FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

ANTIVIRAL DRUGS ADVISORY COMMITTEE (ADVAC)

Wednesday, December 14, 2011
8:00 a.m. to 5:15 p.m.

FDA White Oak Campus
White Oak Conference Center
Building 31, The Great Room
Silver Spring, Maryland
Meeting Roster

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Call to Order

Introduction of Committee

DR. CARGILL: Good morning. I would first like to remind everyone present to please silence your cell phones, Blackberry, and other devices, if you have not already done so. I'd also like to identify the FDA press contact, Ms. Patricia El-Hinnawy. If you are here and present, please -- there she is. Thank you very much.

Good morning, again. My name is Dr. Victoria Cargill, and I'm the acting chair of the Antiviral Drugs Advisory Committee. I will now call the meeting of the Antiviral Drugs Advisory Committee to order.

We will go around the room and please introduce yourselves. We will start with FDA and Dr. Edward Cox, to my left, and go around the table.

DR. COX: Good morning. Ed Cox, director of the Office of Antimicrobial Products, CDER/FDA.
DR. BIRNKRANT: Debbie Birnkrant, director, Division of Antiviral Products, FDA.

DR. SINGER: Mary Singer, medical team leader, Division of Antiviral Products.

DR. CHAN-TACK: Kirk Chan-Tack, Medical Officer, Division of Antiviral Products.

DR. MARGOLIS: Harold Margolis, currently the chief of the Dengue Branch at the Centers for Disease Control.

DR. HENDERSON: D.A. Henderson. I'm at the Center for Biosecurity, University of Pittsburgh, located in Baltimore.

MR. RAYMOND: Daniel Raymond, Harm Reduction Coalition, the acting committee representative.

DR. STRADER: Doris Strader, Division of Gastroenterology and Hepatology at University of Vermont.

DR. CONNICK: Elizabeth Connick, infectious disease, University of Colorado-Denver.

DR. CARGILL: Victoria Cargill, director, Minority Research and Clinical Studies, Office of AIDS Research, NIH.
MR. TRAN: Paul Tran, the designated federal official for the Antiviral Drugs Advisory Committee.

DR. MURATA: Yoshi Murata, infectious diseases, University of Rochester.

DR. VAN DYKE: Russell Van Dyke, pediatric infectious diseases at Tulane University in New Orleans.

DR. GLENN: Jeffrey Glenn, Division of Gastroenterology and Hepatology at Stanford University.

DR. RELLER: Barth Reller, Infectious Diseases and International Health at Duke University.

DR. LYONS: Rick Lyons, director of Infectious Disease Research Center at Colorado State University.

DR. GOETZ: Matt Goetz, infectious diseases, VA Medical Center-Los Angeles and UCLA.

DR. MAGILL: Alan Magill, infectious diseases, currently at the Defense Advanced Research Projects Agency, DARPA.
DR. BOHM: Skip Bohm, veterinary medicine at the Tulane National Primate Research Center.

DR. BENNETT: John Bennett, Laboratory of Clinical Infectious Disease at NIAID/NIH.

DR. CAMARDO: Joe Camardo. I'm the industry representative. I'm with Forest Laboratories, New York.

DR. CARGILL: Dr. Roberts just joined us. I wonder if she could introduce herself, please.

DR. ROBERTS: Dr. Rosemary Roberts, the director of the Office of Counterterrorism and Emergency Coordination at CDER.

DR. CARGILL: Thank you. And if I could advise our members to please turn off your microphones when you finish speaking, it will be very helpful. Thank you.

For topics such as those being discussed at today's meeting, there are often a variety of opinions, some of which are quite strongly held. Our goal is that today's meeting will be a fair and open forum for discussion of these issues and that individuals can express their views without
interruption. Thus, as a gentle reminder, individuals will be allowed to speak into the record only if recognized by the chair. We look forward to a productive meeting.

In the spirit of the Federal Advisory Committee Act and the Government in the Sunshine Act, we ask that the advisory committee members take care that their conversations about the topic at hand take place in the open forum of the meeting.

We are aware that members of the media are anxious to speak with FDA about these proceedings. However, FDA will refrain from discussing the details of this meeting with the media until its conclusion. Also, the committee is reminded to please refrain from discussing the meeting topic during breaks or at lunch. Thank you.

**Conflict of Interest Statement**

MR. TRAN: Good morning. The Food and Drug Administration is convening today's meeting of the Antiviral Drugs Advisory Committee under the authority of the Federal Advisory Committee Act,
FACA, of 1972. With the exception of the industry representative, all members and temporary voting members of the committee are special government employees or regular federal employees from other agencies and are subject to federal conflict of interest laws and regulations.

The following information on the status of this committee's compliance with the federal ethics and conflict of interest laws, covered by, but not limited to, those found at 18 USC Section 208 and Section 712 of the Federal Food, Drug, and Cosmetic Act, is being provided to participants in today's meeting and to the public. FDA has determined that members and temporary voting members of this committee are in compliance with the federal ethics and conflict of interest laws.

Under 18 USC Section 208, Congress has authorized FDA to grant waivers to special government employees and regular federal employees who have potential financial conflicts when it is determined that the agency's need for a particular individual's services outweighs his or her
potential financial conflict of interest.

Under Section 712 of the Food, Drug, and Cosmetic Act, Congress has authorized FDA to grant waivers to special government employees and regular federal employees with potential financial conflicts when necessary to afford the committee essential expertise.

Related to the discussions of today's meeting, members and temporary voting members of this committee have been screened for potential financial conflicts of interest of their own, as well as those imputed to them, including those of their spouses or minor children, and, for purposes of 18 USC Section 208, their employers. These interests may include investments, consulting, expert witness testimony, contracts, grants, CRADAs, teaching, speaking, writing, patents and royalties, and primary employment.

Today's agenda involves discussions of the pathways for development of drugs intended to treat variola virus infection, also known as smallpox, in the event of an outbreak, including the use of
animal models of other orthopoxviruses as potential evidence of efficacy. This is a particular matters meeting during which general issues will be discussed.

Based on the agenda for today's meeting and all financial interests reported by the committee members and temporary voting members, no conflict of interest waivers have been issued in connection with this meeting. To ensure transparency, we encourage all standing committee members and temporary voting members to disclose any public statements that they may have made concerning the topic at issue.

With respect to the FDA's invited industry representative, we would like to disclose that Dr. Joseph Camardo is participating in this meeting as a nonvoting industry representative acting on behalf of regulated industry. Dr. Camardo's role at this meeting is to represent industry in general and not any particular company. Dr. Camardo is employed by Forest Research Institute.

With regard to the FDA's guest speakers, the
agency has determined that the information to be provided by the speakers is essential. The following interests are being made public to allow the audience to objectively evaluate any presentations and/or comments made by the speakers.

Dr. Richard Moyer has acknowledged a professional and financial relationship with Chimerix, Inc., whose product is under discussion today. Dr. Moyer has worked as a consultant for Chimerix, Inc. on studies of CMX001 in the rabbitpox models and currently maintains an open consulting contract with Chimerix, Inc. Dr. Moyer does not currently receive compensation from Chimerix, Inc.

Dr. Mark Buller has acknowledged he owns 20,000 shares of stock options with Chimerix, Inc. The value is unknown, since the stock is not traded. He had a five-year subcontract with Chimerix, Inc. that ended in 2010 in which he was a principal investigator. The original contract to Chimerix, Inc. was from Health and Human Services. Dr. Buller tested SIGA Technologies, Inc. compounds
through a collaborative antiviral testing group
sponsored by the National Institute of Allergies
and Infectious Diseases in which he was the
principal investigator.

Lastly, he was a past member of an external
advisory committee board on two Health and Human
Services contracts to SIGA Technologies, Inc. He
received $10,000 per contract per year.

As guest speakers, Drs. Moyer and Buller
will not participate in the committee deliberations
nor will they vote.

We would like to remind members and
temporary voting members that if the discussions
involve any other products or firms not already on
the agenda for which the FDA participant has a
personal or imputed financial interest, the
participant needs to exclude themselves from such
involvement, and their exclusion will be noted for
the record.

FDA encourages all other participants to
advise the committee of any financial relationship
that they may have with the firm at issue.
Thank you.

DR. CARGILL: We will now proceed with the FDA opening remarks from Dr. Debra Birnkrant.

I would like to remind public observers at this meeting that while this meeting is open for public observation, public attendees may not participate, except at the specific request of the panel.

**FDA Presentation - Debra Birnkrant**

DR. BIRNKRANT: Good morning. I'd like to welcome everyone to the Antiviral Products Advisory Committee meeting today. This is a unique meeting because we will be discussing approaches to antiviral drug development for treatment of established human smallpox illness.

Although naturally occurring human smallpox was eradicated decades ago, concerns have been raised about the possibility that variola virus might exist outside WHO-designated repository laboratories and be used as an agent of bioterrorism. And it's important, also, to comment on the preparedness if such an event were to occur.
We have been working on this topic for a number of years, and we have had extensive interactions with experts in the poxvirus field over the last several years, including an FDA-sponsored workshop in 2009.

Today we are bringing some of these issues to a broader group of experts who have experience with difficult issues related to product development. I also wanted to remind the committee that we will not be discussing a formal application and we will not be taking a vote. Rather, we are seeking advice that can be applied broadly to development programs for antiviral smallpox treatments.

The objectives of today's meeting are to discuss key scientific issues and challenges in the development of antiviral products for the treatment of human smallpox under the Animal Rule because human efficacy studies are neither ethical nor feasible, and recognizing that although the Animal Rule has its limitations, we need to be able to exercise flexibility in this unique situation; and,
to obtain input on potential paths forward for
developing drugs to treat human smallpox if an
outbreak were to occur.

Smallpox drug development is not
straightforward. There are many knowledge gaps;
for example, lack of knowledge with regard to human
pathophysiology and other scientific uncertainties.
There are important differences from vaccines.
Namely, vaccines are used for prevention, and we
will not be discussing prevention issues during
this meeting.

Here are additional examples of distinctive
features of smallpox that may affect drug
development approaches. There has been an absence
of cases for decades. There is a narrow host range
which is considered important to eradication.
There is, again, limited information on the
pathophysiology of human smallpox. There are
notable disease differences between humans and
nonhuman primates, and there are differences
between variola and other orthopoxviruses with
regard to disease characteristics, drug
susceptibility, and host range. And there is a
lack of a previously recognized effective drug,
although several drugs were studied, but none were
found to have reproducible and reliable efficacy
for treatment. Toxicity profiles were also
limiting.

Here are some scientific challenges that we
face in trying to develop a blueprint for drug
approval of an antiviral to treat human smallpox.
Virus stocks are limited to two centers globally.
There are restricted laboratories where these
scientific investigations would occur, and there is
a need for WHO authorization in order to conduct
these investigations. Variola models don't fully
reproduce human smallpox.

Ethical issues preclude human challenge
studies, and questions have been raised about the
relevance of animal data to humans using a
surrogate virus if non-variola virus studies were
performed in a surrogate host.

Although models using other orthopoxviruses
do not reproduce human smallpox, they do add to the
overall understanding of the disease, but it's important to note that viral genetic relatedness has not been a good predictor of wide variations in pathogenicity across viral and host species. We need to consider effects of viral inoculum, exposure route, and strain. Consistent results across multiple models may be helpful, but are they reasonably likely to predict human treatment responses? And this is one question we'll be posing to the committee tomorrow.

I also want to bring to your attention that under the Animal Rule, human studies are still needed for safety, pharmacokinetics, and confirmation of benefit, when appropriate.

The agenda is quite full over the next day and a half. We have selected presentations and speakers who can lay the groundwork for the discussion, starting with an overview of smallpox from an historical perspective, a presentation on the Animal Rule, presentations on preparedness, and multiple presentations on the various animal models under consideration.
We will hear about two development programs and end with a presentation today on challenges in smallpox drug development. Again, I want to remind you that the focus is not on the specific application but on broader issues that current development proposals help to highlight.

I would also like to reiterate that a drug development program for an antiviral to treat human smallpox presents many challenges. We also realize there's a need for flexibility in interpreting the Animal Rule in this unique case. We believe the intent of the Animal Rule was to cover diseases such as smallpox, but conducting animal studies with variola has brought forth unanticipated challenges. It is also important to note that the Animal Rule allows for approval, with restrictions, to ensure safe use.

The questions we'll be posing to the committee tomorrow are summarized on this slide. We would like to have your advice on which animal models could be used for evaluating a drug and information that could be extrapolated to
understand likely efficacy in humans. In addition, we have a question on study design issues and one on the potential role of human data from related naturally occurring diseases. I also want to thank the sponsors, Chimerix and SIGA, for participating in this meeting, as well as USAMRIID with Dr. Goff.

Thank you, again, and we look forward to a productive meeting.

DR. CARGILL: We will now proceed with the first presentation. I would like to remind public observers at this meeting that while this meeting is open for public observation, public attendees may not participate except at the specific request of the panel.

**FDA Presentation - Barbara Styrt**

DR. STYRT: My objective in the next presentation is to give a very brief review of the historical information on smallpox from the perspective of what might be helpful to know for evaluation of new products, what's typically useful to know about a disease when antiviral treatment is studied, what can be gleaned from the smallpox
literature that might help in assessing proposed
treatments for preparedness in case of future
outbreak threats, bearing in mind that the
eradication of the naturally occurring human
disease over 30 years ago depended on massive
surveillance and containment efforts, the presence
of an effective vaccine, and the lack of an animal
reservoir.

The outline of the overview will be the
general clinical description of smallpox and its
outcomes, possible predictors of outcomes,
historical attempts at therapy with its very
different track record compared to the success of
vaccine-mediated prevention, and context of other
diseases and interventions.

The conventionally familiar clinical picture
of smallpox is a representation of a variable
spectrum involving human-to-human transmission, a
couple of weeks of an asymptomatic incubation
period, a few days of nonspecific symptoms with
fever, then an evolving centrifugal rash, macules
to papules to vesicles to pustules, perhaps a day
or two in each stage; then, scabs maturing and
separating over a period of weeks, and then scars.

Some cases could have fulminating or
purpuric course and rapid fatality without the
characteristic rash or could have rash evolution
that was described as different from the usual
lesions, and terms might be used such as flat or
malignant.

Many different case types could occur in a
typical smallpox outbreak. A number of different
classification systems were developed to describe
these case types based on the extent and nature of
the skin and systemic manifestations. Some of
these classification systems overlapped. Some
disagreed with one another, and you will probably
hear details about some of them later on today.
The major outcome of concern, of course, was death.

Some of the less common clinical
manifestations, the case types for which terms such
as "fulminating," "hemorrhagic," or "flat-type"
smallpox were used had extremely high case fatality
rates. However, in large series of cases, or large
outbreaks, these variants caused fewer total deaths than the more common case types with more moderate mortality. A feared sequelae in survivors was facial scarring. Other sequelae, such as blindness, were apparently much less common, but the incidence is difficult to estimate.

Regarding outcome predictors, it would be of interest to know not only what might have been prognostic factors at onset of disease, but also the potential for any surrogate measurements that might have helped to predict treatment-related modifications of outcomes. The next few slides address a few possible predictors.

Viral strain differences between variola major, with case fatality rates ranging from around 5 percent to over 40 percent in different outbreaks, and variola minor, with case fatality rates reproducibly below 2 percent, did have prognostic significance. The distinction between major and minor could be made from outbreak epidemiology or virology, but not from examination of an individual clinical case.
Whether strain differences within variola major affected the outcome was controversial. The route of exposure appeared to have prognostic significance in that percutaneously exposed individuals tended to have milder disease than the more typical exposures where infection was believed to occur via the upper respiratory tract.

The infectious viral inoculum was not really known, was believed to be very small, based mostly on occasional reports of cases arising from remote or indirect transmission of virus. Closer, more intensive exposure was considered to increase the likelihood of infection, but the effect on the severity of the resulting diseases was less clear.

With respect to viral burden during disease -- not what the person was exposed to, but how high the viral burden became after they were infected -- the amounts and sites of viral replication in the incubation period were mostly hypothesized from animal studies with other viruses not directly known in humans, but this was thought to be a potentially extensively complex period of
the disease virologically and immunologically.

At the time of the early rash appearance, throat cultures could yield variola virus, but positive throat cultures were also reported in some contexts of smallpox cases who did not necessarily themselves develop disease. If blood cultures were obtained early in symptomatic disease, virus was sometimes recovered, especially from the most severe cases.

Typical smallpox cases did not have detectable viremia by the time of the characteristic rash appearance, and the extremely limited available data suggested that even some of the sicker patients might have diminishing viral titers in blood over time. Smallpox scabs could contain very large quantities of virus, but those measurements did not appear to correlate with the disease stage and severity in several series.

Host factors influencing outcome included immune modification, such as pregnancy, which was associated with severe disease and mortality.

Vaccination of course was intended to prevent
disease all together, but even in the patients who
did become ill was reportedly associated with
mostly milder disease.

The type of source case, as classified by
the extent and type of rash and severity of illness
could affect the likelihood of transmission, but
cases acquired from a severe case could be either
mild or severe and so could cases acquired from
exposure to a mild case.

The initial clinical manifestations after
the incubation period and before the onset of
characteristic rash were not reliably related to
outcome. High fever and severe pre-eruptive
symptoms could lead to either mild or severe
disease.

Some patients with fulminating types of
disease died before recognizable pox lesions
appeared. Some patients had rapid onset of
widespread lesions over a period of a day from the
first skin findings, some reportedly had a delayed
or poor rash progressing to severe disease, and
some mild cases had extended periods of lesion
development reminiscent of the sequential cropping that's more characteristic of varicella.

Lesion counts were sometimes a convenient classifier, but some authors with extensive case experience emphasized the importance of the rash morphology and evolution and the overall clinical status of the patient as more important than lesion number or extent. For example, cases described as ordinary or benign had less severe illness and mortality relative to the extent of rash than cases described as flat or malignant smallpox. Variola minor cases for the same extent of rash had much lower mortality, less severe disease than variola major, and, similarly, vaccinated cases could have extensive rash but with much lower mortality than unvaccinated cases.

The scars were not simply related to lesion extent either. Survivors of the severe flat smallpox types could have superficial scars, while the classic, deep, pitted, disfiguring scars might become apparent only months after the acute illness. These were attributed to collapse of the
facial sebaceous glands and possibly a contribution of bacterial superinfection.

Given that death was the most feared outcome, it would be useful to have a clear idea of how smallpox killed people. That was unclear at the time and not much clearer in retrospect. A wide variety of hypotheses and clinical impressions were reported, a few of which are listed here, and different processes might have played variable roles in different patients.

A few examples from autopsy descriptions. A couple of series decades apart read that the disease-specific lesions in fatal smallpox cases appeared on the skin and the mucous membranes, and that a variety of associated, but not necessarily virus-specific abnormalities could be found in liver, spleen, marrow and kidneys, and there could be possibly secondary lung complications. These pathologic series did not really resolve the spatial, temporal or mechanistic relationships between the virus and findings outside the mucocutaneous findings.
With respect to preventive and therapeutic interventions, smallpox vaccine of course is the great preventive success story. Treatment of established illness encompassed a wide range of supportive care. Antibacterial agents started to become available in the decades before smallpox eradication and were believed by a number of experts to be of some help with complications and were widely used based on very limited data.

Convalescent serum was tried occasionally with mixed results. Several antiviral compounds were studied in vitro and in animals and moved into clinical trials. And for the most part, attempts at controlled clinical trials in patients with smallpox illness were disappointing and no drug was confirmed as useful for this purpose.

Looking at smallpox in the context of other diseases that might share some clinical manifestations, have related causes, or history of intervention development in the counterterrorism field or the antiviral field, how does the understanding of this disease compare and how might
these comparisons affect approaches to drug development?

Smallpox could actually be confused with a variety of other diseases at each stage of disease development, even by experienced clinicians in outbreak settings. This could imply that many things could look like smallpox under some circumstances. It's difficult to predict how much clinical uncertainty would arise if smallpox were ever to occur again humans, but the potential for diagnostic delay and confusion has to be considered.

The viruses most closely related to smallpox, the other species of orthopoxviruses, have enough antigenic similarity for substantial antibody cross-reactivity and vaccination cross-protection but major differences in host range and in pathogenicity for the hosts they do infect.

Variola was very specific to humans in its natural occurrence. Monkeys could be infected in the lab, usually with lesser disease, but no nonhuman reservoir or source was found in field
studies, a finding that was important to eradication efforts.

