of neutralizing antibody titers that are stimulated by ACAM2000 are slightly lower than those induced by Dryvax. In fact, they were low enough to cause ACAM2000 to narrowly miss its non-inferiority endpoint in a vaccinia-naïve population in the pivotal clinical trials. I'll show a summary of this data in the next slide.

So why make a big fuss about this lower level of antibody? The concern is that in theory you could, depending on how the margins are established for the candidate vaccine and their non-inferiority study, it could allow significant down creep of the immune response and still allow the vaccine candidate to be licensed. And such, it would allow further down creep away from what we consider our gold standard which was Dryvax.

So this slide summarizes the relevant portion of the neutralizing antibody responses that

were reserved during the pivotal ACAM2000 clinical trial. The goal of these studies was to show that the ratio of the GMT for ACAM2000 to Dryvax was at least 0.5 or a log value of -.301. As you can see, the margin was narrowly missed and the vaccine -- sorry, vaccinia-naïve population was narrowly achieved in those who had a previous history of vaccination.

The significance of this matter is really unknown. It could be that the immune response generated by Dryvax is extremely overwhelming and many magnitudes beyond what is truly necessary for protection. Such viewpoints are supported by historical literature reports that tout neutralizing antibodies such as low as 32 may be sufficient for protection against smallpox. I believe Dr. Isaacs will expand upon this information in terms of this historical titer.

In closing, I would like to thank all those who helped me prepare for this presentation, and I'll be happy to take any questions. Thank you.

(Applause)

Okay, with that I'll introduce the next speaker, which is Dr. Inger Damon from CDC. She is the chief of Poxvirus and Rabies Branch.

DR. DAMON: So thank you very much. I appreciate being able to come and talk about some of the work that's been done in our group, looking at using live variola virus as a way to additionally evaluate some vaccine responses in humans.

So this work has been evolving over a number of years and we've primarily focused our work on two NIH-sponsored trials: one which took place in St. Louis University, one at Harvard University. And our interest was to look at -- because of the ease of what we can do inside the containment lab and the notable importance of the vaccine-elicited humoral response and protection -- was to look at the role of the variola plaque reduction neutralization test and what it can tell us about vaccines and the responses that are elicited. And we felt that it was going to be especially important if take cannot be used as a correlation of successful protection from vaccination,

and also it's interesting to look back at some of the historic literature from Downie and McCarthy which suggested that vaccinia neutralization and variola neutralization in assays done in their labs in the '60s did not necessarily correlate with each other.

So what I'm first going to talk to you about is some published work which came from the DMID 02-017 trial and then both looking at how the use of MVA, either intramuscularly or subcutaneously, compared with Dryvax and the elicitation of a variola neutralization and then some unpublished work where we tried to better understand the comparisons that one might be able to achieve between using different virus targets as the neutralization target of the assay.

So the long and the short of it is the study ended up being a little lopsided from what we initially intended. So what we ultimately ended up looking at were 12 individuals who were vaccinated with Dryvax. We simply looked at them at times of peak time post-vaccination, so at day 28 post their Dryvax vaccination. And then 26 individuals who

received sub-cu MVA -- and this is the end IMVAMUNE product -- they received two doses and we look at them at peak times post-vaccination based on the vaccinia neutralization data, so 14 days post their second, so their prime boost MVA. And then we also looked at a fewer number of people, 15 who received intramuscular MVA.

And because the goal of this was as well to compare the vaccinia and the variola neutralization, we used assay formats that were comparable to what had already been done at St. Louis University, so using an assay that had been developed by Fran Newman, which involved a 15-hour incubation in terms of the sera with the virus in order to interpret the neutralization tests. There were a couple of differences, protocols. They used 24-well plates to plaque out. We used 6-well plates. We ended up with a denominator of about 200 virus particle plaque forming units in our control wells. They had less than 50 plaques in their control wells. And in general, we used pretty comparable positive controls

in terms of using a VIG.

So what I'm going to describe is some of what we looked at in looking at the variola neutralizations. So we did both descriptive data analyses and then analyses of significance. So we in general used non-parametric statistics, so we didn't assume that this was a normal distribution. We did frequency analyses. We looked at percent reduction at each dilution, and we did log-linear transformation to calculate both 60 percent and 90 percent PRNTs, as well as geometric mean titers at a 60 percent or 90 percent PRNT.

And then we also tried to control for individual variability that might be seen which GMT might not give you as much insight on, and so looked at fourfold and eightfold rises in pre-vaccination and post-vaccination sera calculating out 60 percent and 90 percent PRNT because that's the endpoint titers that St. Louis University had already done with their vaccinia data.

So this is some of the descriptive data.

And what you can see is that there is an overall trend to a higher percent variola plaque reduction in individuals vaccinated with MVA versus those vaccinated with Dryvax. And it's only statistically significant, however, at the 1-to-40 dilution.

If you look at the 60 percent neutralization data, again you see the same sort of trend that those vaccinated with MVA in general have higher (inaudible) variola 60 percent neutralization titers and endpoints, but nothing achieved statistical significance in comparing groups. If you looked at 90 percent plaque reduction neutralization in terms of just numbers of individuals who were able to reach that at various endpoint titers, you get higher endpoint 90 percent PRN titers in people vaccinated with the MVA regimens in general. The significance doesn't achieve significance but it nears it at the 1-to-40 dilution in comparing MVA sub-cu administrations in Dryvax. And as well with IM versus Dryvax.

If you look overall at geometric mean titers, so this is at the 60 percent neutralization

level, there's a trend towards higher GMTs with variola PRNT titers with MVA regimens than Dryvax regimens. If you look at the 90 percent level for GMTs, you achieve statistical significance in these individuals post-vaccination. So that's a 0.03 with a Wilcoxon rank-sum and a P of 0.02 between the MVA and Dryvax regimens.

And then if you look at -- we also looked at fourfold and eightfold rises with both 60 percent and 90 percent endpoints. And what you can see is that a higher proportion of those vaccinated with MVA subcutaneously achieved a fourfold rise versus those vaccinated with Dryvax. And that also achieved statistical significance.

So what our overall observations here were that a two-dose MVA regimen was as effective in the elicitation of a variola -- and this is just looking at mature virions. So as Dr. Moss went into, there are two forms of virus that you really are interested in how the vaccine-elicited immune response will recognize those two forms of the virus. So this is

just looking at mature virus virions.

And they are as effective as what you see in the elicitation from a Dryvax regimen at peak times post-vaccination. And some of our analyses actually would suggest that the MVA regimens are superior in the elicitation of a variola PRNT neutralization response. And that's interesting to think about in light of one of the studies that came out from Huw Davies in collaboration with Dr. Moss' lab looking at protein arrays and looking at what you saw in people vaccinated with MVA versus Dryvax. And what they saw in the subset of individuals with MVA is that the elicitation of responses to the L1 and D8 proteins on the mature virion were often more robust or more frequent in individuals vaccinated with MVA. And the limitation of our study, obviously, is the small sample size.

So we also then went on further -- and this is work that is in final stages of analysis now -- to look at and compare how vaccinia or variola fared when they were the target of the neutralization assay. So

using the geometric mean titers at a 60 percent or 90 percent level there's no significant difference between the 60 percent GMT target comparison but we had to exclude a number of the variola PRNT endpoints because the endpoints were extrapolated beyond our last point where we had actual data at a 1-to-1,280 dilution.

If you look at the 90 percent GMT target comparison, we do see some significant differences in terms of the endpoint, the geometric mean titer that's observed between when one uses vaccinated Dryvax as the target of the neutralization assay versus the variola strain Solaimen which we used at CDC. These are the differences here so, and these all are statistically significantly different.

One of the questions that we have been trying to address is whether the differences are due to either variances in the viral preparations, and so we've assessed the quality by looking at particle to PFU ratios, so looking at what combination of material may actually be viable virus versus non-viable

particles. And at least in the preparations using real-time PCR assay, the particle-to-PFU ratios -- this is not variola Dryvax, it's variola Solaimen -- the particle-to-PFU ratios were apparently similar and the variola preparations made at CDC and the vaccinia Dryvax preparations made at St. Louis University.

And then if you look, sort of try to do a pair-wise comparison. So again, trying to assess the reliability, how well does a vaccinia neutralization response in an individual correlate to what the variola neutralization response would be. We looked at this using the 90 percent neutralization readout. And what you can see using a non-parametric analysis, so a Spearman correlation coefficient, there are weak correlations that are seen as weak-positive. And if you look at that, however, on a graph you really see that you're really looking at pretty much a scatter plot.

So here, just looking at a subset of some of the data, you can see that there may be a trend of -- based on which regimen you are vaccinated with. So

whether you were vaccinated with MVA or Dryvax, there may be a trend. But overall, if you compare all variola neutralization, 90 percent PRNTs to Dryvax, there isn't a good linear correlation. And that's also shown by looking at your R-squared value which is 0.014. So this is also looking at Dryvax. Again, looking at Dryvax versus variola with a smaller subset of the individuals, so looking at the scatter.

So the second study we looked at DMID 05-010. We were interested in this for two reasons. Well, actually three. So in this study they vaccinated with MVA, which was the ACAM -- this formulation, ACAM3000. They didn't have a Dryvax or ACAM2000 comparator arm but they did challenge at a six-month time point after vaccination was completed in a subset of their volunteers in the study. Just prior -- unfortunately, not everybody completed that phase of the study because at that point in time there was a removal of Dryvax from utilization.

So they also in this study, in addition to the intramuscular and subcutaneous routes of

administration, looked at an intradermal dose sparing administration of MVA. So we were interested in looking at that as well. So, again, because of time and what we can do within the containment lab, we initially looked at a subset of individuals from the study who received the higher dose regimens of MVA. So received two doses of MVA, day 0 and day 28, either 10^8 subcutaneous route or the 10^7 intradermal dose sparing route. And then a subset of these individuals went on to receive Dryax challenge at six months or a placebo. And there was a placebo arm in each of these as well.

So I'm going to briefly show you just to remind people of the data which came out of these two papers which were done under the auspices of Raph Dolin, but Lindsey Baden was one of the primary senior investigators in recruitment of the trial and has been our primary collaborator on this.

So they looked at neutralizing antibody responses using essentially a luciferase readout. So they have a recombinant virus with luciferase. They

measure neutralization. Instead of doing it by a plaque reduction neutralization assay but by looking at the ability of virus found with sera from people who have been vaccinated and then look at the ability of the virus then to an intracell and be read out in a luciferase assay.

And so what you're looking at is their data with either a WR vaccinia luciferase construct or an MVA luciferase construct. And what the neutralization responses are after essentially two weeks after your first dose of MVA and then watching it boost after your second dose. And it's interesting to notice that the neutralization readouts with the MVA luciferase are in general higher than with the WR vaccinia.

So what their data suggested was that the ACAM3000 preparation of MVA was safe and well tolerated. It was immunogenic in eliciting anti-vaccinia antibodies and T-cell responses which I'm not going to show you. And that the 10^8, the high dose groups, and 10^7 ID elicited similar neutralizing antibody responses as Dryvax following a prime boost

regimen. And then they also showed which -- in the papers, which I'm not going to show, that it elicited an antibody response to both IMV and EEV antigens.

So what they did at six months after their two-dose MVA regimen, they looked -- they challenged with Dryvax in substantive individuals. And they characterized them based on whether they had a normal response which they called category 3 or a diminished response which either graded out as category 2 or category 1. So essentially a challenge. And also an ability to then measure what the memory antibody response looked like.

So what they found in their studies, and this is published in the Seaman article, is that of those who received placebo, none had an attenuated response and in those who had received the 10^8 sub-cu or 10^7 intradermal, since that's what I'm going to look at with variolas as well, the majority of these individuals had an attenuated response, either a 2 or a 1.

And if you also looked at viral shedding,

that, too, was diminished. So if you had a category 1 MVA response, your viral shedding was lower than here if you had a placebo vaccination and then were challenged with Dryvax.

And what they went -- and what you can see also -- so this is just looking at it -- another way of graphing out that same data, looking at the difference between viral shedding between those who received vaccine and those who received placebo. And one of the interesting findings in this study was then being able to stratify that your overall neutralizing antibody titer -- and this is based on their MVA luciferase readout assay -- was associated with reduced viral shedding and also with reduced take. So stratifying out, if you had the higher neutralizing antibody titer, so the here or there assay is an ID50 of greater than 100, you had less viral shedding and you had a diminished take than if you had an ID50 between 21 and 100 or an ID50 -- a low ID50 of less than 20.

So what we wanted to try to do is to see how

this might correlate with the variola neutralization data. So what we did was took a subset of individuals from arms D and E. So we took 23 individuals and we looked at a total of 144 samples. And we looked at period of time over multiple visits, so this would also, instead of just looking at times at peak vaccination we could also look at the response after one and two doses and then look at the memory response that was elicited through the Dryvax challenge.

So what you're looking at here is a busy slide. Looking at -- so here instead of looking at 60 percent or 90 percent PRNTs, we also wanted to do similar to what the original investigators had looked at. So here we're looking at 50 percent anti-variola PRNTs. And what you can see -- so here's people who got the subcutaneous administration of MVA 10^8 two doses and 10^7 intradermal. And you can see cub-cu most of the individuals get a nice boost between dose one and dose two of MVA. So you see a rise in titer. There were a couple that showed no boost between the first and second dose. So they already had achieved a

high level after the first dose and didn't boost further.

Interestingly, with the 10^7, and again, these are small numbers, almost half of the individuals, once after their first MVA, had pretty high levels or -- and didn't boost after their second dose. And this is something that we're interested in following up on.

And if you looked then looking at what happens between the time that they received their two doses of MVA and then the Dryvax challenge. And so we also have somewhat of a control group so those who only received MVA and didn't get a Dryvax challenge. So in this what you're looking at is the red and the fuchsia color who received MVA without a Dryvax challenge. So those people who received the two doses of sub-cu MVA, in general your GMT post-MVA was about 1-to-217. If you received the two-dose MVA and then got your Dryvax challenge, you saw a nice memory response in terms of a rise of your variola neutralization titer from 1-to-170 to 1-to-1,000 essentially. With intradermal you saw also a similar

memory boost after the Dryvax challenge. And so that's just graphically displayed here. As well, we saw good responses in both fourfold rises in titer and eightfold rises in titer.

So we then tried to do the same correlation back so we provided the variola data back to the investigators at Harvard to then compare with the previous data that had been achieved on diminution of take. And again, because our data is underpowered there does appear to be a decrease, a trend to the titer of 50 percent PRNT trend to association with decreased duration of Dryvax shedding. And this will be work that we try to follow up on with getting more samples from that study to investigate. Again, this wasn't something we were going to do with the study because the strategies for doing the neutralization assays were different, different methodologies. One being a plaque reduction neutralization titer, one being a readout. But it's interesting that if you look at just the GMT anti-variola MV titers, they're actually similar to what was seen with the MVA

luciferase readouts. So whereas the 50 percent variola with 10^7 intradermal MVA was 1-to-222, it was 1-to-201 with the MVA luciferase readout, and similarly, 1-to-213, 1-to-220 with the sub-cu regimens.

So where we are now is to, again, to get more samples to see if we can determine if there is a significant bridging endpoint with the variola neutralization assay and then also to get a better handle on understanding the kinetics of the neutralizing antibody response after the Dryvax challenge and the effect of the vaccination regimen. And finally, beginning to work on EV neutralization or common plaque reduction assays to begin to look at the effect of these immunization regimens on the ability of sera from these individuals to neutralize the extracellular virus form of variola.

I'm going to end here. (Applause)

CAPTAIN NELLE: Are there any questions for Dr. Damon?

It seems we have a very quiet crowd.

So now it's time for a break. We're running pretty much on schedule it looks like, a little ahead of schedule. So how about we take a 10-minute break and come back at 9:35.

(Recess)

CAPTAIN NELLE: If I can have everybody's attention, we'd like to get started with our next talk. I don't have a bell so here's a ding, ding, ding, ding, ding, ding, ding.

Okay. So while everybody is filtering back to their seats, I'll go ahead and introduce our next speaker. This is Dr. Stuart Isaacs from -- the associate professor of medicine at the University of Pennsylvania School of Medicine. He's going to give us some more background information, specifically on antibody responses to smallpox vaccines.

DR. ISAACS: All righty. Good morning.

So this was a huge topic to begin to look at and try and summarize, so by no means is this going to give all the answers to all the questions. And probably raises more questions than it's going to

answer. So a brief summary of what I hope to cover, I focus on antibody responses, look at some of the early measurements of antibody responses. Some of these studies have been mentioned by other speakers, and I'll actually show some of the data. Targets of MV neutralization. As we've all heard, the importance of antibody responses to the extracellular or envelope virus and mention the side issue with LC16m8, one of these next-generation live virus vaccines. And then the potential role of the Fc domain of antibodies as important in protective responses, something that hasn't been brought up yet. And then another topic not brought up yet is antibody responses to immune evasion proteins and I'll use MVA as an example.

So why focus on antibody responses? So historically cellular immune responses were not easily measurable and so antibody responses historically have been looked at. And fortunately for all of us looking at antibody responses, it turns out antibody responses are really important in the protection from a secondary or challenge after vaccinations. A totally

different story on a primary initial infection with a pathogenic pox virus in which both antibody and cellular immunity is critical.

For time, in your handout I have a couple of summary statements about the ectromelia virus challenge after vaccination showing the importance of antibody responses; a slightly different story with WR intranasally with a vaccinia virus strain where it looks like there's an overlap between the cellular and antibody responses on rechallenge; and just use a non-human primate study to highlight the importance of antibody responses after vaccination. So this study from the NCI, from Franchini's group, in which they vaccinated non-human primates with Dryvax and then a month later challenged with monkeypox. And as you see here, with no depletions you get full protection with full survival and undetectable viral loads. Control animals under no treatments all succumb to infection. This is an intravenous monkeypox challenge.

If one depletes B cells at the time of vaccination, so drastically limiting the antibody

responses to vaccination, these animals -- this group does very poorly with mostly high measurable viral loads, too numerous to count monkeypox lesions, and survival of only one of the four animals. And this was different than both the CD4 and CD8 depletions at the time of challenge in which these animals appeared to be very well protected with undetectable viral loads and full survival.

And then as another example of the importance of antibody responses, animals that were not vaccinated but treated prior to challenge with intravenous monkeypox and treated with the vaccinia immunoglobulin, while these animals were all protected from mortality from challenge but they certainly show evidence of disease with measurable viral loads and a wide array of pock lesions showing that they had disease but were fully protected by antibody treatment alone.

So looking back at one older study, a Downie and McCarthy study that's been mentioned in the past, they looked at mature virus neutralization of variola

virus and using CAM, so in days prior to tissue culture experiments. And their work and their summary showed that after either primary vaccination or revaccination of people previously vaccinated, that there's actually a pretty long lived antibody response after a single vaccination in which one could measure MV neutralizing antibodies for up to 40 years from the primary vaccination. And while revaccination you get MV neutralizing titers more quickly than after primary vaccination, in general the levels aren't much higher than after a primary vaccination.

And in more recent years many studies have really recapitulated that finding. And I just use a study from Mark Slifka's group to highlight this using some of our more modern day techniques of ELISAs in which he had a group of about 108 people who were either previously vaccinated once or up to 6 to 14 times and that's what all these color codes mean. But basically, in general ELISA antibody response to virus was pretty stable, really didn't have a half-life or didn't decline over time and could be measurable. All

these black dots are single vaccinations occurring 70, 80 years out from the primary vaccination.

And in some work my lab did with serum specimens that Mark gave us we showed a very good correlation between the MV neutralization with ELISA data. And in Mark's paper and in many other papers and also talked about here, this ELISA titer correlated to this very famous target of a 1-to-32 neutralizing titer that historically showed that people were less susceptible to smallpox infection.

So going back and looking at that study, it's not as crystal -- I don't think it's as crystal clear but this is the data that exists. So this study by Mack in 1972, which was a prospective study that was actually in a bigger cohort of patients looking at an antiviral prophylaxis at the time of exposure, and this study -- this sub-study looked at people who were bled at the time of contact looking at their antibody responses. And this was an outbreak occurring in Pakistan.

So they had 142 subjects who were bled prior

-- at the time -- at the start of the study. And there were just 3 cases of variola virus or smallpox infection in these 142 subjects. And as one sees looking at the neutralizing antibody titer, the three cases of subjects who were bled, there were two additional cases in children who they did not have blood samples from. So it's from this data where this magical 1-to-32 antibody neutralization titer comes from. I was a little disappointed looking at this primary data.

In the same study by McCarthy and Downie in '58, you know, they looked at other markers of antibody responses and hemagglutination inhibition assay was an assay available at the time. And in distinction to the neutralizing antibody responses, the inhibition neutralizing antibody responses were really short-lived. And even upon boosting you get a short-lived boost but then once again a very short-lived hemagglutination antibody response.

It's interesting thinking about what we know now. The hemagglutination inhibition assay is driven

by the A56 protein, which is an extracellular virus protein but also expressed on the cell surface. And we know antibody responses to that protein are actually pretty high and pretty long lived. So exactly why the inhibition assay is just such a short-lived response is probably a function of the assay and I'll talk about a similar finding with another early assay later.

However, using the hemagglutination inhibition assay, they clearly show that, you know, most are protected in the absence of this hemagglutination antibody. This is this Mack study, prospective study. And that even the presence of a low HI titer was not a reliable indicator of protection. So I think the audience could take away and we could tell the FDA that the HI inhibition assay is not going to be a good test for us to use going forward.

So another interesting study, again out of Mark Slifka's group in Oregon, looked at -- he was able to find eight variola virus survivors in the

Pacific Northwest. And looked at their antibody responses because the thought was that antibody response or infection with variola virus resulted in lifelong immunity to re-infection with variola virus. So it would be very interesting to look at the longstanding immune response that one gets to variola -- survivors of variola infection to those who were vaccinated, those who -- and then others who got -- had variola and got vaccinated or people who were just vaccinated twice. And while this doesn't reproduce well, I just highlight the blue triangles which were the variola virus survivors. And basically, the findings were that the antibody responses, long-lived once again in both groups, were essentially indistinguishable.

So if the thought that initial infection with variola virus is lifelong immunity but vaccinia virus vaccination gives long-term immunity and probably immunity from high mortality or severe morbid smallpox, it certainly -- historically the protection people say wanes after three to five years. Full

protection from smallpox. So there is indeed something we're not measuring that distinguishes past variola virus infection from live vaccinia virus vaccination.

So as Bernie mentioned, there have been proteome microarrays to try and identify the targets of antibody responses and work out of Phil Felgner's group a couple of studies I'll just point out here. So they detected antibodies to about 25 different proteins, many likely not involved with virus neutralization. And a consistent theme that is seen is a considerable heterogeneity in the antibody responses to various proteins. And after an initial vaccination, consistently found in at least 50 percent or more subjects were these MV proteins that are neutralizing targets of antibody as well as some EV proteins that I'll talk about a little later. And then after a second vaccination, a boost vaccination, some very highly potent targets of monoclonal antibody neutralization, A27 and L1 were more consistently detected.

And so taking -- Bernie Moss wrote a very nice review on this topic and it's in my list of references at the end of the talk. This summarizes the protein targets in which protective antibody responses, either by various subunit vaccine approaches or monoclonal antibody approaches show evidence of protection. And I think I added one extra target here that wasn't in that review.

So moving on. With the proteome, as well as -- this is from Shane Crotty's group along with Phil Felgner's group, they looked at MV responses in 50 Dryvax vaccinated people. Once again, a very heterogeneous response. They found that H3 was the immunodominant neutralizing antibody target. But if you depleted the serum of antibodies directed against H3 by precipitating out that antibody, it did not alter the MV neutralizing ability of that serum at all. And so leading to the thought there's definitely a redundancy of MV neutralizing activity. And as pointed out in this paper and in Bernie's review, you know, there's a likely presence of many other -- some

other unidentified targets as well as higher order complexes that we're not measuring as individual proteins that have to assemble in an antibody response to that assembled complex on the MV surface might be a very critical target of neutralizing antibody that we're just not measuring yet.

So this audience is pretty savvy based on the lack of questions the speakers have been getting, that they know that MV neutralization is not enough. And all the early work was really to impact the genesis and the protection was really done prior to the demonstration of the significance of the extracelluar or envelope virus. We have to decide is EV extracellular or envelope virus.

And this was really highlighted in work that's been talked about here so far, experiments with inactivated vaccines that highlighted a lack of correlation between a neutralizing antibody response measured against mature virus and protection. But looking at these data, and as Bernie pointed out, these inactivated vaccines certainly protected from

death. And I'll show you the data here. Some of the data from Boulter's study using rabbitpox as a surrogate model in which they vaccinated with live rabbit -- live vaccine, although at a whopping dose, measured neutralizing antibody titers and showed that on challenge the animals had no fever and all were protected from death. Inactivated virus which this study was done with formalin inactivated and using an adjuvant or no adjuvant, you could get whopping MV neutralizing antibody titers that protected from death but still about a third of the animals had evidence of infection. So it kind of means what target of protection are you looking -- are we going to be looking for in our vaccines in weighing safety versus efficacy. And the control animals mostly got sick and most died.

Perhaps the antibody responses are important or this correlation or lack of correlation with the MV neutralizing activity is seen more with passive transfer of sera from animals that were vaccinated with inactivated vaccines in which you could see some

very whopping high MV neutralizing titers that were then passively transferred prior to rabbitpox challenge. And even with this high MV neutralizing titer, all the animals got sick. And in passive transfer experiments there were deaths there, whereas sera from live vaccinated animals that were then passively transferred with much lower titers, some still got sick. Of course, passive immunization is not as good as active immunization. But once again, all protected from death, so, once again, highlighting the lack of correlation between MV neutralizing antibody and protection.

So the audience all knows that EV or the extracellular virus is an important target of the immune response after live vaccinia virus vaccination. And work out of my lab using once again serum specimens from that initial Mark Slifka study, we looked at the correlation between MV neutralizing and EV neutralizing in a plaque reduction assay.

So one thing I really have to note and I'll highlight in red up here, so EV is actually a very

difficult virus to neutralize in a plaque reduction assay using antibody alone. And so my NT30 was set to look at this. So there was a correlation, but we did note that 12 percent of vaccinees who had very good or had good MV neutralizing activity had essentially immeasurable EV neutralizing activity. And interestingly, all these specimens were from people vaccinated greater than 25 years previously. And so thus, for some individuals neither virus-specific antibody measured by ELISA, which I'm not showing, or the MV neutralizing capacity can predict your EV neutralizing ability. And so I think this is a hole in thinking about what type of tests we need.

This is just data reiterating the earlier work that Mark did that basically shows against MV neutralizing you get a stable, long-lived, greater than 40 years without any diminution in your MV neutralizing activity. But once again, the EV neutralizing in a plaque reduction assay, there is a drop-off, a statistically significant drop-off after about 20 years of your EV neutralizing activity.

So as Bernie and others have mentioned, so the main targets of EV neutralizing activity, either by a comet inhibition assay or a plaque reduction assay is A33 and B5. And I'm going to focus on B5 and maybe during the panel discussion or during questions other people could ask about A33.

So work my lab did, which is not shown here, with VIG, we showed that B5 was the major neutralizing target of EV in VIG. And work out of Jeff Smith's lab using individual vaccinated subjects showed that by pulling out the B5 antibody by adding increasing amounts of B5 protein, one can totally eliminate the ability to neutralize EV in a plaque reduction assay whereas adding A33 protein or A56 protein to that same sera did not alter EV neutralizing activity.

And just as a side note, in the same paper they did work also with MV in which -- and similar to the other work by Crotty and other publications that you really can't take away the MV neutralizing antibody if you add A27, H3, L1, you do not change the ability to alter MV neutralization, although Jeff's

group did one interesting thing. They used UV inactivated mature virus particles at various concentrations and showed that the virion can soak up the antibody that's neutralizing mature virus and you can diminish or take away the MV neutralizing antibodies. So the MV particle contains all the information. And so if we knew that we'll be on our way.

So in talking about EV neutralization, since we're talking about next-generation vaccines of LC16m8 and MVA, I'll just point out the issue behind LC16m8 and this vaccine which in animal models shows very good protection in surrogate models has a truncated B5r protein and, therefore, expresses just a short end terminal portion of the protein in which there is some antibody response measured.

So work out of Jeff Smith's lab in which they looked at the sera from 42 non-human primates who were vaccinated with LC16m8, in a primary vaccine they found a very, almost no EV neutralizing activity in that sera. And just to point out, you know, so work

out of my lab along with the group at VaxGen at the time, in a rabbit study in which LC16m8 was used as the vaccine, we were able to show that there was EV neutralizing activity that was just as good as Dryvax. So if this is a difference between species rabbit versus humans, although I suspect it's more a function of the test between the two labs and what our cutoff points are, et cetera. Because looking at Jeff's lab's data, in the 43 subjects who were boosted with LC16m8 who most likely had previously been vaccinated with the Lister strain of vaccinia virus, one sees that they were measuring very low levels of B5 neutralizing subjects years after prior vaccination. And I think this is lower than what he previously reported in another study and what we've also seen. So I suspect it's an assay issue and certainly I think something that moving forward and looking at vaccines, EV neutralizing assays are a big hole in what we're doing.