Camelpox was reported to be the species most closely related to variola, aside from possibly gerbilpox, but is highly specific to camels and only rare relatively mild reports of transmission to humans have been made. Several viral species with broader host ranges are reported as less closely related to variola: cowpox and vaccinia, which caused sporadic cases of localized skin lesions in humans and of course have had historical roles as vaccines against smallpox; and, monkeypox, which was recognized during the smallpox eradication campaign, as a cause of generalized skin lesions. All of these encode proteins associated with immunomodulation and immune mimicry, with different repertoires across species, and you'll hear more about all of them later today.

Most human cases of monkeypox are reported to be associated with animal contact. The descriptions of skin lesion morphology resembles smallpox, usually in the more benign range of the
historical descriptions, with less mortality, less human-to-human transmission, and reportedly more striking lymphadenopathy. The availability of clinical and pathologic information is somewhat limited due, in part, to its sporadic occurrence in parts of Africa, with poor access to health care.

A single outbreak in the U.S. was associated with human contact with prairie dogs apparently infected by rodents imported from Africa. No human deaths and no definite human-to-human transmission were reported in that outbreak, which was apparently associated with a viral clade of relatively lower virulence.

Now, how does the historical information relevant to smallpox treatment compare to the information supporting other types of product development? In smallpox prevention, vaccinia strains used as vaccines have a pretty good historical track record. The most recently approved smallpox vaccine was closely related to the historically used products and could be evaluated in human study of biomarkers considered
relevant to its protective effects, primarily the cutaneous take reaction and also antibody responses.

A preventive vaccine, by definition, is used before the pathophysiologic processes of diseases get into full swing, usually in the absence of active viral replication, and elicits complex immune responses to which readily emerging resistance might be less likely than for a small molecule drug used during established illness.

So, in theory, prevention might be simpler than treatment, but nevertheless, development of next generation smallpox vaccines has been a highly challenging undertaking, as discussed in a public workshop a few months ago.

Two antibacterial drugs have been approved for anthrax post-exposure prophylaxis with animal data as a substantial component of their supporting data. Those animal studies used the anthrax pathogen. The animals developed a disease similar to humans. There has been extensive study of the roles of specific bacterial toxins in the disease
process, and animal data were evaluated in the context of extensive human safety and efficacy data from other serious bacterial infections and with information on the relevance of human pharmacokinetic parameters that are better defined and understood than is typically the case for antiviral therapy.

Two products for protection against chemical threat agents have been approved via the Animal Rule. These typically have a defined molecular mechanism of the toxic substance, animal models using the same toxic substance, and some human data, though at different doses and for different uses. Yet, development of all of these agents to counter threat agents involved much difficulty and debate.

A word regarding current antiviral drugs: The antivirals for chronic HCV and HIV that have been major foci of recent development activity of course have clinical trials based on virologic markers for which you have a lot of data about the behavior of the biomarker in the absence of
treatment -- it doesn't go away -- and you have a lot of information about the magnitude and the timing of treatment-related changes in the biomarker and how they relate to subsequent clinical events, and how these relationships differ between diseases.

Even here, development of therapies is an ongoing challenge and the ability to put confidence in a surrogate, whether that's a biomarker or an animal, decreases markedly as you move into other diseases, especially into acute rather than chronic viral illnesses.

Lacking so many of these factors from other development approaches, the historical information about smallpox treatment, both its sparseness and its complexity, is in a category by itself. Differences from other development pathways could affect issues such as animal rule criteria, study design, and eventual uncertainties about benefit.

So a few considerations for further discussion. The understanding of events in human smallpox is limited. We hope there will never be
any more cases. The situation involves trying to consider the best possible preparedness for a treatment that is hoped to be permanently hypothetical.

The limited available information suggests that variola virus and the resulting smallpox illness were characterized by profound variability in host susceptibility whose determinants were not well defined, like complex and poorly defined relationships between putative prognostic factors and clinical outcomes and a lack of well defined, reliable surrogates to predict outcome. So the historical records and their limitations underscore the challenges of defining pathways for development of new antivirals for treatment of smallpox.

Thank you.

DR. CARGILL: We'll now have our second presentation by Dr. Khan.

Speaker Presentation - Ali Khan

DR. KHAN: Thank you, Mr. Chairman, and thank you for the opportunity to address this committee this morning.
The eradication of smallpox was a signature public health achievement. As we just heard in the last talk, humans are the only host for this virus. They are universally susceptible and, unfortunately, an unintended consequence of the eradication is that since routine vaccinations stopped in the United States in 1972, an increasing amount of Americans are susceptible.

As you heard about the disease manifestations, morbidity and mortality, another key feature of this disease is also its infectiousness. The basic reproductive rate or the R-naught, there are various estimates, but I'll give you a median here of 2, and this refers to how the disease would spread in a community if it was reintroduced into a community.

Now, because we could never certify that this virus has never -- that there is no residual virus in the world in labs besides that in the two WHO repositories and because of increases in synthetic biology that could potentially re-create this virus de novo, the Department of Homeland
Security issued a material threat determination for smallpox, stating the U.S. needed to be prepared for potential reintroduction of smallpox into our communities. Smallpox also remains one of the few named diseases in the redo of the international health regulations as a public health emergency of international concern.

So the Department of Health and Human Services has been pursuing a comprehensive acquisition -- medical countermeasure acquisition strategy to address the threat of smallpox. This includes a series of smallpox vaccines, ACAM 2000, MVA, LC16M8, immunoglobulins, and antivirals, which we'll be spending most of today talking about.

Now, this acquisition and development of antivirals or other medical countermeasures is really just a small component of the broader role of public health in the event of an emergency due to the reintroduction of smallpox into our communities, including epidemiology and surveillance, responsibilities, laboratory support responsibilities, obviously, communications, and
risk messaging, and those others that are listed here on the slide for you to read.

Now, smallpox is one of the threats that CDC worries about as part of its responsibilities to keep American's safe from all public health threats 24/7. Its role specifically in planning includes supporting the Department of Health and Human Services to identify national smallpox vaccine response recommendations and reviewing those vaccine response policy and clinical utilization guidelines with ACIP and our other critical stakeholders. We are also involved in specific targeted activities with MVA vaccine for patients with atopic dermatitis and HIV.

Let me move to the antivirals at this point, and I'd like to take this time to really stress our global responsibility here. The World Health Administration postponed setting a date for destruction of smallpox virus stocks until 2014. Most of you probably know, but let me just remind us that there are currently only two repositories of smallpox in the United States, one here, WHO
repositories, one at CDC, and one in Russia. And all work that's done on smallpox, all research activities, is done under the auspices of the World Health Assembly. And there's been a push to destroy this virus, and the only reason we are allowed to work on this virus is to make sure currently that we have some antivirals available in case this virus is reintroduced into our communities. So this sort of emphasizes this issue of we really have as short limit of time to complete this crucial research and define new ways to evaluate the efficacy of our smallpox countermeasures.

Going to antivirals, there are two obvious uses of antivirals. One is for outpatient treatment for early diseases and for inpatient treatment for moderate to severe disease. However, there are some other potential clinical uses of antivirals in treatment of adverse events following vaccination, use in conjunction with smallpox vaccine as post-exposure prophylaxis, use as post-exposure prophylaxis for individuals who do not
mount an immune response to vaccination or refuse vaccination, and use in treatment of other orthopoxviruses, such as monkeypox, which we just heard about and, as you know, is endemic in portions of Africa.

Now, use of these antivirals would require that we answer some critical questions, and those questions would include the effect of these antivirals, the duration of treatment on shedding of the virus from infected patients and/or secondary transmission; the antiviral effect on the efficacy and immunogenicity of vaccination; the assessment of combination therapy versus monotherapy with antivirals with different mechanisms of action; the valuation of emergence of viral resistance over time; and the studies needed to support use in pregnant women and children.

What I'd like to do is give you a list here on two slides of what we consider the desired characteristics of a product, of an antiviral product. The first is sufficient efficacy, safety data, and dosing information for treatment of
symptomatic disease in adults, children, pregnant women, and other at-risk populations. And here, as in other circumstances, CDC prefers to see sufficient data to support use in pediatric and adult patients for an EUA and not an IND for children, and this is the same for various other diseases such as anthrax, for example.

Both adult and pediatric formulations, the ease and the route of administration, simplify dosing, a broad window of effectiveness to use a drug; a suitable safety profile; a limited regimen, preferably approximately 21 days or less; shelf life, a minimum of three years, ideally, five years or more, since we would have to maintain this for ad infinitum, potentially; storage at room temperature; CGM manufacturing requirements met; efficacy against other orthopoxviruses; efficacy in preventing decreasing secondary transmission; and, then, obviously, cost and manufacturing scale issues.

There are also regulatory considerations for us. On behalf of the American public, we maintain
the strategic national stockpile within the
Department of Health and Human Services. And
inclusion into the strategic national stockpile
should be based on sufficient clinical safety and
efficacy data to be released and used under an EUA
during an emergency for all populations.

Multiple regulatory mechanisms complicate
public health responses for us; for example, having
an EUA for adults and having an IND for kids. INDs
require additional tracking mechanisms for release
and use, and INDs require informed consent and IRB
approval at various levels.

This would make response to a national -- I
won't say national -- a global public health
emergency very difficult, and FDA input is
essential to us as we determine the release and use
priority, one antiviral over another, for example.

Those regulatory considerations are a
component of the broader public health
considerations as we think about which drugs to
stockpile and how much to stockpile, including
consistent processes for acquisition and planning.
that occur within the public health emergency medical countermeasure enterprise process, and then the response planning that is required for these that include issues such as the timing of effectiveness, the route of administration, clinical recommendations, and guidance, the prioritization and allocation policies, regulatory mechanisms, and the overarching smallpox vaccine response strategy.

All of this is just to remind us, again, that this is more than just acquisition of a drug. There's a much bigger enterprise which we call sort of the end-to-end process of public health to make sure we identify what's going on in our communities, and at the end of the day, put a drug or product in somebody's mouth and make sure it works and monitor if it's working and it's having a public health impact.

Let me leave you with this. We have a narrow timeframe to complete this critical live viral research to obtain approval. Development and research with smallpox antivirals should not only
consider the data needed for pre-EUA and approval, but also those studies needed to inform public health policies and clinical utilization. And, finally, a successful outcome will require continued coordination and integration of efforts between FDA, CDC, NIH, BARDA, and others within the emergency medical counter-enterprise.

Thank you very much.

DR. CARGILL: We will now hear from Dr. Kovacs.

Speaker Presentation - Gerald Kovacs

DR. KOVACS: Good morning. I'd like to thank the FDA and the advisory committee for coming together today and tomorrow for a very important and timely discussion on antivirals for smallpox.

My name is Gerry Kovacs. I'm the director of the Medical Countermeasures Division for CHEM BIO RAD NUC in the Office of the Biomedical Advanced Research and Development Authority within the Office of the Assistant Secretary for Preparedness and Response at Health and Human Services. I've been asked by the FDA today to
provide you an overview of HHS' antivirals program.

This provides you an overview of my discussion this morning. I'll begin with a brief overview of the threat, how we classify the threat, how important we think the threat is, what HHS has done in developing a strategy to counteract smallpox, both from the vaccine and biologics side to the antivirals; how we've developed the requirements for antiviral drugs and the specific target product profile that we're working on; the two antivirals for discussion today, one developed by Chimerix and the other one by SIGA, and where they are in the development plan; our stockpiling strategy for these two drugs; and, finally, a reiteration of the challenges that you'll be hearing throughout the course of the next two days.

Smallpox is a real threat. It has been defined by the National Institutes of Health and the CDC as a Category A threat agent. That means it has a great potential for massive health, economic, social, and political disruption. Importantly, it was also recognized by the
Department of Homeland Security and declared a material threat to the United States.

It's important to note that vaccinations were halted in the early '70s, so anyone under the age of 40, approximately 40 years of age, currently has no immunity to smallpox. It was mentioned earlier that the mortality rates are varying from 30 percent in ordinary smallpox to 100 percent in the hemorrhagic and flat forms.

The R-naught value for smallpox is not big as it is with measles, but it does spread from person to person. And, importantly, there are no FDA-approved drugs for treatment of smallpox. This is why it is considered a Class A threat agent and a material threat by DHS.

Dr. Khan described the efforts by the World Health Organization to retain the virus stocks for important studies that need to be conducted for the approval of antiviral drugs and the licensure of vaccines. It's important to note that with the advent of molecular technologies, it is a real possibility that the virus may not come from the
two repositories, as we expect, but rather from the genetic engineering by rogue molecular biologists.

This describes in a nutshell HHS's strategy for the development, stockpiling, and the approval of licensure of medical countermeasures for smallpox. This program started before 9/11 and it very quickly grew after 9/11. In support of this program were both HHS and DOD and the various agencies that support the development of these types of drugs and vaccines.

We currently have in the stockpile sufficient vaccine to vaccinate every American. We have products as well, in the form of VIG, vaccinia immunoglobulin, to treat the complications of vaccination with live vaccine. And we also have a vaccine that we've developed for the immune-compromised, which is highly attenuated and safe to use in that population.

Important to note here is that within the national stockpile, we have drugs that are licensed and we have drugs that are not yet licensed, but presumed safe and effective enough to be used in
the event of an emergency under emergency use
authorizations. And these include the Aventis
Pasteur Wetvax, the modified vaccinia Ankara,
smallpox vaccine. What we don't have in the
strategic national stockpile are antivirals to
treat smallpox disease. That's the topic of
conversation for these discussions.

What we do have are emergency INDs, we have
sponsor-held INDs to do clinical trials with these
two drugs. What we don't have is emergency use
authorization or, as you know, approval for these
products to be used.

The requirements for antivirals originated
very early on, and what we have now as a
requirement is not for one smallpox antiviral drug,
but rather for two. We realized, as virologists,
that resistance is something that we have to deal
with in developing antiviral drugs. We also feel a
need to develop two antiviral drugs at least to
counteract the different modes of action -- with
different modes of action to counteract the
different pathologies that might be seen in
hospital settings when people come in with smallpox
disease. And from a preparedness standpoint, it's
important to note that two antiviral drugs
mitigates the risk of depending solely on one
manufacturer to provide us with drug.

The requirement that we work under was
developed through a material threat assessment that
was generated by the Department of Homeland
Security. The material threat assessment was
generated based on a mass casualty event in a large
metropolitan area, such as Washington, DC or New
York City. It did not take into account monkeypox
in Africa or the adverse events that we would see
upon vaccination with live replicating virus.

The modeling that HHS and DHS have done have
taken into account the age of the population; by
that I mean anyone under 40 has no significant, or
any for that matter, immunity towards smallpox; the
R-naught value, the response times that we will
have to have in place in order to respond
effectively in the event of an attack and the
at-risk populations. The requirement was finalized
by the Public Health Emergency Medical
Countermeasures Enterprise in 2007, revisited in
2010, and revalidated.

Dr. Khan described some of the specifics
that we would like to see in an antiviral against
smallpox, and some of these are reiterated here.
So I'd just like to focus on two aspects of this
slide, and they are that for a drug to be
considered for procurement within our office, it
has to meet a requirement to be able to manufacture
at least 1.7 million treatment courses of the drug.
This is based on the material threat assessment and
the public health consequence modeling that we've
done within HHS and DHS.

Importantly, we are not seeking approval for
a drug to treat monkeypox or the adverse events of
vaccination with ACAM 2000 live replicating
viruses, but rather for the exclusive treatment of
smallpox in individuals who come in contact with
the virus.

These are the two programs that are most
advanced at HHS for development of antiviral drugs.
You will hear a lot more from the sponsors about the specifics of these two drugs over the next day or two. They are Chimerix and SIGA. Chimerix and SIGA are developing antiviral drugs with very different modes of action, which is what we want.

Chimerix's drug is a DNA polymerase inhibitor that not only inhibits the DNA polymerase of orthopoxviruses, but other double-stranded DNA viruses, as well, such as adenovirus and herpes.

SIGA has a very specific molecule that attacks a protein involved in the morphogenesis of poxviruses, and it's specific only for orthopoxviruses. Both drugs have been tested in myriad challenge models that you'll hear about and are presently in phase 2 clinical trials.

Dr. Khan alluded to emergency use authorization throughout his talk, and I'd like to highlight the importance of emergency use authorization on this slide, because it is the underpinning by which we prepare our strategy for stockpiling all countermeasures.

We presently have in the strategic national
stockpile five medical countermeasures that are not yet licensed or approved, but may be used under EUA. What we would like to have are two antiviral drugs in the strategic national stockpile that may also be used under EUA.

EUA is the trigger that we use to begin stockpiling of drugs. It's not the end state of these drugs, but rather a step towards ultimate preparedness against the threat. And what we need from this committee is very creative ideas of how we can generate the body of data that we'll need to support emergency use authorization in the event of a smallpox attack.

I don't want to reiterate the number of challenges that we face developing antiviral drugs against a disease that no longer exists for which animal models that perfectly mimic the human disease are nonexistent, surrogate models, and so forth.

What we pose to the committee is a nontraditional means by which to approve a drug that has these challenges. I think overall we'll
have to take a holistic approach that takes into account a number of imperfect animal models and datasets that lead us to believe that the drugs will be useful and non-harmful in the event of a smallpox attack.

In closing, I'd just like to restate that HHS is very committed to continuing the development of smallpox antiviral drugs, for we feel that this is the last piece of the puzzle that we need in order to have an arsenal of medical countermeasures to protect us against smallpox if it ever rears its head again.

We have built the requirements for antiviral drugs based on classified and non-classified information, and what we need are at least two antiviral drugs to treat 1.7 million individuals who may become infected with smallpox and need treatment. And what we need from the FDA and the advisory committee today are creative ideas of how we can stockpile these drugs prior to approval.

Thank you very much.

DR. CARGILL: Thank you, Dr. Kovacs.
At this time, clarifying questions from the committee -- I'm sorry.

Dr. Roberts?

**FDA Presentation - Rosemary Roberts**

DR. ROBERTS: Good morning. I want to thank the organizing committee for the work they've done to put this on. They've been working now for several months, and I thank them for giving me the opportunity to present a high level overview of the Animal Rule. So I'm going to talk about what it is, the requirements, a little bit about the essential elements to address efficacy under the Animal Rule, and then safety information.

What is the Animal Rule? It's a regulatory mechanism to approve drugs or license biologics when human studies are not ethical or feasible. So you can see, based upon the presentations we've already heard today, smallpox would be a potential threat that could be studied under the Animal Rule.

What is it not? It's not a simplified or expedited route to development of drugs or biologics compared to the traditional pathway, and
I think most in the audience today, unlike five or six years ago, would agree it is not expedited, it's not an easier route.

The requirements. Now, in the guidance and in the rule itself, there are four requirements or tenets that are laid out by which the agency can obtain evidence to move forward for regulatory action, and they include a reasonably well understood pathophysiologic mechanism of the toxicity of the substance. In this case, the substance is variola virus. You already know that we have a lot of questions about the pathophysiologic mechanism of its toxicity, how it works in an immunomodulatory/immunomimicry way. And so right there is a question of the first requirement.

The effect is demonstrated in more than one animal model. I don't even have to talk about a well characterized animal model here. What you are going to see is a presentation on several animal models and how information from all these models may be put together to help us to come forward with
a regulatory decision.

The study endpoints should be clearly related to a desired benefit in humans, and the rule outlines enhancement of survival or prevention of major morbidity. And enhancement of survival is a challenge in and of itself because we know that the human smallpox, for the majority of people, variola major had a mortality rate of somewhere 5 to 40 percent. So it's a real challenge to have a model in an animal that mimics that and tried to show a survival benefit.

The data or information on the kinetics and pharmacodynamics of the product and other relevant data allow selection of an effective dose in humans, and there'll be more talk about this as we move forward.

So as I just mentioned to you, there are a lot of uncertainties in the various tenets of the rule, and many have pointed out -- Barbara pointed out in her historical perspective and Debbie has pointed out -- there are a lot of unique characteristics and features about variola virus,
what we know and what we don't know, what we'll be able to study in the future, and what we can continue to try to glean from the history. So it is very unique, and the agency recognizes that uniqueness.

So I just want to briefly touch on the various characteristics of the threat agent that we've outlined in the draft guidance of 2009, that those who develop animal models and sponsors who bring products forward using these animal models need to address in their presentations to the agency.

The challenge agent; and we would typically expect that that challenge agent would be identical to the agent that infects humans. And in this case you know that variola virus is going to be a real challenge and has been a real challenge in order to be able to use it in the animals since the only natural host for variola is the human.

We would want the challenge agent to have the pathogenic determinants that we see in the etiologic agent that affects humans. Typically,
for a virus, we like to see that it's a virus that
came from a lethal infection, that that virus has
been passaged very little, and that it has been
standardized in the way it is grown up and how it
is handled with the exposure.

The route of exposure, we would like to see
the route of exposure be the same as the way the
human is infected and we want to be able to
quantify that exposure.

With respect to host susceptibility, the
species should be susceptible to the threat agent,
and you've already heard that that's going to be
very difficult in this scenario with variola.

The response to the etiologic agent in the
model should be similar to the response seen in
humans. For the natural history of the disease,
Barbara has given you the information that we know
about human smallpox, and we would like to have the
animal model be as faithful as possible to what we
know about the human disease and condition, and we
have to recognize that we do have a lack of
relevant human data.
With respect to characterizing the product that will be the medical intervention, I just want to highlight some key areas here. We need to know the pharmacokinetics in the unaffected animals as well as unaffected humans, and that we can obtain. We would also like to see pharmacokinetics and pharmacodynamics in affected animals and humans, but we're not going to be able to see that in humans; so that any data that we can obtain from relevant human diseases to help us to fill in this gap we would like to see sponsors provide for the agency.

Design considerations. Remember that the adequate and well controlled animal efficacy studies that are done under the Animal Rule replace the phase 3 clinical efficacy studies that are done under traditional drug development. So we want to see that these products that are being studied for animal efficacy, that the studies are done adequate and well controlled, with randomization, blinding, and a statistical plan that is adhered to and agreed upon by the agency prior to the study being
conducted.

The study design will be indication-dependent and clinical scenario-dependent, and we would ask, depending upon your indication, that the timing of and triggers for intervention are defined and that they can be confirmed as to their relevance to the model.

The route of administration would be the route we would expect in the human population; the dosing regimen, endpoints, again, enhancement of survival or a decrease in major morbidity. The studies need to be done with good laboratory practices, and there has to be consideration for the supportive care that would normally be done to humans with the disease.

For safety information, the considerations are that under the Animal Rule, the human safety studies can be performed in healthy volunteers as long as there is not too great a risk to the human volunteer or that the information that we would obtain from the human volunteer would appear to be relevant for people that have the actual disease.
state and would get exposed to the product. So sometimes we have to seek other appropriate populations.

With respect to the size of the safety database, it depends on if you're dealing with a new biologic or drug for which we have no information on safety. This will be the first time it's being introduced into the human population or whether we have an approved product that we may have human safety on other commercial indications and preferably that that human safety information would be done at the same dose and duration that is to be used for the medical countermeasure indication. Also, the size of the human safety database will vary on the indication sought.

For treatment, in general, the sponsor will have to study less patients for human safety because of the risk-benefit. And if it's going to be a prophylactic claim, especially if it's a post-exposure prophylactic claim, where we might be exposing individuals who think they were exposed, but are actually the worried well, we have to make
sure that there is an appropriate safety profile
for everybody that might receive it in a
prophylactic indication. And then of course, it
needs to be studied for safety at the dose and
duration that is indicated for the medical
countermeasure indication.

So, in summary, CDER believes that the
Animal Rule can be used in the setting of smallpox
for therapeutic products. We recognize that there
are unique circumstances for smallpox and that
these need to be taken into consideration, and CDER
is prepared to be flexible as we look at these
products that are brought forward for smallpox
therapeutics.

Thank you.

DR. CARGILL: Thank you, Dr. Roberts.

We'll now hear from Dr. Smith.

Guest Speaker Presentation - Geoffrey Smith

DR. SMITH: Good morning. Thank you for the
invitation to come to FDA and make this
presentation. It's actually 30 years and 60 days
since I first came to this part of the world as a
very green and young post doc to work at the NIH in Bernard Moss' laboratory, and it was at that time that I was introduced to poxviruses, a field which I have been very enthralled with and I'm still working with 30 years later. That's why I'm here.

So I've got 19 minutes now to tell you about orthopoxviruses, their properties, phylogeny and spread. So this is what I'm trying to do; a little bit on classification, and then some of the properties of these viruses, and then I'm going to talk about vaccinia virus, how it replicates, and, particularly, how it spreads, and talk about a new mechanism which we discovered and published last year on how this virus can spread faster than was thought possible hitherto by a new mechanism. And, finally, I should have at least one slide that devotes to anti-poxvirus drugs, the subject of this meeting.

So the genus orthopoxvirus is one of eight genera of the chordopoxviruses and it's the one most studied because of variola virus, the cause of smallpox. And these other members of the family
are listed here. Many of these have now been completely sequenced with one or more strains, and they are very similar in their genomes overall.

They're also morphologically indistinguishable such that even by the electron microscope, you could not easily tell one from another. And importantly, they're all immunologically related and they confer protection against subsequent infection by any other member of the genus. And, of course, that was the basis upon which Jenner first used the cowpox virus as the vaccine to prevent smallpox in 1796.

So looking at the phylogeny, this shows several members of the orthopoxvirus genus, and at the top you can see there are several strains of variola virus which cluster closely together. And at the time this slide was made, the two sequences that were available of camelpox virus, at about 1 o'clock, were the closest viruses known to variola. Taterapox, or the gerbilpox virus, is about equidistant from variola virus, as is camelpox.
Other members of this genus cluster quite closely together. So the two strains of ectromelia virus that are shown here, at about 4 o'clock, cluster closely together, as do the two vaccinia strains, shown about 9 o'clock, and monkeypox, shown at the bottom, is within the orthopoxviruses, about as divergent from variola virus as any orthopoxvirus is. And that was of some reassurance to the World Health Organization, who were concerned that following the cessation of vaccination against variola virus, following the eradication of smallpox, monkeypox might become more prevalent in the human population and evolve into a more smallpox-like agent.

The only other point from this slide I want to make is that the two strains of cowpox, shown in purple, are actually quite divergent and sufficiently divergent to be reclassified as separate species. And, indeed, more recent data with several other strains of cowpox have shown that these other viruses that are called cowpox, some of these are also quite divergent and may
indeed eventually be classified as separate orthopoxvirus species.

So now for the characteristics of these viruses, this is an electron micrograph and shows that the size of these viruses is big as viruses go. So dimensions of 200-by-300 nanometers is large enough for these variants to be visualized by a microscope, not using the electronic microscope. So they're big, a large and complex virus particle. They have double-strand DNA genomes of about 200 genes. It varies as bit with the different strains of virus, and, indeed, the different species of virus.

Unusually for large DNA viruses, poxviruses have evolved to replicate in the cell cytoplasm, and that means that they need to bring with them their own enzymatic machinery for the transcription of the virus genes and the replication of the DNA, and they do that. And they also have many virulence factors, and these proteins are not essential for the virus to replicate, but they confer on the virus the ability to survive and be
replicated and transmitted from one host to another.

Another interesting feature of orthopoxviruses is that from each infected cell during the virus replicative cycle, there are two types of infectious virus produced. The first we call intracellular mature virus, and this represents the majority of infectious progeny that are produced. This is very stable and essentially this is what makes up the smallpox vaccine when freeze-dried. It is surrounded by a single lipid bilayer. But there is as second form of virus called extracellular enveloped virus, which, although only a minor component of total infectivity, is very important to virus spread, and this is surrounded by a second lipid envelope in which there are antigens encoded by the virus which are absent from the IMV surface. And, therefore, this virus is biologically and antigenically different to the IMV form. And if you want to have an optimal vaccine against an orthopoxvirus, it is better to have antibodies that are targeted against...
the proteins both on the surface of the IMV and the EEV form.

This is a genetic map of an orthopoxvirus, and I've chosen camelpox virus as an example. And each of these horizontal lines represents about 25 kilobases of DNA, and the large arrows at the end indicate the inverted terminal repetition so that genes at the left-hand end within that region are deployed and are repeated at the right end.

I'm going to introduce the genes to you and the proteins encoded by them according to their function, and what I want to show is the positions at which genes encoding proteins or particular functions are clustered.

So here is the code at the bottom. And if we take genes encoding proteins affecting RNA or DNA metabolism, or the assembly of virions, the structural proteins that go to make new virions, you can see that the genes encoding those proteins, in the two shades of blue and the green, are all located in the central part of this genome. And bioinformatic analyses have shown that for all the
chordopoxviruses, there are approximately 90 genes that are conserved in every virus and which are all present in the central part of the genome. And those genes will represent the genetic basis or a minimal genome of an ancestral poxvirus from which the present day poxviruses have evolved. And with that evolution, extra genes have been acquired and bolted onto the end of the genomes, and those extra genes, which often encode host range or immunomodulatory factors, give the viruses their particular host range virulence and tropism.

So, now one slide to overview the morphogenic pathway of the virus and how these two distinct forms that I've mentioned are produced. This initial diagram shows a microtubule with a virus core moving on it, moving deep into the cell after the virus has first infected a susceptible cell. And then within the cytoplasm, close to the nucleus in some cases, there is a virus factory established in which one can see by electron microscopy the different stages of replication.

The first form produced are crescents, which
are composed of host-derived lipids and virus-encoded protein. And these grow and encapsidate the virus genome to form immature virions of spherical or oval shape, and these then mature via a process of proteolytic condensation to produce the first infectious form of virus, called IMV.

The IMV particles are then transported from the factory on microtubules to an area within the cell, which is sometimes close to the microtubule organizing center, and at that site, that virus is wrapped by two additional layers of membrane in which viral proteins have been embedded. And that wrapping process produces a triple-layered form of virus, which we call intracellular enveloped virus, or IEV. And that virion is then also transported on microtubules to the cell surface. And when it reaches the plasma membrane, its outer membrane will fuse with the plasma membrane to expose a virion on the outside of the cell by exocytosis.

That virion is called cell-associated enveloped virus, or CEV, and its function is to induce the polymerization of actin from beneath the
plasma membrane at the position at which that virus
sits, and that growing actin tail will propel the
virion away from the cell in search of new
uninfected cells to infect. And as I will show
you, mutants that lack the ability to induce these
actin tails are highly attenuated and only spread
slowly. And lastly, some of the virions which have
reached the cell surface, rather than inducing
actin tails, may simply detach and be called EEV.

So when we talk about vaccinia virus entry
and attachment, we have to define first which form
of virus we're talking about, whether it's the IMV
form or the EEV form, because naturally as they
have different surface proteins and, indeed,
different numbers of membranes, they have different
problems, topological problems, in gaining entry to
the target cell.

For both IMV and EEV, they're known to bind
to different cellular receptors, and a number of
molecules, including glucosaminoglycans, or GAGs,
laminin and others have been proposed.

We know that in the process of penetration,
the IMV form, with its single membrane, can enter in one of two ways. It can either fuse directly at the cell surface, releasing the core into the cytosol, or the virion can be taken up by endocytosis and within an acidified vesicle will fuse to the membrane of the vesicle, again, releasing the core into the cytosol.

Now, for EEV, the problem is more complex because the virus has to shed both membranes. And I will show you electron micrographs in a moment of how this may happen, but for EEV, the extra membrane is ruptured at the point of contact where the virion engages the cell surface, and that then exposes an IMV particle which can then bind to and fuse with the cell membrane as for a free IMV particle. And whichever form of virion one starts with, the endpoint is that a core is released into the cytoplasm, able to initiate a new round of replication.

So this is an electron micrograph series of pictures showing IMV entry by fusion at the plasma membrane. And in panel A, in the top left, we can
see IMV particles stuck to the cell surface, and in B, one is shown at higher magnification. And then if one warms up the mixture of cell and virus, one can then see the fusion events taking place. In panels C and D, one can see the continuity of the virus membrane and the plasma membrane. And in the bottom two panels, one can see the virus core now moving away deeper into the cell, leaving its membrane at and continuous with the plasma membrane of the cell.

For EEV entry, we can see in these series of images how the external membrane is removed. So in panel A, we have a virion attached to the cell. In panel B, we can see that at the point of contact, the outer membrane has been disrupted so that now the internal virus, the IMV particle, gains access to the plasma membrane. And in panels C and D, you can see now that the inner membrane has fused with the plasma membrane, releasing a core into the cytosol and leaving the extracellular second membrane outside the cell. This is a remarkable process. It's very uncommon in virology, and the
mechanisms by which it works are, therefore, interesting. But it's a nonfusogenic event to remove this outer EEV membrane.

So in the remaining time, I want to talk about a new method we discovered and published last year for how vaccinia virus, and we believe other orthopoxviruses, can spread more rapidly than had been thought originally. And if you wish to read more about this and, indeed, see the videos that I will show you, these can all be downloaded freely from the Science web page.

In fact, last year, we had a letter from Warner Brothers when they were making this movie Contagion, asking if they could use these videos for their movie, and we said sure. But in the end, they didn't do it.

[Laughter.]

DR. SMITH: So this is a video of a vaccinia virus plaque forming on a layer of susceptible cells, and you can see that the virus spread out rapidly, and the cells in which the virus has replicated are trashed and they look really quite
different to those uninfected cells outside the growing plaque.

Now, this type of live video microscopy then enables you to ask the question how fast is the virus spreading across that lawn of susceptible cells. And by measuring the rate of spread and the number of cells that the virus crosses per unit time, it became evident that the virus was spreading across each cell in only 1.2 hours, which was far too fast given that we knew that the virus takes a minimum of five to six hours to form any new virus particles in each cell.

So how can this be? And by repeating these measurements with different types or mutants of vaccinia virus, one found that in the top three lines where the virus is spreading rapidly, the virus is spreading at about this rate of 1.2 hours for each cell. But in all the mutants shown at the bottom, in which the ability to produce those actin tails is disrupted, the viruses are spreading four or fivefold more slowly and they all cluster together. So actin tails are important for spread.
So what do we have so far? We know that the rate of spread across each cell is too fast and inconsistent with the replication kinetics. We know that mutants without the ability to make actin tails spread more slowly, one cell about every five to six hours, which fits with the replication cycle. And we know that actin tails are important. But because these were reported hitherto only to be produced from the cell surface, once the virus had completed its replication cycle, they could not explain this rapid phenomenon, so there must be another mechanism. So what is it?

Well, if you look at this video, where we're using a GFP tagged virus, and it's spreading across a lawn of susceptible cells, one can see the cells that are producing the virus because these have gone green. The green fluorescent protein from the jellyfish has been fused to a late viral protein that makes one of the capsid proteins of the virus. So that is only produced, and therefore the cell will only go green late in the infectious cycle.

So the green cells are where the viruses are
coming from, and yet you can see on this lawn of susceptible cells, as the virus is spreading from the top right to the bottom left, that there are virions, these individual green puncta, which are several cells distal to the cell which is producing the virus.

So how that can be? How can it get so far so quickly? Well, if you look at the lawn of susceptible cells that are infected with the green-expressing virus at high magnification, one can see in the white box that there are cells which have become infected already, because in the cytoplasm they have these blue cytoplasmic factories of viral DNA. And on those cells, which have not yet gone green, so they cannot have made any virus particles, there are green dots, which are virions, which are being pushed by a red actin tail. So this suggests that the virus particle and the actin tail have derived from different cells.

But how can one prove that? So to address that, we did the following experiment. We made a cell line which would produce red actin. So the
cell would be red. And we mixed some of those cells with some normal cells and created a mosaic of red and normal cells. And then we infected such a layer of cells with the green-expressing virus and looked for a position where we had a green cell where viral replication was taking place adjacent to a red cell which had not yet gone green, and so could not make new virions.

We observed that green cell and we looked for situations where, on that red cell, there were red actin tails pushing green viral particles. And if we could find such a combination, then de facto the virion and the actin tail must have derived from different cells. And we could find that quite easily.

So here we can see a series of frames taken from a live video in which, on the top right panel, you can see that there are red actin tails pushing green viral particles on the surface of this red cell before it goes green.

This is shown more clearly in this video where we have the red cell adjacent to a green cell
in which the virus is replicating. And you can see in the white box growing red, that is, actin tails, propelling virions away from the surface of this cell in which virus replication has not yet taken place. And if you look carefully at this actin tail coming off the cell, it comes back and touches the cell surface, kisses the cell surface, and then pushes off again; in other words, it bounces, so a virus particle can be repelled repeatedly from the surface of the cell.

So what's the mechanism? Well, one hypothesis would be that shortly after the virus infects the cell, it expresses one or more proteins on the cell surface which marks that cell as infected and causes subsequent repulsion of superinfecting virions. And candidate proteins for that would need to be, one, proteins that are on the cell surface; two, they must be made early; and, three, they must be involved in actin tail formation. And consulting the literature, it was evident that there were at least three candidates, proteins A33, 36, and B5.
So the experiment we did was to engineer viruses in which instead of these genes being expressed both early and late during the infectious cycle, we made them only late. So there was no early expression. And we looked at the size of the plaque that those viruses could form as a measure of the rate of spread. And when we did that, it was clear that when these proteins were only made early -- sorry -- only made late, the plaque size -- in other words, the rate of spread was greatly diminished.

So on the left-hand side, one can see the size of a normal plaque in the deletion mutant, shown in black bars, the plaque size is tiny. And in the stippled bars, one can see the size of the plaque formed by viruses in which either the A33 or A36 genes are regulated only by a late promoter. So you need early expression of these proteins for rapid spread. So A33 and A36 are needed, B5 is not.

Are they sufficient? Is that all you need? So to address that question, we made cell lines
which would either make protein or both proteins
and we would add to the surface of those cells
GFP-tagged extracellular virus or intracellular
virus and ask whether actin tails are formed, and,
remarkably, they were. Indeed, within just
15 minutes, we could see that virions were being
propelled away from the surface of these cell
lines, which were uninfected but which just
expressed these two viral proteins. And that's
quantified in this slide, showing you need both
proteins; either alone is not sufficient. It only
works for the extracellular form of virus, EEV, not
the intracellular form, and it doesn't work for
another large DNA virus such as herpes simplex
virus.

So this is the model for the spread. We
took at the top line, the virus goes into the first
cell. It undergoes its complete replication cycle,
and new virions are pushed away from the cell on
actin tails, and they will infect the surrounding
cell. Within that cell, shortly after infection,
the virus expresses two early proteins, A33 and
A36, that form a complex, move to the cell surface, and mark that cell as infected such that when additional virions, still being produced out of that first cell, engage that complex of proteins on the cell surface, they induce the polymerization of actin to propel this virus away from the cell, and it will be propelled again and again until it will find an uninfected cell in which it can enter. That cell, too, will produce this complex of proteins early after infection, and so virions can bounce across several cells to reach an uninfected cell that it can infect. And this, therefore, enables the virus spread to speed up considerably because the virus does not need to replicate in each cell along the way.

So this really redefines how plaques can be formed and it's a novel method of virus spread. Indeed, it's very Darwinian, because it's perfectly logical that a virus would not want to power more virions into the same cell that it already has infected.

The last slide I want to leave you with is
the demonstration that the ability to spread slowly equals low virulence. So in this plaque assay at the bottom, we're looking at viruses on the left-hand side, a wild type virus, in the middle panel, a virus in which one gene, called F12, has been deleted, and you can see that the plaque size crashes. If you put the gene back in again, the plaque size recovers.

Now, if you take that collection of viruses and put them into an animal model, in this case, mice, intranasally, and we measure the weight change or weight loss of those animals at different times after infection, the day of infection is shown by the vertical white arrow, and the green and the red lines show the weight loss attributable to infection with either the wild type or the revertant virus. But compared to that, the yellow line at the top shows the weight loss caused by infection with the virus, which has lost this gene and cannot spread rapidly. And you see that the animals are completely -- finally lose no weight at all.
The degree of this attenuation is illustrated by the blue line, which shows the outcome of infection with a dose of the deletion mutant 10,000-fold greater than of the wild type or the revertant. And there, too, the animals are perfectly well and not losing any weight. So the ability of viruses to spread rapidly is critical for virulence.

Then the final slide looks at the position at which the anti-poxvirus drugs, which we are here to discuss, act in the pathway. So cidofovir is an inhibitor of DNA replication and will stop the formation of new particles, but this excellent drug, ST-246, blocks the wrapping of the intracellular virus to form the extra, so the IEV form, and so essentially is blocking spread, because you need to have that extracellular virus to enable the virus to spread, as I have shown you.

So although ST-246 does not block the replication of the virus in any individual cell, it prevents disease, because it stops the virus spreading.
So I'd like to conclude by thanking the FDA for the kind invitation to come to Washington again, to thank the people in my laboratory who have done the work I've presented to you today, Virginie Doceul, Mike Hollinshead, and to thank the Wellcome Trust and the Medical Research Council for their enduring support.

**Clarifying Questions from the Committee**

DR. CARGILL: Thank you very much,

Dr. Smith.

At this time, we will have clarifying questions from the committee for the speakers. I'd like to ask the panel if you would please raise your hand. Mr. Tran will take your name. And when you conclude your question, if you would please, again, turn off your microphone.

Dr. Van Dyke?

DR. VAN DYKE: Yes. I wonder if someone can help me understand what additional information is necessary to get an emergency use authorization beyond an NDA, and I'm thinking specifically of the comment made about pediatrics and how important it
would be to get sufficient pediatric data to get an EUA.