And so another EV neutralization, in recent years the Fc domain or the functional side, the domain

that interacts with FC receptors and/or complement has become -- is being looked at further. And work here by Shane Crotty's group showed that while VIG is able to neutralize envelope virus, although, you know, these are just small numbers of plaque skirting from 90 to 45, if one includes an active complement during the neutralization incubation period, one could get a much more enhanced neutralization, although Shane talks about this that EV neutralization is dependent on complement. But I think that's just some semantics. And not only in VIG but he also looked at some sera from that individually immunized subjects in which he was able to show that complement enhanced the neutralization of the EV along with some controls there.

Another assay he used in this paper was showing, you know, so not only is complement and antibody neutralizing individual virions in plaque reduction assay, but complement can also, along with antibody, attack infected cells if the target is on the infected cells. And so this is some work in which

a monolayer of virus-infected cells are then incubated with either VIG or VIG and complement and then stained with crystal violet and one, you know, clearly shows that either with VIG or sera from an immune individual one could really attack those infected cells. And on your handout is just a quantification based on the crystal violet.

So what's interesting is this complement idea of the functional domain of FC fixing complement looks really interesting but it was interesting to go back to old data once again that looked at complement-fixing antibodies. This is the McCarthy paper from '58. And similar to the hemagglutination inhibition assay, this was a short-lived response that on primary vaccination you would get complement-fixation titers that then went away. And even upon revaccination you got some initial responses that went away.

So I had to go back and look how complement-fixation assays were done because clearly it looks like complement-fixing antibody is important work. I'm not talking about which subunit vaccines. We have

some data that shows complement-fixing antibodies is really an important part of the protection by antibodies directed to individual proteins. So in a complement-fixation assay, what one's doing is diluting that antibody, adding antigen that binds antibodies, and then complexes. Antibody antigen complexes are made that then can fix -- that can then fix complement. And presumably, non-fixing antibody is not non-fixing complement.

And then the investigators add some sheep red blood cells and incubate that at 37 degrees. And if lysis occurs, there's no complement-fixing antibodies, whereas if you just get precipitation of the red blood cells, that's positive for complement-fixing antibodies. And clearly this test is different than the functional tests that had been looked at more recently.

And I'm going to just finally conclude with some remarks about the host defense modulators that are expressed in orthopox viruses. So one orthopox virus host defense modifier, the TNF receptor -- TNF

receptor or what is called CrmB, this gene is lacking in MVA. And just to use that as an example, as you know, we're thinking about antibodies directed against neutralizing targets but there is evidence that antibodies to other host immune modulators is important. And so I'll use the interferon alpha/beta receptor story from Luis Sigal's lab.

So this is a mouse that was vaccinated intraperitoneally with WR and he shows that there is an antibody response that's generated to the interferon alpha/beta receptor protein. In work, while we're not talking about subunit vaccines, protein vaccination with this interferon alpha/beta receptor actually resulted in full protection from an ectromelia challenge study in the susceptible mice. So this antibody response could be contributing to the protection we see with live virus vaccines.

And then looking at where this interferon alpha/beta receptor protein is, it's a full-length protein in ectromelia, monkeypox, variola, the viruses we're really worried about. It's present in WR. It's

present in one of the clones that were sequenced during the plaque isolation of what turns out to be ACAM2000. And we remember that Dryvax is a swarm of viruses, and so this plaque isolate had this protein, whereas ACAM2000, which was moved forward, has a C terminal truncation of about 100 amino acids, as does the same truncation occurs in MVA, either ACAM3000 or MVA. Interestingly, Lister lacks this gene.

So other issues that I'm not going to talk about. There's some new studies -- this is all out of Greg Poland's lab -- looking at gender difference in MV neutralized -- in antibody responses and, you know, whether this is large enough to make a difference but they do note that females had a higher MV neutralizing titer than males. Also, some work out of their labs showing some potential genetic factors that differ between the races.

So in conclusion, so antibody response is definitely important. Cellular responses likely provide additional modes of protection, so I'm not dissing the cellular immune responses. Antibodies,

titers, and MV neutralization are important but cannot be the only criteria. Antibody titer and/or level of MV neutralizing activity for full protection really is not known. HAI and complement-fixation assays are not useful measures. Antibody responses to EV targets are important but assays to find protective response are not great. The plaque reduction EV neutralization. Common inhibition we didn't talk much about. And where some functional assays, like the addition of complement fits into all this. And the role of antibody responses to host defense modulators are largely unknown.

So I'll stop there and take some questions. Thank you. (Applause)

CAPTAIN NELLE: I'll kick it off and ask a question.

DR. ISAACS: Good.

CAPTAIN NELLE: You mentioned that antibodies to B5 are mainly responsible for EV neutralization. But on the other hand there's not such a similar situation for the MV targets. So one

question is how important in terms of virus replication is B5? Can you make a virus that's devoid of B5? Is it viable or is this something that we should really be paying a lot of attention to?

DR. ISAACS: So, yeah. So, so B5 -- the truncation in LC16 that makes it lack B5 is probably -- is one of the major attenuating features of that vaccine. So a vaccinia virus that -- or poxvirus that doesn't express specific EV proteins like B5 and A33, they do not spread well within a host or in tissue culture. They make a small plaque phenotype in tissue culture, in cell culture, and in animal models are essentially avirulent. And so the lack of B5 was the major attenuating feature of LC16m8. But there were still redundancies in that vaccine that still in animal models make it a very good, protective vaccine.

CAPTAIN NELLE: Bernie, I think you have to use the microphone.

DR. MOSS: Bernie Moss. I think what we should emphasize and I know you know this but I'm not sure all the audience knows it, is that although

antibody to A33 does not score in the neutralization assay, it is in animal -- in small animal models anyway it's at least as potent for protection as antibody to B5. So it highlights the impotence really of the EV neutralization assay in general.

DR. ISAACS: Yeah. Thank you, Bernie. Yeah, so I said I was not going to focus on A33 but the lack of B5 in LC16, it has A33. And as Bernie mentioned, in subunit vaccines and/or passive antibody transfers against single targets, A33 looks as good, if not better, than B5 in certain situations. And whether it's the complement neutralization of virions that are induced by A33 attaching to EV and/or the cellular cytotoxicity of complement where A33 antibodies bind to cells. So thanks for pointing that out.

Yeah. Another question.

CAPTAIN NELLE: When you come to the microphone, be sure to state your name.

DR. FELBERBAUM: Hi. This is Rachael Felberbaum from Protein Sciences.

So I know we're not really focused on subunit vaccines today but I'm getting the sense that we're not really sure what all of the targets would be for a subunit vaccine. There seems to still be maybe proteins and antibodies that are being produced to unknown factors in the active vaccines that we have. And so I'm wondering what your opinion is. I mean, how many different proteins do you think would need to be targeted? And is a subunit vaccine feasible?

DR. ISAACS: Yeah. So, you're speaking to a topic near and dear to me because I do work on subunit vaccines. And, you know, maybe in 5, 10 years when the FDA assembles a similar open panel to talk about subunit vaccines, which I think are going to be even more difficult. We're having trouble wrapping our hands around live virus vaccines that have so many different targets and redundancy that, you know, how we're going to seek approval of protein or DNA-based or other recombinant-type vaccines against specific proteins is going to be more difficult. But how many is better?

You know, so in thinking about subunit vaccines, from a production and commercial standpoint, the less complex the virus is, the easier it is to go through the regulatory process and understand how the vaccine works. Whereas, having more targets reassures you that it might be more effective in a wider population. So work certainly that Bernie's group has done and others, so if one focuses on the target, the protein targets on MV and EV, clearly you need one of each. You know, you get some protection with just an MV target and some protection with either B5 or A33 as a target. But when you put one from each group together it's even better. So I think the minimum is going to be two. Some might say three or four would be the safer way to go.

I'd be curious if others had any thoughts on that topic, too.

DR. SUN: This is Wellington Sun from CBER.

Has anyone done any work on the antibody dependent cellular toxicity from these vaccines?

DR. ISAACS: Yeah. No, there's probably

some older literature in which people looked at that. And now there's more work being done with complement mediated lysis. But there isn't a tremendous amount of literature on cellular -- antibody-dependent cellular toxicity.

Bernie, are you -- I see your brain is working there. Am I remembering that right?

So, yeah, I don't -- that's an area that has not been fully explored either.

CAPTAIN NELLE: Any other questions? Okay. Thank you very much, Dr. Isaacs.

DR. ISAACS: Thank you. (Applause)

CAPTAIN NELLE: Our next speaker and the final speaker for this morning's session is Dr. Freyja Lynn.

Dr. Freyja Lynn is also from CBER and today she'll be talking about the Assessment of Effectiveness of Smallpox Vaccines: Immunogenicity Assay Considerations, otherwise the issue is we're talking about this antibody bridge or immunological bridge that we will need between the human studies and

the animal studies.

MS. LYNN: Good morning. First of all, I have to say that, yes, I am with the Division of Bacterial Products and you might ask why am I talking about viral vaccines. And, in fact, I'm talking more about assays in this talk than really the viral vaccines itself. And I was with the Office of Biodefense at NIH, which is how I initially got involved in working with smallpox vaccines and looking at both assays and animal models. And I think this will be a really good chance to sort of build on what's already been covered, and in fact, there are a couple of slides I can actually skip over which is good. But essentially I want to focus on using the assays and how do we use assays to provide evidence of effectiveness. And then what specifically are we dealing with with smallpox assays. And I think this will also wrap into this afternoon when we start talking about animal models.

And, you know, Tim, this morning, kind of focused on two approaches. And I think that's how I

wanted to organize my talk and compare how we would use immunogenicity assays in a non-inferiority path to licensure versus how we would use immunogenicity assays if we wanted to go through the Animal Rule. I suspect the ultimate answer to the path will be a combination or a more complicated situation. But I figured if I could just sort of divide it out we might be able to discuss specific assay issues. So this is sort of how I'm going to run my talk.

Now, people talk about surrogates of protection and correlates of protection. And there's disagreement within the field as to what those terms mean. So I just want to define them for today, for me, for potential use later on in the day. I don't claim that these are everybody's definitions but in my mind a surrogate of protection actually explains the mechanism of protection. And you're really lucky if you have one of these. They're generally obviously shown in the clinic and it makes paths to licensure extremely easy because you can simply use your surrogate of protection.

A correlate of protection is also generally show in the clinic and it correlates with protection but it isn't necessarily the only mechanism of protection. It probably is an important mechanism but probably not the only one. But again it helps to facilitate paths to licensure when you have correlates.

What I think we have in the case of smallpox is immune measures which may correlate with vaccination but haven't been shown to necessarily predict protective immunity.

Now, all this in this slide has been covered already. I just want to mention again the importance of knowing that what may have been appropriate for an old vaccine, for example, ACAM2000 or even going back to Dryvax or even going all the way back to cowpox, may not necessarily be appropriate for the vaccines that we're looking at in this day and age, especially as we move away from live viral vaccines into subunit vaccines. And I think that's a really important thing to keep in mind when we try to figure out how we're

going to use assays to draw bridges between vaccines or between models.

So again, I decided I would divide the talk up into two major focuses, one looking at an approach that would use non-inferiority to the licensed vaccine which would, in the case of where we are today, look at non-inferiority to ACAM2000. And the second approach would be to try to bring in the Animal Rule and see what we could bring -- take from the Animal Rule that would help us assure that the next generation of vaccines are going to be effective in the clinic. Again, as was pointed out earlier, when you're dealing with the Animal Rule, you have to make sure that you remember we're trying to protect humans and not animals.

So let's start with non-inferiority with the licensed vaccines. And at this point I think the first -- the first issue, what do you compare to, is easy. You compare to ACAM2000. But when it comes to what assay do you use, what are going to be your appropriate immunological endpoints, and following

from that, what are you going to set for your criteria for non-inferiority are more difficult questions that I think is one of the reasons we're here today.

So what data do we have that starts to let us figure out where are we in terms of the assays and what are we going to use for assays? And this is a paper that's been mentioned earlier looking at the immune or the antibodies induced by vaccination with either MVA or Dryvax. And these are human responses. And again, we just look at the hotspots and we can see that if you immunize with Dryvax you have a much more diverse set of antibodies than if you just immunize with MVA. And in fact, depending on what antigens you look at, you can either find a response to MVA or not find a stronger response to MVA as you would to Dryvax. And this is just to introduce the complexity. As we've heard before these are big viruses with lots of proteins and it's a really complicated picture.

These are data from Inger's lab that again are using what we would all love to use, which is variola. And again, as she's pointed out, you can get

some really beautiful data using variola that I think are very reassuring to all of us even though as has been mentioned it's not a correlate of protection that's been shown in the clinic. But it is comforting. It is a neutralization assay. It is our pathogen. But as she has pointed out, we're limited in resources. These are difficult assays to do and she's pointed out that in fact these are difficult assays to encompass both types of the virus. And so I think we may think about doing maybe a subset with variola for comfort level but I don't think we're going to do our major clinical studies.

So we're going back to, well, what antigens can we use? And this is a paper from Sharon Frey's group and the VTEU study that was done. And I think this is one of the studies that Inger looked at -- presented some data from. But essentially they did a variety of different regimens with MVA and they also did -- came back and did a challenge with Dryvax. And they tested the assays either using an MVA as the antigen of the neutralization assay or using Dryvax.

And this is just antibody titer over time. And what's interesting is that although I don't know that these assays were fully validated or if they could be optimized or changes could be made, but depending on whether you used MVA or Dryvax, you can see that in particular this group, which is lower when you use MVA, is higher when you use Dryvax. And I think the members of this audience will go, well, yeah, duh, you know, that's probably -- we can have lots of explanations for why that's true but again, even when we talk about some of these other antigens that we'd like to use, we have to be really careful.

These are data courtesy of Bavarian Nordic where they actually looked at two probably more related strains, the WR versus the Dryvax, and did Group C, which is the same group that we saw in the previous slide coming out of that NIH study. And again, just looking at mean titer over time, and you can see that the WR gives a substantially lower response, although the pattern. So again, if you're looking at a pattern of response you might be okay in

using either of these. But if you're looking at magnitude of response you'd have to be very conscious of which antigen you're selecting.

And then one of the probably under-discussed assays is the ELISA. Because, in fact, the PRNT may be functional but if it's not been shown to correlate, we don't really know how to interpret those data probably any better than we know how to interpret an ELISA. But even when you start using ELISAs you have the same -- you're going to have some of the same issues if you -- either you use MVA, in this case Dryvax. This comes out of the same paper as some of the earlier data. And I think it's important to look at this early response here where depending on the antigen you use in the ELISA, you get a very different profile of response.

So if you're going to use these ELISAs to assess clinical inferiority, we're back to these questions. What assay do you use if you don't have a clinical correlate? Again, you know, a lot of people want functional assays. Some people would probably

prefer the PRNT. But again, we don't know if it's any more relevant. I've shown that, you know, vaccine strains versus a human pathogen, you have to be careful what you use because you might get a different answer. And again, what difference is meaningful? If we don't know what the relative value across the vaccines would be just on the face of it, then how can we set inferiority criteria? So again, all of these things complicate the picture. And again, as I mentioned earlier, you have to take into account the mechanism of protection. If we had an assay that actually addressed the mechanism of protection or could get a correlative assay, we probably -- it would make this whole thing a whole lot easier.

So let's talk a little bit about the Animal Rule. And essentially, to apply the Animal Rule you have to develop an animal model that you're confident is reasonably relevant, and then you have to figure out how to bridge the animal efficacy to the human dose. And this is what Tim was talking about, that wonderful number four of the Animal Rule. And that's

where it starts to get tricky.

Now, one of the things we've done in the past, in particular with anthrax, is we've started trying to propose things that you could do to do this kind of bridging. Now, again, these are not requirements of the FDA. These are just possibilities. And one is to use your animal model to determine a correlative of protection. And if you do that and you have species-neutral assay that you can then apply to the humans, you can actually bridge the correlative of protection in the animal model to your immunogenicity in humans and actually start to think about how you could try to make an estimate of how effective the vaccine would be in the human population based on the immune responses. Again, I can't emphasize enough that in order to make this as easy as possible you really do try -- you really do need to try to get a species-neutral assay.

And can this be done for smallpox? Well, this is a study that was done at OBRA or by OBRA at Battelle. And this is where we were just looking at

the multiple doses of MVA, looking at the PRNT titer versus what we were calling a maximum clinical assessment score. Now, the higher the number here, the sicker the animals are. And because we had very few deaths in the vaccinated animals, we couldn't really do a feet up, feet down kind of analysis. So we just -- these clinical assessments include things like the number of lesions and the fever and lethargy and all those various signs that we look for in the animals. And I think you can see that overall there is a general relationship between an increased PRNT and a decrease in the maximum clinical assessment score.

Now, the biggest problem with this study is that it's confounded by dose because, in fact, we also see a really lovely relationship between the higher the dose, the lower the clinical score. And I'll talk a little bit about this later. This is something you have to be very careful of when you're trying to determine correlates of protection. You need to come up with a model that you can actually take away other

confounding factors like dose so that you can really -- so that you can relate your correlate to protection rather than dose to protection or some other confounding factor.

Okay. PRNT, as I said before, assay we're comfortable with. It's functional. We may not know what antigen to use but if we can come up with a reasonable antigen it makes everybody happy. Now, a lot of functional assays are on the face of it species-neutral. And I'll give an example a little bit later on with the toxin neutralization assay we use for anthrax. And so the first question we have to ask ourselves if we're going to apply a PRNT to an Animal Rule and we're going to try to approach it through this kind of bridging, again, not givens at all, but we'd have to say, ask the question, is the PRNT species-neutral?

And these are some data from -- again, from Bavarian Nordic that might indicate that the PRNT is not as species-neutral as we might like. And this is looking at a human immune serum, actually VIG, and a

non-human primate immune serum. And this is a difference between two different vendors for fetal calf serum. And so when they use the assay using this vendor, the two titers that they get for these two sera are pretty much the same. When they switch to HyClone, suddenly the human titer increased quite a bit. And this to me indicates that what affects one species in this particular assay may not affect another species in the same assay. And so your assay conditions become incredibly critical and a little bit unpredictable in terms of trying to draw a bridge between a non-human primate and a human titer.

This is a similar kind of study using human and NHP, changing the cell line from either -- from a vero cell line to a human cell line. And although the differences are subtle, you can see that moving from non-human primate to human increases the human titer but decreases the non-human titer -- the non-human primate titer. Again, this is an indication that this assay is going to be, I think, more difficult than we might have anticipated in terms of being able to

bridge among the species. And this is just one aspect of bridging between the species in terms of the assay itself that we would have to look at to reassure ourselves.

So in terms of trying to think about how we're going to bridge between the animal and human, again, I'm focusing primarily on, you know, PRNT versus ELISA because I think those are probably at this point our two most practical assays that we might use. The PRNT, we have to make sure that it's species-neutral. We have to choose our virus, which may or may not be appropriate -- I mean, what may be good for one product may not be good for another. And as I showed in an earlier slide, which I didn't actually point out, but as we were getting down to the level where we were getting low enough doses of MVA, we were actually very much bottoming out on the PRNT assay sensitivity. And so if we have to go low enough with MVA in order to get doses in which we have protected and unprotected animals, the immune responses measured by PRNT may be too low. And so the

PRNT assay by itself may not be sensitive enough.

Now, the ELISA comes with its own set of problems. Inherently, ELISAs that are run with conjugate sitters species-specific are inherently not species-specific. But there are methods that you can use that we've actually used for some other pathogens including plague where we needed to come up with a species neutral assay and it can be done. There are a variety of approaches. Some are better than others and some are more successful than others. But again, we're concerned with the choice of antigen. And they're also just some very basic practical kinds of issues, even when you're using, for example, a killed viral prep. Bavarian Nordic has talked about that, you know, they've seen major differences in crude versus a purified prep. Apparently, poxviruses are extremely sensitive to purification. And so they've gone with a crude prep. But they're having even batch-to-batch consistency issues. So you have to be extremely careful about how you bridge your reagents and how you batch your studies and how you again are

going to run assays to compare, if you're going to go either non-inferiority or Animal Rule, that you batch your assays so that they are internally consistent and can be internally compared.

So where are we right now in terms of the animal model? NIH is still -- and others are still working on trying to gather some additional data. Right now I know of at least one study that's doing some dose ranging using MVA to try to find a dose that will give you both protected and unprotected animals. If you can find that dose, then you can actually look at correlates of protection that are independent of dose and it gets rid of that whole confounding factor. And so that's the ideal. And we know that that's probably going to be extremely difficult to do with Dryvax or ACAM because we tend to get an all or nothing response. But we're hoping that we can do it for MVA or that they can do it. Now that I'm with FDA it's NIH doing it.

And then there's going to be a lot of work that's looking at the assessment of the immunogenicity

in the animal models, again, probably focusing on ELISAs and PRNTs to look at which assay is more sensitive and which might actually correlate with protection so that at least for MVA we can start to think about how we can build a bridge from there, from the animals to the other -- to the human situation. And again, looking at species neutrality and making the point one more time that every single correlate or every single vaccine candidate may have its own correlate. And so we may not be able to generalize, for what is good for MVA, what is good for ACAM2000, may not be relevant for some of the subunit vaccines.

Now, believe it or not, it can be done. Tim actually alluded to these data and showed how the approach was being taken for anthrax. And these are actually some of the anthrax data in the non-human primate model. And this is just a logistic regression looking at the probability of survival versus the toxin neutralization assay titer. Now, TNA it turns out was kind of a slam-dunk for anthrax in a lot of respects because anthrax is generally a toxin-mediated

disease. And so if you can come up with an assay where you're looking for antibodies that neutralize the toxin, it's probably going to be an important mechanism of protection. And I think the data to date suggests that in fact it is an important mechanism of protection. However, it's not a surrogate, and I think it's important to recognize that. If you look here you'll see some animals that survive that had very little antibody. And over here you have some animals that died that had quite a bit of antibody. So it doesn't explain everything, but the data do correlate. We can get a very nice regression line. We can get really tight confidence intervals. And so we can start to build some assurance that when we look to bridge these data to humans that we actually are probably going to get a reasonable estimate of how effective we think the vaccine will be in the human population.

Again, we're not trying to estimate efficacy necessarily; we're just trying to show a reasonable likelihood of clinical benefit. And I think these

kinds of data help reassure us that we can actually do that.

So to wrap up, again, just sort of talking about non-inferiority versus the Animal Rule, non-inferiority everybody likes because it's based on clinical data and it's based on a comparison to a vaccine you're pretty confident in. And so, again, there is a real comfort level with that. But again, I think we're really confounded by trying to figure out what immunogenicity measure we're actually going to use to assure ourselves that we're measuring something that's relevant to protection to both of the vaccines that we can actually do a fair comparison and set endpoint criteria. Again, the selection of the assay and the selection of the antigen.

The Animal Rule is reassuring because you can actually demonstrate efficacy. You can challenge animals and see if they survive or not, see if the vaccine protects them or not and I think that's very reassuring. You can start to work on figuring out what assays are relevant because you can manipulate

the model so that you can look at correlates of protection and decide if you can measure something that does relate to protection that you might be able to then bridge to humans.

The question is always what is the relevance of the animal model? And one of the things I think we need to keep in mind is that no animal model is going to be perfect, and there are always going to be some limitations to any animal model we use. But models are extremely useful if we interpret them with reasonable scientific judgment. And so I think, you know, animal models are just -- could be an incredibly important part of any licensure for this kind of a vaccine.

The other assumption we have to make is not only that the assay is pan species but, in fact, that the immune response across the species is similar enough so that when we measure an antibody in animals and we measure the same antibody in humans, that it is sort of equivalently relevant. So if I measure, for example, in the anthrax case, if I measure toxin

neutralizing antibodies in animals, are they the same essentially as a toxin neutralizing antibody in humans? And I think there are plenty of data out there in the anthrax case that, in fact, is true and we can be secure in that. But again, you know, this is another leap that we have to make.

So, with that I'll end and take any questions. (Applause)

DR. GUPTA: Rajesh Gupta, from CBER.

I think despite the complete understanding of the protective mechanism, it seems that the antibodies play a major role and that may even like choose to license vaccines in the absence of clinical efficacy of the trials. And also due to the lack of robust assays (inaudible) immune responses, I think the antibody assays are going to play a major role. But what we heard at the same time the (inaudible) test is susceptible to the (inaudible) assay, the quality of the antigen that (inaudible) has more genomic copies than the actual live virus or the quantity of the antigen, how much it is used in the

assay, and also some (inaudible) the endpoint is taken, like the 50 percent reduction of plaque forming units or 75 percent or 90 percent.

So I was just wondering that if there is a need to standardize an assay so that everybody uses a similar format. And the second suggestion is that if there is a need for a standard in this assay, what internal control? Like the vaccinia immune globulin has been available for some other suitable preparation which can be used as a standard or as an internal control on the assay so that you can at least compare the results from different labs.

Those are my general comments. But specifically on the last presentation I have a couple of questions. First, is there any correlation between the ELISA and the (inaudible) test? And secondly, the data it showed on the -- I think the protection levels and the protection antibody, I think that looked very similar to what we had seen for diphtheria or other diseases when they tried to see the protective levels. And what I think was concluded was that there is no

absolute level of protection which can give you a protection in animals or humans but at certain levels, like for tetanus and diphtheria, the protective levels have been defined at 0.01 International Units. So the pathogens cannot propagate or, in fact, or cannot (inaudible) like go from host to host.

So in this case you may have -- in (inaudible) may have more than 0.01. In diphtheria, particularly, they have shown that people with even one unit that got diphtheria, like very similar to the data we showed. But 0.01 International Unit is considered a protective level.

So coming back to the smallpox study. So if an assay can be standardized in terms of some units or microgram of antibodies, maybe that will also help in defining some kind of level which can prevent the infection of virus to person-to-person transmission.

MS. LYNN: Right. Okay. Lots of thoughts there.

First of all, I'm not a big fan of protective levels. Just because of standardization

issues as well as some other issues. And I think if we're going to go with an Animal Rule, which is where I think we would start to work on something like a protective level, I think we're much better off going with the kind of analysis like a logistic regression like we're doing with anthrax because there we're able to take into consideration the entire distribution of the responses in the humans to a vaccine and allow us to kind of get a broad idea of effectiveness. I'm not sure that the data are going to be clean enough unless we get a surrogate to be able to set a cutoff level.

I think Inger showed some data on correlation between PRNT and ELISA. Did you? I'm trying to remember now. And I don't know, have you done correlation between --

DR. DAMON: (inaudible)

REPORTER: Go to a mike, please.

MS. LYNN: I'm forgetting.

DR. DAMON: I think the data I showed was the data from the Seacrest paper which looked at the correlation between an MVA luciferase neutralization

assay and the amount of viral shedding after a Dryvax challenge.

MS. LYNN: Right. Okay. Okay.

DR. DAMON: Yeah, so that (inaudible) regressions.

MS. LYNN: And I don't off the top of my head remember a graph but I think that's a tricky thing to do because of the -- in terms of the antigen. And I don't think I've seen a really good comparison between the same antigen used in ELISA and the same antigen used in PRNT. But again, I'm not a smallpox expert so somebody in the audience may have done that and know the answer to that question.

DR. ISAACS: So I showed -- I think there is a good correlation between an ELISA antibody response and the MV neutralizing activity. Many groups have shown that for MV neutralization.

DR. GUPTA: That will depend upon what antigen you use in the ELISA. Right? You gave examples of B5 or other (inaudible).

DR. ISAACS: So these are a whole virion and

most of that was done. So you're right. If you pick a specific protein there could potentially be differences.

MS. LYNN: Right. And I caution, again, comparisons or correlations between assays like that because there's a lot of other things that play into it, including the variability of the assay. And trying to put a correlation coefficient and say, you know, I have a correlation coefficient of 0.8, therefore, my correlation is good, is a little misleading because it only really looks at one aspect. And the correlation -- in fact, the linear regression is probably not entirely appropriate for these kinds of analyses anyway because you don't have a dependent and an independent variable and other issues.

So I think you have to be really careful when you're trying to correlate assays and figure out why. And I'm not sure we have an assay that we're confident enough in in terms of being a correlated protection to want to bring another assay onboard and say, okay, this is the assay I'm happy with so now I'm

going to bring in another assay, compare it to it, and move to the other assay because it correlates with my first assay. I don't think we even have a first assay to look at. And, you know, if we're going to go with an immune measure ultimately, if that ultimately happens that we can't come up with a correlative protection so we're going to use an immune measure, then my personal bias is go with the simplest one you've got that's the best assay, that's the most reproducible which would probably be something -- some sort of species neutral ELISA where you could do a lot more standardization.

Standardizing a PRNT I think is going to be tough. I think you can introduce standards like the VIG but trying to get methods together and do a standardization, it's hard for pneumo ELISAs so I can't imagine how hard it would be for smallpox neutralization.