So what's involved in that process?

DR. COX: So maybe let's just back up just a little bit and talk about EUA and NDA. So the standard that we're looking for in an EUA is that the known potential benefits outweigh the known and potential risks. So it's, in essence, a lesser standard than the NDA standard, which is substantial evidence of efficacy.

So you think of an EUA as sort of a step along the way, if you will, as data is being acquired. But you're specifically raising the question about special populations' data and information that would help you to understand appropriate dosing in those special populations. And I think if we're -- as a drug is being developed, gathering information to be able to essentially meet those standards for those special populations so that we know an appropriate dose and we can understand if there may be potential additional risks in those patient populations would
be part of gathering the data.

Now, in a setting where you're developing a drug using an animal rule approach, it can be particularly challenging because of issues around pediatric studies. So in these settings, there may be other ways to try and approximate or using pharmacometric tools to estimate pediatric dosing.

So hopefully that has helped some. I think it's a fairly complex question. There are a lot of different things that you're bringing up, but I guess the general point is the EUA is generally looked upon as being a sort of step along the way. The standard there is somewhat lower, and in order to be able to appropriately use the drug in special populations, you'll want to be able to understand dosing and adverse effects in order to be able to do so in an informed way as you look at the various different -- at the standards along the way as you move from EUA to NDA.

So hopefully that helped some, but there are a number of different things involved in the question that you're bringing up.
DR. CARGILL: Dr. Henderson?

DR. HENDERSON: There are a great many questions that I have, but 15 minutes is not a long time to devote to clarifying things. Let me come to one particular problem that keeps bothering me, and that is we have a unique situation here where we have a virus, smallpox virus, which seems to grow only in humans, very different from other pathogens that we're dealing with. And we have no human -- no animals that replicate or seem to be closely related to the immune system of a human's in handling organisms.

It sounds like a very difficult problem to come up with some sort of a test which somehow or other is going to have some merit and fit with what would be the Animal Rule.

Now, when the Animal Rule was written, and a lot of work went into that, I don't think this was really anticipated, this particular problem. But I didn't have the impression that the Animal Rule was chiseled in granite, that there are other possibilities. We could look at the Animal Rule
and perhaps come to other approaches for approving
the use of some of these drugs, these antiviral
drugs. And Moses did not bring the Animal Rule
down and deliver it to us. We have the power, I
think, to change it ourselves, if I'm not mistaken.
And it seems to me that this was the conclusion
that was reached by the international expert
committee which looked at the question of licensing
of drugs.

I wonder what the response to this is,
because this has puzzled me very greatly. It seems
to me like we're in an impossible situation.

DR. COX: Thanks, Dr. Henderson, for the
question. So let me try and step through this one,
too. It seems like we're going to have a lot of
complicated and complex questions today.

So the issue of the Animal Rule -- and the
Animal Rule is in our regulations. It's written
there. It was done through the rulemaking process.
So that's what's in our regulations. And I think
at the heart of your question is really a key
issue, and I think one of the key issues that we're
here to discuss today is that smallpox virus poses some unique challenges. We've heard that in the discussion so far. And in that setting, there are risk-benefit issues around this that need to be carefully considered. And as Dr. Roberts presented in her slides, we can look at the Animal Rule, given some of the unique aspects of what's going on with smallpox, some of the unique challenges it poses, and can look at that scientifically and exercise flexibility, where appropriate to do so, and doing so keeping risk-benefit in mind, keeping the characteristics of the issues that we're dealing with here and how uniquely challenging it is.

So we're gathered here today really to hear your advice on these issues, to understand what we can learn from the animal models that have been done or can be done and try and get to a understanding of a body of evidence, in essence, that will allow us, with the inherent uncertainties that will be there, to predict how a drug will perform in humans in treatment of smallpox.
So that really is -- Dr. Henderson's question is right on the mark, and it really is why we're here today to get your advice on this very topic.

Recognizing uncertainty, recognizing the risk-benefit of the issue, recognizing the ability to use flexibility here, we're looking for your assessment of the data that we have and thoughts on additional pieces of work that could be done that would further bolster our ability to make inferences as to how the drug would perform in humans.

DR. HENDERSON: Let me just respond briefly to say the background material provided and certainly the briefings today have been very extensive, and I very much appreciate them. I'm just looking at this and thinking about it and talking with some of my colleagues. We can learn all sorts of things by doing more experiments.

I also think of my own experience in 9/11 when we were threatened with intercepted intelligence thinking we were going to have a
second event, and caught with very little smallpox
vaccine and not very much we could do about it, and
being desperately concerned we move quickly to try
to get a product.

Now, in this case, I would look at -- an
antiviral product for smallpox would be extremely
valuable if we had an attack with smallpox. And at
this point in time, we're going through a
considerable number of hoops, and have already gone
through a considerable number of hoops, to try to
meet the requirements of an animal rule, which is
an arbitrary rule. And I just ask the question,
shouldn't we have -- I think, as the international
group recommended, as to whether we need to rethink
what it is we're doing on this rather than trying
to identify now yet more various approaches that
will really impede development and the possible
realization of an antiviral drug.

DR. CARGILL: I think that Dr. Henderson
raised a number of fair questions, and I think
Dr. Cox has responded to them. And my
understanding is that that really is the focus of
our time tomorrow. We have truly open time to
begin to really get into the meat of these matters.
I believe our time for today -- and these are
important points and I'm not dismissing them. I
want us to be sure we come back to discuss them.
But I also want to make sure that as we build our
knowledge base and our common understanding of
these things, that we also have an opportunity to
ask clarifying questions as we go along the way.

So I would ask the panel, if you have
clarifying questions, to raise your hand so that we
can begin to make sure we all have these pieces of
information in a very complex area to have a very
robust discussion tomorrow.

[No response.]

DR. CARGILL: Then if not, I do. I have a
question for Dr. Khan. It's on slide number 10.
And my question is really very straightforward, but
I was going to ask you, in that slide, I'm not sure
I heard you correctly, is this the listing in order
of importance or just the listing? That was the
clarification I needed.
DR. KHAN: Just the listing.

DR. CARGILL: Just the listing. Thank you.

Yes, Mr. Raymond?

MR. RAYMOND: Thanks. I had a question, I think, for Professor Smith. We heard at the beginning from Dr. Styrt that what we know about smallpox is that percutaneous exposure is associated with less severe disease. And I just wondered if there was any sense from the other orthopoxviruses of why that might be and how that's modulated.

DR. SMITH: So the question, if I understand it, comes down to why does infection by the intradermal route rather than infection by the natural route, the respiratory system, give a less severe infection? Why does vaccination work? Is it safer than variolation, or why does variolation work?

Well, I don't think the answer is known. There are plenty of examples, though, in either human or veterinary medicine where, if you give a pathogen via an unusual route, the outcome is
different. I think if we had a full understanding about that, it would certainly help the deliberations of this committee, but I don't have any words of wisdom to offer to explain that.

DR. CARGILL: Thank you.

Dr. Camardo?

DR. CAMARDO: Thanks. I have a question for Dr. Kovacs. It's the slide on the minimum requirements for procurement. I see capability to manufacture and deliver 1.7 million treatment courses within five years. That seems like a small number and a long time. I just wonder how we got to that -- or how you got to that number and the timing?

DR. KOVACS: If I understand your question correctly, how did we get to the 1.7 million treatment courses?

DR. CAMARDO: Yes.

DR. KOVACS: That was based on the material threat assessment and the public health consequence modeling that we did. That's the estimated size of the population that we believe would need antiviral
therapy. And the five years is stipulated by legislation under Project Bioshield. A product can't be -- you can't contract for a product that cannot be licensed within that timeframe.

DR. CAMARDO: Can I ask -- yes, sure, I guess I can.

DR. CARGILL: Please.

DR. CAMARDO: Licensed then would mean either the EUA or full NDA, or you can't -- that may not be a question for you, but it's somewhere between one of those?

DR. KOVACS: The legislation states licensable or approvable.

DR. CAMARDO: Licensed and approval.

Can I just -- sorry, but we won't have a chance to see the modeling you've done, and I'm just reacting to it. It seems like -- is that a point threat or -- I mean, it seems like if you can make a little bit of smallpox, you can make a lot of it. It just seemed like a small number to me. That's the reason I reacted. But I assume it was based on a model.
DR. KOVACS: That's correct.

DR. CAMARDO: All right. Thanks.

DR. CARGILL: Dr. Van Dyke?

DR. VAN DYKE: I also have a question for Professor Smith. Thinking of variola minor and major, could they be differentiated virologically? Are there known genetic markers that you can tell the difference between those two viruses?

DR. SMITH: No. The genomes are known. I have examples of both groups. And it is not possible to determine from the differences that have been shown what is the cause of one being more virulent than the other. One can only do that by experimentation, and such experimentation, fortunately, is prohibited.

DR. CARGILL: All right. Then I'd like to remind the panel that our guest speakers will also be available tomorrow if you come up with additional clarifying questions.

In the meantime, we will now take a 15-minute break. And, panel members, please remember that there should be no discussion of the

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meeting topic during the break amongst yourselves, with any member of the audience, and we will resume at 10:10 a.m. promptly.

(Whereupon, a recess was taken.)

DR. TRAN: Dr. Buller, if you could go ahead and take this.

**Guest Speaker Presentation - Mark Buller**

DR. BULLER: Good morning. Today I'm going to split my talk into four sections, and the first part will be sort of an extension of what Geoff Smith talked about, a little bit more about the orthopoxvirus genera. And then I'll compare and contrast the similarities and differences between mousepox and smallpox. And then I will give you some more information about the mousepox model in mice, as well as pointing out the important role that route has in the pathogenesis of this and, I would argue, all models. And then I will finish up describing some different biomarkers we've looked at as triggers for therapeutic intervention in our model, and try and give you a feeling for which ones worked and which one didn't.
This, first of all, is not the orthopoxvirus family. It's the orthopoxvirus genera. They act like a family in the sense of a human family, and they're very closely related. Species are 95 percent identical at the DNA sequence level, ectromelia versus vaccinia versus variola. And then if you go to the actual species, strain variation within a species is about 99.5 percent identical. So there's very, very little variation. And I think this suggests caution if one wants to take the antiviral studies from the HIV and HCV areas and apply them to pox for estimations of durability of a poxvirus antiviral.

For example, with HCV, the species is broken into about six serotypes, and there's only 28 percent identity at the sequence level. If you go down to a subtype, the sequence identity rises to about 80 to 85 percent. And even within the subtype, isolates will only have 80 to 85 percent identity -- sorry -- 90 to 95 percent identity. And if you take the evolution of HCV in a single person who's infected, the sequence identity of
variola over 40 or 50 years is higher.

So these are different viruses, and I think you have to look at the poxviruses from a point of view of robustness of the development of antiviral a little differently than HIV and HCV.

The other thing that Geoff talked about was the central conserved area of a genome. All of the orthopoxviruses are co-linear. So the genes in one are in the same place or approximately the same place as another. And the areas at the flanks that Geoff talked about as being bolted onto that central conserved area I call host-response modifiers, but you could call them a number of different names, they include gene products that block the host anti-apoptotic pathways; they include gene products that target different immune responses to infection, binding proteins for cytokines and chemokines; and they include gene products antiviral effector pathways within the cell, and indeed a lot of other pathways also. And they can actually be equivalent to 10 to 20 percent of the genome.
So each orthopoxvirus encodes a lot of these host-response modifiers, and each species has a different complement or pattern of these open reading frames that do this. So you can look at the orthopoxviruses as a very closely related family with very small differences having an effect on the pathophysiology of the interaction with the host.

Another point to make is that, to my knowledge, most, if not all, orthopoxvirus infections in a natural host are acute, and we have to keep in mind that the successful antiviral therapeutic is synergizing with this immune system to clear the virus in the evaluated models that I have seen.

This is an intimidating slide, but it's really quite simple. It's sort of very much like the one that Geoff showed. In this case, it is the cowpox GRI genome that's being shown in three different pieces. And the light green boxes are referring to genes that are conserved among all of the different orthopoxviruses, and they're named on
the far left of the slide. If you can't see it very well because of the size, the variola virus isolates are at the bottom.

The yellow boxes are open reading frames or genes that are truncated -- I'm sorry -- the dark green are truncated and the yellow are fragmented, and the red boxes indicate genes that are lacking in that species.

The two antiviral candidates that we're talking about today, ST-246 and CMX001, target two genes that are in this conserved area. ST-246 targets the F13 and CMX001 targets the polymerase, as we heard before. And these genes are highly conserved among all of the orthopoxviruses and, indeed, both drugs have shown to be extremely effective in vitro against all of the orthopoxvirus species, as well as against variola.

This is a phylogeny of the orthopoxvirus genera that I want to spend a little bit of time on, and Geoff showed you a similar one in his talk. The first thing I wanted to mention is that the -- right here where we have taterapox, cowpox
and variola, sequence identity does not suggest
similar in pathophysiology in that camelpox does
not cause human infections, and taterapox we've
spent quite a lot of time studying, and the most
robust animal model we've been able -- small animal
model we've been able to generate is an intranasal
infection of the mouse, the SCID mouse lacking an
immune system, ten-to-the-sixth plaque-forming
units, and you get death in 68 days. This is not
what I would call a robust model.

If you look at some of the other models or
viruses here, vaccinia virus has been examined
against both candidate antivirals in mouse models.
Rabbitpox is about four levels down. That is a
variant of vaccinia virus, and its host is the
rabbit and it has been used to qualify the two
candidate antivirals.

Cowpox, as we work our way down, is used in
a mouse model and has been of limited utility in
studying these two antivirals, but has been used.
Monkeypox is probably the virus that's been used in
the most varied numbers of models. It's been used
in a prairie dog model, a squirrel model, a
dormouse model, and, of course, monkey models, and,
also, recently, has been shown to infect a mouse
strain, a CAST/Ei mouse, which may be a very good
model for evaluating therapeutics.

The very bottom model is ectromelia. As
we've talked or people have alluded to in the
discussion, variola virus is specific for humans,
and the model that has been most often used with
variola is an IV challenge in the cyno monkey, with
about ten-to-the-eighth or ten-to-the-ninth PFU.
And you'll get the sense from the rest of my
comments, through the ectromelia side of things, I
don't think that IV challenge models have much
merit.

This is a slide that I'd like to spend a few
seconds on. And this is a distribution of the
sequenced genomes of variola virus from a
similarity point of view. And do not look at the
bottom lower portion, because we've talked about
that. But if you look at the larger figure and the
A grouping and the B grouping and C grouping, the A
grouping is the West African isolates of variola. This is all from Esposito's paper. The B grouping is the Brazil isolates, and this is this variola minor that you heard about. And the C grouping is the clade that contains most of the virulent vaccinia virus strains.

But what I want to point out is if you look here -- and I hope that you can see it -- there is the group of strains that are in magenta, and the case fatality rate -- you'll probably see it better on the handout. The case fatality rate is between 1 percent and maybe a little higher, and this is a second indication of the evolution of a variola minor-like strain coming from a separate clade.

So from this data, we know that these highly transmissible, low virulence variola minor strains have evolved at least twice, once in clade B and once in clade C. And they're all very, very highly related. And as Geoff mentioned, even though we have examples of these viruses, it's not clear what the genes are that are responsible for the high virulence.
This is a summary slide comparing mousepox with smallpox, and the similarities are listed above. Both require low inoculums to initiate a severe infection with high mortality. In both cases, the virus can be detected in the pre-exanthem period. In both cases, the primary lesion is likely -- in the experimental version with mousepox and in the natural infection with smallpox is likely in the upper respiratory tract as there is no primary lesion in the lung on autopsy of smallpox victims.

There is a rash that can be detected in both cases. In ectromelia, it's more strain and route-dependent. And both of these are natural pathogens for their host. The important comment there is that those host response modifier genes that I mentioned to you before, they will be tailored to the natural ligand if the virus is the natural pathogen of the host as opposed to a ligand that it may or may not recognize because of the evolutionary differences of the natural host.

The differences are listed below, and
there's really two. The first one that's often mentioned is the tropism of ectromelia to the liver, where variola virus does not have significant liver pathology. And I would only argue that the close relatedness of all of the orthopoxviruses to me suggests this is a true observation, but it's really an observation on top of a lot of commonality in the pathogenesis. Both have a predilection for lymphoid tissue and both have, as I mentioned before, very, very close evolutionary history from a similarity point of view, which usually means function to me.

The bottom point is quite important in that the mouse disease course is a lot shorter than in the human situation, with mortality occurring 7 to 12 days after infection versus 18 to 22 or longer for smallpox.

The mousepox models we've used are three. The A strain mouse gives a lethal disease by all routes of infection, with six to eight days for mortality to be observed. The LD50 is less than 1 PFU. What this means is that the mouse is a more
sensitive indicator for measuring infectivity of the virus, and if you do electron microscopy studies of the inoculating suspension to count particles and then relate the particles with the virions to lethality, you can convince yourself that the lethal dose is one to two particles in the A strain model. There's little or no immune response to infection; there's no rash. So it's a highly lethal model and both of the antiviral compounds have been tested in this model.

The SKH1 strain of mouse we evaluated because it is a mouse that lacks fur and allows you to study the evolution of the rash. In this model, we get an extended disease period of 10 to 12 days. The LD50 is about 100 PFU. There is a robust immune response to infection, and rash develops between seven and eight days after infection.

This is an example of an SKH1 mouse with a rash at 18 days. The mouse has recovered, but you can see it's a systemic infection with a well distributed rash.

The mousepox model that probably best
represents smallpox is shown here. Again, we have high mortality following a low volume virus infection of about 90 PFU for the LD50. If you give the virus by the skin, then the LD50 is a lot higher. This also is similar to smallpox in humans. As we heard in the discussion period, if you put variola in the skin, the case fatality rate might be less than 1 percent versus 30 percent if it's inhaled. So we have a similar kind of pattern here.

The mouse is very immunoreactive to the virus. And we can see rash, but it's really dependent on route and strain. And by the intranasal route, we don't consistently see rash, and I'll mention this later. Time to death is 11 to 12 days.

This is a schematic of the idealized infection or how I envisage the infection of mousepox. There's a very small introduction of virus into the cornified epithelium or, for experimental model the respiratory mucosa. And the virus replicates locally very similar to what Geoff
showed you for vaccinia virus. And during the replication and spread from cell to cell, it elaborates a lot of these host-response modifiers that I mentioned before. And the ligands that they target are shown in red in the slide. So at the very top you see the type I interferons are targeted, IL-1, IL-18, TNF and chemokines.

Some of these molecules are soluble. They're excreted from the cell. They bind the target ligand and stay soluble. In the case of chemokines, that would interrupt the chemokine gradients. The cells would not be able to chemotax to the site of infection, and others will actually bind to the glucosaminoglycans on the surface of the cell or the extracellular matrix and form up a shield of molecules that will sop up the host cytokines, and one example is shown in the next slide courtesy of Antonio Alcamí.

This first slide shows this protein -- I hope you can see it with the lights. But there's a faint green stain around the surface of the cell, and that is the type I interferon binding protein,
binding to an uninfected cell. So this protein has the ability to bind to the surface of the cells through GAGs.

This next slide shows you what we think is going on, or Antonio does. So in use here is a vaccinia virus as the example virus. And the dark cell in the middle, it is infected and is blocking the cellular pathways, as mentioned, by E3L and K3L, which target anti-interferon activity within the cell. And then this protein, the type of binding protein, is created from that cell. And you notice, to the left and the right, it's binding back to the surface of the cell, and there is absorbing the interferon alpha beta that's being synthesized.

So this would allow it to set up a foci of infection where the host response either muted or blocked, yet the foci infection is continually making virus which is leaving the foci and systemically seeding elsewhere. And so you can understand why I'm a little adamant about IV being sort of a route that would negate this ability of
the virus to set up a foci of infection early, and then that can be manifested in systemic spread below the detection of the host.

This next slide is a slide from Luis Sigal's lab, and it's just showing the same thing, but from an actual experiment. So this is a liver section through the liver from a mouse infected with Moscow strain of ectromelia five to seven days after infection. And the far left panel, where it says anti-luciferase or anti-luc, that is an antibody that is binding to a virus reporter. So all the green cells show you which cells are infected.

The panel to the right is in red and that is showing you the cells which are reacting to an anti-interferon binding protein. And when you merge the two, you see that there are a lot of cells that are clearly not infected, but are binding this antibody, which says that the viral proteins on the surface. So you see how this could protect the foci infection from the host response.

DR. CARGILL: Excuse me, Dr. Buller. You have three minutes remaining.
DR. BULLER: Okay. That's fine.

DR. CARGILL: Thank you.

DR. BULLER: Thank you.

This is a couple of slides just to emphasize the issue about the viremia being shortened if you give it IV. Self-supporting activity I talked about. The division molecules in the past slides is gone. The virus, if it's intracellular mature virus that Geoff spoke about, is rapidly inactivated, and so you're reducing your effective challenge dose. And you also end up jumpstarting the immunoresponse because these virions are coated with complement. And, of course, the virus is seen in different cells and the infection may be aborted or may be fully replicative, but through different cell types. And these are the references.

This is the intranasal route, and I'm just showing you -- sorry -- this is the comparison of intravenous with intranasal and to show you that the LD50 is different. This shows you that the level of virus infectivity is higher in IN versus IV, and, most importantly, the cell response to
generation CD8 positive T-cells with gamma in
response to an IV infection is a lot higher than
against an IN, again, saying that route is
important.

This is showing the IN route that we use and
the infection in the upper airways and in the nasal
turbinates. This is just to show the location of
the lymph nodes that we take out, and these are
analyses -- this is a summation of the mortality
from sub-Q versus IN that I spoke about, and it's
lethal by IN and resistant by the sub-Q. These are
infectivity titers in different organs. This is
the mandibular lymph node in the top left and then
different tissues from that.

Here is showing you that, in the mandibular
lymph node following infection, in blue you have
the IN response, And I put in here the sub-Q
response to show you that the interferon gamma
produced early correlates well with protection, and
you don't see that in a lethal model. Antigen
presentation is shown here. You don't see it
following IN infection, but you do see it following
sub-Q in the footpad. And this is one of the final
data slides just to show you that interferon gamma,
again, is earlier in a subcutaneous or protected
infection versus the intranasal, late. The right-
hand slide is just a marker of liver necrosis.

These are the biomarkers I mentioned. We've
looked at all of these, and of the ones that seem
to have a possibility of use, and I'll mention them
in a second, are measuring genomes and possibly
weight loss, we've extensively looked at trying to
make rash work in an ectromelia model and we can't.
We can show efficacy of the two drugs out to six
days post-infection, but the rash doesn't present
itself until seven to nine days.

From the public literature, there is another
example with rash being detected in the rabbitpox
model at five days. They can protect with ST-246
at four days. Death is at six. I'll be surprised
if rash could be used as a therapeutic indicator in
this model. The third model is monkeypox IN, in
prairie dogs, and there it showed very, very good
efficacy, a study from Inger Damon.
So two of the studies did not suggest that rash would be do-able in these models, and it may be that this could possibly be representative of a human situation that if somebody had smallpox and they're diagnosed based on rash, rash appears starting at 15, 12, 14 days, but really the characterization of the rash, smallpox and not chickenpox, can take up to a week. So you may not get to treating somebody until 18-19 days after infection, and that may be actually too late.

This is just show you a weight loss graph that suggests that weight loss might possibly be used as a trigger for intervention in this model, noting that we can protect at six days, and there's a good difference between infected and uninfected at that time point. And this just shows you the genome copies in saliva versus blood. Genome copies in saliva could also be used in this manner.

These are some references on what we've evaluated in this model, and then the conclusions are here. I mention that there are a lot of similarities between the two, that route is very,
very important, and we could not use rash as a trigger.

Then, finally, I think from my point of view that the therapy efficacy in a number of low dose respiratory challenge models will provide the strongest evidence of potential effectiveness against smallpox, and I don't believe that the cyno IV variola model adds to that.

Thank you very much.

DR. CARGILL: Thank you, Dr. Buller.

Dr. Moyer?

**Guest Speaker Presentation - Richard Moyer**

DR. MOYER: Well, first, let me thank you for the invitation to speak here today. It's a privilege for me. And I hope in so doing I can convince you of some of the values of the rabbitpox virus, rabbit model of poxvirus infections.

The genesis of these studies actually stems from the fact that when we were interested in really looking at host pathogen interactions, it became obvious about eight or nine years ago there were a plethora of animal models out there. And,
in fact, I would argue that even today, this is a problem. And I think the field, not only ours, but others, would benefit from standardizing the animal models, sticking with what they are, and that way everybody's evaluations of this and that would be relatively similar. But you can see they're all over the place in terms of dose, in terms of virus, and this is a very confusing thing.

So we decided to go back and look at this from scratch, and we asked, first, what makes a good animal model for smallpox. Well, it's pretty obvious. You need to recapitulate the essential elements variola, including infection pathogenesis transmission. You want a low dose of virus which progresses to lethal disease. You'd like to mimic the natural routes of infection. You'd like a prodrome. You'd like systemic dissemination of the virus, and you'd like a lethal infection.

Most importantly, the feature which you don't hear much about is this last one, which is a natural aerosol transmission -- natural aerosol transmission of virus in naive sentinel animals,
which, in itself, leads to progression to lethal
systemic disease.

Now, I'm going to have two main objectives
in my talk, and I want to tell you what they're
going to be. The first is to describe the
rabbitpox/vaccinia virus infection of rabbits. And
I would argue -- and there may be others, but I
would argue this provides a very simple and novel
way in which to evaluate new vaccines and
unexplored roles of antivirals, particularly in
controlling spread, which has not gotten almost any
attention. I think that's something that really
needs to be addressed.

The second part of my talk is going to show
how you can exploit these models to learn something
really kind of interesting. And in our case, it's
the elucidation of the role of the skin in poxvirus
infections. And what I'll try to convince you is
our findings illustrate the importance of the
scarification procedure itself, irrespective of
whether you have virus there or not, in providing
protection, and this information may support other
vaccine platforms in addition to those for poxviruses and, frankly, I hope I will convince you it's fortunate that scarification was used as a part of the smallpox eradication campaign. In other words, nature favors the ignorant.

So here is what was known in the literature before we got cracking on this. Rabbitpox virus is 95 percent identical to vaccinia virus and the smallpox vaccine. It was originally identified as a lethal epidemic in rabbit colonies in the 1930s in the Netherlands and little work in vivo has been done on this since the 1960s. And the classic literature also suggests that unlike rabbitpox, vaccinia virus is not lethal in rabbits.

Now, what caught our attention was the fact that it wiped out rabbit colonies, which argued very strongly, knowing nothing else, that there was a significant spread from animal to animal. So this caught our attention.

Now, what I'll attempt to convince you of is rabbitpox virus overcomes many deficiencies of other orthopoxvirus models. It's fatal in 18 to
14 days, so it's shorter than, say, variola would be; but if you're paying animal fees, this is not a bad thing. And it generates systemic disease with virus in liver, spleen, lymph nodes, along with blood. You get dermal secondary lesions, severe respiratory disease, and, most importantly, you get natural aerosol transmission between rabbits.

Now, here is the way we chose to do these, and this goes to my argument about standardization of how to do this. We use rabbits, 9-week-olds, fairly young. Both flanks are shaved. There's an intradermal injection.

Now, why do we do this? We did this because we wanted to be absolutely sure, as much as possible, that we controlled the dose that the rabbits got. And that's one of the major problems with intranasal injection. You know what you're adding, but you don't really know what's going in there. Also, you'll see in a few minutes that you don't have to worry about this causing lethal intranasal infection, because it surely does.

Okay. Rabbits are modified daily.
Euthanasia criteria are defined by the NIH and we obey them, and that is our indicator of death, even though it's not death.

Now, this is a rabbit infected with rabbitpox virus intradermally at ten-to-the-third PFUs. And you can see the progression going along the top here. From day 3 to 5 to 7, that's primary lesion development, and viremia scored very nicely in the rabbit by shining a light behind their ears.

You can see, as indicated by the arrows, I'm not sure how well you can see it from there, the secondary lesions become very prevalent from day 5 to day 7. The rabbit begins, occasionally, depending on whether they get secondary supervening bacterial infections, they begin to get all signs of respiratory illness. The lungs are toast. These are hemorrhagic lungs; you can see in another picture there. And, of course, you don't get this kind of infection unless you get dissemination within the animal because no animal ever died of injection of virus into the skin. So that one is one of the criteria that is immediately fulfilled.
by this model. All right.

Now, this is a quantification of that infection, and if you do it the way we did it, it's perfectly reproducible. We've done it. Others have now done it, and they get exactly the same results. The disease itself is heralded by a temperature spike, beginning about day 3 to 4. Weight loss begins to drop at that time. Survival begins to go down at day 8 to 10. So the rabbit has essentially been gutted and is moribund within eight days after infection. And I should add, even though we use 1,000 PFU, just for convenience sake, the lethal dose is well below 100. We do this simply because it's convenient and doesn't matter in terms of the disease we produce.

On the bottom part of this graph is a time course of development. Secondary lesions appear about day 5. There's a collapse in temperature prior to death, an interesting feature. Severe respiratory distress begins about day 6, and the rabbits are pretty much gone. This N is 25. It's probably now closer to 75 since this graph was
So to summarize, 100 PFUs is 100 percent lethal within nine days. We've never had a survivor; little difference in pathogenesis, frankly, other than speed between ten-to-the-second and ten-to-the-sixth PFUs. Severe respiratory distress we don't always see. Sometimes the rabbit die before they get it, but it's not often.

Hemorrhagic lungs, the titers in the lung of the virus are out of sight, ten-to-the-seventh to ten-to-the-ninth PFU per gram. But it raises the question, can the virus be naturally transmitted from animal to animal.

Here is how we tested this. We have two models for this. One is non-contact transmission. What I have here are two cages. On the left, as you see it, are the sentinel animals. On the right are index animals, and that double arrow in the middle is a space between the two cages. In addition, we have what we call an inter-lapine aerosolized poxvirus dissemination device, which is a $10 fan from Office Depot, which sits on the side
of this cage, and we don't blow enough air to simulate a Category 5 hurricane, where the bunnies are hanging on the cage with their paws. This is just a gentle breeze lofting from the index animals to the sentinel animals.

So it's a six-inch separation. The index animals are sacrificed at eight days post-infection and gotten out of there, and this is an experiment we originally performed in quadruplicate and since many other times.

What you see is beginning somewhat later, which is what you would expect, because the rabbits have to be viremic and have virus in the lungs, presumably, to get the spread. Beginning about day 5 through day 13, the disease begins to develop in the sentinel animals.

The lungs are shown on -- we don't have that. Oops, it doesn't show on this one. There it is. The lungs on the right show that there's considerable damage in the lungs, as you might expect. And so the aerosolization and the aerosolized spread is totally natural with about 30
to 40 percent reproducibility; 30 to 40 percent of
the sentinel animals will get sick.

Now, this is not very efficient if you're
studying this in a laboratory. It just is costly.
So what we decided to do is see if we could develop
a rabbit-to-rabbit transmission with animals in the
same cage.

Now, what I don't show you here is these
animals were fitted with Elizabethan collars so
they could not nuzzle each other, they couldn't do
any of these things. The bedding was changed three
times a day. So I would argue that this is a
simulation of an aerosol transmission, but you
can't absolutely rule out some weird kind of
spread, but I'm convinced it's aerosolized.

We did this in a similar fashion. We had
animals in the same cage. One animal was infected
with 1,000 PFU. The index animal was sacked at
eight days, and this was performed repeatedly.

Here is an observation of what we saw, and
this compares contact versus non-contact sentinels.
And you can see by just perusing and comparing non-
contact to contact that the symptoms develop with
roughly the same kinetics as they did when the
animals were in separate cages. There's not much
difference at all.

What the advantage of this is, as shown
here, is in contact transmission -- I use that term
loosely; I don't think it's contact, animal-to-
animal spread, I think it's aerosol -- you get
100 percent of the sentinel animals infected. So
it becomes a reproducibly tractable system for
studying effects of both vaccines and antivirals on
the process of spread, which is very useful.

It's 93 percent lethal. We had our first
survivor of rabbitpox ever in this experiment.
Non-contact transmission is roughly, as I have
here, 31; it's roughly 45 now. It has also been
100 percent lethal. And like the non-contact
animals, the first sign of infection is a huge
temperature spike.

So, in summary, the rabbitpox virus model
capitulates many of the essential features of human
smallpox, extremely low infectious dose. And by
the way, to get aerosolized infection naturally, the relative effective infectious dose to sentinel animals has got to be extremely low. That's important. You get lethal systemic disease, natural aerosol transmission. And facile aerosol transmission, as I said, suggests that the infective infectious dose required for transmission is very, very low. And this model has been used by a number of people to study their favorite antiviral drugs.

Now, what's interesting about this -- and I'm going to shift to part 2 here -- is if you review the literature, there's a dichotomy, and the dichotomy is shown here. Remember, rabbitpox and vaccinia are 95 percent identical based on sequence. They aren't that dissimilar.

Now, rabbitpox virus is fatal. That's funny -- do you have funny lines, too? Yes. I have funny lines -- fatal in eight to 14 days, systemic disease, natural aerosol transmission, but vaccinia virus, the literature is replete with the various experiments that say exposure of vaccinia,
or rabbitpox, for that matter, we've done that, all
the animals survive. That's what the literature
says. A single lesion heals in eight to 21 days.
You get protection. In other words, the rabbits
are immunized against subsequent infection. And
rabbits, of course, are used by researchers to
generate vaccine and antibodies all the time. So
what's going on here?

So we decided to look at what was going on
with vaccinia. So we got WR and we did an
intradermal infection. And lo and behold, the
animals went down like stones, just like they did
with rabbitpox virus, in total contradiction to the
literature. And this just shows you the profile of
that infection. It's very similar to what I showed
you for rabbitpox. Primary lesion on the top.
There's the viremia. They make much better
lesions, for some reason, than does rabbitpox. And
you can see the animals begin to crunch up and not
look so hot at about day 8.

So what was surprising is the infection was
actually virtually indistinguishable from that we
saw with rabbitpox virus given intradermally. And these are the kinetics. They look really pretty similar. I won't belabor them. That's the point.

So we thought to ourselves, well, how can this possibly be? Okay? So we began to wonder about the route of delivery of the virus. And if you go through the literature on these viruses that precedes 1970, everybody pretty much introduced vaccinia into animals by scarification, except for extreme models, which nobody uses anymore.

Now, intradermal injection is an interesting thing. It's very, very shallow. It's just under the epidermis. In fact, you can see you typically get a bubble of liquid when you first inject it. That's an indicator you got it right.

Now, scarification on the other hand, you take a needle and you jab it like crazy into the skin and if you're a human getting immunized, as you have to in the laboratory, against smallpox, they do it until you bleed. So it goes through, all the way through, and you get little drops of blood.
So the next experiment we did was look at vaccinia scarified into the animals. The infectious dose was five-times-ten-to-the-ninth, which is seven logs higher than we typically use for intradermal. Now, this was administered through a bifurcated needle, and we can all do the math. There's about 10 microliters or so in a bifurcated needle, so these animals are not getting five times ten-to-the-ninth PFUs, but they're getting a lot, and a lot more than they would get through the intradermal route we typically use. And here you see it. It's exactly what was reported in the literature.

You get minimal secondary lesions. These lesions heal quickly. Just exactly what it says in the literature. Totally different outcome than we see when we do this intradermally.

So this is kind of a quantification, in crude terms of that, comparing the disease and survivals that you see in terms of intradermal at ten-to-the-third and scarification at ten-to-the-ninth, intradermal. Now, this is with vaccinia,
these particular experiments, not rabbitpox. You see the dose is -- most of them die with vaccinia, but with scarification, we lost one animal out of about 30. And the average clinical score, which is a composite of temperature, eating habits, urine, feces output, a whole bunch of clinical parameters, respiration, is much, much, much more severe when you do intradermal injections than you do with scarified.

So for some reason, the act of scarification -- not the act, but introducing the virus via scarification leads to a totally different outcome than when you do it intradermally.

Now, this just shows you that scarification, per se, does not impede dissemination of the virus. And I won't go into this anymore, because of the time restrictions of this talk, but we find very little difference in dissemination from the primary site to the organs. In fact, most animals that are scarified, as I did, actually develop some viremia and secondary lesions, but the animal doesn't care,
and they clear. The animal is fine.

So, now, why does scarification result in such a mild disease despite ten to 1,000-fold the amount of virus delivered? So we asked the question, does the act of scarification itself induce an immediate nonspecific response, like inflammation and swelling at the inoculation site, and does this very early response alter disease pathogenesis.

Here is how we did this. First, scarification, we noticed very quickly, introduces inflammatory cell infiltration. And you can see by a comparison of these upper left two panels, at 48 hours, there's extensive inflammation, inflammatory influx into the primary site, and, intradermally, there's basically nothing. And at 72 hours, it's kind of the same, but it's this very early response of scarification, very quickly, that introduces a nonspecific influx of cells. So we like that.

So what we did is we did an experiment called a ring scarification experiment. I want to
explain this, because I think it's important. What we did was first we scarified the skin in a circle in about a 2-centimeter ring with no virus, just PBS. And then we intradermally injected ten-to-the-third PFUs, which is our standard lethal dose of vaccinia, within the ring, which would go into that -- which would give essentially not at where we scarify, but within the region surrounding the region that was scarified.

We did this in two ways. In the first way, we scarified first and intradermally injected, and then we turned it around and intradermally injected first, and then we followed that with a scarification. And I'm talking about quickly, within two to three minutes of each other, very quickly, about as quickly as we could do it.

Now, when we did this, first, we scarified first, because we didn't know any better, and intradermally injected, and most of the rabbits survived. This is scarification with an empty needle.

Now, this graph compares the differences in
the order, and, remember, these are within two to three minutes of each other. And what you can see in the triangle line on the left, which is survival, that if you ID first and then scarify, that the rabbits die, essentially. But if you do it the other way around, you have a very high percent of survival, and that's also reflected in clinical score.

So what does that mean? What it means is that there is something going on very, very rapidly through the act of scarification that has a very significant impact. Now, we were lucky, and we were lucky in the sense that it happened so fast at the site of inoculation, there was no time really for any cellular influx.

So what's going on there is due to cells which are resident at the site of inoculation, at least that's our theory. So what's there? Well, we didn't know. But what we did, it turns out, Agilent has a rabbit chip which has not been marketed, but you can get it if you beg, plead and borrow. And there are about 30,000 genes on this
chip. So what we did is we isolated the lesions, isolated the RNA, et cetera, et cetera, et cetera, and we probed these chips to see what was going on and whether we could see anything very rapidly after infection.

Here are the results. Don't comment on the orange and blue, that's Gator colors. Anyway, what happens, on the left, orange, which is intradermal, scarification on the right, orange is like controls. So there's really no difference that you can see in this lower half of the figure up to about 24 hours, and certainly 24 hours before things begin to kick in. But if you look at the scarification, within four hours, there's been a major gene change in terms of expression at the site of infection. It's like lickity-split; and so, whatever scarification does, it triggers a whole change in the gene schema, which has an effect on whether these infections are going to be lethal or not.

So what are the genes? Well, this is the one of the most interesting listings. And I call
your attention to the red brackets, are the most interesting genes within the collection. And what you can see is a bunch of complement components that are massively upregulated. There are some interleukins, like interleukin-1 beta, which is almost a hundred-fold upregulated, and things like that. Interleukin-1 beta, interleukin-6, and interleukin-8 are upregulated extremely rapidly. There are some major histocompatibility antigens which are upregulated, and there are toll-like receptors out the gazoo, and TNF, all which are upregulated just like that. And I would propose that it is that process which confers -- the manifestations of that process which are what determine whether the animals are going to survive.

So what's there? Well, the most likely cells we think that are responsible for this are the keratinocytes. These are the outermost layers of cells and skins. They are involved in host injury to cutaneous injury, namely, burns, UV light, and, of course, scarification, which is an injury. In response to injury, the cells'
activation are known to activate massive changes in transcription via NF kappa beta, and they harbor a variety of goodies that are resident within keratinocytes, which include interferons, toll receptors, and other antimicrobial substances.

Now, if you go to look at the molecules that are expressed by the keratinocytes -- and I'm not going to go through them all; you can't read it, and it's a long list, and I'm time constricted -- there are the interleukins at the top, the interferons are there -- we didn't see that, actually -- toll-like receptors, other receptors. It smells like what we saw when we did the arrays. So it doesn't prove that they are keratinocytes, but I would suggest that it is the keratinocyte with a minimum of data.

So what we think is going on here is when you scarify animals, even with an empty needle, that you break the skin barrier and you induce this response of the keratinocyte -- we'll say keratinocytes -- to get these influx and rapid upregulation of a number of these genes, which are
immunomodulatory and actually serve to control the infection. And those components then act on these resident cells, which are up here in grey on the left, to mediate their effects.

So, in summary, what I've done is I've tried to convince you that the rabbitpox model recapitulates most, if not all, the essential features of human smallpox infection. Now, I didn't present it, but we have -- you've seen, for the natural spread, we've done it more controlled with intranasal infection. There it's as lethal intranasally as it is through the skin intradermally.