DR. GUPTA: I agree with you on the protective levels. I did not mean that we have to define the protective levels because in the end if you

have that kind of regression, that's why I think it serves the purpose. But on the ELISA, I would say that if it does not correlate, I agree also that the correlation options are very misleading. So you have to define a certain level that at this level if you get the ELISA titer and the nucleation titer, I presume that nucleation test is still considered as a gold standard. So if you start with ELISA (inaudible) like immune response or immune conversion, then you may be getting very irrelevant antibody response with the ELISA if that (inaudible) antigen is not chosen appropriately or showing some kind of a correlation if the nucleation is considered as a gold standard.

MS. LYNN: Right. And I'm not -- and again, and I think other people might agree that I don't think the PRNT is a gold standard. It's what's been used. It's the functional assay. But, you know, has it been shown to correlate? I think the data would suggest it has not. And if it doesn't correlate, then I don't make the assumption that because it happens to be functional that it's better than a nonfunctional

assay if neither of them correlate. So I think we have to look for the correlation first.

Any other questions?

CAPTAIN NELLE: All right. Thank you. Thank you, Freyja.

Next I'm going to ask the panel members to come up front and join me at the stage here and we'll begin our panel discussion.

Okay. To facilitate discussion during this session we have a number of questions that I hope will generate lots of discussion.

The first question that we have is what are the major regulatory issues that must be addressed for licensure of next-generation smallpox vaccines? And in particular, if you note any particular gaps in our knowledge that we need to fill in order to proceed down the road to licensure on these vaccines.

MS. LYNN: Okay. I'll start since I'm on a roll.

I think we're limiting this to effectiveness.

CAPTAIN NELLE: Right. That's correct.

MS. LYNN: Right. That's where we're going.

I think the major regulatory issue is the assay. And no matter which way we go we have to be reasonably assured that the vaccine is effective. And no matter which regulatory pathway we take, we need to have an assay that we're comfortable with that we think is relevant.

CAPTAIN NELLE: Do we have enough knowledge about the mechanism of protection to actually select one assay as being better than the other? Certainly. We want an assay that has some relevance in protection but what does the panel believe in terms of our background knowledge? Do we have enough knowledge to say that we can go with just antibodies or do we need to be looking at other mechanisms, other cellular mechanisms? Or what are the thoughts there?

And I'm going to pick on Dr. Isaacs to answer this one. Sorry.

DR. ISAACS: Thanks. I think there's going to need to be more than one assay. Now, whether --

I'm not going to really comment if we think there needs to be a cellular immune response assay because in thinking about these live attenuated vaccines, I think they will generate some type of cellular immune response and there's a bevy of targets out there for that.

But the question is about, you know, do we need more than one assay for antibody responses? I think we do because some of the data we saw here today where MVA looks better than Dryvax in some assays, and I think that's, as most of the audience knows, has to do with a dose -- the dose of MVA that's being used for vaccination, 10^8 PVU of virions being injected versus a 10^5 dose of a thousand times more virions. And if the assay is just looking at MV-specific proteins it will make MV look better in some of those MV assays which I think the data showed. So I do think there's going to need to be more than one.

DR. BURNS: Yes. I'm Drusilla Burns from CBER. And I am not a smallpox expert. I'm on the panel because of the experience I've had with anthrax

and using the Animal Rule.

And I would just -- from a regulatory perspective though, once you start adding more than one assay, the complexity in trying to bridge from the animals to the humans or even for non-inferiority in a human population becomes very great. So I think you do have to think about this very carefully. Is it something that's really you have to have? Do you have a lot of confidence that that would give you a lot more information that is necessary to have? Or does the scientific community as a whole, the smallpox community, feel that there is one assay that even though it may be called only an immune measure is reasonable enough to give you that reasonable likelihood that the vaccine would be affected?

DR. MOSS: Bernie Moss. So we all agree that the assay for MV is not complete and that the assays for EVs are fraught with difficulty. So one possibility would be to use two ELISA assays, one which would have MVs. I won't get into which MVs yet. And the other would be to take at least two proteins

from the EVs, which are known to induce protection in animal models. And I would throw out the A33 and the B5 protein. And to try to see if a combination of two ELISAs, one which would be good for all of the surface proteins on the MV and two of the EV proteins. And I think that would have a better chance of making a standardized assay than some of the functional assays.

MS. LYNN: Yeah. I also wanted to follow up to both those comments in terms of multiple assays, and actually Ed may be able to speak to this as well. When we were doing the modeling for anthrax we were actually modeling, adding to the statistical model what might contribute to protection. And so you want the antibody response to be the only thing that contributes to protection. And what we found was that in fact there was a dose effect. There were some other effects because of the multiple different scenarios we were using but I'm not sure that you couldn't do that kind of modeling with two different assays and then again bridge that back to the humans. I think more than two assays gets to be difficult but

I think that kind of an approach, a two assay approach like that, makes a great deal of sense. And I think the statistics could do it.

DR. BURNS: Right. I should say, I mean, we certainly have other vaccines where we use more than one assay. Pertussis, for example, we look at several different assays. It's doable. It's just the complexity increases. But, yes, I totally agree.

CAPTAIN NELLE: Any other discussion on choice of assays that we would use for bridging? Any other thoughts or concerns?

DR. MIDTHUN: Karen Midthun, CBER. I guess another question I have is following up on the suggestion that Dr. Moss had that one could look at an ELISA that might encompass MV on one hand and responses to some EV antigens on the other hand. What kind of thinking goes into figuring out from which virus those particular antigens should be derive?

DR. MOSS: Well, we all know the best one, but I'm not sure that Inger would like to make enough variola virus and test the loss of infectivity in

order to distribute MVs to laboratories. So I don't know. Unless one can do more comparisons, I would tend to use the Dryvax or the ACAM2000 because that is licensed and we believe that the antibodies to that virus are protective. If we would use MVA or some other virus, we wouldn't even have that knowledge. So I would choose ACAM2000 probably.

DR. GOLDING: So just as a follow-up, while ELISA may be ultimately, you know, the easier to establish and to standardize, et cetera, then how do you then perceive bridging it to a protective titer? Would you do a passive immunity? I mean, you have to have some proof of concept to really validate any given -- ELISA test is correlating with *in vivo* protection.

MS. LYNN: I think to correlate it, if you want a real correlate we're going to have to go to the animals and try to find what assay correlates with protection in animals. So you can't -- I mean, obviously, we can't do it in humans. So we would have to go to the animals.

And if we find that it correlates, then it makes us very confident. Unless -- I think passive studies have been done that suggest that antibody is important in protection against smallpox. I don't think I would try to set a protective level or try to do a correlate analysis based on passive transfer because it is only one -- it is only antibody and in particular for viruses I think we know that it's not going to be the whole mechanism of protection. But I think you could do a correlate with ELISA. That's one of the things I think people are working on. And then you'd have to make sure again that you had a species neutral assay so that you're pretty confident that comparing -- hopefully comparing against a uniform standard, then you could start to draw some parallels between antibody levels and just circulating antibody between the different -- between the animals and the humans. And I think that would be probably the easiest approach.

DR. NUZUM: So Ed Nuzum, DMID/NIAID. I have to make the same caveat that Drusilla did. I'm

not really a pox person either. So my comments also come from our experience with anthrax. But, you know, in the animal models for anthrax we've talked about a small animal species and large animal species where the small animal would be where you get your statistical power and then NHPs is kind of verification of what you see in the small animals.

So we didn't have as much of a problem with anthrax as far as identifying which assay. For smallpox, obviously we do and there's lots of different ways we could go. Maybe we could think in terms of proof of concept data for multiple assays. You know, EV ELISA, MV ELISA, you know, I think this MVA variola neutralization is very impressive. To me that's a highly functional assay right there but we know we can't do all samples in a pivotal study in that assay. But the fact that it works is huge to me. So if you have that and then you have basic, more proof of concept data in other, you know, maybe other multiple endpoints that are doable and standardizable and then settle -- as the data from these multiple

assays come to get and settle on one assay that would be used for the pivotal studies and the BLA submission. An assay that is standardized is meaningful based on correlation data and so forth. So kind of, you know, a pyramid approach using different pieces that kind of come together.

MS. LYNN: We have actually experience with that in the past where you do most of your clinical data or your nonclinical data with your workhorse assay and you save your functional assay to do a subset. And so there are precedents for doing those kinds of tiered analyses as long as you're really confident you understand what the assays are saying. So from an assay standpoint that's doable.

CAPTAIN NELLE: Would there be value added in terms of taking sera from the clinical studies and using those in passive transfer studies and doing challenges in the animals?

MS. LYNN: I think anything you do that shows that you understand something about the protection and the relevance of the assays, the better

off you are. Again, I wouldn't set a protective level or do a correlative analysis based on passive transfer but I think that's reasonable and I think some of those kinds of things have been done.

DR. BURNS: If I could just add to that, I agree totally that doing some passive studies may give you information about mechanisms and that sort of thing. You do have to be careful about levels of antibodies, as Freyja just said. And we found that in the anthrax case that it took much higher antibody levels to protect by passive immunization than by active immunization. So you just have to be careful about how you interpret the data and what data you consider to be critical and what is informative.

CAPTAIN NELLE: I think the data, that data that Dr. Isaacs presented about, you know, using live virus versus those that are inactivated definitely raises the question down the road, even though we're not talking about fourth generation vaccines, you know, certainly those would not work in such a case, but I think we also have to take into consideration

that when we're talking about the third generation, undoubtedly and unknowingly, we're setting a precedence down the road that will have to be dealt with by other vaccines also. So another consideration to consider.

MS. LYNN: For some of the later vaccines where you're talking about subunit vaccines, in fact, in my mind the situation gets a little easier because you understand a little bit more about specifically what you're giving and potentially how that's going to protect. And if you're looking for a virus for neutralization, obviously you're going to pick a virus that has those antigens on it. It's more complex than that obviously but I actually think that when we move forward into subunit we may end up having an easier time dealing with some of these issues.

DR. ISAACS: The problem though is, you know, the subunit vaccines oftentimes will give you much better antibody responses than the live virus vaccine. You know, we could get much, much higher. So in many of these assays the subunit vaccines will

certainly look better than the live virus vaccine but I still think that is a difficult road ahead even showing that.

DR. KOVACS: This is Gerry Kovacs from HHS.

If we could just step up a little bit from the assays themselves and look at the regulatory issues a little bit more holistically. I'm hoping that we're not taking a binary approach towards licensure here, whether it's non-inferiority or Animal Rule. But rather looking at the licensure of next-generation vaccines sort of as a combination of the two. Because I don't think we're at a point in our scientific understanding of the vaccines or the modalities by which they protect to be able to make either decision. I think what we're left with is a potential data package that leads us towards a better understanding of what these next-generation vaccines are capable of doing in terms of protecting individuals against smallpox.

I think there was a comment made earlier about -- I think it was Bernie who showed the list of

contraindications to the current vaccine. The Center for Disease Control is working on modifying its concepts of operation and its utilization policies for smallpox vaccination. The trend, the thought right now is that next-generation vaccines would not be used in a ring vaccination setting but rather in individuals who are not at the foci of infection. So we have to consider now the risk-benefit ratios of administering next-generation vaccines versus ACAM2000 as well.

DR. ISAACS: I totally agree with what Gerry just said about that this clearly is going to need both the assays and the Animal Rule because what the Animal Rule will do would be quickly eliminate any vaccines that might look great on assays but are ineffective. So this whole issue with the inactivated vaccines that weren't fully protective, you know, which would look great in the assays would be eliminated. So this certainly has to be a two-tiered approach.

DR. GRUBER: My name is Marion Gruber. I'm

with the Office of Vaccines.

I would like to make perhaps a comment rather than asking this as a question. We've heard this morning and Tim introduced these two concepts. I realize since seeing this new generation or third generation vaccines based on the Animal Rule solely or, you know, non-inferiority comparison to licensed product or a combination thereof, and you just made the comment that you would hope there's not a binary approach to that, so the either/or, but this will be a combination.

So what I'm struggling with, however, is let's say we're doing this and we have the animal model and we have the assays that we can eventually bridge to some immune response in the animal and then you do the non-inferiority study where you compare, you know, your investigational vaccine to the licensed product. The Animal Rule approval in itself, that path has a lot of complexities and it's not always a black-and-white decision when looking at the data I could imagine. Now you add the clinical study, the

non-inferiority study.

So the problem, the regulatory problem that could arise is how do I weigh the evidence? Let's say the data is such that you have wonderful protection in the animal model. You can show that if you vaccinate the animal model and you challenged, you have protection that, you know, the assay works. You can even, you know, bridge by some means to an immune response in the human, but then you do your clinical study where you do the non-inferiority comparison to the licensed product and all of a sudden all bets are off. How do you reconcile that? And that, I think, is what we struggle with, you know, from a regulatory perspective.

DR. KOVACS: Marion, I realize that wasn't a question but I'll give you an answer anyway.

We do both. That, in my opinion, is what we should be doing here with this vaccine. We shouldn't be taking an approach toward Animal Rule or non-inferiority, but opening up every door that we can to look to see what's behind it.

MS. LYNN: My own personal opinion is I'm very concerned about depending too much on clinical non-inferiority when we can't do the efficacy study. And when we're relying on an immune response when we have two vaccines that may have different mechanisms. I think we can do it. I think we can probably get some good information. I think it probably would make everybody more comfortable if we didn't see a huge diminution of immune responses in MVA if we were going to look at MVA versus ACAM2000, for example. But I just think we have to be really careful about interpreting those data. It would be nice if we could do both vaccines in the animal model and show that both have the same correlate. If both have the same correlate then you've got the basis to a great non-inferiority in the clinic. But I think the data are still out on that.

DR. MESEDA: Clement Meseda, CBER/FDA.

My question is specifically to Dr. Damon. I wanted to know what the capacity of the CDC is for variola PRNT and whether there's any potential for

expanding that capacity.

DR. DAMON: So I think in considering the amount of dilutions and the number of sera, so in general when we looked at these trials we've set up experiments where we take on sort of no more than about 150 sera specimens from an individual and then do the appropriate dilutions. We run reference standards on each assay so that we have some idea of the reproducibility and some quality assurance and quality control but it will take roughly a month to two months to get well characterized data that we feel comfortable going through to analysis with doing that type of work. So scaling it up with additional priority -- research priorities would mean that we would have to balance the priority of various work that needs to be done with variola.

DR. SUN: This is Wellington Sun from CBER.

I want to get your opinion on the issue of given all the difficulties and pitfalls inferring form the animal model to what will go on humans in terms of effectiveness. Can you give us some insight into what

would be the relative importance of doing these non-inferiority studies in the animal model versus doing it in humans in terms of immunogenicity by whatever measure you have?

DR. ISAACS: If I understood the question, I'm thinking that, you know, in the animals you have the advantage of doing both at the same time. You would be looking at the immune responses and then their response after challenge. So you're saying potentially in nonhuman primates to potentially do a broader non-inferiority just looking at immune responses without proceeding with the challenge. And I think the capacity to do that ends up being easier to do in humans.

DR. SUN: Actually, I was referring to looking at the investigational vaccine with ACAM2000 in the animal model as compared to doing a non-inferiority in humans, how would that -- I mean, would that -- what are the relative merits of doing one or the other, or both?

DR. ISAACS: I think in most -- certainly

the pivotal studies will be a comparison with the approved, you know, the currently approved vaccine. So I see shaking. More shaking. Maybe no.

I would suspect, you know, I would suspect a lot of the work leading up to it will be done with a comparison. I know a lot of the preclinical work certainly is done to show that your vaccine is working as well as the currently approved vaccine.

MS. LYNN: To do a true non-inferiority in and on human primates and get the kind of statistical power you want is going to be difficult because we're just limited on the number of monkeys we can have and have them challenged. I sort of would refer to what Ed was talking about and in turn respect to what Gerry was talking about in taking a holistic approach and using both. I mean, you can get I think enough numbers in nonhuman primates to reassure yourself that you're not seeing a huge loss in protective activity with a new vaccine. Then, hopefully, you can go into the clinic and show either based on correlates or based on whatever best data you can that you're

getting enough of a response in humans to make yourself believe that the vaccine is also going to work in humans. And, you know, unfortunately I don't think we have all the data right now to tell us exactly how all that's going to work but as a general approach I think we're going to need every piece of evidence we can get, like Gerry was talking about. You know, doing the animal studies, doing the human studies, and putting together a package that says we are really sure or we're really -- not really sure but we're confident that the product will provide clinical benefit.

DR. KOVACS: Gerry Kovacs. I think it's a good question based on the fact that the current label on ACAM2000 has a Black Box. So it cannot be used in people who are not at-risk for smallpox infection. So it behooves us to work very, very closely with the Department of Defense on this study because they are the only ones who have the population that we would need to conduct a vaccine trial of this nature. So we are working with DoD on this and hopefully we will

reach a point where the protocol is acceptable to the Food and Drug Administration for non-inferiority.

DR. ISAACS: But Gerry, you're referring to doing it in a mechanism that doesn't require civilian volunteers to do this study. You're saying to use the exiting population.

DR. KOVACS: Right. So the limitation to doing a non-inferiority trial is that we can't do it in civilians. We can only do that trial with the assistance of the DoD. Just so everyone knows. It's not a trial that we can do at St. Louis, for example. It has to be done with the DoD and military personnel who theoretically are at greater risk from smallpox than we are.

DR. ISAACS: Why is that? I actually wasn't aware of that.

DR. KOVACS: Now you are. (Laughter)

DR. ISAACS: So why is that? Because for other studies we get civilian volunteers doing things so I guess I don't understand that.

DR. KOVACS: I think the -- and Cindy

Kelley, if you can correct me if I'm wrong here, the Black Box was placed on ACAM2000 due to the unexpected high rate of myopericarditis. And so we don't want to be vaccinating individuals who are not at risk for smallpox. Is that right?

DR. DAMON: So the only other group of people who are routinely being vaccinated in addition to the military or first responders are essentially lab workers who work with infectious orthopox viruses that are replicative and there is some continued vaccination of public health personnel who are going to be involved or targeted for a potential need in a smallpox response. At this point in time that's a fairly small nonmilitary population. So that could be why Gerry mentions that the military would be a key component of doing any human, non-inferiority trials at this point.

DR. JIANG: Yeah, my name is George. George Jiang from Booz Allen Hamilton.

I have a question regarding the current FDA proto vaccine ACAM2000 and how the vaccine works to

protect people. You know, from today's presentation it's very clear for me, similar to other vaccinations, smallpox vaccine can protect people through inducing antibody response and cellular immunity. And from Dr. Inger and Dr. Isaacs' data, the antibody response seems more important than the cellular immunity because cellular immunity provides an additional model of support for the protection.

However, the data from you is also a clear indicator that the antibody response can be seen as early as about two weeks after vaccination. So my question is -- actually, it's about CDC's recommendation for post-exposure prophylaxis. CDC recommended on its website that ACAM2000 can be used within 72 hours after post-exposure to protect people against the disease. If the antibody response can be induced as early as two weeks, what is the rational for that recommendation that 72 hours can protect people against the disease or mitigate the disease's severity? Can the panel give comment and have a consensus on this question?

DR. DAMON: So I think this is based on a couple of small studies during the end of the eradication program, which looked at use of post-exposure prophylaxis of contacts of patients with smallpox. And again, it's not possible now to stratify out some of the data. So some of the studies vaccinated people between 7 to 10 days after contact with somebody who had smallpox, some up to 2 weeks, and some within a week window of time. And there is, depending -- so it's difficult to really stratify out the time but there is some benefit in terms of a decrease in smallpox disease and those contacts who received vaccination post-exposure and hadn't received it before. The greatest benefit though is in the study which also included individuals who were revaccinated in the post-exposure time period. So that's really the published data that's out there. It also comes from WHO consensus statements on the utility of use of the vaccine in vaccinating contacts of patients. And so really it's -- what you're looking at is pathophysiologically what you're

probably looking at is the dynamic between giving the vaccine in a way such that you're probably stimulating Langerhans cells, you're giving it through the multiple puncture or close to an intradermal-type mechanism. And so you are eliciting the antibody response in a timespan that's quicker and you begin to get extensive replication of the variola virus itself.

DR. JIANG: Thank you.

DR. ISAACS: I'll also add to that. So Bernie showed the incubation period after exposure, too, and so that's on our side with quick post-exposure prophylaxis.

MR. CULPEPPER: Randy Culpepper, Martin, Blanck, and Associates. I provide support to DoD Health Affairs.

What would be the impact if DoD suspended their smallpox vaccine program?

CAPTAIN NELLE: Gerry?

DR. KOVACS: Altogether you're saying? If --

MR. CULPEPPER: Yes.

DR. KOVACS: Yeah. Well, I guess the HHS would have to conduct this non-inferiority trial in civilian populations and we'd have to work with CBER on seeing how that protocol could be conducted.

MR. CULPEPPER: Would that be possible with the Black Box warning?

DR. KOVACS: Say --

MR. CULPEPPER: Would it be possible with the Black Box warning on the ACAM2000? As you mentioned earlier, Gerry, you were saying that we can only use the ACAM2000 on populations that are "at risk" of exposure to smallpox. So would you be able to find a civilian population that you could do those non-inferiority studies on?

DR. KOVACS: I can't speak for the agency. I would have to defer to CBER on that.

DR. MIDTHUN: Karen Midthun from CBER.

I think the concept of doing this in the DoD population is that currently they are among those for whom the vaccine is recommended and routinely used. So if you can use it in that context, then that makes

sense. You know, clearly if there weren't such a population that had been defined then, you know, we would find another mechanism to do this. You know, clearly it's very important when you do a study to convey to the individuals what the risks associated with it are and I think that, you know, obviously, you know, one would find a way forward. But given that it is routinely administered to certain populations it makes sense to conduct the clinical study in that population.

CAPTAIN NELLE: We're kind of skipping around between questions so we're not necessarily going in order anyway so we're going to continue that them.

One thing we'd like to go back to is what is the value of *in vitro* variola studies in evaluation of these next-generation smallpox vaccines. We heard from Dr. Damon's talk, a lot of this has been done. And I guess to kind of start off this discussion, one I think area in which, you know, we've seen data from the clinical studies, would it be informative to also

when we're doing these pivotal animal studies to also take some of these samples and do *in vitro* neutralization with variola to complement what's already been done for the clinical samples?

DR. ISAACS: And I'll also say that, you know, the future variola testing is going to be really important for subunit vaccines to then actually show the sera from animals or humans work against variola virus. So I think these studies are, you know, obviously capacity prevents you doing it on everyone but as Inger was saying, subsets are certainly reassuring information.

DR. KOVACS: I think that's the key word there, reassuring, because they're not going to be pivotal in nature. They're not going to be done under GLP. So what we're going to be left with is good data that supports the indication but not one that would be used in a traditional licensure path for any vaccine. In terms of capacity you have to ask Inger.

MR. FISHER: Robert Fisher, CBER.

Dr. Damon, a question. What would the

capacity of CDC be for actually performing some of these studies in a subset of different variola strains?

DR. DAMON: So I think for a subset of different variola strains I need to look -- first we would need to make sure that we had WHO approval. So that becomes one issue. So far those protocols have been approved. I think in terms of capacity, again, it can be done but it takes time and we just would need to reconfigure resources. And I think these are the types of things that we would want to talk about in terms of from the previous experience what makes sense to put together a statistically sound study design so that we can put together the right package of data to make sure that the experiment is done well.

DR. WEIR: Jerry Weir, CBER.

To follow up on that, would you have to get specific WHO approval for the experiments?

DR. DAMON: Yes.

DR. WEIR: And how long might that take?

DR. DAMON: It usually takes anywhere

between one month to three months.

DR. HINDS: Allison Hinds, Joint Staff National Military Command Center.

What's going on in the world for next-generation smallpox vaccines outside of the United States?

DR. DAMON: Yeah. So I think, I mean, I think a lot of the discussion this morning has focused on MVA, which is currently being manufactured by Bavarian Nordic. The other next-generation, depending on which hat you wear, vaccine would be considered LC16m8, which Dr. Isaacs also mentioned. So that is licensed in Japan for use. And then I think investigationally as researchers, there are many researchers internationally who are looking at various subunit vaccines or DNA vaccine-type strategies, but nothing that's moved into manufacturing-type capacity.

DR. GRUBER: Marion Gruber, Office of Vaccines.

I'd like to go back to this question, what is the value of *in vitro* variola studies and the

evaluation of next-generation smallpox vaccines? And I think the FDA put this question there for a reason because we're looking at sitting vis-à-vis at the table with a company and have to tell them what is the critical data package to support the safety and effectiveness of your product? And we heard earlier on it should likely be a combination of the Animal Rule model pathway and nonclinical -- non-inferiority studies in the clinic. And I just heard, well, this would be nice supportive data but not really pivotal to really, you know, look at effectiveness. And then I heard that really needs specific (inaudible) or approval and it could take months. And these are somewhat complex assays.

So I'd like to push this a little bit. I mean, we heard earlier on, we need a nice complete data package but again, from a regulatory point of view, we need to know is that a "must know" or is it a "nice to know." And so what really is the value? Listening to how complex these studies are and what we already heard on recommendations what should be in the

data package, I think we really have to try to answer that question.

DR. DAMON: Well, you may consider that I have a vested interest in answering this question but, I mean, I think -- I can't answer from a regulatory standpoint. I can answer as a scientist and as a clinician that having an assay done that demonstrates that the sera from -- or the product of the vaccination has some effect against the virus target that you're looking at gives me a great deal of comfort and confidence in it. Whether that should be part of a pivotal study for regulatory review, I can't put that hat on and answer it because one would consider that I have vested interest. I mean, both ways. I mean, it certainly would be a considerable amount of capacity needed to try to do variola PRNTs and moving even noninfectious virus outside of the containment facility to be used in other laboratories as ELISA antigen standard is fraught with complexities because the fact that the genome is present in that material and the full length genome is not allowed

outside of the collaboration centers themselves. So there are many issues but that would be my opinion as sort of wearing my research hat and my clinician hat.

DR. BURNS: Could I follow up with another question on that to you? What role do you think proof of concept studies versus the more pivotal studies could play?

DR. DAMON: Yeah. And I mean, I think that that's sort of the approach that our group has taken so far that, you know, we feel that contributing this information to the peer review literature in terms of some of these, you know, preliminary evaluation studies gives additional confidence in the fact that these vaccines will have good activity against the virus that they're ultimately targeted against.

MS. LYNN: I also wonder, you know, we've got an assay we're not entirely sure of but it is functional and it is against the pathogen versus the animal model where it's a model and it's not humans and it's not even variola. And I think it's going to be a combination of, as we said before, of data that

are over -- when you look at every single piece it may not be sufficient but if you look at the overarching message that the data ultimately tell you, so I guess I would say it kind of depends a little bit.

DR. KOVACS: I think there are clear limitations to the assay itself and moreover, the fact that it's being done at the CDC with variola virus. So I think calling it a pivotal assay is a nonstarter. And I think what we have to start thinking about is since these products are nontraditional in essence, we have to think about them in very untraditional ways. I said earlier, you know, it's a complete picture of the state of the science today, which is as far as we're ever going to be able to get. But there's nothing precluding us from saying in whatever phase four commitment stages we may end up, that we can continue to do studies to support the eventual -- hopefully never -- the use of these products in humans.

DR. R. ROBERTS: Rosemary Roberts, CDER/VA. And clearly this is not my area of expertise at all.

I'm on the therapeutic side.

But I'm somewhat confused as to exactly what the regulatory pathway is here to get the next vaccines licensed. There's talk about using the Animal Rule and then there's this clinical non-inferiority study that is -- I thought some of the slides said was pivotal, but it's not required. So what is the regulatory pathway?

CAPTAIN NELLE: I think that's what we're trying to establish today. (Laughter)

DR. R. ROBERTS: Okay.

CAPTAIN NELLE: I think the question is whether or not what portions of the pivotal studies -- what they're really going to be. Do we need to -- I think everybody is in agreement. I don't want to speak for anybody so please speak up if you disagree. I think the main component that we're trying to address under the Animal Rule is what is the protective level of antibodies? Or if we choose another immune marker, what is that protective level? And I think in terms of doing the non-inferiority

study of whether or not that's actually pivotal is a question that I don't think we've resolved. And one may consider that not to be pivotal but to be supportive depending on if you're looking solely in the animals or solely in the humans. You could easily see also making it pivotal in the animals. Those are questions that we're trying -- that we're struggling with and the hope is by having this discussion today we'll be able to come to some type of conclusion and preference.

DR. R. ROBERTS: Well, I still think it's pretty confusing from an audience standpoint. I mean --

CAPTAIN NELLE: The problem becomes if you go truly Animal Rule approach only, you're left with the question of how does it compare to the licensed vaccine that's already out there. And I think everybody would like to know that information. So I think at a minimal my feeling is that such non-inferiority studies would at least be supportive and provide us with another piece of information so we can

feel comfortable in using these vaccines.

DR. R. ROBERTS: Okay. Thank you.

DR. J. ROBERTS: Jeff Roberts.

MS. LYNN: Just one quick comment.

DR. J. ROBERTS: Oh, go ahead.

MS. LYNN: What I'm hearing as a consensus is that there is no one path and it's going to be -- unfortunately, it's going to depend, which does make it very confusing. But I think the openness that I'm hearing to a combination of data that, you know, if you can do non-inferiority in animals, which may not be a pivotal study but would provide data that would suggest that the vaccine is efficacious, you could do a pivotal efficacy study in animals under GLP. And if you can get a correlate you can use that piece as well. If you can take that correlate into a human study, then that allows you to do a really good non-inferiority with a caveat that it's going to have to be in the military. You know, it's going to be limited in terms of what you can do because of the limitations on the population. So, you know, what I'm

getting out of this is an openness, a recognition that the science isn't perfect, but the recognition that it's going to be, we have multiple tools at our disposal here to bring forward a case of reasonable likelihood of clinical benefit. But it's going to be a multi -- because we can't do any one thing perfectly, we're going to have to do a lot of things to provide an overwhelming or sufficient amount of information.

Anyway, that's my take on what I'm hearing. I don't know if that helps.

DR. J. ROBERTS: Jeff Roberts, Office of Vaccines.

Just listening to the difficulty with using variola wild type virus in these assays, it makes me want to circle back around to which input virus we're going to pick for, particularly for the PRNT assay to go forward with. And I know we touched on it a little bit earlier but I'd like to drill down on it a little bit more. I wonder if the panel members would be willing to like name a strain, for example, and to

think about it a little bit more deeply, particularly would you think that the proportion of EV to MV and whatever these preparations are should be a substantial part of what we consider for which input virus to go forward with?

DR. ISAACS: I'll try to answer for Bernie.

So I think Bernie's rational proposal to put forward was that -- so one of the ELISAs would be a mature virus preparation that would encompass the large number of MV neutralizing targets and then for EV one would use some type of purified proteins to do those two assays. But with that said, your question is still valid. So what MV form, and I think Bernie suggested it be Dryvax or ACAM2000. And you know, the question about the B5 and A33, there is amino acid differences between variola virus and various vaccinia viruses. So potentially one could use variola virus proteins as the -- for those two proteins made in a recombinant fashion.

DR. J. ROBERTS: The other question I had, to switch gears completely, is one of the things we

think about in licensing a product is the duration of efficacy. And we rarely talk about that for this, you know, these potential products for obvious reasons. But I wonder if we have any blind spots on that. Does the panel have any thoughts on that? What needs to be done to demonstrate duration? And if that has any impact on how the animal models are designed and carried forward.

DR. MOSS: Well, in the nonhuman primate models there are data that says, for example, MVA is still protective against monkeypox for more than two years. So it is possible to get duration data.

DR. ISAACS: And that's going to be an important subunit vaccine question because the durations I think are going to be much shorter. But then the use for what we're trying to use the vaccine for I think has to be weighed against that also.

MS. LYNN: This is one of the issues I think that distinguishes HHS from Department of Defense because for HHS we're looking at probably -- this particular vaccine, we're probably looking for, you

know, post-exposure prophylaxis. We want to be able to get protection quickly so that we can contain an outbreak versus DoD, which is definitely interested in duration because they're looking at trying to maintain long-term immunity in their troops. So this is -- for every biodefense vaccine, this is a constant sort of tug. But, you know, my experience is that, you know, HHS really is trying to fulfill HHS needs and where we can overlap with DoD and do both things, that's great. But, you know, licenses have to come in with an indication and that's what we'll take and what FDA would take into consideration in terms of the kinds of data that one would need.

DR. CHALLBERG: Mark Challberg, NIAID.

I'd like to return a little bit to the issue of the value of *in vitro* variola studies yet again. It seems to me that if you listen to the data that Stuart reviewed and also some that Freyja reviewed, I mean, the PRNT assay, although it is a functional assay and from that regard would seem to give us a little bit more comfort about the utility of the

assay, the fact of the matter is the data don't support that assay as being any better correlate of protection than various other immunoassays like ELISAs. So Bernie suggested that the best assay might be a combination of a MV and EV ELISAs. It's just not clear to me that if the PRNT assay using Dryvax as the target virus is not a particularly good correlate, why is it that the PRNT assay using variola is an any better correlate? I just don't see that there's any data to support that view. And I wonder if it would be worth spending some time developing an MV ELISA using variola antigens or if that's just more trouble than it's worth. So maybe you could comment on that.

DR. MOSS: I think it would be good, unless Inger has already done it, to do the same kind of comparison of the PRNT with different strains of vaccine virus and variola. And to do that with ELISA and see whether it equalizes out all the different orthopox viruses.

Inger?

DR. DAMON: Yeah. So we do have an MV ELISA

for variola and we use purified virus for that. I don't know that we've -- I'd have to look back at our data. I know we haven't done it with any of these clinical trials sera. I think we've looked at it anecdotally with sera from animals in various immunization regimens. So yeah, that could be something that would be worthwhile to do a more rigorous look with some of the sera from some of these trials.

DR. MOSS: (inaudible)

DR. DAMON: We -- at this point in time there is one lab that does have the B5 and A33 equivalent antigens of variola in a baculovirus system. So it would be -- we haven't done it, but it has been done. So this is Roz Eisenberg and Gary Cohen's group.

DR. ISAACS: I mean, so yeah, they're at Penn and we worked together on the subunit vaccine. And we haven't rigorously looked at it but a polyclonal response between the vaccinia and the variola proteins look pretty similar. But the

statistics and stuff is -- I stay away from. So I don't know about that.

But to go to Mark's question about functional variola assays, I agree with -- I'm agreeing with you with these live attenuated vaccines but I think with subunit vaccines I think we would need the live variola to show that these responses, both against MV and especially EV and a Comet reduction assay or something, that would be reassuring again.

DR. FINN: Theresa Finn, Office of Vaccines. And I should also preface what I have to say by saying I have no pox credentials at all.

So, and I'd like to -- one of the questions that Tim has -- it's, I think, the third question -- is how should current licensed smallpox vaccines be used in the evaluation of the next-generation smallpox vaccines? And one of the slides that Dr. Damon showed was a slide where you had taken these people who had been vaccinated with MVA and then challenged them essentially with -- it was with Dryvax, I think. And

it was very interesting that there was an attenuated response in those folks who had got MVA at certain higher dosages. So I'd like to ask the panel if you think there's any utility in incorporating that sort of an assessment in clinical evaluation of next-generation smallpox vaccines and maybe as well as or in lieu of some of these assays that we're talking about -- immunogenicity assays.

DR. MOSS: There would still be the same problem with civilian population, that it's not licensed to use it.

DR. KOVACS: I think so. I think the only way that we could do that would be to incorporate an ACAM2000 challenge to the non-inferiority study. So we're looking at an inverse correlate of protection relative to what ACAM provides you in the form of a take. It's difficult to measure the size of the diminution of the take but I think it's feasible.

CAPTAIN NELLE: Okay. If there -- I think we'll conclude session one at this point. It's now a few minutes after 12 o'clock. We're scheduled to

reconvene at 1 p.m. Even though this workshop is sponsored by the Food and Drug Administration, we will not be providing any lunch as previously stated. So you're all on your own. Thank you.

(Recess)

DR. DAMON: In my third hat as moderator for the second session, I'd like to introduce Jennifer Cann from the Integrated Research Facility at NIAID. Excited to hear her talk again. Got to meet her last November when she came to do some presentations -- which may be a little similar to this -- when we started to review the non-human, the primate model, with variola virus.

DR. CANN: Thank you.

Yes, so we will kick off the afternoon, the animal modeling session, talking about the variola virus challenge models. I'm going to specifically stick to the non-human primate models, just in the interest of time. And you'll understand why as I start getting through the slides.

This is just a brief overview of what we'll

be covering over the next half an hour. I feel like I'm a little bit preaching to the choir, and I really don't need to have an introductory slide discussing why we need animal models with smallpox. But in the interest of providing some context, I will devote one slide to an introduction.

We'll then delve into about the past 105 years' worth of variola research in non-human primates. And then we'll wrap up with just a few slides by way of discussing, talking about the elements of a good animal model of smallpox.

Okay, so why do we need animal models of smallpox? I think these days, when we talk about the orthopox models, we tend to think more about the monkeypox, the cowpox, the rabbitpox, ectromelia, and so on. But it's important to remember that smallpox, in and of itself, is its own virus. And we do need models of it in order to have any type of confidence in our medical countermeasures.

The regulatory reasons I'm not going to touch on. I think everyone here is familiar with the

Animal Rule. That's why we're here. So I won't say more about it.

The scientific reasons, I think, are beyond the scope of this talk. And they're well covered by the Institute of Medicine's 2009 report. So I won't talk about that either.

But I do just want to make a couple brief points before we launch into the animal model discussion.

The first point I want to make is that variola virus is a unique orthopox species with unparalleled host specificity and pathogenicity in its natural host. And I realize that's a very fact, and everyone knows that. But the reason I point it out is that this makes this an extremely difficult disease to model.

When we think about the disease that we're trying to model -- that is, naturally occurring smallpox -- at its most basic level, it's composed of two fundamental components: the variola virus, and the human. In animal modeling, we remove the human from

the equation and replace it with an animal model -- thus having an animal model of human smallpox. Again, a very basic concept.

The reason I bring it up is to point out that if we also remove variola virus from the equation and replace it with monkeypox or rabbitpox or cowpox or anybody-pox, we're no long modeling human smallpox. We're modeling human monkeypox or human cowpox.

And I don't mean to imply that there isn't important information to be learned from those models. There most certainly is. But variola virus, again, is its own unique orthopox species. So all of that information that we learn from those other models we can't have utmost confidence in unless we apply that same regimen using the variola virus. And for that reason, we're going to review what we know about the variola virus models that have been used in the past.

So, without further ado -- there's a fairly rich body of literature out there. It's easily divided into 20th century research and 21st century research. It's no surprise that most of the research

falls into the 20th century.

This was the most complete list that I could compile, searching the literature. I was able to lay my hands on all but one of these papers.

As I read through the papers, I pulled out the ones that I felt like could best inform a discussion of animal modeling. And those are the ones that you see kind of highlighted here. These are the ones that we'll go through. I put the others here for your reference, so that you can also go through and look at them. And in the 21st century we have three papers using the actual variola in non-human primates. And we'll talk about all three of those, as well.

Okay. So the first one we're going to start off with is a 1906 publication from Brinckerhoff and Tyzzer. This was a collection of four papers, so, Parts 1 through 4. We're only going to talk about Part 2. Part 1 was specific to vaccinia, so it didn't fall within the range that we're talking about today. The Part 3 and Part 4 really were not very informative. They were immunologic studies and such,

using very old technology. So I didn't feel like they were as informative as Part 2.

So we'll focus on Part 2. Don't feel like it's limited. This is a very long paper. It's actually 10 separate animal modeling experiments. So they use various non-human primate species and administer the virus via different routes of administration. So it's a good place to start, and we'll just delve right in.

The first model that they used was a cutaneous inoculation model. They used primarily macaques. Is should also say, as we go through the old literature, in many cases a lot of the variables that we take for granted as being reported today were not reported. So if you see question marks anywhere in the kind of nuts-and-bolts of the studies, it just means that I couldn't find that information in the paper no matter how hard I looked.

So for this paper they used 65 macaques. They used both cynomolgus macaques and pig-tailed macaques. They never clearly come out and say how

many cynos versus how many pig-tails. But as I went through this and added up the numbers, the best I could infer is that of the 65 macaques, only 5 were pig-tails, and 60 were the cynos.

They used males and females, but they weren't clear with regards to exactly which studies involved how many females and how many males.

They also used two orangutans. Both of those were females. And among all of the non-human primates, ages were not reported or even estimated.

Virus strain and dose is another variable that, of course, we are used to paying close attention to. Not so much in the distant past. So it all of the Brinckerhoff and Tyzzer studies, they really don't report a strain or a dose. They simply report that they collected inocula from clinical cases.

They don't get any more specific than that except, in this case, to say that 9 out of 10 of those clinical cases were severe variola vera, and 1 was mild.

Elsewhere, as we go along, the only other

thing they report with virus strain and does is that in one of the studies you'll see they actually compare pustular contents as an inoculum, versus the crust content. And we'll get to that.

But for the cutaneous inoculation model, they took this inocula from the clinical cases and they administered via cutaneous excoriation and direct inoculation. So they made 6 to 12 excoriations per subject, it wasn't clear. And then they looked at the development of the gross appearance of the skin lesions, this nebulous "constitutional reaction," which is not very well defined, but I think we would all agree that today we would say a "constitutional reaction" in a non-human primate would be lethargy, anorexia, you know, kind of sitting hunched in the cage. So a "constitutional reaction."

They also measure body temperature, reaction of the lymph nodes -- which, I assume this is just a, you know, a palpation of the peripheral lymph nodes and some sort of measure of lymphadenopathy. And they did a little bit of histopathology.

What they found was that, in the macaques, 77 percent of them developed generalized exanthema at 7 to 10 days post inoculation. Of those, 72 percent developed exanthema by day 9. The distribution was typical of what you would expect of smallpox, that is to say that it was centrifugal, no lesions developed on the trunk or on the haired surfaces of the lateral surfaces of the limbs.

They also measured body temperature, and that's what you're looking at here on this graph. They found that body temperature consistently rose from day six to eight, post inoculation. And that was then followed by the cutaneous lesions within 24 to 48 hours.

If we compare this to the human condition, what we see -- and this is something that we see consistently across the non-human primate models -- we see that the actual prodromal period is shortened. So we wouldn't -- in the human case, we tend to say that the prodromal period lasts, on average around 12 days. Of course there is a range. But in the non-human

primates, that seems to be shortened by three or four days.

The constitutional reaction -- even though this was a rather nebulous endpoint, I was at least grateful that they compared it directly back to the human disease, so it left us not guessing how it would have compared. They say that the disease was not as severe as it is in the human condition.

And then the limited histopathology, they looked at the skin and they looked at the lymph nodes. The skin lesions, of course, have been well described. They had typical lesions. And in the lymph nodes, they had some sinus histocytosis, edema, and mild hemorrhage. Notably, there was not a lot of lymphoid hyperplasia in the lymph nodes, another indication that this was a mild form of the disease.

They also saw no other significant lesions.

In the orangutans it was different story. Now remember, they only had two orangutans. Both of them died. One died at day seven and one died at day eight. The animal that died at day seven, they didn't

provide any additional information as to the cause of death or the clinical picture of the disease. They said that this animal developed typical skin lesions and axillary lymphadenopathy, and that it died of an "intercurrent infection."

And this is another recurring theme throughout the literature. As far back as we can go, not only with the animal models, but also with the human disease, we find that a lot of times the clinical course of disease is potentiated by some sort of secondary bacterial infection. I don't think we have a very good handle on it, but you'll see that come up again and again and again as we go through these studies.

In the second orangutan, they said that some skin lesions developed, but that they weren't enough to be classified as generalized exanthema. That animal, as I said, was found dead on day eight, also had axillary lymphadenopathy. At necropsy, they found cecal and colonic hemorrhage. And they cultured the heart blood. They were able to culture a bacillus

that they identified as belonging to the group of organisms causing hemorrhagic septicemia in animals. No more specific than that, but important nonetheless.

Likewise, on histopathology, they found bacilli in all of the organs. And they state that they found no variola, specifically -- which I think is important.

Okay. So that's the end of the cutaneous model.

The other thing I would like to point out, actually, with the macaques and the cutaneous model -- remember, they developed very mild disease. The thing that struck me when I read this is that what they've done here is actually variolation of the macaques. So, if you were looking for a model of variolation, this actually would be not very bad. If you think about the situation when variolation was done in humans, most of them developed mild disease and then recovered without any further symptoms.

So, while this is not an appropriate model of ordinary or classical smallpox, it actually is a

very good model of variolation -- so far as we can tell with this limited information.

Okay, so they went on to do a keratitis model. This model, they also used cynomolgus macaques. Again, the virus strain and doses unknown. They simply excoriated the cornea, and then directly inoculated the animals. All of the animals developed a localized keratitis, but none developed system lesions or generalized exanthema.

They also did what they call a "mucous membrane model," again using cynomolgus macaques. They made shallow incisions in the soft palate, the nasal septum, and the inner surface of the lower lip, and then directly inoculated those areas. All of the animals developed localized lesions, but only two developed generalized exanthema. So, again, a very mild form of the disease.

Moving on to the intratracheal models, this will particularly be of interest to some of you as we start to move into the more recent literature using the other orthopox models that have been administered

intratracheally. I thought this was an interesting comparison.

On the left, this is what they did first. And this was a tracheotomy model. So they actually performed a surgical tracheotomy and directly inoculated into the lumen of the trachea. They only used to cynomolgus macaques for this. And what they found was that the animals actually developed fairly severe disease. They developed pyrexia by day 7, generalized exanthema within 24 to 48 hours after the pyrexia, a marked constitutional reaction, and 1 animal actually died, again, of a concurrent bacterial infection.

So they had some success with this model. But what's not up here -- and they do report this in the paper -- is that they had tremendous problems with healing at the surgical site. So they had extensive poxvirus lesions in the skin at the surgical site, and they could not get it to clear.

So they then went to the non-tracheotomy intratracheal model. In this case, they used cynos

and pig-tails -- and this is where the five pig-tails come into play. And instead of doing a tracheotomy, they simply went in through the oral cavity, excoriated the mucosa of the proximal trachea, and then directly inoculated the animal.

In this case, the animals also developed a more severe disease -- pyrexia by day seven, moderate generalized exanthema, and a marked constitutional reaction. Of importance to note, though, is that this only occurred in the cynomolgus macaques. None of the pig-tailed macaques developed any type of lesions or reaction whatsoever.

They then moved a little deeper into the respiratory tract, and did a lung inhalation model. There were again two types of lung inhalation models. The first one involved five monkeys, five macaques. They don't specify that it was a cynomolgus macaque, but adding up the numbers, trying to put it all together, this was the best guess that I could come up with.

They collected the virus, again from a

clinical case. In this case they specify that they collected from a vesicle. They then took the vesicle contents, put them in water, shook it up, put it in an atomizer, and then sprayed it into the animal's mouth. This was not successful. None of the animals developed any form of disease whatsoever.

So they decided to get a little more aggressive, and they again, presumably, used cynos -- again, got a virus from a clinical case. And this case, they collected from pustules and from crust. And they actually used an intratracheal tube and mouth pipetting to blow the pustule or the crust contents into the lungs of the animal.

I should also say that before they did this, they actually did a small pilot study to ensure that this intratracheal route of infection would result in particles' being deposited into the lower airways. And they did that by taking some spores, coating them with new methylene blue, and then blowing them down into the lungs. They did that on one animal. They report that the animal died immediately. And on

necropsy, they found that the lungs were entirely blue. So they interpreted that as successful administration of their, you know, fake inoculum, and they went ahead and did it with these pustule and crust contents.

What they found was that all of the animals developed profuse generalized exanthema, pyrexia, cough and this nebulous "constitutional reaction." Or two or three of the animals that received the pustule contents. Of the animals that received the crust content, none developed any type of disease or fever whatsoever. So mixed success.

They also did a fomite model. They used both the macaques -- the cynomolgus macaques -- and an orangutan.

For both animals they collected blankets that had been used by smallpox patients for greater than 24 hours. They then took those contaminated blankets and placed them in the cage with the animal. They left them in the cage for 16 days. After 16 days, they still had no evidence of an infection, and

considered this model unsuccessful.

To prove that, they then went back and vaccinated the animals. And in that case, they found that four out of the five animals exhibited a primary take to vaccination.

The remaining animal had died. They don't say why. They don't cite a secondary bacterial infection, but they do say that there was no evidence of variola virus infection at necropsy.

For the orangutan, there was also no evidence of infection after 18 days. And, again, a primary take in response to vaccination.

And then, to wrap it up -- this is that last of the Brinckerhoff and Tyzzer marathon studies -- they did a monkey direct smallpox-patient exposure model. They took five cynomolgus macaques. They put the macaques in the room with smallpox patients for 16 hours and then removed them. You could never do this today, right? They then noticed that there was no evidence of infection after 17 days. And a primary take in three out of five of those animals in response

to vaccination. The other two animals, there was no reaction whatsoever.

So, again, just good evidence to confirm what we know, as far as the macaques being less susceptible to infection than the human.

Okay, so then we jump forward 50-some years to 1960, when Nicholas Hahon and Benjamin Wilson published a paper on the pathogenesis of variola in cynomolgus monkeys. So the old, the scientific name for the cyno.

They used 13 animals per experiment, and did three experiments. They used the Yamada strain of variola virus, and they administrated it via aerosol. And I know the aerobiologists in the room want to know more details about that, and I would love to tell you. But they really don't go into any details. They say that they created an aerosol, and that each animal -- they calculated that each animal inhaled 5×10^4 infectious units by determining how many infectious particles were in each aerosol drop, and then comparing that to the tidal volume of the monkeys. So

probably not very precise.

They also had four controls in this study. And those animals received an aerosol of 20 percent normal CAM suspension. They also state in the "Materials and Methods" that 95 percent of those particles were less than 5 microns in diameter.

The results -- the incubation period, similar to what Brinckerhoff and Tyzzer had found, the incubation period was about 6 days, again, versus an average of around 12 days in humans. Constitutional symptoms were mild: pyrexia, again, and generalized exanthema, with the typical distribution and progression of the lesions as you see in humans.

They did a little bit of pathology, and the skin and mucosal lesions were consistent with variola virus infection and inclusions and all of that. They did actually, at this point, start pointing out the shortened prodromal period, or incubation period, and the earlier onset of the fever and exanthema compared to the human disease. It's interesting, though, that this remains consistent amongst most of the non-human

primate models.

Hahon then went on to work with Malcolm McGavran the following year, and published another study on airborne infectivity of the entire variola-vaccinia group. So this study involves not only variola, but vaccinia, rabbitpox, cowpox and monkeypox, as well as a variola minor strain.

I only report the data for variola, but I can tell you that generalized exanthema only developed in the animals that received variola or monkeypox. So the rabbitpox and the vaccinia did not produce lesions in those animals.

Even in the variola animals that developed lesions, they said that the lesions and the constitutional changes were mild. So I think we're seeing a theme develop there, whereas at least by most of these aerosol routes of exposure that we're using, we're pretty consistently getting disease that falls on the mild end of the spectrum.

This is from a review paper that Hahon published, also in 1971. And I put it in here. I

thought it was actually a nice kind of demonstration of what had been done to date.

So you can see that they actually looked at lots of different models. You know, they weren't really looking at them in models in the sense that we look at them, they were more concerned with, you know, natural reservoirs, or animal reservoirs of the virus. But, nonetheless, it can be useful for us.

So, all the species, different routes of inoculation, including intratesticular, and there's an intracardiac, as well.

And then they had the signs of infection. I think the most important thing to point out is that in all of these models, the animals either developed localized lesions or generalized exanthema, with fever. So it's not that we're not able to induce the disease with variola in the non-human primate. It's that we tend to get a more mild form of the disease.

In 1966, Westwood, *et al.*, published this study. And I thought this actually was really interesting. This was also a respiratory model. This

used the rhesus macaque. They specifically say the Indian-origin rhesus macaque, and I give them kudos for that. There were 109 animals in the study. So it's not entirely clear what there specific aims were, but they were certainly powered to detect a lot of differences.

They used the Higgins strain of variola virus, and they generated an aerosol using the Henderson apparatus. So, using this apparatus, they claim that at least 98 percent of the particles were less than 1 micron in diameter. The actual dose, however, is not reported.

What they found -- and I think this is actually really striking -- is that only two of the animals died. This is actually probably the one study that has the best reproducibility of them all.

So the mortality rate was very low, 1.83 percent. And the remaining animals that did not die developed moderate disease. So, again, fever, lethargy, anorexia, again, around day five, day six post-infection, very consistent; and the typical

cutaneous and mucosal lesions within 24 to 48 hours after the fever developed.

They also state that all of these animals -- the 107 animals that lived -- fully recovered. They didn't say what that timeframe was and when they recovered, but it was a self-limiting disease.

Noble and Rich, in 1969, also reported on aerosol transmission of smallpox in the cynomolgus macaque. This paper is not entirely clear on the method. I'll try to explain it to you as best I understand it, but the numbers do not add up when I tried to make sense of what they did and reconcile it with the "Materials and Methods." So bear with me.

They had a total of 22 cynomolgus macaques. They used the Harvey strain, at 5×10^6 pock-forming units. They had two separate groups. One group had five animals, one group had two animals. All of those animals received the virus intranasally.

Then they took 13 orthopox-naive animals and co-housed them in a cage with these intranasally-infected animals, in just a regular standard cage. In

the second group, they also co-housed orthopox-naive animals with the intranasally-infected animals, but instead they used an aerosol isolation chamber.

And that's what they're trying to demonstrate here. Essentially what this is, this is a closed container with an air source which starts here, and a fan. So the air blows from this end of the chamber, through the far side, and then out. They put the intranasally-infected animal upstream of the naive animal and turned the fan on.

It's in the reporting of the results that it becomes a little unclear where the numbers fall out. So, again, bear with me.

All of the animals that were inoculated intranasally had a seven- to eight-day incubation period. They all developed a fever, lethargy, anorexia, and typical skin lesions. They also reported that some had occasional gingival bleeding and melena.

Of the naive animals, those that were put into the standard case with the IN-infected animals

also developed clinical signs and lesions same as those with the IN route. I thought this actually was very striking, especially given Brinckerhoff and Tyzzer's experience with the contact with the patients and no luck whatsoever. So this study kind of struck me as a little bit odd.

They also said that with the standard cage route, that they were able to transmit that through six serial passages in the naive cage-mates. And this is where the numbers -- it's hard to make the numbers add up. So if there are 13 animals total, and 5 were inoculated intranasally, these animals were exposed serially. So an infected animal was put in, developed disease, removed. Another infected animal was put in.

I can't make it all completely add up to 22. But they say they could take it through six serial passages, and it failed at the seventh passage. No disease developed in the seventh animal.

Those that were in the isolation chamber -- which was only two naive animals -- both of those animals were reported to have developed clinical signs

and lesions the same as the IN route. They specifically say that the lesions were "extensive."

So this paper goes a little bit in contrast to the others that we reviewed this far, in that the disease seems to be a little more severe, a little more readily transmitted, especially via this respiratory route that's been unsuccessful in the past.

Noble published another paper the following year. And in this study, he again looked at animals exposed by an intranasal route. But he looked at New World and Old World monkeys. So he looked at the cynomolgus macaques, he looked at African greens. So the Old World species. The New World species he looked at were the spider monkeys, the wooly monkeys and the capuchins. I think it's interesting that there are no marmosets in this study, but I'll let Dr. Mucker speak to that later on this afternoon.

They used five different strains of the variola virus. Four of those were variola virus major, one was minor.

For the studies, Studies 1 through 4 used the major strains, and they were administered intranasally at 1.5 mL per animal. And you can see the concentrations here. The concentration was not reported for the Pakistani strain.

For the variola virus minor, that inoculum was administered intramuscularly and intraperitoneally. The dose was not reported.

In these studies, in the Old World monkeys, either no or only mild disease and lesions developed. So this is in direct contrast to Noble's previous work, which said they developed severe disease and they were able to readily transmit it to naive cage-mates. The New World monkeys developed no disease at all.

And the variola virus minor strain developed moderate disease in one out of the two cynos, and no disease in the remaining species or the final cyno.

Okay, so that brings us up to the 21st century. So all the 20th century stuff was either very early on, or in the 1960s, 1970s. After that, as

you all know, smallpox was eradicated, and we tended to lose interest in it -- until 2001.

And this is a study that was published in 2004 by Dr. Jahring's group in PNAS. He also chose to use the cynomolgus macaques. They had 36 animals. They used 2 strains of variola virus: either the Harper strain at 10^8 or 10^9 PFU, or the India 7124 strain at somewhat lower doses, 10^6, 10^7, 10^8, and 10^9.

In this case, the virus was administered intravenously, both for the Harper and the India. There was a smaller subset of animals, of just eight animals, where they administered it intravenously and via aerosol.

What they found was that those animals that received it IV and via aerosol, they developed very rapidly fulminate disease, with almost uniform lethality. So seven out of eight of those animals died. And they report that the end-stage lesions resembled the human disease. They don't go into any detailed descriptions of the clinical progression or the pathology, but they do say that it looked like

end-stage lesions.

In those animals that received the virus only via IV, they found a differential effect. The animals that received the high dose -- the 10^9 PFU dose -- there was uniform lethality. They all died. And they all had lesions that were consistent with the hemorrhagic form, the more severe hemorrhagic form, of the disease.

However, in those animals that received lower doses -- less than 10^9 -- they had no lethality, and they found that the disease severity, as assessed via the skin lesions, positively correlated with the disease dose. So, again, they had 10^6, 10^7, 10^8, and those all followed a regular dose response curve.

The time course of the disease that developed, by day two, they had cutaneous erythema and hemorrhage. By day three, early vesicles and pustules, fever, cough. And then, of those animals in the 10^9 group, they died between day 3 and day 13.

So, in comparison to the animal models that used the respiratory or the intranasal routes of

infection that already have a more naturally shortened prodromal period, here we have dramatically shortened it by giving the virus by the intravenous route.

Their final interpretation of their findings was that the disease type was determined by dose. So, specifically, they concluded that those animals that received 10^9 PFU developed acutely lethal hemorrhagic smallpox. And those that received the lower doses or the more moderate doses, developed less severe, ordinary light disease, and recovered.

I'm going to talk about one more study, and they we're going to do a follow-up to this study.