Intradermal injection of vaccinia is lethal, unlike what we had expected to see based on the literature, and that's certainly contrary to historical reports, but it does put it in line with what we see with rabbitpox. And what it emphasizes is how important it is to standardize these models so that everybody is on the same page.

Scarification of vaccinia is not lethal.

The physical act of scarification for its
protection of infection by what we propose to be a 
keratinocyte response, and we propose that so that 
everybody can get out the bazookas and shoot us. 
It is fortunate, however, that scarification, where 
it's used, is a normal part of the eradication 
program. Who would've thought? I wouldn't have, 
but it turns out that may be a very important 
control of a successful vaccination, at least as it 
was originally done.

Does scarification have a role for 
administration of vaccines and other agents? And 
the last point I would argue, which is not shown on 
this slide, is the rabbit model for animal-to-
animal spread offers a very nice, convenient way to 
evaluate antivirals on that process. And I would 
predict that the amount of antiviral -- I don't 
care which one you're using, the amount of 
antiviral drug that you need to control spread is 
far less than you would need to control the 
infection in index people or animals because the 
infectious dose for spread is so low.

So thank you, and I'll be happy to answer
questions, if there are any. I'm done.

DR. CARGILL: Thank you Dr. Moyer.

Mr. Mucker?

Speaker Presentation - Eric Mucker

MR. MUCKER: Thank you. I really appreciate the chance to tell you about susceptibility of marmosets to monkeypox virus and what we've learned since the last presentation, which, to be honest with you, it really isn't that much, but here we go anyway.

So the question is why develop another model, and the answer is kind of obvious. The current models utilizing variola and monkeypox require an unnaturally high dose to induce a severe disease. And, again, this contradicts what we know about variola in that they think it's somewhere around 100 PFU, 1,000 PFU.

I'm not going to get into the actual routes because I think each of these routes have their place, depending on the indication, depending on the real world scenario. But we've decided that maybe we can do better, maybe we can lower this
dose, maybe we can get something that looks more
like an ectromelia-like or a rabbitpox-like model
in the dose being that low. So in conjunction with
that, we're hoping to get a more extended
incubation or more extended asymptomatic period,
which would be, again, reflective of what we know
about variola.

So we decided to go somewhat of a different
route. Instead of looking at maybe different
isolates of monkeypox or maybe changing from a cyno
to a rhesus, we decided to go with, hey, let's try
a new world marmoset or a new world primate and
marmoset.

The reasons for choosing a marmoset were if
you go back in the literature, back as far as 1982,
there was an outbreak of what they termed a
tanapox-like species that was in a colony. And I
just want to point out that the tanapox did not
kill the primates, and this goes to the differences
between poxviruses, because what I'm going to say
next is the fact that there was a cowpox-like
isolate in a new world colony that basically
obliterated the colony. And now they use it in the Cal-Pox model, C-A-L pox, and in marmosets, and that's the Matz-Rensing and Kramski papers that are cited here. And there's also a newer Matz-Rensing paper that's not cited on here that goes more into the pathology. But the main point being that there are differences between poxviruses, and the marmosets themselves, at least in terms of something like tanapox, can mount a response and survive.

So what are some of the other advantages to marmosets? One of the nice things about marmosets is they're small. They require less test article. So when you're evaluating compounds, you could do so more economically. Any absorption issues you may have in a macaque model -- I should say absorption/metabolism issues, and I think this will become evident with CMX001, where they were having some -- I think there were metabolism issues in cynomolgus macaques -- this provides another avenue for those compounds.

There's obviously a couple more listed on
the "why use marmosets," but I'm going to go over to the "why not," because these are questions that most people like to ask.

The biggest thing is bio samples. You do not -- these animals are roughly 400 grams. You do not get a lot of bio sample off the animals. And my response to that is usually that they're smaller, you can house more of them, you can arrange to collect the bio samples you need in your design.

The limited reagents, I don't consider them limited reagents. I consider them untested reagents. If you go to NIH's website, they have reagents for different nonhuman primate species, and they're slowly becoming available -- well, I should say tested. Some of the kits have been out for other animals, for humans, for whatever.

One of the problems we ran into was the special diet and housing. This actually took a long, long time to get up and running. But I don't know if other facilities would run into the same issues that we ran into, but that is actually a
consideration.

Our design was actually kind of simple. We have been working with the IV model at USAMRIID, the IV macaque model at USAMRIID for -- well, since I have been there. That's been like 10 years or so. And I know they were working on it before then. But the idea was to recapitulate the IV model, switching up a host, and that's basically what we did here.

Our major correlate was mortality. To me, it doesn't get any more severe than that. And the basic design was an N of 3. And, again, we used the same virus that goes into our IV macaque model. We looked at hematology, quantitative PCR. Again, the quantitative PCR assay is the same assay. Weight, temperature, and, again, this was every three days, and this was due to the size of the animals and kind of the unexpectedness of knocking these guys down every day.

Just real quickly, I want to orient you to this figure. This is basically a summary of the study. I don't know if I mentioned it or not. We
really consider this study more of a pilot study, a
stepping stone, but I just want to reemphasize that
if I didn't say it previously.

So anyway, in this figure, this is basically
a heat map. If you look, the darker color, the
later in the day for each of the columns, and these
are some of what we consider correlates of
infection, so lymphoadenopathy, rash, viremia, and
death.

On the far right, you'll see how the animals
were grouped. On the far left, there are the
individual animals. At the bottom is the range for
each of the groups. And at the very bottom of the
slide is the mean of those values you see in the
top right of the slide.

Where I want to call your attention to is
basically the 48 PFU at the mean -- let's see. So
the 48 PFU, and you'll see that we are basically
asymptomatic for -- depending on whether you're
going to consider viremia. And when I say viremia,
I do mean QPCR. We did confirm in the 48 PFU that
you do pick up virus, but those numbers haven't
been put together yet. So I just wanted to say
that is DNA via QPCR.

But anyway, the length of time these animals
remain asymptomatic is closer -- I'm not going to
say it's exact -- is definitely closer than what we
could do with the IV models and any other monkeypox
or variola model. And we're looking at basically a
range of about 8. Again, if you're looking at
viremia, that's not really an outward clinical
sign, but as late as 11 for lymphadenopathy, which
is a sign of monkeypox, but maybe not so much
variola. And we are getting death as late as 15,
but, on average, about 14. Again, this is an N of
3, so more studies have to be done. But, again,
this more or less aligns with what we know about
variola in that you remain asymptomatic for at
least eight days or as long as 14, 17 days.

I'm not going to beat this over the head too
much. I just want to point out a couple things
about the survival. Number one was we actually had
to skip a group because of the intensity of the
infection. We lost animals roughly about day 8 in
the first group. So we actually skipped a dose.

So that's why there's not -- there's a dosing group missing. It's not exactly a log reduction.

The second thing I want to point out, too, is, again, this is an N of 3, but how closely these animals succumbed to disease with one another. You're looking at within a day, for the most part, except for the highest dose. Again, these animals went out to day 15.

Some clinical parameters. We looked at temperature, and temperature was on, for the most part, an every three-day basis. And in hindsight, we could have designed this better. We could have used the drop-ins where you can actively, in real time, gather temperature and activity data. Because marmosets tend to fluctuate temperature so much, whatever temperature increase we actually saw in the data is taken with a grain of salt. And even with the data the way it is, there's no real discernible fever. We did see a decrease in temperatures as the animals started to succumb to disease.
In terms of weight, animals fluctuated about 5 or 10 percent throughout the study, with the exception of a few animals, and it varied. There were some in the highest dose group and there was, I think, one or two in the middle-of-the-road dose group. They probably gained weight because they were retaining fluids or they had some sort of fluid imbalance.

In terms of QPCR, strikingly high viremia or DNAemia, we were looking at somewhere between ten-to-the-ninth and above. So I think it was as high as eight-times-ten-to-the-ninth. This is roughly two logs above the IV macaque model. Again, I didn't mention it, but in the other groups, this is dose-dependent. You could see the shift in the curves, shift in days, with 48 PFU, the black lines, being able to detect that about, on average -- we had one animal at day 6 and two animals at day 9 detectable above the limit of quantification.

Hematologically, like what -- if you dig through the literature a little bit, but like you
would see with variola in I think it was Akita in 1925, is mobilization of white blood cells. There wasn't really much more than that that I could pick up from the literature, other than this increase in lymphocytes and white blood cells seems to -- the increase is actually more pronounced in early hemorrhagic cases.

The last hematological parameter I'm going to talk about is platelets. We saw acute thrombocytopenia. Animals dropped -- and these are actually the values themselves, but they dropped a huge percentage, some of these animals going from 700 or so platelets to less than 100. And, again, this has been described in the literature for human smallpox, moderate to severe cases of smallpox and especially hemorrhagic cases of smallpox. This is a feature.

What we saw in conjunction with this decrease in platelets is these cutaneous manifestations, which are very hemorrhagic in nature and, with that in mind, were also dose-dependent. Basically, the higher the dose,
the more generalized, the more bruising effect we had, where decreasing the dose, you got more discreet type petechial ecchymosis. And with the disease course in mind, the manifestations, the rash around day -- anywhere between day 9 to day 14 and the quick onset to death, the decrease in platelets, and now the cutaneous lesions, it looks a lot like early hemorrhagic smallpox. And in this figure, left is the marmoset, the trunk of a marmoset, and on the right is actually some pictures I pulled up off of the PHIL website, and, as you can see, they look fairly similar.

Histologically, the lesions basically look like variola or monkeypox lesions, with the exception -- and I'm going to draw your attention more to the figure on the right. Most of the skin that was taken had this hemorrhaging in the dermis, which is not a pronounced feature of ordinary smallpox. But other features, such as the epithelial proliferation, the degeneration necrosis, crust formation, was seen in certain sections of the skin.
From an organ side, basically, there were some classical monkeypox-like lesions, again, in the testes, in the esophagus, lungs. You can see the necrosis on the lungs. These, again, are what we typically see in the IV macaque model.

Things that were not -- I'm not going to say they're not present; I'm just going to say they're not as pronounced and much more aggressive in the marmoset model is, again, this hemorrhaging effect, this subpleural hemorrhaging in the lungs, submucosal hemorrhaging in the urinary bladder, and scrotal hemorrhaging.

In terms of the liver, from a gross standpoint, it basically looked enlarged, pale, friable, nothing really that amazing. From a histological standpoint, it was basically inundated with intracytoplasmic inclusion bodies. There was degenerate to necrotic hepatocytes, as well.

Again, we looked at these intracytoplasmic bodies to make certain they were not an artifact or anything crazy, and it turns out that, yes, these are viral factories, and you're basically looking
at a virus, orthopoxvirus.

So, conclusions. Again, we have by far the lowest dosed model utilizing monkeypox. It is uniformly lethal, and, in these aspects, along with the incubation period, is a lot more reflective of smallpox. We do see these high genome levels in the blood. I could probably argue that one either way in terms of is that really more useful than something that has ten-to-the-eighth genomes in the blood. But in terms of your assay, that'll probably strengthen some. Any variability in terms of, like, drug treatment and whatnot, the more you have above your limit of detection -- or your limit of quantification, I should say.

I mentioned some of the classical manifestations, the epithelial changes in the skin, the testicular inflammation, the pleura inflammation. One thing that's not on here that I actually just received the e-mail from my pathologist is the lymphoid tissue. Basically, there was lymph node hemorrhage, and in 17 of the 18 monkeys, the one monkey being a very abbreviated
course because it died of something unrelated to
the pox infection -- but in 17 of 18 of these
animals, there was some hemorrhaging in one of the
lymph nodes, whether it be mandibular, axillary, so
on and so forth.

So some of the things we don't normally see
in typical or ordinary smallpox or the IV model is
the hemorrhage. It's basically the intensity of
the infection and the pronounced features within
the liver. It's not that -- again, in the IV
model, you get no lesions in the liver, and I
should say IV macaque model. It's not that you
don't get lesions in the liver; it's that these are
a lot more pronounced. This is a lot more
aggressive.

Again, I've kind of put the argument forth
that this looks a lot like early type hemorrhagic
disease, but there's obviously some other things we
would like to do if we were to move forward with
this model, which leads me to what the future
holds.

Again, we need to wrap up, complete the
pathology. I think we're probably going to skip
the plaque titrations of tissues, since these
animals were not perfused. I'm not sure what
that's actually going to tell you. And since we
are doing IHC, or immunohistochemistry, we'll
actually know what's going on in each tissue.

I don't know if I really hammered it home,
but we sampled every three days. What we like to
do is set up groups to sample -- to kind of fill in
those sampling gaps. And I did mention before
about future studies involving some sort of
telemetry-like device.

The main thing, too, if we're going to
continue on down the monkeypox pathway, we'd like
to alter the route. Again, the IV model was a case
in point, a stepping stone for something that,
again, looks more like variola and what we know
about variola, and that would be a natural route.

Again, this model hasn't been through any
series of treatments or we haven't tried
vaccinating the animals. So, again, this is the
new kid on the block. But if, basically, I could
say I could pick one thing on this slide to try, it would be the last one I have on there, and that's variola virus. I think if you are going to license any antiviral and state that it is active or its indication is against smallpox, that you should test smallpox. Until we get to a point where we're actually doing something with the host itself and not the virus, I think that's going to have to be the way.

A lot of people I'd like to thank, and I notice one or two people were left off here. I'd like to thank Peter Jahrling. He actually got us our first round of marmosets to use. I'd like to thank Brett Taylor, who, between Brett Taylor and Suzette Tardif, basically got us set up at USAMRIID to use marmosets, and Kay Jordan, as well, and to the viral therapeutics branch who helped me out with these studies. The person not on here is our pathologist who we're using in IRF, is Lou Huzella.

Thank you.

DR. CARGILL: All right. Thank you, Mr. Mucker.
Our next speaker will be Dr. Challberg.

**Speaker Presentation - Mark Challberg**

DR. CHALLBERG: Thank you. I'm Mark Challberg. I'm the program officer for extramural poxvirus research at NIAID.

I'd just like to start out with a couple of acknowledgements. First of all, I'm going to describe work that's a very big project, too big to be carried out at one lab. And so it was carried out at the four laboratories that I've shown here by the indicated investigators.

This work was designed and overseen by my colleagues at NIAID, listed here. And I really need to acknowledge at the outset that this work is based on and certainly informed by a lot of work that had been done previously at USAMRIID by John Huggins, Peter Jahrling and his colleagues, and that work, as you just heard, continues today.

So just a little background. NIAID initiated the monkeypox model development program in 2004 to support development of next generation medical countermeasures. We selected monkeypox
amongst the available orthopox models because monkeypox causes disease both in nonhuman primates and in humans that is at least superficially similar to smallpox in humans.

I think it's important to note that the disease that monkeypox -- well, that monkeypox is not typically a disease of monkeys. The natural reservoir is not monkeys. But natural infection of monkeys does occur in the wild, and natural infection of humans does occur in the wild.

Now, at the time we started this work, most of the experience with the NHP orthopox models, both monkeypox and variola, used the IV route of exposure, but in discussions with CBER, expressed interest in evaluating respiratory routes of exposure to support vaccine development. So we decided to carry out a series of studies using the intravenous, intratracheal, intranasal, and aerosol routes to see if we could find the best model for evaluating countermeasures that recapitulated many of the aspects of human smallpox.

So here's the overview for the rest of the
talk. I'm going to talk briefly about some characteristics of human smallpox that you've already heard probably ad nauseam just as a way of evaluating animal models. I'm going to talk about the development of these nonhuman primate models comprised of monkeypox virus infection by different challenge routes. And I'll briefly talk about the use of one of the respiratory route models to demonstrate efficacy of an antiviral drug, and then I'll have a couple of conclusions.

So here's a slide that was put together originally by Dr. Henderson and Dr. Breman, published in the New England Journal, and it summarizes clinical manifestations of smallpox, and I just want to make a few quick points.

First of all, as you've already heard, the natural route of infection is by a low dose of variola virus into the upper airway. Following infection, the virus replicates in the infected cells and then spreads out to various organs throughout the body. While that is happening, the infected individual is basically asymptomatic; so
there's a long asymptomatic incubation period while a lot of virus replication is going on.

At the end of that asymptomatic period, there's a brief prodrome, which is an elaboration of nonspecific symptoms, fever, malaise, and so forth, and then, finally, the elaboration -- after the prodrome, the elaboration of smallpox-specific symptoms, and mainly what we're talking about here is a characteristic skin rash.

I'd like to say a little bit more about the skin rash. As Dr. Styrt mentioned this morning, or earlier this morning, clearly, the relationship between number and type of lesions and the severity of disease is complex, but I think -- but one can make a few general points.

If one looks here at simply what's going on with ordinary smallpox, which in this outbreak comprised some 80 percent of the cases, when you go from ordinary discrete to ordinary semi-confluent and ordinary confluent, where basically the difference between these is really just the number of lesions and kind of the diminishing space
between lesions on the skin, that case fatality rate goes from around 10 percent to upwards of 60 percent. So clearly there is a relationship in ordinary smallpox between the number of skin lesions and the severity of disease.

So, to summarize, we think a perfect animal model would include these characteristics via low dose of infection by the respiratory route, along a one to two-week asymptomatic incubation period; systemic disease with multi-organ involvement, characteristic skin lesions, and in such a way where the severity of disease correlates in some way with the severity of rash.

Finally, and I think this is important, we view this last item on this list as sort of a reality check for animal models. The one thing we know about smallpox is that it was eradicated using a very efficacious smallpox vaccine. So I think it's important that we don't set the bar so high in an animal model or make the disease so severe that we can't protect with a vaccine that's known to be very efficacious in humans.
So all these studies used cynomolgus macaques. We carried out dose studies to characterize the dose response, reproducibility, disease progression, and pathogenesis of each route. We began the study of each route by range-finding studies, basically, dose ranging studies, to assess disease progression, and we wanted to ask whether it was possible to use telemetry or other clinical observations to characterize what was going on.

At the end of the dose ranging studies, we carried out serial sacrifice pathogenesis studies, and, again, this was a large project. It was not possible to carry out all the studies at one lab. So we had each of the respiratory routes evaluated at a single lab, but all labs participating in this study carried out an IV challenge study just so we could make sure that every laboratory got approximately the same results when they did the same experiment.

Initially, these studies were designed to establish challenge doses that resulted in severe
disease in 90 percent of the animals. When we started, we really didn't quite know what to look for. We were afraid that if we used mortality as an endpoint, that we would set the bar so high that we would not be able to protect either with vaccine or therapeutics, so we decided to use an endpoint of severe disease. But at the same time, we also realized that mortality as an endpoint is complicated by the fact that euthanasia decisions basically have to be consistent with the policies of each facility's sensitivities and IACUC sensitivities. So it's a little bit hard to make them completely consistent.

As I mentioned, all the animals in the dose ranging studies had telemetry implants to monitor temperature and heart rate and so forth, and we were trying to look for a way of evaluating the severity of disease in real time using these telemetry implants. That wasn't incredibly successful, I think. Of course, we also collected information on clinical signs, clinical pathology, and so forth.
So what do we mean by severe disease? The top four bullets were the definition of severe disease when we started, basically, death or moribund sacrifice, of course; overall clinical assessment using a standardized clinical assessment score sheet; and a severe rash. And any one of those features would part of the definition of severe disease.

As we went along with these studies using the respiratory route, we realized that one of the characteristics of infection by the respiratory route in this model is severe respiratory symptoms. So we also included the final bullet, which was designed to capture severe disease manifested in a respiratory infection.

We used uniform challenge material. In fact, all the challenge material that was used by all the labs was made at a single facility. This was the Zaire '79 strain that you've already heard about, isolated in 1978 from a 1-year-old boy who was severely ill, but who did not die of the disease.
Sequence analysis of this virus supports the assignment of this strain to the Congo Basin's clade of monkeypox, which is thought to be the more virulent of the two known strains of monkeypox. And we prepared two master and working stocks from independent scab material, and, basically, there was no difference between the results we obtained with each stock.

So here's an example of the range-finding data using the IV route. As you can see, there's a nice dose response curve. At low doses, there was essentially no -- none of the monkeys got severe disease, and as doses increased up to one-times-ten-to-the-eighth, essentially, 100 percent of the monkeys got severe disease.

The other thing to note -- a couple things to note on this slide. First of all, that using the IV challenge route, there is at least a general relationship between the number of lesions that manifest and the severity of disease. So when you go from ten-to-the-fourth, where there's very few lesions, no severe disease, to ten-to-the-eighth,
where there's many lesions, 400 is clearly an underestimate. They just stopped counting after 400. So there is a relationship here between lesion number and disease.