This was a study that was published in 2009, and this was the efficacy study -- one of the efficacy studies for ST-246. I included it because they did have a control group that did receive variola virus, so I thought it was important, for completeness, to include it.

They also used the cynomolgus macaques. They had two controls. Those are the controls that we'll be talking about. And for those of you that are

interested in the ST-246, here is the information for who got what, when.

They used the Harper strain of variola, again an intravenous route, at 10^8. So this was designed based on the previous study that determined that 10^8 would result in ordinary disease.

What they found among the control animals was that, similar to what was previously reported, the skin and mucosal lesions first developed between days one and four post infection. And they report that the disease was of enough severity that they had to euthanize the animals at day 12 post infection.

So the results from this are similar to the Jahrling, *et al.*, study in that 10^8 produced consistent ordinary smallpox. So Jahrling's group then designed a follow-up study to their 2004 study. And, again, this was based on the results they received from that study.

They stuck with the cynomolgus macaques. They again used the Harper strain and again used the 10^8 and the 10^9 PFU doses. This was designed to be a

serial sacrifice study to look at the pathologic progression of the disease. And as such, one of the goals of the project was to classify the disease type based on its clinical presentation and the gross and histopathology.

What they found in this case was that it was just not as clear-cut as it was the first time around. In the 10^8 PFU group the results were very consistent. All of the animals developed exanthema or ananthema within 5 to 11 days post infection, and the disease type was easily classified as ordinary or classical smallpox.

It was not quite so straightforward for the 10^9 PFU group. In that group, three of the animals died prematurely. They had no classical vesicular or pustular lesions. They had only mild to moderate petechia.

The other animals -- the other 6 animals that survived -- developed what looked like the disease that occurred in the 10^8 group. So 6 of these 10^9 PFU group animals developed ordinary or classical

smallpox, and they were just euthanized according to schedule. The three that died early, however, had lesions and a clinical presentation that was consistent with hemorrhagic smallpox.

So from this study, it made it clear that the type of disease that developed -- at least in the cynomolgus macaque IV model -- is not solely dependent on viral dose. So we know dose plays a role, but it's not the only factor that determines what type of disease develops.

So that's the literature.

So by way of discussion, I thought that as we kick off this afternoon talking about the various orthopox animal models, it would be good to kind of have a few minutes' discussion on what actually makes a good model of smallpox. What are those elements that we're looking for? And I think everyone would agree that the first thing we need is a clearly defined target.

Smallpox has a very wide range of clinical presentations, as you all know. In fact, it really is

not one disease, but really three diseases. You know, there is the minor disease, that's either asymptomatic or extremely mild. There's the major ordinary, classical disease, which is more severe and more debilitating, but generally self-limiting: 30 percent mortality, 70 percent of people recover.

And then there's the far end of the spectrum, where we get into the hemorrhagic forms of the disease which are, you know, acutely lethal, but not nearly as common in the population.

So when we think about animal modeling, really, the first thing we need to do is to find what it is that we're trying to model. If we're looking at vaccine efficacy studies, and the endpoint is to determine their effect on mortality, then we want an animal model that falls more on this end of the spectrum, and has a very high mortality rate. If, instead, we want to see how a particular vaccine, or get some idea of how a particular vaccine is going to react more in the general population, then we're looking at more like this type of disease.

And I think it goes without saying that it's really not possible to model all of the forms of smallpox with just one animal model. I mean, it's impossible to have a disease with 30 percent mortality and then, at the same time, have a disease with 100 percent mortality.

And so we really -- rather then saying, well, I'm modeling smallpox, we need to say, are you modeling variola minor, variola major, or very severe smallpox? Define the purpose that we're using the animal model for.

So if we look at this and we think about all of those whirlwind of papers that I just ran through, we can kind of classify them as to where they fall out on the spectrum of smallpox disease severity. As I've pointed out going along, most of them fall on the more minor end of the scale. But I think it's important to recognize that there are good examples that fall more closely to the ordinary disease itself. And then, of course, the IV models, we know they fell out down here on the hemorrhagic spectrum. So we really do have

options. It's a matter of determining what it is we're trying to model, and what factors we need to put together to make that model.

And that brings me, really, to the second element of a good animal model, and that's one of reproducibility.

I want to point out that if you look at, for example, Brinckerhoff and Tyzzer, in Brinckerhoff and Tyzzer studies -- granted, it was 10 different studies -- but they actually found that some animals responded in a very minor way, some responded in a little more severe way, and some -- the orangutan -- actually developed hemorrhagic and died.

The same thing goes for some of the other papers. Jahrling, *et al.*, from 2004, the 10^8 dose produced ordinary disease. The 10^9 dose produced hemorrhagic disease.

Wahl and Jensen, a similar sort of thing. Most of the animals developed ordinary disease, but three of them developed hemorrhagic disease.

And so I think where the literature is

falling short thus far is on reproducibility. So it's up to us to really look at this closely and try to determine which combinations of the known variables result in which forms of smallpox.

We do an excellent job of paying close attention to the virus strain. We know there are differences in virulence among the different strains.

Virus dose, we know it plays a role. It's not the single, sole determinant of disease type, but it does play a role.

We know route of administration plays a role. We know route of administration is important for regulatory reasons, as well.

So those things are all important, and we do a very good job of addressing those.

Where we fall short is more on the host side of things. So we consider, you know, a host species. What is our host species model going to be? The cynomolgus macaque gets used a lot. But we have some indication that there's differential disease susceptibility among different non-human primate

species.

So it seems there are other examples of orangutans' being exquisitely sensitive, of orangutans' being in the zoo in Jakarta and exposed to patients who were shedding the virus, and they developed the disease -- developed hemorrhagic disease -- and died. Versus the rhesus, which seems to be a little less -- or there's some evidence that it might be a little less susceptible than the cyno. And in these studies, in the Brinckerhoff and Tyzzer studies, the pig-tails didn't develop any disease at all. So there may be some just differential susceptibility among the hosts that we need to consider as we design the studies.

We also, of course, need to consider host immune status, host age, sex -- is sex important? Even if we don't know if it's important, we should at least randomize for it and report it, so that it's in the literature and we can later go back and try and figure these things out. Pregnancy status, we know that was a big risk factor in the human condition.

And then, of course, there's a whole slew of unknown variables that likely do play a role. The secondary bacterial infections, as I said, I challenge you to find many examples in the literature where secondary bacterial infections do not confound either the data or the actual clinical disease in the human.

And what about the endogenous viral infections? You know, we know there are endogenous herpes viral infections in people and macaques. What about the gamma herpes viruses?

The retroviridae. You know, for instance, if you're trying to model the disease, you want to see how the vaccines will react in the HIV-positive population, then you want to use a model with SIV or SHIV. SRV type 2D is another important one. That's endemic in the macaques population, and it simply doesn't get reported in the literature whether or not the animals are positive or negative.

And I realize that some of these things -- the B-virus is involved in this, as well. You can get B-virus-free animals. You can get SRV-free animals.

I realize they take more time and money, so it's not always feasible to do those things. But I think it's important that even if we don't go out and specifically select SPF animals for these studies, we need to at least run the serology and report whether they were positive or negative, and be aware that this might be having an effect on the type of disease that we're producing.

The polyomaviruses are another good example. You know, they're endemic in people, they're endemic in the macaques. We don't routinely run serology for them in the macaques, but it is possible.

Chronic skin diseases are another big one. We know that chronic skin diseases in people are a risk factor for more severe disease. They're a contraindication to vaccination. And we know that the non-human primates -- chronic skin diseases are common in non-human primates. Yet when we do our studies, we don't give them a thorough physical exam specifically looking for chronic skin diseases, and excluding those that are positive.

Animal origin is another one. I think specifically about the rhesus and the Chinese origin versus the Indian origin rhesus, and all the literature out there that shows how these two different animals that originate from different parts of the world respond differently to different infections, how their immune responses are different. And I'm sure there are many others.

I think, essentially, the important thing to keep in mind is that anything that is a risk factor in the human disease becomes a variable in the animal model. And I think that, looking at the literature -- not just the variola literature, but the other orthopox models, as well -- we fall a little short in characterizing our host factors, and trying to control for them or randomize for them, or what have you.

So, I think that that's all I have to say.

(Applause)

DR. DAMON: We have time for some questions.

So our next speaker is Dr. Mark Challberg from NIAID.

DR. CHALLBERG: Thank you, Inger. It's a pleasure to be here to talk about some of the studies that we've performed the past several years on the monkeypox model for smallpox.

I just want to start out by acknowledging that this was really a large project, and included the participation of a lot of people. My colleagues at NIAID, of course, and the investigators at the various CROs who actually performed the work: Southern Research Institute, Battelle, Health Protection Agency in the U.K., and the Lovelace Research Institute.

So this project was initiated in 2004. And at that time, most of the limited work that had been done with non-human primate models with smallpox had been done with the IV route of exposure. And we were having a lot of discussions with our colleagues at CBER, trying to come up with an approach to licensing new-generation smallpox vaccines. And they expressed an interest in models that used a respiratory route of exposure to evaluate vaccines, because as we all know, human smallpox is generally transmitted by the

respiratory route. So we decided to carry out a somewhat systematic study evaluating exposure of cynomolgus monkeys to monkeypox, using the intravenous, intratracheal, intranasal and aerosol routes of exposure.

Before I get started describing these studies, I'd just like to return a little bit to a couple of the themes that Jennifer discussed.

So this is a slide Inger showed already, just broadly outlining the course of human smallpox. And I think the main point, if you simply look at the main elements, kind of a bird's-eye view of human smallpox, what are the characteristics? Well, it's, as I already said, infection is initiated by the respiratory route. There's a long asymptomatic incubation period. Symptoms are first noted by the beginning of a fever and then characteristic lesions develop.

So it turns out -- and I think one of the main points that I'm going to try to show -- is that it's not possible to recapitulate every element of

this disease progression using a single route of exposure of monkeys to monkeypox. So the exact course of disease depends on how you give the virus, and basically how much virus you give.

The other point I'd like to make is that we don't know a huge amount about human smallpox, and I thank Inger for the next couple of slides. But if we just, again, look at a bird's-eye view, if you want to evaluate -- the main method by which smallpox was classified was on the basis of the skin lesions, primarily. So if you look at the various manifestations of smallpox, going from not so severe to the most severe, the main thing that characterizes these types of smallpox, I guess, is the number and types of lesions that humans develop.

And, in fact, you can look a little bit more closely at that. If you just look at the classic types of smallpox, classic smallpox, then you can see that there's a correlation between the number of skin lesions and the case fatality rate. So if there's very few lesions, case fatality is about 10 percent.

And that progresses in an upwards manner so that the flat type of smallpox is really -- or ordinary confluent smallpox, in which there are so many lesions they coalesce together has a much higher case fatality rate.

So in a broad level of looking at human smallpox, we can say that there is a correlation between disease severity and the number and types of lesions.

All right. So now the studies on monkeypox in non-human primates. The goal of the studies was to characterize the dose response, and reproducibility, disease progression, and pathogenesis of each route of infection.

And what I'm going to do is present a very broad overview of what we saw.

We carried out range-finding studies to find out how much virus we needed to actually produce disease. And then once an optimal dose was decided upon, we carried out a serial sacrifice to determine the course of disease in the animals.

Each respiratory route was evaluated in a different lab, but every lab carried out at least one small study using the IV route, so that we would have some basis for comparing results from lab to lab.

So when we started, it was not clear what the endpoint of the perfect animal model would be. We were a little bit worried that if we aimed for a uniformly lethal disease, that we would be developing an animal model in which the challenge was so severe that it would not be possible to actually protect those animals from disease by any vaccine or therapeutics.

So we decided to have the endpoint, or to try to develop a dose regimen in which we used as a criterion the amount of virus needed to give severe disease to 90 percent or more of the animals.

As this progressed, it became clear that there was not a great deal of difference between what it took to develop an SD_{90} and a lethal disease. So we tended to describe the studies more in terms of mortality. But at the outset, we were looking for a

model in which we could produce severe disease, at least severe disease in 90 percent of the animals.

So, the definition of "severe disease" was initially based on what we knew about studies that had been done with the IV route. And as we went along and learned a little bit more about the other challenge routes, then we found that we really had to modify our definition of severe disease somewhat.

The euthanasia decisions were based on, of course, the IACUC's recommendations at each facility. And I would say, my own opinion is that if one has death as an endpoint, that's generally, nowadays, going to be dependent on euthanasia. And it's actually easier, I think, to develop a consistent set of criteria for severe disease across different laboratories than it is to develop a consistent set of criteria for euthanasia. Because every laboratory has a somewhat different sensibility about, you know, when you should go in and euthanize and animal. But that's probably a subject for another workshop, I would say.

All right. So, as I say, the definition of

"severe disease" was initially based on what we knew from the IV route. Basically, we were just looking for body temperature, either very high or very low, a high degree of weight loss, a listless or sluggish appearance, and severe -- a huge number of lesions.

The challenge material we had done was made up by ATCC and was distributed to all the laboratories. So every laboratory used exactly the same challenge material, again, to rule out one element of difference and one element of irreproducibility between labs.

The challenge strain was the Zaire 79 strain. This was isolated from scab material from a one-year-old boy who was severely but not mortally ill. So he had a typical monkeypox infection.

Sequence analysis of the strain supports the assignment of this strain of monkeypox to the Congo Basin's clade monkeypox which, as many of you know, is considered to be the more virulent of the two known clades of monkeypox. Unlike some other Congo Basin isolates, but like variola, or some isolates of

variola, the Zaire 79 strain has a truncated interleukin-1 beta receptor homolog. The impact of this truncation on virulence of this strain is really not known.

So, I'm going to get into the results. So here's the initial range-finding study, done with the IV route. You can see that there is progressive -- a dose-dependent increase in mortality and a dose-dependent increase in the number of lesions. So the number here, the second number in this column, denotes the peak lesion counts, the average peak lesion counts in each group.

Of course, one of our goals was, as I mentioned, was to assess the reproducibility of each one of these models. And so I'm just going to go very quickly through the results from IV challenge from the other labs. And I think you can see, without my going through them, that fundamentally, each lab, using the same challenge material, titered in common laboratory, basically everybody got the same result.

So at the same dose of virus, given IV,

basically the disease progression or the mortality was about the same.

So now moving on to the aerosol route, here we find, again, a dose-dependent increase in mortality. But now an interesting finding, which turns out to be true, I think, of all the respiratory routes: there really isn't a dose-dependent increase in lesion count or in the number of lesions. In fact, if anything, at the very high, uniformly lethal, doses of monkeypox given by really all the respiratory routes, there is a decrease in the number of skin lesions.

So, unlike human smallpox, where the virus is transmitted by a respiratory route, in the monkeypox model in the non-human primate, the respiratory route does not give a dose-dependent or a disease severity-dependent increase in the number of lesions.

Again -- okay, again we got approximately the same result doing a very similar aerosol at a second lab. Basically, the results were almost

identical, with a 10^5 dose giving a uniformly lethal infection in 3 animals.

So one of the issues that we were initially worried about was whether it was possible, with the aerosol route in particular, to have a reproducible challenge of monkeys in which the actual presented dose taken up by individual monkeys was reproducible. And this shows -- this actually shows the results from the serial sacrifice study, in which we infected a number of monkeys initially at a target dose of 1×10^5 PFU, and then these monkeys were sacrificed at various times after infection.

But what I want to point out is that the technology for aerosol delivery, I think, has gotten to the point where it's possible to pick a target dose and then come really close to that target dose in a very reproducible manner. So the actual presented dose to these monkeys -- as I say, the target dose was 1×10^5. The presented dose ranged from about 5×10^4 to a little over 1×10^5. So this is a very reproducible way, now, of infecting non-human

primates.

This is the range-finding study from the IT route. Again, we see a dose-dependent increase in mortality. And again, we really don't see a dose-dependent increase in the number of lesions.

And finally, this is the results from the intranasal route. Again, a dose-dependent increase in mortality. But here we didn't get uniform mortality even at the very highest dose that we presented.

And this is shown a little bit more clearly here, when the SD_{90} from each of these routes was calculated, the IV route turned out to be about 2 x 10^6, by IT 1 x 10^6, aerosol about 1 x 10^5. But by IN, we couldn't -- the calculated SD_{90} was greater than the amount that we were actually able to deliver. So we really have not done much with the intranasal route.

So I want to go very briefly over the pathogenesis studies, just to make a couple of points.

This really summarizes what I've already said, that there is -- although it's not captured here quite so well, there is not a correlation between

severe disease and the number of lesions. But each route of infection gives a very similar result for the amount of virus in the blood at peak levels. So each route of infection produces a disseminated disease that's manifested by a viremia.

There is a little bit of difference in the amount of time that it takes to develop disease. As you might expect, the IV route causes disease to appear a little bit more quickly. But even with the other respiratory routes of infection there is not a prolonged incubation period. Fever begins at about day four. Peak weight loss shows up at around day 10 to 14. And the animals often die at around this time.

Clinical signs are not particularly remarkable. Again, I want to make just one point, and that is that unlike the IV route, all of the respiratory routes are characterized by severe respiratory symptoms. So the animals all developed cough and they all developed respiratory distress, and basically, they all die of bronchopneumonia.

This just shows a summary of the

histopathology results. The only thing of note here is that there's clearly infection of the lungs, obviously in the respiratory routes. But you see that also in the IV route, although the pathology noted in the lung by the IV route obviously is not enough to cause huge numbers of respiratory symptoms.

And, again, continuing on that theme, if one now looks at the presence of virus in various tissues by immunohistochemistry, what you see is that in each case, this virus is widely disseminated. You can find it in virtually all the tissues. It takes a little bit more time for the virus to spread out by the aerosol route, but eventually it ends up widely disseminated.

So, conclusions are that following challenge by all respiratory routes, there is disease severity that is challenge dose-dependent. The number of skin lesions is not challenge dose-dependent. And, in fact, at the highest doses, there's very few skin lesions.

In each of the respiratory routes there are

symptoms of respiratory distress and the development of bronchopneumonia. And, as I've said, in each case the virus is highly disseminated throughout the body of these animals.

Challenge by both the IV route and the aerosol route is reproducible, both study-to-study and lab-to-lab. I say that about the aerosol route but not the other two respiratory challenge routes, IT and IN, simply because we've only looked at it closely in the aerosol route. But certainly, the IT route, in which we've done some more studies, is quite reproducible study-to-study in the same lab.

With all challenge routes disease progression is accompanied by wide virus dissemination, as I've already said. And compared to the respiratory routes, some of the characteristics of disease with the IV route are more like human smallpox. So, again, disease severity, in the case of the IV route but not the respiratory routes, disease severity correlates with lesion number, and there's minimal respiratory signs of infection.

So, as interesting as these studies are for potentially studying the pathogenesis of monkeypox, that's really not the reason that we set out to do them. What we really want to use these models for is to test vaccines and, potentially down the line, therapeutics.

So I want to just spend the last couple of minutes just showing the results from one challenge -- vaccination challenge study that we finished not too long ago using the aerosol route.

So the IV route has been used in a number of published vaccination studies. Bernie's published a couple of studies, and Stuart discussed another study that was published several years ago, so I won't talk about those. But I just want to show you that at least the aerosol route -- and certainly we've done other studies with the IT route -- that it's capable of at least reproducing what we know about the vaccine, the licensed vaccine, that exists, ACAM2000, which is known to protect against human smallpox.

So this is a study that was carried out by

HPA. Six animals in each group. They were vaccinated either with one dose of ACAM2000 -- this is group B, one dose of MVA -- or two doses of MVA. And what you can see is that in the control group, unvaccinated group, all of the animals died. There were no survivors. The ACAM2000-vaccinated group, all of the animals survived. There were no deaths.

And the animals vaccinated with a single dose of 1 x 10^8 MVA, most of the animals -- 4 out of 6 -- survived, but 2 came down with the disease severe enough so that they died. But the two doses of MVA completely protected these animals from death. So, at least at a high level, this animal model seems to be useful to be able to evaluate vaccines.

And these results are shown in a little more detail, in which we plotted the euthanasia score, which is just another way of describing the clinical assessment. Again, in the control group, unvaccinated group, all the animals had very high clinical assessment scores, euthanasia scores. In the 1 x MVA group, the animals were somewhat protected. Again

there were two animals in this group that did not survive. But the clinical assessment of the ACAM2000 and 2 x MVA groups was very similar, and basically none of these animals manifested any signs of disease whatsoever.

So, I think I'll stop there. I'll be happy to take any questions.

Thank you. (Applause)

DR. DAMON: I'm going to ask you a question, Mark. Moderator prerogative. Is there any data from this study on the immune response to the non-human primates prior to challenge? In the studies?

DR. CHALLBERG: Yes.

DR. DAMON: Okay.

DR. CHALLBERG: And, you know I mean, it was very, the immune response was very similar between the ACAM2000 group and the 2 x MVA group, using the assays that we have.

DR. DAMON: And the one-dose MVA group?

DR. CHALLBERG: Yeah, they had an immune response, but not as high.

DR. DAMON: This is ELISA-based assays and T-cell responses.

DR. CHALLBERG: I think both ELISA and PRNTs were done. Yeah.

DR. DAMON: Other questions?

MS. GRUBER: This is Marion Gruber. Perhaps we can -- well, I'd like your input, but perhaps this is a question for the panel. You tell me.

When I look at the data that you presented, and looking at the different route of exposures, IV and inhalational, and the outcome and the results of these studies -- in thinking about a pivotal study in an animal model to demonstrate effectiveness of a vaccine against challenge with a, you know, smallpox agent -- let me put it this way -- would you think it's important to really evaluate different routes of exposure, or do you think it's sufficient to settle on one route of exposure?

DR. CHALLBERG: Well, I guess I think that certainly, if you want to think about the various respiratory routes of exposure, that there's no reason

to do more than one. I think the results, broadly speaking, were very similar using all three of the respiratory routes of exposure. And there's no reason -- there would be no reason at all to evaluate a vaccine with more than one of those.

I think a case could be made for using, say, the IV route and one respiratory route. The disease manifestations, the monkeypox, the non-human primates by the respiratory route, I think is a little different than human smallpox. I mean, these animals of bronchopneumonia -- which I don't think is -- which is not thought to be the main way that humans died of smallpox.

And so, to my mind, it would make sense to evaluate it using -- if you wanted to get a spectrum of disease, if you wanted to have an animal model that incorporated all of the aspects of human smallpox as we know it, the only way that you could do that would be to use more than one animal model, I think.

DR. DAMON: Okay. Thank you very much, Mark.

I guess since the time is getting late, our next speaker is going to be Eric Mucker. Soon I will be able to introduce him as Dr. Eric Mucker.

MR. MUCKER: So, first of all, I want to thank you for inviting me for this talk. And second, I want to thank Dr. Cann and Dr. Challberg. They saved me a lot of time and introduction.

So, without further ado, I want to tell you a little bit about marmosets, a New World primate, their susceptibility to monkeypox. And hopefully, eventually, I'm testing with variola.

So the question is, why develop another model? As I again pointed out, there are other models utilizing monkeypox and/or variola. I listed a few here. The other one that's certainly out in the literature is the intrabronchial model. There's actually a couple different intratracheal models, one just recently by Dr. Goff which uses a microsprayer. The other is bolus route.

So I guess, in terms of what links these, why do we need another model, is they all require

unnaturally high doses of virus to induce severe disease. And I guess a lot is interpretation of what we consider severe disease. If you go by WHO's determination, and try to apply that to a non-human primate -- I've been working with the IV model for many years. And, for instance, 100 lesions, 200 lesions on a monkey via the IV route, the animals get a little bit sick, but, you know, they basically shake it off.

So, anyway, what we were considering "severe" is something near mortality or mortality. And this is to help support the animal efficacy rule.

So our aims were fairly clear. We were looking for a uniformly lethal model, NHP model of monkeypox and, hopefully, with the data we can gain from this move on to try to utilize variola.

As a graduate student, I wanted a little bit more out of it. I wanted an increased incubation period so I can look at pathogenic events. That would have just been a bonus.

So the question is, why marmosets? There's

multiple reasons to use marmosets. And there's actually some literature that points to their susceptibility. Gough reported an outbreak in 1982 in a New World primate colony, where six animals died of what looked to be -- well, let me back up. Six animals died. Unrelated events post a poxvirus infection that looked like cannapox, which gave us hope that they were, A, susceptible, but, B, that they could probably be vaccinated, they could mount a defense against a poxvirus.

Max-Rensing reported what's called "calpox," in 2006. It was an outbreak in a colony where it basically caused very severe disease in the animals. So, again, pointing out the differences between poxviruses, poxviruses are, you know -- thank god they have the homology they do, that way we get vaccines. But they are different. And just recently, Kramski took the calpox from the Max-Rensing outbreak and developed the model in marmosets.

So, what other reasons are there? They're an up-and-coming model for a lot of things. Dengue

was the newest paper out, Lassa virus.

And there are also -- because of this, they're used in behavioral studies. The number of reagents are increasing. I don't want to step as far as saying that there are as many reagents out there as there are for cynos, or macaques in general, but they're getting there. And it's an idea of testing the cross-reactivity. And I use that very, very, very loosely because usually, in my mind, anyway, cross-reactivity doesn't always give you the right answer when you're using a human antibody for a monkey protein.

But, you know, in terms of why not to use it, they're very small, less than 500 grams. You don't get a lot of biosample out of them. And, again, there are a limited number of reagents, compared to, relative to what's out there for macaques.

I threw the special diet, housing considerations, just because we went through about three or four years of trying to get marmosets at USAMRIID before we actually kicked off our first

study. And that was actually a main concern for USAMRIID.

So our design is fairly simply. Basic dose down strategy, based off of 5 x 10^7, what we used for the IV model, to compare apples to apples. And also, to keep our apples-apples, we used the same strain, the same challenge material we used for the IV model, which is monkeypox Zaire 79.

Basically, our criteria was the animals had to weigh more than 300 grams. We had three animals per group and they were all adult male. That's what we could get. And, again, this was a pilot of sorts.

So, basically, the parameters we wanted to look at was hematology. We used a Coulter quantitative PCR, we used, again, the same validated assay that we used for the IV model.

Weight and temperature, I'll have a couple comments on that later. And again, we sampled every three days, just because the animals were so small that we couldn't get a lot of biosample out of them.

So this is basically everything you need to

know about what we did. On the left, you'll see the animal number. On the right, you'll see the back titer challenge. And across the top, you will see the clinical manifestation that we were looking at. And this is actually "day post infection," the numbers.

The colors indicate -- the darker the color, the later the day. So, in a way, it's a heat map of day post infection that we saw clinical symptoms.

And I guess the main thing to point out from this is all animals succumbed to infection, in all groups. From lymphadenopathy, rash onset, viremia -- everything was temporal, dose-dependent.

(inaudible) ranges, they're not really all that informative, but there are ranges on the bottom. And every single one corresponds to the small number. For the most part, the small number is the highest challenge group. We do see some anomalies, such as lymphadenopathy.

The other thing I wanted to point out was how late in infection these guys do go out to. They can go out to day 15. And it's going to be kind of

hard to see, but we'll be going through each one of these individually. But you actually, in the lowest dose group, the first clinical sign, the first external sign, is lymphadenopathy or rash on day nine, which means we're getting -- at least from the presentation -- we're getting a long incubation period, a nine-day incubation period.

The bottom is just the mean of these groups. So, again, survival. Again, all animals succumbed to disease.

The other thing I want to point out in this graph is the fact that if you look -- again, this is only an n of 3 -- the animals tended to be -- they tended to succumb to the disease fairly close to one another. And again, the does-dependent temporal onset of death.

Clinical parameters, basically we looked -- again, like I said before, we looked at temperature, we looked weight.

In terms of temperature, there was no discernable fever. I cannot say there wasn't a fever.

I think future studies, what we need to do is do, like the DSI draw bin, where we're constantly monitoring temperature. Marmosets have a tendency to diurnally fluctuate in temperature. I read somewhere it could be up to seven degrees. So a better way to monitor this is with a draw bin. But we did see a general trend where, as the animals got closer to death, we saw what could be expected, is the decrease in temperature.

In terms of weight, there was about a plus-or-minus 5 percent fluctuation, just in general. Some animals actually increased weight, probably a fluid imbalance. They're not processing fluids properly. But for the most part, the few animals that increased weight, maybe an animal or two that decreased below that 5 percent.

One of the most striking features of the disease is the viremia as interpreted from PCR. Almost all animal groups got up to about -- over 10^9, but about the same, depending on whether they were euthanized or whether they succumbed to the disease.

The other thing I wanted to point out again, it is, you know, the lower the dose the farther you push out. We actually did not pick up -- and it was in one animal that we did -- until day six.

We did go back, since I put this together, we went back and plaqued out a couple of animals in the lowest challenge group, and there was viable virus at about -- again, I still have to put the numbers together, but about 10^7, 10^8.

Hematologically, again, what could be somewhat expected of a viral challenge, in that you're getting mobilization of white blood cells, and a high percent change in lymphocytes. That doesn't tell us what's going on locally, but that is what we saw.

And, again, it's kind of harder to discern, but you could see, again, a temporal change based on dose.