The other thing to note here, and this turned out to be true in all the studies, is that in the end, there really wasn't much of a difference between severe disease, as we defined it, and mortality. So almost all the monkeys that were defined as having severe disease also died or were euthanized. You can see there are a couple of monkeys here that we were defined as having severe disease that did not die, but that generally was not what we saw.

This is an example of the aerosol -- and I'm not going to -- don't be worried. I'm not going to go through all of the challenge routes, but I am going to sort of focus on the aerosol route because it turned out to be a very reproducible way to cause disease in these monkeys. And the other thing to point out is that while NIH certainly were probably most concerned with human-to-human
transmission of variola, it's also true that if variola is weaponized, then infection of humans will probably be via the aerosol route. So we thought it was important to focus, I think, in the end, among the available respiratory routes, on the aerosol route, because that was at least a potential method of infecting humans at some point.

Again, there is a reasonable dose response by the aerosol route. In this case, at the lower infecting dose, all the monkeys got severe disease, at least by the updated definition of severe disease, but not all of them died. The other thing to note here is that unlike the IV route of infection, there is not really a correlation between disease severity and lesion number. In fact, if anything, there's a reverse correlation. And I think this probably reflects the fact that at the very high doses, the animals die from respiratory symptoms before they have time to elaborate skin lesions. It's basically a race, and as the dose goes up, respiratory symptoms start to win out over skin disease.
As I already mentioned, the aerosol route is very reproducible. We were a little bit worried about that when we started. This just shows the actual presented dose given to all of the monkeys in the serial sacrifice study that was done by the aerosol route. The target dose here was one-times-ten-to-the-fifth. And the mean presented dose, actual calculated presented dose that these monkeys got was about seven-times-ten-to-the-fourth, and with a very tight standard deviation.

So just to summarize very briefly what we found from the pathogenesis study, as I indicated, the -- well, the first thing to note is that in none of these models is there a long asymptomatic incubation period. Disease starts very quickly, and it seems to be a relatively compressed course of disease relative to human smallpox.

As I already mentioned, we only see a correlation between lesion count and disease severity in the IV route. It is important to note here that there are high levels of viremia obtained by infecting these monkeys with all the routes,
and -- so I'll just end there.

This is an overview of the clinical signs by these routes, and the one thing to note here is that by the IV route, the main clinical signs are really nonspecific signs that we expect to see in human smallpox, namely, lethargy, inappetence. I mean, basically, these monkeys just really feel bad.

There are no respiratory signs that one can document by the IV route, and that's in contrast to what we see with all of the respiratory routes. So the disease by every one of the respiratory routes is characterized by these respiratory symptoms that I've already told you, and that's due to really a bad case of bronchopneumonia.

Nevertheless, even though the disease at the later stages is primarily a respiratory disease, it's possible to demonstrate that virus replication is occurring in many tissues in these animals. Basically, you can find it almost wherever you look. So even though the respiratory route of infection in these monkeys with monkeypox gives
primarily respiratory disease, it is, nevertheless, disseminated and there's multi-organ involvement.

So, in conclusion, if we just compare the IV and respiratory routes, both these routes gave reproducible study-to-study and lab results, particularly when a single source of virus stock was used. Disease progression, as I've already mentioned, is accompanied by wide virus dissemination regardless of route. But we think the characteristics of disease with the IV route, at least at the later stages, are somewhat more like human smallpox because the disease severity correlates with lesion number, and there's minimal respiratory signs.

Now, I want to very briefly talk about an experiment that we did to evaluate therapeutics following aerosol challenge. You're going to hear, I think, probably a lot more this afternoon about evaluating antivirals in monkeys given monkeypox by the IV route, but we just wanted to make sure that since the disease was quite a bit different using aerosol challenge, that we could actually
demonstrate efficacy of an antiviral.

So this is the study we did. We had five groups of monkeys that were infected with one-times-ten-to-the-fifth, target dose of one-times-ten-to-the-fifth PFU of monkeypox virus by the aerosol route, and then groups of monkeys were treated starting at day 1, 2, 3 or 4 with ST-246, which you'll hear about more this afternoon, I'm sure. And treatment was -- then after the onset of treatment, the drug was given once a day for 14 days.

This slide simply shows the survival data. You can see that initiating treatment in these monkeys even out to four days following infection resulted in complete protection, at least as evaluated by survival. And, in fact, if you look at the clinical signs, which I won't show, these monkeys were protected from essentially all clinical disease. The ones where treatment was started day 4 had started to show some signs of respiratory involvement, but that did not progress after the initiation of treatment.
So this kind of sums up where we are with these models in relationship to the ideal characteristics that we would like to see. So in none of these models were we able to use a low dose of challenge virus. With the aerosol route, essentially 100 percent lethal dose was at one-times-ten-to-the-fifth. While certainly not a low dose, I think it kind of gets close to a dose that might actually happen in nature, so I gave that a plus/minus.

In none of these models did we see a long asymptomatic incubation period. As I've already mentioned, all of the respiratory routes yielded primarily a respiratory infection characterized by bronchopneumonia. Only in the IV route did we see a correlation between disease severity and lesion number. All routes showed disseminated disease, and, importantly, in every one of these models that we tested, it was possible to protect these monkeys from disease using licensed ACAM 2000 vaccine.

So we think that disease course for the IV model resembles human smallpox, at least at some
level, from the time of lesion appearance until death or recovery. And while the later stages of disease progression following the aerosol challenge do not reflect the human disease, it does serve to capture a more natural route of infection, and it also captures an anticipated -- at least one anticipated threat.

So monkeypox models are well defined and reproducible both lab-to-lab, day-to-day. But these models are designed to have a much higher mortality rate than naturally-occurring smallpox or monkeypox. So I think it's reasonable that the exact pathogenesis is not going to be exactly the same as either human monkeypox or smallpox.

So although no one route of infection of nonhuman primates with monkeypox produces a disease with all of the characteristics of human smallpox, we think a combination of the IV and respiratory challenges collectively captures several key features and provides a range of disease pathologies for which antiviral efficacy can be tested.
Thank you.

DR. CARGILL: Thank you very much.

Dr. Damon, please.

Speaker Presentation - Inger Damon

DR. DAMON: So I'm the last speaker between clarifying comments and lunch, so I'll try to be clear.

What I'm going to try to do is, in a very short period of time, review an extensive amount of material. I'm going to try to quickly touch on some of what Dr. Styrt already touched on in terms of review of clinical classification of human smallpox. I'll be doing a more extensive review of the nonhuman primate variola challenge studies, really focusing on work that's been done since 2000, and then review work that we've undertaken over the past year to follow up on the Institute of Medicine's recommendations that we develop a process and develop an approach to see whether the nonhuman primate model using variola as a challenge can be further refined.

So I show this slide, which is quite similar
to what Dr. Challberg showed, merely to emphasize once again that although disease with the hemorrhagic forms of variola was almost uniformly fatal, and flat disease had a very high case fatality rate, the majority of cases of smallpox were ordinary phenotypes, characterized in this case using the WHO classification, and therefore the majority of deaths, as well. Even though the case fatality rates were lower in these types of disease, the majority of deaths were in ordinary disease.

Now, Dixon, his Textbook of Human Smallpox, published in 1962, really tries to carefully clinically describe different manifestations of human smallpox, and he classified them in at last nine different criteria and really differentiated them, whether they were what he termed fulminating or malignant disease or benign disease. And benign disease would be more similar to what we consider to be ordinary disease by the WHO classification in that you have the classic rash presentation in benign forms of disease.
The case fatality rate, he also demonstrated, varied with these various clinical types from a case fatality rate of 100 percent with fulminating or purpura variolosa, which was a hemorrhagic form of disease, to a higher case fatality rate of 70 percent with malignant confluent disease. And then within the malignant forms of disease, various degrees of the classic rash presentation, a more edematous presentation of rash, so more akin to what the WHO classification would have called flat disease. I think, with regards, he also looked at whether there's the initial fever, which is cartooned in Dr. Challberg's slides, and then whether secondary fevers, as well, were developed during disease.

So if you look at the literature and also read Dixon's writing, he really hypothesized that there was a different disease pathogenesis between fulminating, malignant and benign disease. And he characterized them essentially that the mortality with fulminating disease occurs very quickly, one day to three days after illness onset. With
malignant disease, there's more of a longer time
course in terms of the morbidity associated with
disease, a significant weight loss, and mortality,
when it occurred, it occurred about two weeks after
illness onset, whereas with benign disease, with
the more classic rash presentations, secondary and
tertiary fevers, perhaps bacterial superinfection,
and bronchopneumonia was observed in some cases in
elderly populations.

If you looked at hematologic parameters in
these different diseases, the major findings that
were reported were thrombocytopenia and
leukocytosis, which was characterized in
fulminating forms, essentially seeing very early
lymphocyte precursors come out and absence of
neutrophils; and, in malignant disease, low
platelets, again, and leukocytosis with
neutropenia.

It's important to note also in the prior
series that Rao had published on, that if you
looked in those individuals who had hemorrhagic
forms or this fulminating form, that prior
vaccination was largely unprotective against
mortality, whereas in other forms of disease, prior
vaccination was protective against mortality.

If you looked at some of the laboratory
parameters, as has been mentioned before, virus
could be found in both scabs, so skin lesions,
quite long and protracted during illness, as well
as in vesicular pustular fluid from the classic
rash formation. Virus was also found in throat
swabs, to a lower degree in throat secretions,
which is likely the form of transmission between
human and human, is respiratory droplets.

So if you look at viremia, virus was rarely
recovered from blood or serum, and this has also
been discussed by Dr. Styrt; only early in disease,
usually prior to rash onset. However, virus was
readily recovered in blood or serum in hemorrhagic
cases in high titer and persisted until death. And
in these studies, virus detection was all by growth
on chorioallantoic membrane of eggs.

As well, the WHO bulletins reported in the
mid 1960s, in addition to looking at the low
platelet counts or thrombocytopenia associated with hemorrhagic forms of disease, also began to look at some of the coagulation disorders and characterized decreased prothrombin and circulating antithrombin with early hemorrhagic forms of smallpox.

So there have been a number of laboratories and groups that have attempted to study variola infection in nonhuman primates. These are summarized in a nice series by Brinckerhoff, et al, in 1906 in terms of the early 19th century studies, and then in the 20th century, a number of labs, including Hahon's lab in the 1950s and Brinckerhoff and Tyzzer began to look at a number of different nonhuman primate species and looking at variola challenge.

A number of different routes were chosen, intranasal, aerosol, using one to five micron particles, intratracheal, intratracheal scarification, dermal scarification, and oral pharyngeal mucosal scarification.

A number of strains of virus were used. In the early studies, they used primary human clinical
material, either through vesicular fluid or
ground-up scabs to challenge primates. In later
studies, they used virus that had been
characterized through growth on chorioallantoic
membrane. The majority of these studies focused on
Yamada and Lee, which are two strains isolated from
the Orient. Higgins was used in some rhesus
studies, rhesus macaque studies.

Challenge doses of the virus were between
ten-to-the-fourth and ten-to-the-sixth when they
were titrated. The earlier studies were not
quantified; they were just simply primary clinical
material that was used. And a number of strains of
nonhuman primates, the majority of studies looked
at cynomolgus macaques of the Philippine origin,
and there were also some studies done with pigtail
macaques, which appeared to be far less susceptible
to infection; a few studies with baboons, which did
suggest that these were susceptible to infection
and mortality, but there was also bacterial
superinfection, which made it unclear what the
cause of death was in these systems, as well as a
number of studies with rhesus macaques. For the
members of the advisory committee, some of these
studies are summarized in the appendix slides.

When you look back at this historic
literature, though, it's hard to really try to
reproduce any of this data. There's minimal
information available -- in addition to not knowing
what titer or virus was used in certain cases,
there's minimal information on the age, weight, sex
of the animals. We do know in Hahon's studies from
the 1950s, he used very small cynomolgus macaques,
1.4 to 3.5 kilograms.

Clinical disease outcome measurements
included activity, cough, respiratory symptoms,
shivering, fever, rash morphology, mortality, and
laboratory involved some measurements, viremia in
the post-1950 studies, as well as pathologic
examination on autopsy.

Essentially, the bottom line results were
that they could develop some febrile rash illness
in these primate species. The illness depicted was
usually described as being milder as what was seen
in human smallpox. The incubation period, in
general, was between four to six days, and
bronchopneumonia was noted with aerosol challenges
of the animals.

So in 2000 onward, there were a number of
attempts -- these are all studies that were done at
the CDC. USAMRIID scientists worked under the
auspices of the World Health Organization at the
CDC with us to develop a number of these model
systems.

So cynomolgus macaques were the species
used. There's a slide later on which sort of
reviews some of the weights, sexes and ages of the
animals, in general. Those were varied. The
challenge viruses were some of the historic ones
used, so Yamada and Lee. Harper was also used,
which is very similar. It also groups into this
Eastern Asian cluster that Yamada and Lee do, as
well as a strain from India, 7124, where case
fatality rates associated with these were between
25 percent and 30 percent, on average, in looking
at the historic literature.
So virus preparations, in general, were very similar to what was done with the nonhuman primate monkeypox studies. So these were viral lysates. These were material grown, in this case, in BSC40 cells.

Route of challenge was either aerosol, aerosol intravenous, and then later settled on intravenous challenges. The initial aerosol studies used a Collison nebulizer, and four each animals were challenged with either Yamada or Lee strains at ten-to-the-8.5 PFUs. And what's described in two pieces of literature in the peer-reviewed literature are transient fever and subsequent rash.

So further studies really then began to focus on looking at different doses around ten-to-the-eighth challenge dose. So there were studies done -- and this should be Harper, not Harvey; I apologize -- with Harper in India 7124, challenging either intravenously or intravenously with aerosol. Fever was documented within three days of challenge. Early evidence of hemorrhagic or
petechial disease in the skin by day 2, and then
the development of more classic rash-type lesions,
vesicles, pustules, and also cough by day 3.

Animals died early and there was near
uniform lethality seen with this high dose
challenge. So with the Harper strain, IV and
aerosol, three to four animals died between days 4
and 6. All animals challenged with the India
strain intravenous and aerosol died most between
day 3 and 4, so just at the onset of rash lesion,
one out at day 13, and similar studies with five of
six of India IV, very early, between day 3 and 6,
and one survived out to day 10, and 3 of 3
challenged IV with the Harper strain. In the rare
animals that did survive past day 7, greater than
500 lesions were observed.

Other hematologic parameters were profound
leukocytosis. This was characterized in the PNAS
paper as being primarily a monocytosis, so 20,000
to 40,000; mild to moderate thrombocytopenia, but
some animals died before developing this; evidence
of coagulation disorders and DIC through D-dimers
and fibrin deposition; and, elevated cytokines, listed here.

There was hepatocellular and renal tubular degeneration. Virus was found in throat prior to onset of rash or at onset of rash, and viremia was quite high, 100 to ten-to-the-sixth plaque-forming units when monocytes were -- mostly in monocytes and found in buffy coats.

If you looked at a ten-to-the-eighth IV challenge -- so this was done just simply intravenously, not intravenous plus aerosol -- again, this is Harper and India strains; again, early onset of fever, within three days of challenge; rash then developing in a classic maculopapular vesicular pustular manifestation as early as day 3; the increase in number of lesions between day 3 and 6; and, a classic centrifugal distribution. Mortality was one of three of animals challenged with Harper and zero -- none of the three animals challenged with the India strain succumbed during the 28 days that they were observed in the study.
All animals had greater than 500 lesions. There was laryngeal involvement on pathologic analysis. So that's interesting. That's also something that Dixon observed in his studies, and profound leukocytosis, mild thrombocytopenia, extensive deposition of virus material in all tissues, so virus DNA in all tissues, and viral DNA in throat prior to onset of rash.

If you look at the DNA in the blood in this case, there were quite high levels of viral DNA found. The onset was very early, as early as day 1, and increases with time. There was some weight loss seen in this animals and profound anemia was demonstrated by day 11.

In a subsequent study that's come out looking at a time sacrifice study looking at the ten-to-the-eighth and ten-to-the-ninth IV challenge -- so this came out this year in PLoS ONE. So, unfortunately, it's difficult to glean. Some of the information, we don't have the weight or sexes of the animals tabulated in this case, but you can see, looking at sort of what's seen over
So the virus in this case is first seen in
the spleen and the liver, then in the oropharynx
and the tonsils, that the rate and the magnitude of
virus dissemination in organs is higher and in more
tissues and cell types in the ten-to-the-ninth
challenge than the ten-to-the-eighth challenge.

Anemia was documented in both model
challenge doses, and one of the observations of the
discussion of the paper was that hemorrhagic
disease in the ten-to-the-ninth challenge study was
noted more often in females and often was noted
with concurrent bacterial superinfection and was
another potential marker of vaso-vascular
dysregulation and capillary leak. Hypoalbuminemia
with a normal total protein was noted in both model
challenge doses.

So this is in terms of what has the intravenous model, what does it look like in characterization with what we believe we know about smallpox in humans. So if smallpox in humans, if we have, on average, a 10 to 12-day incubation period, followed by onset of fever, followed by, in ordinary disease, the classic onset of rash illness and hemorrhagic disease, this would be much more attenuated and you wouldn't see the normal rash progression in most cases.

This is adapted from a figure in Huggins, et al., published in 2007 or '08. You completely circumvent the incubation period, and you're beginning with what we believe through the pathogenesis studies, with ectromelia studies, is you're really beginning at the secondary viremia, and then seeding organs, including the end organ of the skin.

But there are resemblances with these two different models to human smallpox. So if we compare to human hemorrhagic or fulminant disease,
with the ten-to-the-ninth IV challenge, the time to
death of 3 to 12 days post-illness onset is very
reminiscent. This prolonged viremia is
reminiscent. The leukocytosis and thrombocytopenia
are also reminiscent evidence of DIC. What's
dissimilar is that when the rash manifests, it does
manifest with a typical progression and the
leukocytosis is reported in the PNAS paper as a
monocytosis, not a lymphocytosis.

Additional information that was gleaned from
the ten-to-the-ninth in the PNAS study were that
there's pro-inflammatory cytokines seen early in
disease, which is very supportive of a sepsis-type
model, and viral antigen, virus in organs,
including endothelial tissue, as seen.

In the ten-to-the-eighth challenge, there
are both similarities to human malignant semi-
confluent smallpox, as well as similarities to
human ordinary disease. So the case fatality rate
with the Harper strain would be more reminiscent of
what one would see with the human malignant semi-
confluent disease, whereas if you considered the
rash progression, which is very typical of benign or ordinary form of disease, the case fatality rate would be expected to be much lower in that circumstance. The rash manifestation and presentation is very similar, though, to what you would expect with benign confluent or ordinary disease.

Then if you begin to break out the data -- so this is looking at some of the appendix online material with the challenges with the India 7124 and Harper strain -- these are the ten-to-the-ninth or ten-to-the-eighth challenges, you can see, in general -- and this sort of depicts out what I've told you previously -- the mortality that's seen, for instance, in this case, with a ten-to-the-ninth is, again, as was suggested by the PLoS ONE paper, the younger or the female animal challenged with Harper at ten-to-the-eighth at 2.9 kilos versus the animals of 4 kilos, or the male of 10 kilos, is the animal that succumbed to disease. And you can also see that there's sort of a wide range of weights of animals that were used
in the study.

So that work was presented to the Institute of Medicine in 2009 as part of the review of the smallpox research agenda. It's also been discussed extensively with the WHO advisory committee on variola virus research, and really has led to the questions that the IOM first posed in 2009, which is can the model be improved to better resemble human smallpox, either to provide insights on disease pathogenesis or interventions and identify ways in which the predictive value of the model for testing therapeutics and vaccines can be approved, both focusing on reproducibility of the model and also trying to encompass some of the tenets of the Animal Rule.

So we put together an external panel. This is largely people who have not been part of the process to date, although a few members of the Institute of Medicine 2009 panel were asked to provide that continuity. We asked people with both BSL-4 experience, as well as primate model experience, to review the data both with the
nonhuman primate model, as it's been developed with monkeypox, and some of the work that Dr. Challberg presented, some of the more recent work also that Dr. Mucker presented, some of the studies that Dr. Goff has recently presented with intrabronchial nebulized challenge of monkeypox, and look at whether those refinements in the monkeypox challenge with nonhuman primates could be applied to variola, as well, and also consider route of challenge, strain or species of virus, preparation of virus, challenge dose, strain or origin of the animal species, the health-age-weight assessments, and then really focusing on disease outcome measurements and how you begin to standardize this.

DR. CARGILL: Dr. Damon, excuse me, you have three minutes.

DR. DAMON: Okay.