Lastly, for hematology, platelets, changes in platelet counts. On the top, you'll see this is a percent change. On the bottom are the actual numbers for each animal. Basically what we're seeing is a

fairly severe drop in platelets, a thrombocytopenia.

I think I counted 11 of 18 animals were below 150 x 10^3 platelets per microliter. But, again, this correlated to what we saw in terms of disease. I shouldn't say "correlated," but reflected what we saw in terms of skin manifestations.

The skin manifestations themselves, again, dose dependent. At 2.4 x 10^7 it was more generalized hemorrhage. By the time we got down to 48, they were more focal, signs of some papules. It never progressed farther than a papule, I want to point out. But everything in between was a mix.

Histologically, the skin looked like what we would kind of expect for a poxvirus infection in a small proportion of the slides. Mostly what we saw is dermal hemorrhage, which is more indicative of non-classical smallpox.

Other lesions that we see in the IV monkeypox models and other monkeypox models are necrosis in testes, or necrosis of the epithelium. I might add that, in terms of gross findings, this is

one of the few gross findings we saw, in the esophagus. It was interesting that we didn't see a lot, in terms of necrosis, in terms of gross examination. But we did see a lot in terms of histological examination.

Again, lungs -- you see that there's areas of necrosis and pleural inflammation.

Some non-classical features, a lot of hemorrhages. There was a lot of hemorrhaging in multiple organs. Here I gave you some examples of the scrotum. You can see the hemorrhaging, subdermal hemorrhaging, or submucosal hemorrhaging in the urinary bladder, and hemorrhaging in the lungs.

In terms of the liver, the liver was large, friable. A lot of hepatocytes were -- excuse me, there were a lot of intercytoplasmic inclusion bodies in the hepatocytes. We wanted to make sure that this wasn't like some sort of crazy feature, that they actually wore a virus, so we did EM once in formalin-fixed tissue, and you can see the various virulence, poxvirus virulence.

Real briefly, in conclusion, so what we have is a low-dose, uniformly lethal, NHP model. This could be fairly advantageous. I personally -- I'm in the viral therapeutics department or branch, and we see this as a kind of good sign for therapeutics.

But in terms of vaccines, it could be very useful for testing robustness of vaccines, in terms of escalating the amount of virus to challenge-vaccinated animals. The nice thing about the marmoset model is you're starting out so low at a lethal disease that you can only go up, in terms of the challenge inoculum.

Also, the sensitivity to monkeypox may carry over to, let's say, live or attenuated vaccines. This is all, of course, speculative, but it could be a good model for adverse events, for vaccines, for live vaccines.

Again, I went over the high genome levels in the blood. The other nice thing about the model, again, we didn't see clinical symptoms until day nine. And in NHP model with monkeypox or variola, this is

actually pretty good. This would actually give -- again, in terms of vaccines -- a possible model of post-exposure vaccination.

Again, I went over all the manifestations that are classic of monkeypox or human disease, and the hemorrhaging, which is not part of what we consider classical smallpox -- just a guess, we obviously need more studies -- but possibly an early-type hemorrhagic disease.

The long incubation and the quick onset of lesions, and the fairly short duration before they die after the onset of lesions actually is somewhat reflective of what's been described for early hemorrhagic disease.

What we'd like to do, number one, finally finish out the study. That would be great, and that involves completing the pathology. I think all the slides have been read, and we're just putting together the data now, which -- I can't remember if I put Lou Huzella on the first slide, but he definitely deserves credit for -- he's reading all the histo slides.

Quantitative PCR and plaque titrations of tissues, I'm still up in the air about whether or not we want to do this, being that there was so much virus in the blood. I'm not 100 percent sure what this will tell us. And since we're doing immunohistochemistry anyway, I'm not sure we're going to get to this. We'd obviously like to fill in the data gaps. We were sampling every three days. We'd love -- and that's the nice thing about marmosets, you can get a lot of them in a room. We'd love to set up different groups for different days.

And, again, the IV administration of monkeypox was just the beginning, proof of concept, let's say. But what we would like to do is actually go something like along the lines of aerosol or intranasal, see if we can actually extend that incubation period longer. And obviously we have to show some sort of predictive behavior of the model, and this involves vaccinating or treating.

And finally, you know, this will probably be all through everybody's talk is that, you know,

everybody wants to go to CDC and try their animal model with variola. Hopefully it will happen. I think we've given some good evidence that possibly -- it's not a given, it's definitely not a given. Just because it's susceptible with monkeypox doesn't mean it's susceptible to variola. We know that.

And that is basically -- I'd like to thank Dr. Jahrling and IRF. They really helped. Peter was involved in getting us primates. And, again, Lou Huzella, who was looking at our slides and wrapping up, and helping us write the paper.

I'd like to thank Brett Taylor, he was our veterinarian. He's now -- I'm not 100 percent sure where he went, but he's no longer with USAMRIID. He was integral in getting marmosets to USAMRIID.

Suzette Tardiff definitely helped out in terms of background information need to get the marmosets' housing information, general care, stuff like that. Same with Kay Jordan and the Viral Therapeutics Branch at USAMRIID for supporting the efforts.

Thank you. (Applause)

DR. DAMON: Why don't we take one question, and then go on break and come back for the last two talks before the panel meeting.

Any questions?

DR. NALCA: Aysegul Nalca, USAMRIID.

Did you see hemorrhage in organs at the very high doses? Or did you see it in the very low doses, too, like 48 --

MR. MUCKER: Very low doses, too.

DR. NALCA: Okay.

MR. MUCKER: And it was multiple organs.

DR. NALCA: Okay. Thanks.

MR. MUCKER: The only thing that actually looked different was the skin manifestations. Again, we're still pulling together the data to really cement what we've seen.

DR. DAMON: Great. Why don't we take 10 minutes and come back at 5 to 3:00.

(Recess)

DR. DAMON: Sorry to cut the break short, but

some people have planes to catch at some point today. Why don't we come back?

Okay, so our next speak is Dr. Nalca, from USAMRIID. And we'll be talking about -- we'll be moving now to small animals, into rodents. We'll be talking about an aerosol animal model for rabbitpox in New Zealand white rabbits.

DR. NALCA: Thank you, Inger. Good afternoon, everybody.

Okay. So far we heard about non-human primate models for poxviruses. And now we are going to move to small animal models. And I'm going to start with the rabbitpox model. So, before we move into model, I just want to give you a brief introduction about the rabbitpox virus.

The rabbitpox is classified in the poxviridae family. It's part of orthopoxvirus genera. It transmits through aerosol. It is very infectious among rabbits. It causes severe respiratory disease in rabbits. And the disease course in rabbits is very similar to monkeypox and smallpox in humans.

And rabbitpox outbreak was first reported in 1930, at Rockefeller Institute, and it caused more than 50 percent of fatality in lab rabbits during this outbreak. And then in 1940s, another outbreak happened at the University of Utrecht. And later, in 1960s, in USA also, there was another Utrecht-strain outbreak.

In the studies that I'm going to present today, we used rabbitpox Utrecht strain. Apparently Rockefeller strain is not available any longer.

So, before we move to studies, I just want to give you a brief overview about aerosol exposure that we are doing at USAMRIID.

So, okay, let's do like this.

Before we do aerosol exposure, we measure the respiratory fraction of each rabbit with the whole body plethysmography. Rabbits are unanesthetized for the plethysmography, and also for the aerosol exposure.

And then, we do muzzle-only aerosol exposure. As you see here, nose and mouth in the

aerosol chamber. And then the small-particle aerosol were generated with three jet collision nebulizer running at 7.5 liters per minute.

We used automated bioaerosol exposure system, ABES. ABES was developed at USAMRIID almost a decade ago. It's a computer controlled aerosol system. It provides improved control and data acquisition. It's really easy to use. It's color coded, you know, just we attach everything as color matching. And it has standardized data recording and archiving. It's GRT compliant and it integrates all aerosol functions in the one platform. As you see here, it has pressure control, humidity control -- everything is one platform. And it provides improved dose control and dose calculation. As Mark mentioned, we get very reproducible results with this system.

So first I'm going to talk about the natural history study that we have done with the rabbits.

We had two groups for this study. Each group had 20 rabbits. The first group was exposed to 75 PFU aerosolized rabbitpox virus, and the second

group, 100-fold higher -- 7,500 PFU aerosolized rabbitpox virus. We chose these doses according to our previous studies, that these doses provide lethal disease in rabbits.

Rabbits were observed twice a day for clinical signs of the disease. Body temperature and weights of the rabbits were recorded each morning. I have to make one correction here. We used telemetry implants for this study. And we were able to record temperatures 24/7, but we also recorded, you know, temperatures every morning in order to score these animals and decide for the euthanasia.

And blood collected every 12 hours until day 10 post exposure. We had to stop on day 10 because of the limitations on the blood collection, because these animals are small. Since we collect every 12 hours, we couldn't go more than 10 days. And then we did complete blood counts, chemistries and PCRs -- real-time PCRs -- on these bloods.

So let's look at the presented dose and survival. Here, our target dose was 75 PFU and 7,500

PFU. As you see, the doses were very tight. They were a little bit higher than what we targeted, but they were very tight, except these three animals. And at 7,500 PFU, again it was very tight dose range this group of animals got.

When we look at the survival, all high-dose animals succumbed to disease by day seven. And for low-dose animals, 3 out of 20 animals survived the challenge.

So, the incubation period for aerosolized rabbitpox disease is two to four days. It is dose-dependent. If we give them very high dose, it's less than two to four days. If we give them low dose, very low dose -- when I say "high dose," "low dose" it is 200, actually -- or study shows at 200 PFUs kind of mark. If we go less that 200 it is more than 2 to 4 days incubation. If we go more than 200 PFU, it is around 2 to 4 or sometimes, you know -- again, it depends on the dose -- less that 2 to 4 days incubation. It depends on how high we go from 200.

And temperatures begin to increase on two to

four days post exposure. We see temperatures reach 40, 41 degrees centigrade. And we always see anorexia. Animals stop to eat at day two, three.

And then we see facial cervical edema. Ocular-nasal discharge is very unique features of the rabbitpox disease. You see here "ocular discharge" and then here "nasal discharge."

And then disease progress, we see lethargy, weight loss in these animals. And through the end, you know, they're not eating and they have dehydration, diarrhea and dyspnea and/or open-mouth breathing. And then animals succumb to disease.

So we see pock lesions. Again, it is dose-dependent. If we give the rabbits very high dose rabbitpox virus, before they can develop the lesions they succumb to disease. If we give them the low dose, we always see the pock lesions.

The only thing, the lesions progress macule, papule. We never see vesicles. And then they move to -- it's not real scab, it's kind of, you know, just before scab. And then they disappear.

Here, again, you see cervical edema, ocular-nasal discharge. And here is the lesions at the papule stage.

Again, the lesions around eye -- these are all kind of messed up here -- around mouth, and then at the very beginning of the lesions, pock lesions.

So as I said, we used temperature implants for these animals. For the high dose, we started to see increase in body temperature around day 2, and it reached 41 degrees. And then it stayed high and, you know, we lost some of the animals around day six, and all animals succumbed to disease by day eight or so.

And then for the low dose, we started to see increase in body temperature around four, and it stayed high and three animals survived. And the temperatures came back to normal levels.

We did CBCs for these animals. And normal values for CBCs, 4.6 to 10.2. And here you see, for the high dose, we started -- we observed increase in white blood count around day six or so. For low dose, it was around day seven, eight. And both doses, they

reached the values that they were higher than the normal range.

So when we look at the neutrophils and lymphocytes, we saw the flip -- while neutrophils were increasing, for both groups lymphocytes decreased.

And we did real-time quantitative PCR. And we used pan-Orthopox assay for the whole blood. And, again, we collect blood every 12 hours, and we did the viral load for every 12 hours. And we started to see increase in viral load for the high dose group around day three. And for the low dose group it was around -- after day three. It was around day four or so.

So this is another presentation for the viral load. I just put some examples, 10 animals for each group here.

Here, this is the low dose, this is the high dose. As I said, at day three post exposure a.m., one animal was positive for viral load. And then for day three post exposure p.m., four animals were positive. And by day four p.m., except one animal, almost all animals were positive for viral load, and it was high.

And when we look at the low dose, they started to show viral load in blood around day four post exposure, a.m., and half of them were positive day four p.m. And then all of them are positive day five post exposure.

So we did necropsy on all these animals. As you see, lungs were very edematous, and they were frail to collapsed. There were some hemorrhagic areas. And there was multifocal bronchopneumonia, and that was due to pulmonary edema. And this was most of the time the cause for the death.

So when we look at the histopathology results, we saw necrosis of respiratory epithelium. You see the antigen staining. And there was this (inaudible) here, which is multiple nuclei in the cytoplasm which had cell division.

We saw viral antigen in all different organs: spleen, mandibular lymph node array. Gonads, actually were one of the first ones that we see the viral antigen, after the lungs.

Severe rhinitis was present in several

rabbits. Here, this is the normal nasal passages. And then when you look at here, this is when animal dies, and when we do the necropsy, as you see, there's edema as virus replicates, it kind of blocks the nasal passages so animals cannot breathe. So they started to breathe open-mouthed, too.

So after we have done the natural history studies, we decided to look at the differences between large-particle and small-particle.

As you know, smallpox transmits through large-particle aerosol. So it was important to see if there is any difference between large-particle exposure and small-particle exposure.

So as I mentioned previously, we used three-jet collision nebulizer for small-particle aerosol generation. And then we used spinning-top aerosol generator -- STAG -- for large-particle aerosol generation. And small-particle aerosol, we get between 1 and 3 micron particle size. And for the large particle, it is more than 5 micron, less than 10 micron, the size.

So our doses, for this study target doses were 2 PFU, 20, 200, 2,000 and 20,000. And we had 10 rabbits in each group, for each dose, and large-particle and small-particle.

Let's look at the small-particle first. And, as you see, we got very good results for the presented dose for the small-particle aerosol exposure. But we had some mechanical problems, so all the animals in large-particle, all dose groups, got one load lower than whatever we intended to give them. For example, this 20,000 PFU, we wanted to give them 20,000, but they got around 2,000. And similar for the other groups.

So these animals, we didn't use telemetry implants. These were just chipped, and we checked the chips every morning. And we started to observe increase in body temperature for the high-dose groups for the large-particle on day two or three. And for the low-dose groups for the large-particle, there wasn't a whole lot of change in body temperature throughout the study.

When we look at the small-particle, for the highest dose, 20,000 PFU, we started to see increase in body temperature around day two, three. And then animals succumbed to disease on day six, when they got moribund and they are ready to be euthanized, body temperatures drop very sharply.

And for the lower doses, when we look at the 20 PFU, we started to see increase in body temperature around day 4 or 5, and then it was high for several days, and then it came back. Because, as I said, three animals -- sorry, it was previous study, small dose groups survive in small-particle aerosol.

So changes in bodyweight, we didn't see a whole lot of change in bodyweight for the large-particle aerosol for the low doses. Actually, they gained weight. As you see, at the very high dose there was a decline, and these animals succumbed to disease. And for the second high dose, some of them succumbed to disease and the others came back and gained weight.

For the small-particle, the doses 200 and

above, they lost weight and they succumbed to disease by day 10. And 5 PFU and 20 PFU, they survived. And there was some lethality in 20 PFU.

Here is the survival. As I said, 200, 2,000, 20,000 PFU all succumbed to disease with the small-particle by day 10. And we had 45 percent survival with the 20 PFU. And then we had 90 percent survival with 5 PFU with the small-particle aerosol.

When we look at the large-particle aerosol, all the animals got 3,000 PFU succumbed to disease by day seven. And 25 to 30 percent of the animals got around 700 PFU -- hold on, did I say it correctly? They survived -- yes, 25 to 30 percent. And then when we look at 22 PFU, 60 percent, 55, 60 percent survived, there was one animal that succumbed to disease very late. And 2 PFU, it was around 90 to 100 percent survival.

So here, another presentation for the survival. Again, for small-particle, 5 PFU, 9 out of 10 survived. For 20 PFU, 4 out of 10 survived. For 200, none of them survived. Again, 2,000 and 20,000,

none of them survive.

And we look at the large-particle, 2 PFU, all of them survive; 20 PFU, 6 out of 10 survive; 700 PFU, 3 out of 10 survive; 3,000, none of them survive.

So, we did necropsy on these animals. We collect the tissues and we look at the viral load with PCR on these tissues. Again, the virus were all over for large-particle and small-particle. And it seems like small-particle, the values were high. This was the serial sac study. So when you look at it, it was almost at the 10 times, 10 to 12 PFU levels for the small-particle.

So, if we summarize and compare with the smallpox -- I want to divide it to rabbitpox small-particle, rabbitpox large-particle, and compare with the smallpox ordinary type.

We don't know the dose for the smallpox, how much the people get and get sick. But, as I said, for the rabbitpox small-particle, 200 PFU is the mark. And since we know this for the small-particle, I used the same amount for the large-particle -- 200 PFU.

So transmission routes, all aerosol. And particle size for smallpox large, rabbitpox, as I said, small and large, we did incubation period for smallpox, 7 to 17 days. For 200 PFU, 2 to 3 days. For large-particle, it's four to five days.

For this model, you know, progression is very fast compared to smallpox, it's much faster progress. Incubation period is much shorter, and the disease period is much shorter than the smallpox disease. Again, prodromal phase was two to four days for smallpox, and then small-particle, zero to two days, and large-particle zero to two days.

And clinical signs, we see fever, oropharyngeal lesions, skin lesions for the smallpox. Similar clinical signs for the small-particle rabbitpox. And we have also nasal-ocular discharges and dyspnea. And as I mentioned, skin lesions for small-particle is dose-dependent. If we give them less than 200 PFU we see skin lesions. But if it's higher than 200 PFU, animals cannot develop the skin lesions, they succumb to disease very quickly.

For the large-particle we had very similar lesions, like the small-particle rabbitpox. But most of the time we were able to observe the skin lesions, different than small-particle aerosol.

So when we look at the case fatality rates, smallpox, ordinary type, is around 30 percent. Small-particle rabbitpox 100 percent for this dose. And for this dose, large-particle rabbitpox around 30 percent.

So, at the end, large-particle aerosolized rabbitpox and smallpox resembles, as a model, much better than the small-particle rabbitpox. But also, small-particle rabbitpox is important, because if something happens, if variola virus is used for the bioterrorism event, it's going to be the small-particle aerosol. So we need to keep in mind that small-particle rabbitpox model also important to test therapeutics and vaccines, too.

So, when we look at the advantages, its aerosol route is the same route as smallpox transmission. It has dose-dependent disease progression. And very low-dose virus causes

generalized disease and fatality. As I showed you, you know, if you want lethal model, 20 PFU rabbitpox virus causes fatality in rabbits.

And limitations, the main limitation is absence of immunologic reagents for assays. For example, I want to do cytokine testing, and it's very difficult to find commercially available kits to do cytokine testing for rabbits.

And lack of immunodeficient and/or gene knockout rabbit strains, it's going to be more and more. There are some out there, but right now, not a whole lot. And it has accelerated progression of disease with high dose of virus. It's very short term if we give them very high dose of virus. And, as I said, absence of pock lesions with high exposure dose.

So I would like to thank the Aerobiology Division. A lot of people worked on this project, and Dr. Don Nichols from Pathology, and veterinary medicine people. And to NIAID for funding, also for the very useful discussions.

So, thank you so much. And I'm ready for

the questions. (Applause)

DR. DAMON: Any questions?

Yes.

DR. ISAACS: So I remember when Dick Moyer worked on this that he looked at lesions in the ears.

DR. NALCA: Right.

DR. ISAACS: Did you guys see anything there?

DR. NALCA: In the ears, we haven't. But also remember that Dick Moyer is doing the intradermal route of exposure. I don't know if it makes the difference. But we haven't see lesions in ears.

DR. DAMON: Thank you.

So our last speaker before we move to the Panel session is Dr. Mark Buller. And he'll be talking about some work on mousepox -- or ectromelia -- challenge models.

DR. BULLER: I would like to thank the organizers for inviting me here to talk about the mousepox model. I have a confession that I had to remove one slide from my presentation because it

didn't make much sense to me after I looked at it. And I thought it maybe wouldn't make much sense to you, either. And I've rearranged a couple of slides, also.

Ectromelia is a virus that has only really been isolated in animal colonies. So all of the strains of ectromelia were isolated over the years from animal colonies that housed mice. There is no real knowledge about its natural history in the wild.

With that said, I want to talk to you today about three aspects of this model. The first part will be taking some features from variola infection of people and using them as a criteria to build the mousepox models that we use. I'll then compare and contrast, generally, the smallpox disease with mousepox. I'll then describe three different mousepox models that we use, and try and give you sense of the utility of each one.

And then the main part of my talk is to emphasize the importance of route of infection. And you've heard from a number of the other presenters

that there's no perfect model. And we have to somehow get enough experience with different models where we cover all the bases.

The strength of the mousepox model -- which I'll foreshadow here -- is that you get significant disease and very uniform mortality from a graded dose to below 1 PFU per mouse up to 100 by the intranasal route. The other feature that makes it very advantageous is the reproducibility of the disease. So someone, I think this morning, mentioned about doing studies where you have a high power and one challenge dose, and have survivors and lethal infections vis-à-vis a vaccination. That's something that can be done with these models.

Okay. Something that a lot of us may or may not realize is that all of the information that's written about the incubation period of smallpox, or the eclipse phase, comes from mousepox. And I've highlighted here the two articles which have contributed to that: one by Fenner in the 1940s, and then one by Roberts in the 1960s. And we really don't

understand the early events of smallpox -- or mousepox, for that matter.

This is my view of the infection process, sort of the natural course of variola virus in human, and what drove the construction of the model the way we use it.

First of all -- it was mentioned previously, the previous speaker -- it's thought, from epidemiological data, that the infectious form of variola virus a large-droplet aerosol. And that can be mimicked by large-particle aerosol or, in our hands, we use the intranasal route just for convenience.

And in saying that, the other information that suggests it's an upper-respiratory route infection is that there was very little evidence of involvement of the lung early on in disease, and primary lesions in the lung of smallpox victims were rarely, if ever, identified.

The actual cell type that's infected in variola is not clear. Candidates would be alveolar

macrophages. And from studies with vaccinia virus you could also envisage that the apical or basal lateral surface of the epithelial cells in the lung could also be targets. The vaccinia studies suggest that the infection most likely will be through minor abrasions through the basal lateral surface, although no studies have been done on other orthopox virus, to my knowledge.

The virus would then replicate locally. And from the vaccinia studies, again, the vaccinia growth factor, which is related to epidermal growth factor, would provide a milieu whereby the cells proximal to the lesion are maintained in a uniform tissue layer. So you don't get a total destruction of the epithelial lining in the area proximal to the lesion.

Another black box is how the virus gets from the primary site of infection, the respiratory tract, to internal organs. Again, from most pox models, it's thought to be flushed through the lymphatics into the venous system and seed internally. I'm not sure if it's free virus or virus in cells. Once the internal

organs are infected, it's again not clear the route by which the virus gets back to the cutaneous epithelium or the mucosal epithelium for transmission.

And then what probably can be inferred from our understanding of vaccinia replication in most models and *in vitro*, is that the extracellular envelope virus is released from the apical surface of the cells in the respiratory tract, and then are taken by the respiratory gases out of the individual's mouth, and then if there's a contact close enough, that person's infected.

Okay, the other part that I mentioned was the infectious dose of ectromelia being very low. And ectromelia is not alone in that. One of our models is less than a PFU, and then there are the B6 models around 100. But rabbitpox and WR in European rabbits is a very low infectious dose of 15 to 50 PFU by either aerosol or intradermal routes.

And if you look at epidemiological studies of at least two outbreaks with variola virus after it had been eradicated from the areas in question, in the

Meschede Hospital outbreak, and Aralsk outbreak in the Soviet Union, then it can be inferred that variola has a potential to have a very low infectious dose, and my estimates are probably less than 50. But that's not based on any hard data.

So the similarities between mousepox and smallpox are listed here. This one, I think, is important. And the fact that the virus can actually be detected in -- Thank you -- gases. This is again work by Roberts.

And I mentioned earlier that there's no involvement of the lung. And then you get a characteristic rash which is dependent on the strain of mouse that you're using.

Differences are also very important. And smallpox has a very different pathology than mousepox. Mousepox presents with major pathology in the drain lymph node and the liver and spleen. And, of course, as pointed out by others today about the animal models, usually the disease course is shorter, and in the case of mousepox, there's no exception. We get

death, depending on our model, 7 to 12 days, whereas death with ordinary smallpox would be 18 to 22 days after infection, roughly.

Now I'm going to describe a little bit about the strains.

This A-strain, which we use usually for vaccine challenge studies, is extremely reproducible. The BALB/c mouse is another mouse strain that has these sorts of characteristics but is not as reproducible as the A-strain.

With the A-strain mouse given a dose of virus, all of the animals will die within six to eight hours of each other -- not days, but hours. And that makes a very reproducible model. This is also the model that has extremely low LD_{50}. It's less than a PFU. So you're down to a small number of particles.

The downside is that when these animals are succumbing to infection around six to eight days, you really see no activation of immune response. It's almost quiescent, whereas the other models that I'll describe to you, you start to see a robust immune

response superimposed with the infection process.

The SKH1 strain of mouse is a mouse strain that has a mutation so that the hair on the coat does not mature properly. So as it reaches adulthood, the animal has no hair at all, so you can observe rash. And so we've used this, or evaluated it as a model for therapeutics. And it's got similar properties to the B6 mouse model. It has an LD_{50} of about 100 PFU, and extended disease course. You do get an immune response to infection. And the next slide shows you the rash. But we find it -- not irreproducible, but not very reproducible. So we don't really work with this strain very much.

This is an SKH mouse, about 18 days. And you see typical lesions on the side.

So this is the model that we've spent most of our time working with. It's the C57 Black/6 intranasal mouse model. And it has -- getting back to this idea of route being important. And we heard Bernie talk about variolation being the first vaccination against smallpox in ancient times.

This model, if you give the virus by the skin, gives you asymptomatic disease. No mortality, no disease signs. If you give it by the IV, IM, IP routes, you get a pretty good mortality. And here is the LD_{50} by this route, in this strain. We used a very low volume of inoculation -- 5 lambda each nares -- and that confines the infection to the upper respiratory tract.

So this is what I alluded to. If you use a sub-cu or intradermal or pericutaneous route of infection, the LD_{50} shifts to above a million PFU. Immunologically, it's very reactive. We don't see consistent rash with this model from an IN route. And the time to death is about 11 to 12 days.

Now, this is just to give you a brief vision of how I think an ectromelia infection occurs, and the dynamics of it. So with ectromelia, it's usually through cornified skin. So the respiratory challenge is not thought to be the natural route. The natural route is supposedly through skin abrasions. And the virus we see replicates locally to where it's

injected. You can mimic this by interjection or scratch because the virus is so infectious for the animal.

And it will replicate here. And another very interesting feature of the poxvirus -- and we heard earlier today about these immunomodulatory or immune-evasion molecules -- most of them have a feature that allow them to bind to the extracellular matrix. So you end up getting a foci of infection. And all of these ligands that I've got in red, the virus has a binding protein, most often, or another way to block their action.

In the case of the binding proteins, they adhere to the extracellular matrix around the foci of infection so that they have almost like a defensive shield against either the cytokines being generated or, if there are interferons, being able to bind to the receptor.

So you see how it's very different than if you gave it IV. If you give it IV, you potentially could neutralize this ability of the virus to set up a

stealth-like infection in the epidermis.

We can look at an ectromelian infection and stain this whole layer with antigen and the dermis, and there are no inflammatory cells in the dermis at all. And that's because the integrity of the capillary, the vasculature, is being maintained, but these different molecules -- like the chemokine binding protein -- disrupt the gradient. So there's no signal leaving the lesion to infer to the circulating lymphocytes that there's infection going on.

Again, you lose this with an IV route, but you have it if it's a local route infection. So that's the point I wanted to make here. And then, with time, lymph is flushing through here. The virus comes through the lymph node. You get an infection. And if you believe the original idea, then the virus would come through the draining lymph node, through the lymphatics, and cause systemic infection.

So I just wanted to list five different ways that, if you give a virus, or this virus, by the IV

route, you have the potential to either jump-start the immune system -- which would be important if you're looking at a vaccine scenario -- or cause a different pathophysiology. And then I will follow it up with three examples of data from an IV versus an IN infection, and then finish off with some comparisons between IN and sub-cu.

So one is, you get an instantaneous viremia, so you take away the incubation prodrome. And I alluded to this in this last slide, that by giving an IV injection, you really circumvent, possibly, the action of some of these host-evasion molecules.

The virus that most often people use for infecting animals is the intercellular mature virus that's highly susceptible to complement. So you can drop the titer of inoculum tenfold within minutes if you mix it with complement.

In doing so, you change your dose. But, in addition, you coat the virus with complement fragments, which then can facilitate its taking these particles up in complement receptor type 2, expressed

on APCs and, therefore, may affect the rapidity with which you get a recall response, if you have vaccinated the animal. Virus inoculum productively and abortively fix different spectrum cell types. The types of cells you find circulating, of course, are very different than the types of cells you find in the skin, whether it be the epidermis or the dermis.

So this is the three pieces of data, comparing IN versus IV routes.

The first thing I want to show you, on the left side here is IV. The LD_{50} is very different. The LD_{50} is around, say, 10,000 PFU here. And the LD_{50} here is around 100. The day of death, for 50 percent of these animals it is a lot earlier -- it's around three days -- where it's spread out more here in the IN situation.

If you look at virus infectivity in tissues, on the left we have the IV panel. And here, we have lower amounts of virus infectivity in spleen, liver and kidney at seven days post infection. And we have higher amounts here in the IN. IV lung and IN lung

are about the same.

And then finally, to support that argument that perhaps you can jump-start the immune system a little bit by giving it IV versus IN -- and I don't want to focus on this today, for the sake of time -- but if you look at this panel here, this is analysis of the CD8 cells that are in blood, their ability to make interferon gamma after being stimulated with virus APCs at seven days after infection.

And what I'll show you, which is, very clearly, if you add no virus on re-stimulate these cells, you don't get any interferon being generated. But if you put in virus, stimulate them, you get a significant, a greater amount of CD8 T cells expressing gamma when the inoculation route is IV versus if it's IN.

In the next series of data, I want to compare the intranasal and subcutaneous routes of infection. And normally, with most pox, people use the footpad as the subcutaneous route of infection. And if you do that, and you're comparing with IN,

you're comparing, possible -- well, you are comparing different lymph nodes, and it could be that the anatomical difference between the lymph nodes is important. So, to rule that out, we did a model where we harvested what we thought was the drain lymph node from an IN and sub-cu route of infection, shown here. And what we did is we infected -- for sub-cu we'd infect on the bridge of the nose; for IN, you'll go in the nares.

And this is an example of an IN infection. And you can see at day 3, with 1,000 PFU, the little foci of infection on the nasal turbinates. This is the opening of the nares. And then -- this is superimposed on a bright field with the fluorescent. And then if you wait five days longer, you see it enhanced, a replication of virus. But you notice it hasn't spread down here.

Under these situations, we're starting to see virus in the lung at, say, three or four days after infection, but that's from hematogenous spread. It's not through mechanical spread down the

respiratory tract.

This is just to remind me to tell you that we spent a lot of time pulling all the different lymph nodes out from this area, and then comparing the amount of virus in the lymph nodes following IN versus sub-cu on the top of the nose, and the number of virus-infected cells.

And at the conclusion of all that work, we decided that this group of lymph nodes right here, the sub mandibular complex -- which has got two or three lymph nodes that are very hard to separate, so we take them as a unit -- was draining both sites. And, on occasion, we use this, the trachobronchial lymph node, or popliteal as a control. I don't think I have that data here, though.

So this is, first of all, just an LD_{50} to convince you that what I told is true: that intranasal is lethal and the LD_{50} is, say -- here we've got, oh, 25, 30 percent survival, with 300 PFU, so it's around, you know, 100, 200 PFU. In subcutaneous, you go up to 3×10^6 on the bridge of the nose, and no

effect.

This is looking at infectivity. I've got to apologize. It's from the same experiment but I took it from two different presentations, and the color-coding is different between here and elsewhere. So bear with me.

We also put a control in here which was scratched, because the concern was that when we saw virus in the drain lymph note, and did their lymph node earlier following sub-cu, we thought that that might be due to the pressure of the fluid in the syringe pushing it more into the lymphatic. So we compared that with just putting virus in a needle trough, which was the scratch.

But what you can see here is whether it's sub-cu or scratch, the virus appears very, very quickly in the drain lymph node, where the IN is about a day behind. And that's very reproducible.

These studies were done with very high-dose virus so we could respond. So this was about a million PFU. And once we're happy with doing it at

that level, we'll back it off down to something more physiological.

Looking at liver, kidney, blood, and spleen and lung, the color coding is different here. Lung: IN is blue, sub-cu is red, and then scratch is green. But the point to make is, in most of these panels, you'll see the virus appear earliest after sub-cu or scratch and later with IN. But then the IN titers will be higher later on in disease.

This is showing you lymphoid populations in the drain lymph node from the sites of infection. The first panel at the left here is looking at GFP-positive cells that are in the lymph node as a percentage of CD45 cells, I think. And this is just a reporter gene that's in the virus that allows us to score GFP.

And the interesting thing here is, although beforehand we saw the intranasal route having a delay of a day or two, there's very few cells that are staining for GFP infection in the CD45 cells that are coming out of the lymph node population. And there's

a lot more from the sub-cu. We're not quite sure what this means, and whether the virus that we're seeing -- we do see significant virus here in the sub-cu, in the IN-infected nodes -- whether that's infecting different cells. That would not be reflected in the CD45 population. CD45 means leukocytes, basically.

And then if you work you way down here, with the CD4 and CD8 as a percentage of cells in the lymph node, they go down with time, although the total number cells is going up. And the only cell type that really is going up is the B cell, and that's shown here.

And we don't see much difference between the response of these populations from the IN infection versus sub-cu. The only real response we see is with neutrophils, where we see a spike of neutrophils early in this lymph node following sub-cu.

Again, a difference between the routes. There's commonality, there's also differences.

Now, this is looking -- this is one of the glories of working with the mouse, because we can

measure all these different things. And this is measuring just a small subset of cytokines or chemokines that are being synthesize.

So in the interferon gamma case -- and a lot of times an interferon gamma production early will correlate with protection. Sub-cu we see it very, very early, and then we start to see it come up with the IN. And then this is where the animal is starting, you know, to become sick and we see a fall-off. I'm not quite sure why we see the fall-off, but it does appear to be some sort of regulation.

NCP1, again you see it very, very early. Later, with sub-cu, later with IN, and in smaller amounts. And the same thing with IL6, RANTES, and then KC.

So here we're seeing a very, very different response in the drain lymph node from IN versus sub-cu. And this is highlighted by this next panel, which is a summary of some gene-expression profiling we did, where we looked and just scored the number of genes that are up-regulated or down-regulated following IN

or sub-cu.

And the point I want to make here is that there's very little in common overlap between the two until you get out to day two. So you're getting stimulation in the node, but when it comes from the IN route, the genes being stimulated are very, very different.

And when you look at the kinds of genes that are stimulated, these ones look like an immune response sort of set -- interferons, (inaudible) receptors, activation markers -- where there's a whole hodgepodge of genes that are being stimulated with the IN that don't really correlate with what you would expect a mouse to be doing just trying to protect itself.

And this is down-regulation, same story. Totally different sets of genes, again highlighting that, depending on the route. And here we're looking at the same lymph node, we're just giving the virus to that lymph node from two different routes.

This is looking at APC function of the

mandibular lymph node. And I don't have the proper control, which is sub-cu mandibular. So here what I've got is the popliteal. So this would be what we would expect to see in the mandibular, after a sub-cu infection, but we're using the popliteal.

But you see that the intranasal route, there's actually no real APC function going on, at least out to three days after infection. This is where something's happened.

And this is just a gross indicator of the immune reactivity of the animal, showing spleen, mass, and that with the footpad this would be appropriately the same with the sub-cu in the nose. You see an expansion of the cells in the spleen, whereas with IN, there's very little reactivity in comparison.

And I think this one of the final data slides here. This is looking at serum interferon gamma, and alanine transferase activity in blood, following sub-cu, IN, and the same kind of story.

With sub-cu, we see very rapid interferon gamma come up, and then it comes down. With the IN,

we see a delay it comes up. But, interestingly, it appears to be no real regulation of control that we saw with the gamma from the sub-cu. And it keeps going up, except the animal dies.

This is just to show you a surrogate for liver necrosis, in that in the IN route that we're getting tissue destruction in the liver.

And this last slide here is just summarizing models that have been used, mousepox models that have been used to evaluate different vaccines. And the references are all here for you.

And then this is another model that I just wanted to bring to your attention. And it's been developed in Bernie Moss's lab with Pat Earl and Jeff Americo, and it uses a very interesting mouse strain called the CAST/EIJ, which is a wild mouse that's been bred in captivity at JACS. And it's one of the few mice strains that's susceptible to monkeypox. So if one wanted to do rodent studies with a monkeypox virus, then this might be a very good strain to use.

And the conclusions are self-evident. My

punch for route being super important in choosing the model one uses -- or at least have it in the combination of models that are used to evaluate the vaccine.

The last is that there is also a new model for monkeypox, since monkeypox seems to be the virus that best approximates smallpox in people, and clearly has a role in the licensure of therapeutics or prophylaxis.

And I'll stop there for questions.

(Applause)

DR. ISAACS: Mark, Stuart Isaacs. Nice summary.

So, looking at your very first slide, where you had -- I was really curious to see that you actually named extracellular viruses the "transmitting virus." And I'm very curious about why you said that. Because my vision, and I think I've read elsewhere, that the pock lesions in the mucocutaneous are just filled with virus. And I had always thought that it was dying to dead cells that then released mature

virus that's spread from host to host.

So I was very curious about the --

DR. BULLER: Yes, it's really the efficiency with the way that the extracellular virus is released from cells. It's pushed out, it's active, it's occurring very early on in the infection process, and it's simply that.

I don't know if it's intact extracellular virus because of the sensitivity of the membrane to rupture. And it could be that if it was an EV particle that had a ruptured membrane, it would give a larger aerodynamic drag on the particle and make it even larger than it would have been, which might, you know, cause it to again localize in the respiratory tract versus the deep lung.

DR. CHALLBERG: So, Mark, I'm interested -- so, clearly, there's big difference, as you noted in your conclusion, you get a big difference the pathophysiology in the host response depending on the route of infection.

Have you ever seen a route of challenge

where you don't get ACAM2000, where a vaccine is not efficacious? I mean, is there that much difference between routes, that prior vaccination of the animals is just not effective?

DR. BULLER: Well, I guess the way I'd answer that is the simple answer is no, I haven't.

But the concern that I would have is if you are doing a challenge with one challenge dose, and the challenge dose is borderline with the LD_{50}, then if you had a route that somehow accelerated the response, the memory response to infection, then you might get protection, but you might not see that if it was done with another route.

So if you did a study where you were doing -- and this gets back to the earlier model, where you could ramp-up the challenge dose -- if you could use two or three different challenge doses in the study, I think that would take care of that kind of issue.

So I guess I would like to see more attention paid to a challenge dose that's expressed in the form of LD_{50}s, and one that at least is reasonably

-- 10-fold, 20-fold higher than the LD_{50}.

MR. JIANG: Yes, I'm George Jiang, from Booz Allen.

My question is about the slide for cytokine. So, how were those cytokines measured? Did you measure cytokine from the lymph node cell culture or from the homogenization in situ?

DR. BULLER: The --

MR. JIANG: Interferon gamma.

DR. BULLER: Yes. That was measured as a total homogenate of the lymph node. So the lymph node would be isolated into some buffer, and then the whole thing would be homogenized.

MR. JIANG: Thank you.

DR. BULLER: And clarified, and then measured.

DR. DAMON: So I guess we are going to convene the panel for final discussion.

Okay. So, I guess the first question that was hoped to be addressed today is the advantages and limitations of our current animal models, really

focusing on the ones presented today, which involve the respiratory challenges, non-human primates, with monkeypox, the historic comparison to work and more recent work that's been done with variola challenge in non-human primates, and the marmoset model that Erik presented. And then moving on to the small animal, rabbitpox in rabbits, and then Dr. Buller's presentation, just now, on ectromelia in mice. And what might be used to support, specifically, Animal Rule Approval of next-generation smallpox vaccines.

Mark, I think you sort of started us off in some of your summary comments, in terms of just thinking about the advantages of some of the mouse models.

DR. BULLER: Yes, I think the major advantage is power, and the number of reagents that are available.

The ectromelia model, as I suggested, has got a few flaws, but one thing is, it's very reproducible.

And so if one wanted to correlate or try and

make correlations to -- getting back to what Bernie was saying this morning -- measurements on the antibody response in the vaccine to key proteins on the outside of the envelope virus of the mature virus, and then relate that to survival or not-survival from a fixed dose, with a large number, and you could probably do that with ectromelia.

So I think that's the real power of ectromelia is that reagents are available. It's very reproducible. And it recapitulates two key features -- well, three key features of the disease: low-dose infection, IN infection, or upper respiratory. And you could do a large part of aerosol if you wanted. And then giving a rash in the right situation.

DR. DAMON: Jerry?

DR. WEIR: Can I just add something? And actually a question for Mark -- Buller, that is.

One of your last slides you flashed up a lot of bullets of studies that have been done with various vaccines.

DR. BULLER: Mm-hmm.

DR. WEIR: I think that's probably one of the advantages -- but I'd like to hear your opinion -- that some of the small animal models like the mouse and mousepox have already shown to be pretty predictive of the effectiveness of a vaccine. Would you agree with that?

DR. BULLER: Yeah. Yeah. In fact, what Mark was alluding to about seeing a vaccine that didn't work, if we use dose-sparing approaches, where we ratchet down the amount of vaccine, we can get it to a point where you get certain animals that survive, certain animals that die.

If you use it at the optimal amount -- there's only one study, and I can't really comment who it was, one vaccine candidate that didn't work well at optimal levels in the ectromelia model.

DR. WEIR: So you can make these models very rigorous, then, in the challenge sense.

DR. BULLER: Yes.

DR. NUZUM: Maybe, can I ask -- are we allowed to ask questions, too?

DR. DAMON: Yes. (Laughter)

DR. NUZUM: So while we're on the small-animal models, I mean, generally we have multiple species and multiple routes, and so there's lots of combinations, potential variables. So while we're on the small-animal, I mean, what I heard about the rabbitpox model, some of the advantages were similar. Obviously, mice are going to give more power.

So, between those two, what -- I mean, we're going to need to make decisions on some things to move forward with. And so, just between those two, what would you say?

DR. NALCA: Okay. First of all, I want to make a comment that I think orthopoxviruses are, as far as I know, the only viruses that used to develop this much animal models. You know, we see animal model developed in almost every year, there's a new paper, new route, new animal species used. But none of them are perfect, you know. IV model, non-human primate model is a good model. It causes a lot of lesions. But the IV is not natural route of

infection.

So coming to rabbitpox, it's a great model. If you ask me, it's a great model for proof of concept studies. It's an aerosol route, it's a natural route.

But it has, as I said, some disadvantages. Not a whole lot of reagents and the disease progression is very quick. And we see the lesions sometimes, depends on the dose. If we give them, like 10 LD_{50}, we don't see the lesions. But we want to give them 10 LD_{50} in order to test vaccine.

But, you know, we are not talking about therapeutics. But it's a perfect model for the therapeutics to see that, you know, giving less than LD_{50}, and it provides you longer time period, and you can start, you know, treatment later time point. It's a great screening model.

So, as I said, none of the models are perfect. But rabbitpox, if you ask me, it's great proof of concept studies, small-animal. And it's the natural route, aerosol route.

DR. WEIR: Back to your rabbitpox models,

can those studies be done in numbers such that one can get a rigorous statistical evaluation of the effective -- like, if you were comparing two vaccines?

DR. NALCA: Yes. We have done 32 animals in just one setting. You know, we can do tiers. It can be done. You know, we can get big room, too, to do the bigger number of animals.

But when you think that you have to look at these animals individually, check the lesions and everything, it's going to take observation, like three, four hours in the room. So it's the only, you know, downside of it.

But definitely it can be done. And aerosol can be done in one day, too, because we can use different lines at the same time. You know, they can be aerosolized at the same time, too.

DR. BULLER: I might just add, one difference between the rabbitpox model and the ectromelia model is the ectromelia model would represent a heterologous challenge, in that the vaccines you're talking about are based on vaccinia

virus in the vaccine, and ectromelia, of course, is a different species of orthopoxvirus.

Rabbitpox is essentially vaccinia. So that's more of a homologous challenge. So that would be another difference between the two models.

DR. DAMON: Stuart?

DR. ISAACS: Yeah, hi. So, in thinking about animal models for vaccines, you know, our colleagues working on therapeutics, so is there a model that's going to potentially be developed that can be used for both, so that we don't have to be making -- as Aysegul mentioned, we don't have multiple models for everything.

Is there one or two models that would work for both therapy post exposure, or once disease develops -- therapeutics -- that then we could also use for prophylactic vaccines?

Just something to think about.

DR. BULLER: Well, I could add a comment to that. With CMX001 and SIGA's ST-246, the models that have been looked at are -- when they came to wanting

to generate the preclinical data -- were ectromelian mice and rabbitpox in rabbits, and then going to non-human primate.

And with the Chimerix compound, CMX001, it would be the therapeutic. PK and PD is very important -- pharmacokinetics, pharmacodynamics. And the primate is not representative of the human for that compound. So that's an example where the non-human primate really doesn't work, for at least one therapeutic.

DR. DAMON: So I guess to go to one of the advantages that was pointed out is pre-existing data or studies, where vaccine has already been tested in these models. And I guess -- I mean, within monkeypox challenge in the non-human primates model, that's certainly been done to some extent.

DR. CHALLBERG: Yes, I think -- you know, to my mind, the main advantage of a non-human primate model, particularly for vaccine studies, is the fact that the immune system of non-human primates is probably more similar to humans than that of many

small animals.

So I think, you know, if we want to find out whether a vaccine really works or doesn't, then we probably have to -- and the efficacy of a vaccine is 100 percent dependent on the workings of the immune system. It seems to me that, you know, we have to do some studies in non-human primates.

To my mind, the greatest disadvantage of the non-human primate models that we've looked at with monkeypox, and certainly it's true of variola models, it's just since these viruses are not natural infections of monkeys, it just takes a very high dose -- it takes a high dose of virus to cause a severe or lethal disease.

And for that reason, the kind of studies that Mark was talking about, where you do studies that are, you know, 100 or 1,000 times challenge, with 100 or 1,000 times the LD_{50}, are simply not practicable. And not only that, the disease that is produced by challenges at that high a dose -- even if you could get them at that high a dose -- is maybe not

representative of human smallpox in the same way that a challenge with lower dose is.

So I think it's -- you know, but it's what we have. But I think, ultimately, some sort of non-human primate model really is important for evaluating particularly vaccines.

DR. DAMON: I guess, then, to go to the challenge-dose question again -- I mean, if the feeling is that a high, 100 times the LD_{50} might not give a realistic approximation of disease, would that also be tending to develop a disease spectrum in whatever animal that more approaches the equivalent of a human hemorrhagic smallpox? In which case, the historic data would suggest that prior vaccination wasn't protective against survival from hemorrhagic disease. So it might be a tough bar for a vaccine model to have to pass.

DR. BULLER: Can I talk to that?

What I was really referring to was examining the robustness --

DR. DAMON: Okay.

DR. BULLER: -- of the vaccine protection you've already demonstrated at the low dose. And that way you get a sense of how robust it is.

Mark was alluding to, or asked me the question about, had I seen failures with the ectromelia model? And we worked with some, I think it was ACAM2000, and we went up in the A-strain mouse to 10,000, 100,000 times the LD_{50}. And it couldn't break through the vaccine protection. It just gives you a way to be able to evaluate it. I'm not proposing that you're trying to evoke the proper disease. It's just the robustness.

But I could add one more point about the mouse models, and the -- and I'm not an expert this, but the advances they've made in humanizing mouse strains is really quite remarkable. And we've done some studies, they don't have B-cell lineage in them, but they have the HLA DR1 and A2 to replace the mouse equivalent gene sets.

So you can actually measure, in those strains -- now this would be T cells, you know, BC,

4C, D8, epitopes that then could be looked at in the same human population and you compare that back and forth. And they may have it on the B-cell side, but I'm not sure. And we've done some work with that, to try and transition into human.

DR. NUZUM: Yet another model.

DR. CANN: I'd just like to add a few words to what Mark had to say about the advantages and the limitations of the non-human primate model.

I think with any animal model it's always easier to come up with limitations than it is to come up with advantages. And when we use non-human primates, we almost always list the advantage as being the genetic relatedness to the human being, and the similarities in the anatomy and physiology of normal immune systems.

But when we look at the limitations, I think it's interesting that what are advantages in the small-animal models -- meaning you have the statistical power to answer the question you're trying to achieve, and you have the reproducibility -- those

are the very disadvantages we have in the non-human primate model.

We have serious problems with reproducibility within individual studies. Almost every study, you can pull out one or two animals that respond differently. And by that, I mean they develop these fatal infections that more resemble hemorrhagic disease and they die. But it's impossible to predict, at the start, which of those animals is going to do that. And that's really kind of where we fall short.

Then when you look at the statistical power numbers, everyone here knows, monkeys are expensive. Per diems are expensive. So, you know, these are -- I see those as the biggest limitations, in addition to the high doses that are required, and those sorts of things.

MR. MUCKER: And the models you're referring to? You're referring to macaque models?

DR. CANN: I'm just -- the Old World primate models, yes. I'm sorry.

MR. MUCKER: Because most of the things that

were mentioned, for instance to CMX001, one of the advantages of something like a marmoset model is it is a little bit different than macaques. It's a little bit different than (inaudible). Maybe it'll fill that niche.

The other thing, you were saying about high doses, again, marmosets are an NHP. Most people look at them as little fuzzy gerbil-ish type things, but they are an NHP.

DR. CANN: I would just add that with regard to the New World primate models, in general, any sort of disease that you're going to be looking at the reproductive physiology, particular to the female reproductive physiology which is very negatively impacted in smallpox disease, it's almost imperative that you have an Old World primate model to investigate that, simply because the reproductive physiology is so vastly different in the New World primates.

MR. MUCKER: Correct.

DR. CANN: But otherwise, I agree.

DR. DAMON: Jerry.

DR. WEIR: So how realistic do you guys think the actual variola non-human primate model would be to develop? And how much more would that add to all of the other models that we have?

DR. DAMON: Well, I mean, I think -- and that's an active area of discussion. So I think it's certainly worth trying. So I think some of the approaches that the working group that we've asked to help us think about these issues has suggested to try a couple of respiratory routes of challenge again.

So probably using -- I think Eric mentioned in his talk the bronchoscopic approach to doing respiratory challenge. And I think really what their advice was to really try to think about whether it's possible to develop the model in such a way that you prolong the period of time where animals are asymptomatically infected. So you prolong that period of time to better understand the disease pathogenesis. I think it probably would be -- I think those changes to the model will be more of that, and you can also

hopefully give a lower dose.

So, I think by extending the window of asymptomatic disease gives you a better chance to evaluate therapeutics. It may not have a particular benefit for vaccine studies, other than it potentially -- as Mark showed with the mouse studies -- affects the way the virus traffics, and what the immune response is being developed during that period of time.

DR. NALCA: I have one comment. I don't think that bronchoscopic route will not extend the incubation period or non-symptomatic. Because that route, you know, you give it directly to lungs. You are skipping the upper respiratory system. So it's totally different than the aerosol route. And if you give through aerosol, obviously it's going to replicate at the upper respiratory system and then it will move to lung, it's going to be much longer than just giving to lungs.

DR. DAMON: Right. And so I think the head-on aerosol is also the other recommendation to try

that approach.

I think, practically, though, that will be a little bit more lengthy to develop the capacity in the current containment lab at CDC. And, in addition, sort of a number of other recommendations, in terms of trying to additionally standardize and sort of make the model more reproducible, since some of the data Dr. Cann showed, in terms of the variable mortality that we've seen with the 10^8 IV challenge dose.

So are there ways to better standardize the virus preparations and compare them? Also standardized in terms of the health assessments, and then put together more standardized criteria for clinical disease severity scoring, which would then lead to euthanasia criteria.

So those are sort of the spectrum of recommendations that are coming out.

DR. CANN: I agree those are good recommendations, but I also agree with Aysegul.

I think that, especially in the more modern literature with regard to the variola virus non-human

primate models, the upper respiratory tract just really hasn't received the attention that it seems to deserve.

You know, if you look at the pathology of the lymphoid system in its entirety, even in the IV models -- so these are, you know, animals that receive the virus in a lower limb, there still seems to be a predilection of the virus for the lymphoid tissue of the head and neck. And I think it's an important change that's been overlooked, given the natural history of the human condition and the importance of the upper respiratory tract, and particularly the lymphoid tissue in that upper respiratory tract.

I agree that the IV models and the models that are aggressively pulmonary create, they seem to create an artificial viral-associated bronchopneumonia that differs from the bacterial bronchopneumonia that we've seen historically in human smallpox cases. So it really adds a confounding -- it confounds the entire pulmonary system when the virus is deposited in the lower respiratory tract.

DR. DAMON: Eric?

MR. MUCKER: I think just one of the comments I have is not only, you know, deciding, when we're talking about model, is what we want the model to look like, what's acceptable. In other words, what symptoms do we want to see? What are the endpoints?

And I guess that's more of a comment/discussion point than anything.

DR. CANN: Yes, I agree with that, as well.

I think, you know, in any discussion of any animal modeling for any disease, you know, one of the most important things is that the animal model is only as strong as the study design. So if you don't have a good solid design, with very specific aims, a good purpose, you know, specific endpoints, then you can't expect the animals to start talking and tell you, you know.

So it really requires, I think, a lot of just good, hard and fast scientific rules at the planning stage.

DR. DAMON: Okay, let's move on to the next

question. So --

DR. WEIR: I'm sorry, Inger --

DR. DAMON: Sorry?

DR. NUZUM: -- before we do, maybe let me just comment.

So at least from my perspective, at DMID we've been at this for seven, eight, maybe nine years. And I think from the product developer's perspective, you know, they're seeking some -- I think we're seeking some ways forward, you know.

So we've talked about multiple small-animal species, multiple NHPs. And now there's a humanized mouse, you know, a new NHP model.

I guess my question is, for the experts here in this field -- and I think, assume, we need one small-animal model and one NHP model to go forward -- what is the best in each of those categories? What is the best characterized small-animal model that's also most relevant to human disease? I mean, as far as the basis for going forward.

Maybe some of these other new models need to

be developed, but hopefully, tentatively make some decisions on where to focus product development efforts I think is what we need.

DR. DAMON: Well, I think that is the next question. Well done.

Dr. Weir.

DR. WEIR: Well, I definitely don't have the answer, but I just want to press a little bit on what Ed's saying.

As the folks like you guys who are the experts in animal models -- one of the things I guess I would want to know from you as you talk about the best combination is, if you were to use a combination today, or very soon, are the animal models that are currently available sufficient to answer a basic question for you of would it be predictive of the efficacy of a vaccine?

And I think, for me, that's what I would want to know. I mean, as I said, I don't have the answer. But that's what I would like to know. Because one can continue to develop animal models

forever, and get better and better refined ones. But is there enough today to actually use?

And I actually think there's good news here, based on what all of our presentations. Unlike some diseases, there are really a lot of animal models for orthopox disease. I mean, some things that you might want to use the Animal Rule for, you'd be hard-pressed to come up with two animal models, whereas we've got a lot here.

But the question is, is it sufficient? Is there some combination that is sufficient is kind of what's behind this question.

DR. GOLDING: Can I just make a -- I just wanted to add something to this discussion which I was wondering about.

So, we're usually doing efficacy trial. I think in addition to collecting the convincing clinical endpoints that suggest efficacy, it usually helps us also to validate the assays that show correlative protection then, ultimately, could help us to then bridge to future studies and follow lot

releases and so forth.

So the question is whether any of these animal models that have been described, we heard a lot about the animal modeling in terms of pathology, but we really heard very little about the immune responses -- even when there were some indications of success -- how similar they are to the type of immune responses we see in the human. So, ultimately, it will be very strange if we can only use the animal model for clinical endpoints, then they don't really correlate with the immune responses that we measure in humans.

DR. CANN: Are there any immunologists in the room? (Laughter)

I, too, agree. I have noticed that, as well. There seems to be a dearth of information related to the specific molecular immunology of these poxvirus infections, at least in the non-human primate models. I'm not sure about the small-animal models.

But I agree it's an area that deserves a lot of attention. I wish I had a better answer.

DR. DAMON: Mark?

DR. BULLER: Oh, I was just going to say that with the licensure of ACAM2000, the small-animal models were used not to look at protection, but we're strictly looking for efficacy.

And then, the way I understand it -- and there's people here who probably know it better than I -- is then it was the comparison with Trivax in clinical trials, and the comparison of immunogenicity that was talked about this morning. And I don't think that got down to the level of a correlate of protection, it was more just it had a very similar immune response with the kinds of assays that were used.

So using the -- not that you couldn't do it, but that wasn't how they used the rabbitpox model for LC16m8 or the ectromelia model. It was strictly efficacy.

DR. NALCA: We use, actually, aerosolized rabbitpox model to test Imvamune against Trivax, and we published this study a couple of years ago. And we tested efficacy feed up and down, as well as the

immune responses.

And, you know, in rabbits we saw nice immune responses with the Imvamune, but there was a straggling response there with the Trivax. We didn't get a whole lot of antibody protection. And we couldn't explain very well what was the reason.

You know, Imvamune, antibody responses were higher than the Trivax immune responses in rats.

DR. BULLER: Were you correlating those with different protein targets on the --

DR. NALCA: No.

DR. BULLER: So it's just general --

DR. NALCA: Just general. Yeah, just generalized response.

DR. DAMON: Mark, do you want to talk about some of the work that's been done with the non-human primate and monkeypox in terms of immune assays?

DR. CHALLBERG: Yeah, there's been quite a lot of work done looking at the immune response after vaccination. And certainly, at some level, immune response correlates with protection. There's no doubt

about that.

I think the difficulty comes, with any of these models -- and the non-human primate model is no different -- and the kinds of things that Freyja was talking about earlier today, where, you know, trying to relate quantitatively the immune response in non-human primates to humans is just a very difficult undertaking. I mean, I'm not going to say it can't be done, but I don't think we're there yet.

In terms of these questions, well, I mean, I don't think it's any surprise if I were to say that -- and I think in order -- I think we have enough models to evaluate sort of up-or-down whether a vaccine will work. I mean, I think there's a lot. As people have said, there's a lot of refinements that one could make to every one of these models, but I haven't actually seen any data that suggests that the models as they exist now would not be predictive for evaluating a vaccine.

I mean, as Mark says, you can vaccinate mice with ACAM2000 and protect against a challenge dose of

10,000 times the LD_{50}. That's pretty remarkable, if you ask me. And generally speaking, the vaccines that we know have worked in the field -- well, Trivax -- have protected against the monkeypox challenge. It hasn't been tested in the variola model.

But, you know, the vaccines that we have pretty good idea will work, work in these models. So I personally don't think we need more models.

DR. NUZUM: You know, I think another way to approach this is welcome any input from the audience. I mean, if anybody has any strong suggestions, we'd be glad to hear them.

But, you know, the government's going to need to make some decisions here, going forward. And I think in the absence of a strong reason to do one or the other, or a strong reason not to do something, we're going to make the best call we can. And this is a time for people to speak up.

MS. LYNN: I'm Freyja Lynn. I think one of the things that Mark mentioned, too, brought this to mind. And in discussions that we had at NIH over

strategies or paths to licensure for the products that HHS was trying to develop -- and I think the focus has been on ACAM2000, and the fact that, ultimately, the animal models were used to support that, yes, it is effective. But ultimately, the path to licensure for that vaccine was a clinical non-inferiority.

And so I don't think people who have been working on these models up to now have really been working on them with the idea of Animal Rule in the front of their minds. I mean, I think people have been looking at -- and we see this in the talks -- they've been looking at pathophysiology, they've been looking at dosing of the pathogen. But I don't think people have really looked at the models in the context of the Animal Rule with the kinds of things we were talking about this morning, where you're really trying to look for a correlative protection. People, I think, have just been looking for protection.

And so I think that's something to keep in mind. And that may be an area -- I know NIH is working on that now with the monkey models, the NHP

models. And I think it's something to think about for some of the -- the rabbit model or the mouse model.

I did also have one question about the variola work for the non-human primates at CDC. What's your capacity in terms of size of animal studies for non-human primate studies if you are going to go with a variola challenge?

DR. DAMON: At this point the capacity, the total capacity, would be 24 primates in any one experiment. And that would be full capacity. So we'll start a 16-primate study in mid-October, and that will be the IV 10^8 challenge.

DR. NUZUM: By what challenge route did you say?

DR. DAMON: That will be the 10^8, the standard 10^8 IV challenge.

DR. NUZUM: IV?

DR. DAMON: Yes.

DR. CANN: Inger, what strain of the virus will you be using?

DR. DAMON: That's going to be the Harper

strain, continue to be. The strain that was used in the previous studies with ST-246.

DR. CANN: Do you have any data on the strains that are available at the CDC regarding differential virulence across the strains? Is the Harper strain generally regarded to be more virulent?

DR. DAMON: I think the decisions on the strains to use were based on a couple of pieces of information. One was on this historic data on what previous authors had used. So the Hahon studies and the Noble studies, in terms of what viruses had been used.

And I think also there was some attempt to look at the epidemiologic data and try to understand if any of the viruses that we had in the repository would be predictive of viruses from outbreaks with more severe disease.

And so the viruses, really, that have been the workhorses have either been those which have been used in previous animal studies. So Harper, and then 7124, which you also mentioned in some of the non-

human primate data, was chosen because it's from India and has similarity, and clusters within the India cohort of viruses which were associated -- the Indian subcontinent was, in general, associated with more severe outbreaks of variola major.

Now, whether that's because of the specific nature of the virus itself, or because of population density, it's hard to say.

The other virus that's been a workhorse that's been used -- and somebody asked me the question, as well -- is a virus which we've used in the neutralization assays, which is from Bangladesh, another area with high case-fatality rates? And the reason that was chosen is because it's a virus that had not been passaged in CAM before, and it's been only passaged a couple of times in monkey kidney cells before it was used in studies.

And then there's another paper where we've published some of the phenotypic comparisons, in terms of trying to understand those viruses that appear to make more EV forms of the virus rather than MV forms.

And it's actually interesting, because the greater amount of EV is actually associated with the viruses which are both biologically and genetically characterized as being a variola alastrim minor strains.

DR. CANN: It was brought to my attention during the break that in two of the Noble studies the Harvey strain appeared to be as pathogenic as the other strains that were used, but that it was a lower dose that was used.

And so the question that was asked of me was is the Harvey strain more pathogenic?

DR. DAMON: I mean, that would be the one study that would ask that question and begin to answer it.

DR. SUN: Hi, this is Wellington Sun, from CBER.

This question was actually discussed this morning with the morning panel. But I'm interested to hear from this panel about the usefulness of trying to bridge the animal model with human immune responses

through the use of passive transfer. So that would be, for example, taking serum from vaccinated individuals and then giving it to an animal model, usually I guess it would be a small-animal model, and then challenging those animals with the appropriate -- you know, with whatever poxvirus in their system.

So I'm curious to hear what you all think about that approach. That has been used in the past by CBER for supportive evidence, licensing other vaccines, for example, flavia viruses.

DR. NALCA: Sounds good. If anybody wants to fund it, we are ready to do it.

DR. NUZUM: Well, I don't think I heard all of the question, but I think it relates to the last question on the agenda here. And this already came up a couple times, so I was kind of going to wait until we got to that to say what I'm going to say. But I'll go ahead and say it.

And Freyja alluded to this this morning, about we've designed, and it's in progress, a study in NHPs, a dose-escalation study, dose-down study --

however you want to call it -- where we hope to get a range of protection from little-or-no protection to complete-protection. And this is modeled after what we did in the anthrax work. And it really hasn't been done in smallpox yet.

And depending on how that comes out, if we get a dose-dependent immune response relationship that correlates with protection, we think that will be very valuable in many of these questions. And if it's true in NHPs, we certainly could try it in a small-animal model that we would choose.

But the whole theory is that we will get good protection at vaccine doses that produce an immune response that's at or below immune response levels you get in humans when they're vaccinated. So we developed this concept to humanize dose concept whereby it's a vaccine dose used in animals that elicits protection at response levels, antibody levels, hopefully, that's relevant to human titers from vaccination with the same vaccine.

So if that works -- I mean, that's proven to

be -- and I think it is promising. I mean, clearly, antibody's important and we hope that works. And if it does, it will move us a long way down the road, I think. And we have some hope that the protection will be at levels below the immune response levels in humans. And so that will be very important.

Then the other piece of that is what assay do you use? And obviously, if we had a functional assay that correlates, that's the best. But I think Tim pointed out this morning, the Animal Rule does not require a correlate. So if we can get one, yes, we obviously want to get one. But if we don't get one, we don't think -- I don't think it's the end of the road. You know, a good ELISA to two ELISAs, as we discussed this morning, that relates immune response, it correlates immune response in animals and protection could be very valuable. And the other advantage there is that you use the same assay in the animal study as you do in the humans. Or it's for the same vaccine.

One of the issues -- and Freyja mentioned

this this morning, but I think it bears repeating -- is that in the non-inferiority studies, where you're comparing two vaccines, there's a real challenge there because your assays are different. So, you know, if you don't have the same assay can you really compare? Is your endpoint really the same? So, I mean, there's an issue there with the non-inferiority studies.

So, anyway, we're holding out a lot of hope for this dose-response type study, and we'll see how that works. But if it works, it goes a long way to answering this question.

DR. ISAACS: Dose response to what? Which vaccine?

DR. NUZUM: Well, whichever the test material that we want to use. You know, there's several in development: MVA, LC16m8. Although, you know, if it's a live vaccine there's less likelihood that will work. But for MVA, I mean -- our current study is with MVA.

DR. WEIR: Well, I think you make a good point, Stuart. I mean, one would have to do that for

any vaccine that you're testing or that you're evaluating.

DR. DAMON: Mark, were you going to say something? Mark Buller?

DR. BULLER: Actually, I was thinking of asking a follow-up.

The assay that would be used, would this be -- for the sake of poxvirus -- would it be an ELISA-type assay against the whole immune response to the virus? Or would it be that plus responses against proteins that are known to be important in generating immunity, what Bernie was talking about this morning?

DR. NUZUM: Well, you know, I'm not the best one to answer that. My understanding is it's the assays we currently have, the current ELISAs and PRNTs. If we were to want to address more specifically, like the EV assays discussed this morning, that would have to be developed, is my understanding.

DR. DAMON: And then, perhaps, the one unanswered sub-question on the previous question,

which is: What clinical endpoints? So it sounds like there's been sort of general agreement that some sort of standardized clinical assessment tool and a standardized euthanasia criteria would be the final, ultimate clinical endpoints. So disease severity, an abrogation of that?

Are people thinking rash illness would also -- would people also want rash illness to be a clinical endpoint? And, if so, does that affect what models would be helpful?

DR. BULLER: In the case of a vaccine, I don't see that rash endpoint really is important, myself. What we've used, traditionally, is death. And then weight loss is a very good integrated measurement of the health status of the mouse. And we find that that's very, very -- with an antiviral study, you can titrate in the antiviral and see different levels of weight loss depending on how much is there. So it's very sensitive and, actually, some kind of proportionality.

And then the other would be viremia, if you

wanted a measure of -- or tissue. You know, you pick your tissue. We usually bleed the animals at least one time and do PCR as another measure.

DR. DAMON: Yes, I guess I only mention rash illness because in human smallpox that is an indicator of virus shedding. So would there be other indicators of virus shedding you'd want to look at -- you know, throat swabs -- in terms of transmissibility of virus, and showing that the vaccine is effective in preventing that?

DR. BULLER: Well, Inger, when you mention throat swabs, we just did a long, exhaustive study to try and use rash as a trigger for therapy with antivirals and failed miserably. And during looking at all the different clinical signs and biomarkers that we have in the ectromelia model, the most sensitive is genome copies in blood. We see that about five days after infection. And that made us go and look at saliva. And we can pick up genome copies and following IN infection two to three days after infection in the saliva.

So, you know, if you want something that tells you what the virus is, or the agent, plus gives you a sense that you're early in the infection process, I think PCR is a pretty good assay.

DR. NALCA: I agree with Mark. You know, I also do non-human primate studies, and we test the throat swabs as well as blood, viral load. And it's a very good indicator. And level of, you know, blood viral load, shows us actually indicators if this animal succumbs to disease or not, also.

We didn't test the throat swabs in rabbits, but, again, blood viral load is very good indicator for rabbits, too.

MR. KOVACS: I have a question.

DR. DAMON: Gerry.

MR. KOVACS: Gerry Kovacs. We've talked a lot about how we would determine whether a new vaccine would be more or less efficacious than the existing vaccine, and that's great. And there's been a lot of discussion about that. But another parameter that the agency will have to look at is the safety of next-

generation vaccines, and we haven't talked at all about that.

Bearing in mind that the current vaccine has a black box on it, what does the committee recommend, in terms of evaluating the safety of next-generation vaccines, either in animals or humans?

DR. DAMON: Gerry.

DR. WEIR: I can take that one. And I can let others chime in.

I mean, the reason we haven't discussed it is because of the safety aspect, is because I think all of us are assuming that the safety can be evaluated, and it will be evaluated in human trials. And we'll be able to get that data.

I think this, the reason it's focused on efficacy and all the pathways is because that's the one that's really murky for us.

Yeah, if it goes to licensure, we will have human safety data to support it.

MR. KOVACS: Is it something that you balance, relative to the currently licensed vaccine?

DR. WEIR: I don't know that -- again, somebody else can chime in -- I don't know that it will have to be a direct comparison to that. But I just think you will have to have the safety data, and then be able to understand what the risks would be associated with.

I mean, you could find that a new vaccine is just completely different in some way. I mean, it has a different safety profile. So I think that's why you just will accumulate human safety data and find out.

MR. KOVACS: Fair enough.

DR. WEIR: I don't know if anybody else wants to add to that.

MS. GRUBER: Marion Gruber. Yes, I don't think, Gerry, that I have a lot to add. I just wanted to point out, with every vaccine, if you licensed it in terms of looking at the safety profile you would look at the safety versus the effectiveness.

But what is also important is that you look at the target population. So, you know, maybe the use and the vaccine targets that the population that the

vaccine is indicated for may be slightly different from, you know, a vaccine that is currently licensed. And so you look at the safety profile with that in mind, as well.

So there is no -- I don't think that we would really do a direct comparison to, in this case, ACAM2000. We're going to look at this product on its own, see what the safety data tell us, and see, you know, how is it going to be used and under what scenario and what is the target population.

DR. DAMON: Okay. We're coming up on almost 5 minutes before 5:00. So do the Panelists have any other final comments or questions?

DR. NUZUM: Well, one comment, and then another question, maybe.

You know, coming in today -- and you can tell from my comments I had a kind of a goal to try to make some decisions, or get some consensus, hopefully -- but I just want to mention, in the course of lunchtime I had two conversations that were diametrically opposed as far as what people took away

from the morning session. So then I realized, well, maybe we won't accomplish what I wanted to today. But, anyway, I think it's all good conversation, and good points have been made.

The other thing is, I'd like to discuss a little bit which inhalation route should be considered. That's one thing that we haven't touched on. I think there's a lot of IV data. People say that's much like -- that mimics human disease. But we probably need an inhalational route. And I think I'd like to hear some opinion on that.

DR. DAMON: Jennifer?

DR. CANN: I agree that at least in the non-human primate model I think the upper respiratory tract, as I said earlier, deserves more attention than it's gotten thus far. And I think too much emphasis has been put on the lower respiratory tract, which creates a lot of confounding, artificial lesions that, as best we know, are not present in the human condition.

So I'm actually a very strong proponent of

refining and further developing an upper respiratory tract model in one of the non-human primate species.

DR. DAMON: Aysegul?

DR. NALCA: I agree with Jennifer. You know, the aerosol is the natural route of transmission compared to IT or, you know, intrabronchial or intranasal. So I think we need to focus on more aerosol models.

DR. NUZUM: Just sort of wrap up --

DR. KRAUSE: Could I ask just one more question here? In the context of what's on the board there, it would sort of be nice to hear from each panelist, if it were totally up to you, what species would you recommend be used? What route of exposure would you use? And what clinical endpoint?

Can you just sort of each give your own personal opinion, based on what you know now, would be best in the context of the answers to these questions?

DR. CANN: I can start, but, to be honest, I think, you know, as I tried to say earlier, I think that, you know, there are many different animal

models, and you have to choose your animal model based on what it is you're trying to accomplish.

That said, generically, I think that if you look at the literature, as far as non-human primate goes -- I'm not speaking to any of the small-animal models -- we probably have the most information about the cynomolgus macaque. That said, we also have more reagents and more is known about the molecular immunology of the rhesus macaque. So I guess I would say I would choose a macaque. (Laughter)

As far as route of exposure, again, I feel very strongly that the upper respiratory route in a large-particle aerosol route of exposure deserves a lot of attention.

As far as clinical endpoints, I think that, you know, anything that develops over the course of the disease, whether that be the mucocutaneous lesions, whether it be a bronchopneumonia, should be evaluated.

But, in full disclosure, I'm a pathologist. So my endpoints are on the inside and I look at

everything. So that's my biased approach, I guess.

On to you.

DR. CHALLBERG: Well, my own view is that it doesn't actually matter. I mean, I think, I agree, that we certainly have so much more data in macaques, be it cynos or rhesus, that, you know, it's silly to try to develop that database for another model which is not likely to be any better at this point. But just based on what I've seen from various types of pilot vaccine studies in these models, I think you get the same answer no matter which inhalation model you use.

And because, unlike in the case of therapeutics, where the sort of the course of disease really matters, if we're looking at a severe-disease endpoint, be it mortality or just really sick monkeys, you know, the vaccines work well enough so that the difference between vaccinated animals and unvaccinated animals is huge. You could get 10 people off the street and have them look at these animals in their cage and they would be able to tell which animals are

vaccinated.

So I don't really think it matters.

MR. MUCKER: I guess I'll be the oddball. The question was what's the most characterized? I would have to go with macaques IV model. I think it gives a good severe infection, where the endpoint is something that looks clinically like an ordinary, possibly towards hemorrhagic type, disease course.

In terms of whether to use monkeypox or variola, that would depend on the indication. If the indication says, "is protective against variola," there's only way to test if it's effective against variola. But that's just my two cents.

In terms of a small-animal model, again, kind of torn. When you look at the smallpox literature in general, it's hard to tease, especially the pathogenesis data from the ectromelia model. I think the ectromelia model has been well characterized, but I can pretty much say the same thing about the rabbitpox model.

DR. NALCA: My opinion, you know, at the

beginning, if you want to do proof of concept study, small-animal model is great. We can have big, large amount of animals that we can test. We can do all kinds of different groups. And, you know, we can design the study with a big number of animals. But, on the other hand, definitely we will need non-human primate model to do efficacy pivotal studies.

And then the route of exposure, as you can guess, I'm saying aerosol because it's the natural transmission route. And the large-particle, as we discussed, needs to be definitely done in non-human primate, too.

And clinical endpoints, you know, as we talked, viral load, fever, and also the rash are important.

DR. BULLER: I'll leave the non-human primate to everyone else.

The rodent model, if you want to try and understand correlates protection, then I really believe the mouse ectromelia model -- the mouse is inbred, very reproducible, the reagents -— is the way

to go. And if that's not that important, then it still may be the way to go because it is a heterologous challenge. So you're vaccinating with a vaccinia-type MVA anyway, or LC16m8, and then you're challenging the different species. So that would mimic vaccinating with MVA challenge with variola, where rabbitpox is a strain of vaccinia, so it's a homologous challenge.

But like Mark says, I don't think you can go wrong with either one.

DR. NUZUM: Well, I'll reiterate I'm not an expert in this field. But just based on what I know, I mean, I think going with what's most characterized is a good start.

So, based on that, I guess I'd say the cyno, and mouse ectromelia because I'm assuming one small-animal species, one large-animal species. I'm assuming at least an IV route for NHPs and then probably one inhalational route.

And based on what I'm hearing, probably work needs to be on upper respiratory, large-particle

aerosol.

DR. WEIR: Okay. So I'm not an expert, either, obviously. And being from the agency, I'm not sure I have to give a public opinion. (Laughter) But I will summarize something.

First of all, from what I think I've heard, but also what I think, I do think that there are enough models here that some case can be made. I'm not sure there's only one size that can fit all. I think that probably several versions could be -- a strong case could be made using several animal models. I think that's possible.

My only concerns -- and I think this is what we will have to struggle with -- is that whatever combination of models is chosen, if that's the route that we use, I think we just need to make sure that it's a very rigorous analysis. Because I think all of us know that one can set up an animal model to show different things and one has to be really objective and rigorous about setting that up.

And the final comment is I still think --

and this is a personal opinion -- that somewhere there needs to be some bridging back to a vaccine that we all know and think is protective against smallpox. So somehow, some way, I think that is very important.

DR. DAMON: And I think that point will help you in initial assessments of the model, in fact. So that if you know that a vaccine was effective, or you believe it to be effective because of prior studies, you would expect it to be successful and protective in that animal model then. And that would give you a positive control, in fact, for that you can evaluate that animal model for next-generation vaccine efficacy, or by comparison.

DR. NUZUM: And, you know, I think the comments I made this morning about assay apply again. There are, obviously, multiple models. And I think it's not unreasonable to think we could get proof of concept, or minimally qualified data in more than one model.

But as far as going to licensure, at some point I think we have to settle on something, you

know, one or two, maybe at most, key assays; one small-animal species, one large-animal species, and probably one route of challenge for pivotal studies. And I don't mean to make that decision now, but we need to be planning towards that.

And one thing that's needed here, obviously, is a plan forward. You know, there's a thousand things that can be done. The hard part is doing the right thing, the prioritizing and putting resources in the right places.

So, you know, I think we need a plan. And hopefully this workshop will help.

DR. DAMON: Rosemary.

DR. R. ROBERTS: Can I ask a question? Do you need an animal model that uses variola virus?

DR. CANN: I think, yes.

DR. R. ROBERTS: I'd like to hear.

DR. CANN: More? Well, I think I tried to address this earlier, in the introductory slide of my talk.

But, you know, variola virus is unique.

There's nothing else like it. And to assume that you can just substitute something, even like monkeypox, and get the exact same reaction as you would to variola, I think is a big assumption. And I guess I'm just not willing to stick my neck out that far.

I think that, you know, when you look at animal modeling, you try and keep both the pathogen and the animal model species as close as you can to the human condition. And, you know, we get degrees and degrees and degrees further away from that as we move through different animal species and down into the rodent models.

If you also do that on the pathogen side, then you're taking your model further and further and further away from the natural condition. And at what point do you say, okay, we're so far away that we're looking at a different disease now? And I just don't think we know that information.

One of the things that makes this, at least from a pathology perspective, a very difficult disease to model is that we really don't have a very clear,

modern idea of what the human disease looks like. So we're already at a disadvantage.

If you just completely remove the variola virus from the equation, then I just feel like you're getting into kind of quicksand. It's a personal opinion, but that's how I feel.

DR. R. ROBERTS: Thank you. Do any of the other Panel members -- willing to say whether there's a need for variola virus?

I think this is really a critical question. And the reason is, is that there's talk by the WHO of destroying the lots of variola that we know are in Russia and here in the U.S. And this question comes up: do we as an agency, in order to be able to have further development of vaccines, and for, hopefully, development of therapeutics against smallpox, do we need to retain the variola virus?

DR. CANN: I'll just add a little more to clarify, specifically with regard to testing the efficacy of the new vaccines.

For that, you know, I'm not entirely

convinced that you absolutely have to use variola. I think that it's been demonstrated over the centuries that there's enough cross-protection among the orthopoxviruses that we could, you know, have some relative degree of certainty that if we can induce protection against monkeypox, then we're more than likely going to induce it against variola.

What I'm more concerned about, and where I was coming from in my previous statement, really applies more to the basic research studies and studying the pathogenesis of the disease. Is it necessary to test the vaccine efficacy against the actual variola virus? I mean, I think that's for the group to decide. Certainly the Animal Rule says that it is.

DR. R. ROBERTS: Thank you.

DR. BULLER: My two bits' worth are you don't need variola virus challenges to get licensure for either antiviral or a prophylactic. But I'm in the minority.

DR. R. ROBERTS: Thank you.

DR. ISAACS: I'm not on this panel, but I'll weigh in. (Laughter) You know, so similar to the discussion this morning on measuring immune responses and correlates or surrogates of immunity, you know, I do think variola challenge will reassuring with moving forward.

So I do think it's not going to be used as the pivotal data, but it's going to be part of that data file that is looked at. And it will be reassuring to know that it does protect in a variola challenge model. My opinion.

DR. DAMON: My opinion. For vaccines, I think with the current next-generation vaccines that are being assessed, they are in many ways close enough to our current vaccine in terms of the number of antigens that are expressed, that are on both the MV and EV, that I have pretty good confidence that you can use immunologic data to bridge back, to have some confidence that you're going to have protection against variola, as well, in addition to your animal model studies.

When you get into some of the more antigen-specific recombinant vaccines, I think that's where you may want to have the variola animal challenges.

(Applause)

MS. KELLEY: Well, thank you, everyone. And I want to give a special thank you to Inger Damon. The Planning Committee just came up with all these jobs for her to do and asked her after we planned.

Your agenda says that Dr. Norman Baylor is going to give a few final thoughts and wrap up. However, he is not here. So we have the equally best thing. With us is Dr. Marion Gruber, who is the deputy director for the Office of Vaccines.

DR. GRUBER: Well, here we are. And I have to bring my notes, because as I'd say I've found out that I'm going to be standing here a couple of hours ago.

So I think what I would like to start with, really, is to thank all the folks who came up here today to share with us the scientific data and their wealth of knowledge. And I really do believe that the

discussions that we had today will really help us to further our discussions and our thinking in defining licensure pathways for these next-generation or third-generation smallpox vaccines.

I really want to thank, again, all the presenters today, and the Panel.

So, in terms of the wrap-up and recommendations -- and, of course, I don't know if I should say now, this is non-binding, but from what I heard -- but I think, you know, we heard a lot of interesting suggestions, recommendations, that we will take, you know, back in our discussions internally, and with prospective sponsors and applicants who want to develop and license these products.

So this morning we had an interesting discussion, I believe, in terms of the value and the importance of antibody responses in terms of, perhaps, you know, looking at a prediction of protection, if you want. But that antibody titers and, you know, MV neutralizations are important criteria, but perhaps cannot be the only criteria. We should also think

about looking at antibody responses to EV targets.

We understand that assay issues may present certain challenges. How should assays be used to provide evidence of vaccine efficacy? It's an interesting -- it's a challenging question. Because we heard this morning that the licensure pathway and demonstration of vaccine effectiveness could rest not only on using the animal model provisions, but in addition, and supportive of that, perhaps clinical non-inferiority studies, comparing the investigational vaccine to the already licensed ACAM2000 vaccine. And in this regard we have to ask ourselves what assays to be used and also what antigens to select and what antigen is critical in consideration, especially when you look at PRNT assays.

So the Panel then gave us some recommendations, in terms of number of assays that can be used. Is one assay enough? Is two assays? And mention was made that in terms of an Animal Rule approval it is very complicated even to work and rest on one assay. So it may be challenging to really

base, you know, effectiveness demonstration using two assays.

But then again, a combination of two ELISA assays, looking at the MV and EV, is something that we should further consider. Perhaps it will be good to settle on one assay for the pivotal studies, and supplement with additional assays.

I think the Panel was in agreement that -- and agree, Rosemary, it is confusing -- but using in demonstration of vaccine effectiveness based on the Animal Rule, combined with clinical data, may be the way to go, whereby we have to really discuss. And that's a question that I had this morning. What weight should we give the tools? And here we heard recommendations from the Panel that perhaps the clinical non-inferiority study should be supportive data and supportive evidence for the effectiveness of the vaccine, and that we should focus and really the pivotal data should come from studies under the Animal Rule.

I think we also heard some recommendation

pointed to the value of *in vitro* variola studies. But, again, as supportive, not necessarily pivotal criteria or supplemental information.

In terms of animal models, I think we remember this so much clearer because we just had the discussion. What models should be supportive? What animal models should be chosen? The good news, we have plenty of models available. And there was mention made that perhaps we don't really have to really engage in really developing completely new models because we have models that we perhaps need to further refine or, you know, to investigate a little further. But they are there to give us perhaps the answer.

We heard that non-human primates should really be included and looked at in terms of demonstrating vaccine effectiveness, but that small-animal models, such as maybe the mouse or even the rabbit model, really could give us some statistical power to really look at the effect of vaccines on prevention of disease in these models.

There was mention made that some work should be done to really look at the upper respiratory route of exposure, and perhaps develop some variola models.

What I have not heard today, and I think this is probably based on the fact that -- and I think, Mark, you said it -- that there is a lot of work done in the non-human primate using monkeypox. And there is data to believe or to reassure us that the immune response can be protective. But what's a little bit lacking is investigations and data, really, to see how can we bridge to human immune responses.

And so I have found the last question interesting, that was a bit evaded, in my mind, and that is should animal efficacy studies be bridged to human immunogenicity studies? If so, how?

In my mind, the question is not so much "If so," because that's really what you do. You demonstrate efficacy in an animal model and you look at the immune response induced in this animal model. And then you look at, you know, how can you bridge that to the immune response that is induced in humans?

And perhaps more work needs to be done here.

And that, of course, then raises the very interesting and challenging question about the dose that can be used, or should or needs to be used in the animal model versus human.

So, I think that's what I took home as main messages. Again, I think it's tremendously helpful. I found it very critical that we convene today to have this workshop to hear the scientific data.

And, again, I really believe that this will be very instrumental, the discussion that we've had, to really further develop a pathway to licensure of these vaccines.

Thank you very much. (Applause)

MS. KELLEY: So, once again, I just want to make sure to thank all of our speakers, all of our panelists, and all of you for attending. Have safe trips home. (Applause)

(Whereupon, at 5:15 p.m., the

PROCEEDINGS were adjourned.)

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CERTIFICATE OF NOTARY PUBLIC

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I, Irene Gray, notary public in and for the District of Columbia, do hereby certify that the forgoing PROCEEDING was duly recorded and thereafter reduced to print under my direction; that the witnesses were sworn to tell the truth under penalty of perjury; that said transcript is a true record of the testimony given by witnesses; that I am neither counsel for, related to, nor employed by any of the parties to the action in which this proceeding was called; and, furthermore, that I am not a relative or employee of any attorney or counsel employed by the parties hereto, nor financially or otherwise interested in the outcome of this action.

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