So I'm going to briefly go through some of the draft recommendations. So the route of challenge, the group, in general, felt that since the natural route of infection is by the airway, that monkeypox studies, virus challenge studies,
had indicated that the aerosol dosing could more closely replicate human smallpox in terms of timeline disease, in terms of extending that incubation period, and that there is improvement in equipment which has allowed improved reproducibility, that this is something that should be considered. So either an aerosol or an intrabronchial nebulized would be a first approach.

But for the strain of virus that really that most of the work recently has focused on, India 7124 and the Harper strains, that that should probably continue when trying to do a head-to-head comparison, if that's possible, with an aerosol challenge approach or an airway challenge approach.

Although both rhesus and cynos have historically been used, most of the work has focused on cynos. So the recommendation was to continue there and to try to use captive bred animals as opposed to wild-caught animals and standardize the source of the animals.

Additionally, work focused on some of the recent work out of the Moss lab in terms of looking
at the CAST/E mouse system, as well as models with dormice, which had shown susceptibility to monkeypox, that this is something that could be considered, as well as to consider marmosets.

Finally, that age, weight and gender matching would assist in controlling potential artifacts, and the initial recommendation, or draft, is to try young post-puberty males, 3 to 5 kilos, to avoid some of the estrous cycle considerations, and then move on with both sexes, if indicated.

In terms of preparation of virus, really the recommendation came down to try to use a more purified source of virus, so to use a sucrose-cushioned pelleted virus preparation.

Then, finally, in terms of disease outcomes, to develop a uniform endpoint scoring system which would both be used for clinical scoring, as well as euthanasia criteria, to consider especially if -- to develop capacity to better characterize any lung pathology if a respiratory challenge is going to be used, and to develop good hematologic
measurements.

Also, as previously, measure blood chemistries, virus shedding, not just measure DNA, but also try to measure infectious virus; get a sense of the immune response in these different systems, and on necropsy -- do necropsy and evaluation of defined organs.

So a brief summary. I think, in some ways, the IV challenge with variola does model some aspects of late stages of smallpox rash illness; have some similarities to hemorrhagic and fulminant disease. The rash progression, however, is typical of ordinary disease.

Reevaluation of an aerosol or an upper respiratory challenge may provide a model system with a longer incubation period, so you can better evaluate outcomes at different times post-illness onset using rash illness as a standard for intervention; and to basically try to improve and make the model system more reproducible through the use of standardized reagents and measurements and scoring systems.
Clarifying Questions from the Committee

DR. CARGILL: Clarifying questions from the committee for the speakers at this time, please. If you would, again, raise your hand, Paul will get your name. Thank you. Go ahead.

DR. BOHM: This question is for Dr. Damon. You talked about several attributes of the animals picked for study, such as strain, origin, age. Was there any discussion about MHC Class 1 or Class 2 genotype, bringing that into the picture?

DR. DAMON: I think that might have briefly been touched on, but hadn't been extensively discussed.

DR. CARGILL: Dr. Lyons?

DR. LYONS: Rick Lyons. This question is for Dr. Challberg. Just because it's come up before in some primate models and with other pathogens, can you just comment on the value of any additional supportive therapy in the context of treatment for this disease, and the challenges?

DR. CHALLBERG: Well, we discussed that quite a bit at the outset of the studies, and I
think our approach for these studies was to stay away from supportive treatment as much as possible consistent with IACUC recommendations on site. So some of the sites wanted the use of analgesic in the case of obvious pain and so forth, and we did that if we needed to. Some laboratories didn't require that. But, basically, the only supportive therapy was really augmentation of the food with some tastier food.

DR. CARGILL: Dr. Van Dyke?

DR. VAN DYKE: Another question for Dr. Challberg. I may have missed this, but at what point following infection did the animals develop the rash?

DR. CHALLBERG: Well, in the IV model, the rash develops at about day 4. So we see fever at day 1, rash in a few monkeys, day 3, most monkeys, day 4, certainly all the monkeys by day 5. In the respiratory routes, the development of rash was a little bit more delayed than that, oftentimes out to day 6. And as I said, that presented -- I think that's the issue with rash and the respiratory...
models, is it starts to develop later, and it's kind of a race between the development of the rash and the development of the bronchopneumonia.

DR. CARGILL: Dr. Goetz?

DR. MAGILL: This is a question for, I think, Dr. Mucker on the -- as I understood this, this is a relatively new model, the marmoset model, and this is basically one experiment to date.

MR. MUCKER: Yes, a series of probably six or seven iterations but under the umbrella of one pilot experiment.

DR. CARGILL: Now, Dr. Goetz. I apologize.

DR. GOETZ: This is a question I maybe should have asked this morning. I think Dr. Styrt mentioned briefly the antivirals that had been tried historically. I wonder, do we have any information about what the in vitro activity of those antivirals was?

DR. STYRT: In terms of comparisons, I think I'd leave that to some of the other experts who may wish to comment further, because I've sort of seen it argued in different directions. One could
argue -- but the availability of cell culture data was much less at the time. And when people have tried to look at the same drugs with current culture methods, there have been somewhat mixed reports. But some of the newer drugs do look like they might have more in vitro activity. The animal data from the older studies was extremely skimpy and would be difficult to comment on, and, for that matter, the clinical trials are pretty skimpy, also.

To the best of the ability to evaluate drugs at the time, when they proceeded into human clinical trials, the human clinical trials tended to be disappointing, but it could be argued that one could find things that are more active in the preclinical data now. But how that would relate to what you would actually find in a clinical trial, of course, takes us back to speculation.

DR. CARGILL: I'm sorry. Could you state your name for the record, please?

DR. STYRT: Barbara Styrt.

DR. CARGILL: Thank you.
Dr. Bohm, did you have a second question?

DR. BOHM: Yes, I do. And this is for all the speakers. I just wondered if there's been any other work done in the animal models, other animal models, similar to the rabbit on animal-to-animal aerosol transmission? Has anybody even attempted those studies?

DR. BULLER: We've done work with ectromelia, but it's within a cage and it's intranasal route, so it's not a true aerosol route. But the suggestion was that it was spreading through aerosol droplets, but the studies are not that deep.

DR. CARGILL: Dr. Buller, if you could please stay back up there for me. I also have a question for you. It's on your slide number 13, if we could have that slide up, please.

You made a statement during your presentation of that slide, the importance of contrasting and comparing intravenous versus other routes. And I wonder, since you went through that fairly quickly and it's come up several times,
could you restate that again, please?

DR. BULLER: Well, listed here are five different reasons why I believe that an intravenous inoculation route is not appropriate. First of all, you take away that eclipse period that we've talked about. Secondly, I described all of these immune evasion genes that the virus encodes, some of which sort of produce a protective shield around the foci of infection in the periphery, which I think allows seeding of the animal. That, again, is gone if you do IV.

The virus inoculum in most of these studies, Geoff described the extracellular virus, which has an extra envelope, and intracellular. The extracellular virus has a lot of proteins that block complement activation and deposition on the virus. The intracellular does not. So if you do your study with intracellular mature virus, 10 percent of that dose might be effective. It might even be less than that. So the complement in the blood basically reduces your inoculum by 90 percent within a couple minutes. And in doing
that, it charges the virus with molecules that permit possibly targeted phagocytosis of the opsonized virions, which might jumpstart the immune system. So, therefore, although you think you're giving it to the animal straight, a sidebar issue is that you might activate the immune system faster than if you did it from IN or aerosol inoculation.

Finally, the cells that the inoculum would see initially are very different than the cells that the virus would see through a natural route of infection.

DR. CARGILL: Thank you. I just felt, since we had, one, compromised your time a bit, and, two, you had gone through this and had to accelerate, it was worth going back over that again. Thank you.

Dr. Bennett?

DR. BENNETT: I'd like to follow-up the question that Dr. Magill had to Dr. Styrt. It has to do with the use of thiosemicarbazones, such as Marboran. I don't see that being mentioned anymore. Is that because there was never any promise in vitro or are there other problems with that category of
DR. STYRT: Okay. So this is Barbara Styrt again. And, again, you may have people who can speak to you in a lot more detail about thiosemicarbazones, but I think one of the reasons why they were not pursued further -- two of the reasons why they were not pursued further was that they didn't look particularly promising when they were actually taken into clinical trials of patients with established smallpox illness. There was some debate about whether they showed some promise in post-exposure prophylaxis, but the design of the some of the studies was extremely flawed, and they did have a lot of GI side effects. And the feeling seemed to be that the tolerability was also going to be an issue there.

I think that people have tried looking in vitro at some additional thiosemicarbazone derivatives, but there's a lot of information out there that's at extremely preliminary levels that would be difficult to evaluate in terms of whether there's any serious pursuit in those areas or not.
But, essentially, during the smallpox era, they did not seem to have an adequate risk-benefit balance for anyone to want to keep pursuing them, and then, of course, interest diminished drastically after the tremendous accomplishment of smallpox eradication. But it was toxicity and tolerability, as well as dubious clinical results in terms of efficacy.

DR. CARGILL: All right. Then we will now break for lunch, and we will convene again in this room in one hour from now at 1:30. I would ask that you please take any personal belongings you want with you at this time, as the ballroom will be secured by FDA staff during the lunch break.

Panel members, again, please remember that there should be no discussion of the meeting during your lunch among yourselves or with any member of the audience. Thank you.

(Whereupon, at 12:31 p.m., a lunch recess was taken.)
DR. CARGILL: Good afternoon, and welcome back to the afternoon session. We will now proceed with the first sponsor presentation. I would like to remind public observers at this meeting that while this meeting is open for public observation, public attendees may not participate except at the specific request of the panel.

Both the Food and Drug Administration and the public believe in a transparent process for information gathering and decision making. To ensure such transparency at the advisory committee meeting, FDA believes that it is important to understand the context of an individual's presentation.

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speaking.

Industry Presentation - Randall Lanier

DR. LANIER: All right. My name is Randall
Lanier, and I am an employee of Chimerix. And on
behalf of Chimerix, I would like to thank the FDA
for giving us the opportunity to share our
experience in developing CMX001 as a smallpox
antiviral under the Animal Rule.

Can everybody hear me in the back okay?

I'm going to start with some background on
CMX001, then describe our experience using the four
tenets of the Animal Rule as an organizing
principle. So I'll go through each of the animal
rules in turn and our experience with each of them,
and then end with a proposed development path for smallpox.

The one-sentence summary of CMX001 is that it has broad spectrum activity against double-strand DNA viruses, and this permits simultaneous development of CMX001 for smallpox under the Animal Rule and also for adenovirus and CMV through traditional pathways. But what is CMX001 and why did we think it was a good candidate for a smallpox countermeasure?

CMX001 is a lipid conjugate of cidofovir, and cidofovir is in the strategic national stockpile for smallpox vaccine complications and has long been known to have activity against orthopoxviruses. CMX001 is a lipid conjugate that was designed to improve the dosing, safety, and efficacy of cidofovir.

The structure shown here -- and what you can see in the red circle is the structure of cidofovir. And this has been covalently modified by the addition of a lipid, hexadecyloxypropyl, and this changes the properties of the drug quite
substantially. It's designed to mimic a phospholipid, as you might guess from the structure.

The lipid makes an IV drug an oral drug. Cidofovir is IV; CMX001 is oral. It also eliminates the dose-limiting nephrotoxicity of cidofovir and dramatically increases the antiviral activity of cidofovir. It's important to note -- and I'll probably mention this about six times in the talk, but it's very important -- that CMX001 and cidofovir act via the same antiviral, cidofovir diphosphate. So they both are anabolized to cidofovir diphosphate, which actually inhibits viral replication. CMX001 delivers this much better and improves the overall ADME profile quite substantially.

This slide gives a high level overview of our development. There are more than 50 animal studies in diverse orthopoxvirus models. I'll show you data on some of these in just a moment. It's in phase 2 for CMV and adenovirus. We have a large safety database that includes more than 350
patients treated for multiple viruses in open label studies and more than 400 in placebo-controlled clinical trials. Commercial scale validation of our manufacturing process will be complete in a few months, in the first quarter of 2012. And we have a completed tox package which includes long-term studies with monkeys, where there was no dose limiting toxicity up to 25 milligrams per kg.

Moving to the Animal Rule, tenet 1 holds that there is a reasonably well understood pathophysiological mechanism of toxicity, in this case, of smallpox, and prevention by the agent, in this case, for CMX001. I'm not going to go into any detail on the mechanism of smallpox toxicity; that's been discussed thoroughly already. But I'll just mention that it involves viral replication, resulting in a variety of signs and symptoms and ultimately death in a high percentage of patients, depending on which form of smallpox it is.

CMX001 inhibits smallpox disease by inhibiting viral replication via the DNA polymerase, as shown on the next slide. And this
slide shows CMX001 on the left, which efficiently delivers cidofovir inside the cells where it is cleaved to cidofovir and then anabolized to the active antiviral, cidofovir diphosphate, and cidofovir diphosphate can inhibit viral replication in three ways, all of which act via the polymerase.

If two molecules of cidofovir diphosphate are incorporated into the growing DNA chain by the viral polymerase, the chain will be terminated and replication is stopped. If one molecule is incorporated, there's the potential for the chain, for that first strand, to be completely synthesized, but then the second strand has a very difficult time reading back through that incorporated cidofovir. So there's a block to second strand synthesis if just one molecule is incorporated.

Also, the polymerase is sort of poisoned by the addition of one molecule of cidofovir, such that the DNA strand movement through the DNA binding cleft is inhibited, and the analogy is that of a stripped screw being inhibited and it's going
into a nut.

As has already been described by previous speakers, the orthopoxvirus polymerases are homologous, so it isn't surprising that CMX001 is broadly active against these viruses.

This slide shows the activity of CMX001. The viruses are shown on the left-hand side, the EC50s of CMX001 and cidofovir are shown in the middle, and the ratio of cidofovir EC50 to the CMX001 EC50 is shown on the right-hand side; so it's the fold increase in potency for CMX001. And what you can note here is that CMX001 increases the potency, depending on the system, 24 to 273-fold, with the highest change in potency being for variola major here.

This increased activity is due to the more efficient transport into the cell by the lipid conjugate, leading to more of the active antiviral inside the cell, and we can show this looking at human PBMCs. In this experiment, we incubated human PBMCs with clinically relevant levels of cidofovir, in grey, or CMX001, in green. The
levels shown on the X-axis are in micromolar and approximate the human Cmax. The levels of active antiviral cidofovir diphosphate are shown on the Y-axis in picograms per million cells. And the punch line is that a 100-fold less CMX001 gives sevenfold more of the active antiviral; so roughly a several hundred-fold increase in the active antiviral delivered by CMX001 versus cidofovir, and this rather neatly explains the differences in the EC50s.

Before I discuss the animal models, let's spend just a minute on resistance. Resistance in vaccinia has been examined extensively by several groups, as described on the next slide. And here we're looking at a variety of data on resistance and vaccinia. As expected, the resistance maps to the polymerase gene, and, notably, it was very hard to generate resistance in vitro with more than 30 passages being required to get to tenfold increase in the EC50, multiple mutations required for this increase, and the virulence was inextricably linked to resistance. That is, as the resistance
increased, the virulence decreased, so much so that mice infected with resistant vaccinia could still be saved with cidofovir. Given that CMX001 could be used widely in the event of a smallpox attack, a high barrier to resistance we feel is quite reassuring.

On to the animals. Tenet 2 posited the effect should be demonstrated in more than one species, predictive for humans in the absence of a definitive model. Because humans are the only permissive host for variola, there is no definitive model for smallpox, and multiple models are required. We've used three main models, rabbits, mice, and monkeys, and we will show you mortality endpoint data from rabbits and mice with CMX001.

In monkeys, we've used cidofovir as a surrogate for CMX001 because CMX001 is susceptible to rapid first pass metabolism in this species. This phenomenon is not unique to CMX001, but it does preclude testing of CMX001 for efficacy directly in the monkey model.

The next slide shows high level comparisons
of the models we've used the most. Many of the characteristics of the model stems from whether or not the host is permissive for the virus in question. So if you look across the top, you'll see ectromelia in mice, rabbitpox in rabbits, and variola in humans. The host is permissive for the virus, and the LD50s, either estimated or measured, are quite low. However, in the case of monkeypox and variola in monkeys, the host is not permissive in very high levels. A million to a billion plaque-forming units are required to reach a lethal inoculum.

The routes of infection that we've used are shown, and potential treatment triggers are also shown. The primary one that we've used is the lesions in the rabbit model, but there are a number of other potential triggers that could be used, and some of those were touched on by previous speakers. The endpoint in all cases was mortality, with the exception of variola, where the mortality is too variable to use this as an endpoint.

We didn't put monkeypox in humans on this
slide, but it's probably pretty similar to variola in humans, with the exception that mortality is probably 0 to 1 percent rather than 5 to 50 percent.

The next few slides go into specific models and results for CMX001. The rabbitpox model has some very desirable properties, as I think Dr. Moyer has explained much better than I can. But the few things that I'll say are that they're permissive, which permits a very low inoculum, and that the pathogenesis shares a number of hallmarks with human smallpox, again, as has already been described.

I think it's important to keep emphasizing that the antiviral target is homologous, and this gives us a lot of comfort in trying to extrapolate from these models. Importantly, from a very practical standpoint, the model is quite reproducible and we've now transferred the data that I'll show you that was done in Dr. Moyer's lab at the University of Florida to Battelle and gotten the same results.
Finally, having a robust, reproducible model with reliable clinical signs and symptoms that can act as triggers for treatment we feel is a prerequisite for doing meaningful GLP studies.

This is a slide that Dr. Moyer has already shown. I'm showing it again to emphasize potential triggers that could be used and to say that for the studies I'm going to show you, we've used lesion appearance in the ears as our trigger for initiation of CMX001 treatment.

These are shown photographically on the next slide, just in case anyone missed Dr. Moyer's presentation. In the top set of panels are mock-infected rabbits, so these are uninfected rabbits. And in panels D through F, these are rabbits infected. Panel D shows the primary lesion site from an intradermal infection of rabbitpox, and panel F shows the lesions, and this is what we were using as a trigger for the studies that I'm going to present.

This slide summarizes data from three blinded, placebo-controlled studies, where dosing
was started after detection of lesions in the ears. They were conducted by Dr. Amanda Rice in Dr. Moyer’s lab at the University of Florida, and CMX001 was administered orally at 20 mg per kg either as a single dose or as multiple doses given every other day. Two or three doses were the maximum -- three doses was the maximum. And while it looks like three doses may have been the best, we got 92 percent survival with three doses of CMX001 when dosing was initiated at lesions versus 17 percent for untreated rabbits, there was also a significant prevention of mortality with just one dose, shown in the last row, where 58 percent of the animals survived with a single dose of CMX001 administered at the time of detection of lesions versus 8 percent of the animals who received a placebo. And, notably, the placebo mortality was quite consistent from study to study.

Dr. Moyer has also already touched on this model. We looked at CMX001 in the natural contact model. So this was not the aerosol transmission model where the animals were separated by the six-
inch barrier; this was a contact model where the animals were co-housed. So index animals were infected intradermally, with 100 or 1,000 PFU of rabbitpox, and then sentinel animals were co-housed with the index animals.

Just like in the last study, CMX001 treatment was initiated at the first onset of lesions in the ears. In the 100 PFU infection of the index animals, all of the animals survived, whether they were treated or not. At 1,000 PFU ID infection, all of the untreated animals died, so zero of three survived, and the majority of the animals treated with CMX001, again, one, two or three doses of CMX001, survived.

So our current plan for the rabbit model is to move forward with pivotal studies in the direct ID infection model. We're not currently planning more studies with natural transmission.

The mouse model also has many desirable features, as Dr. Buller has enumerated for you already, but not the least of which is the ability to run large studies under GLP. The studies I will
show used intranasal infection, with very low
inoculum, where the mortality was 100 percent
without treatment. And like with all of the
orthopoxviruses, the antiviral target of cidofovir
diphosphate administered through CMX001 is the
viral polymerase.

Study 530 was run in Dr. Mark Buller's lab
under the direction of Dr. Scott Parker at
St. Louis University. These were A and CR mice
which were infected with 15 PFU of ectromelia
administered intranasally. And as you can see, all
of the untreated mice died, with day 10 post-
infection being the mean day of death, and none of
the CMX001 treated animals died. They were dosed
with a 20 mg per kg loading dose followed by a
lower maintenance dose of either 2 and a half mg
per kg or 1.25 mg per kg administered over 14 days.

The second mouse study examined the effect
of one dose of CMX001 given after the midpoint in
disease, and mice were infected as before and
received either one dose -- all received one dose
on 20 mg per kg CMX001 at day 4, 5, 6, or 7. And
notably, here the mean day of death was day 9. One
dose of CMX001 had a significant effect on survival
up to day 6, but none of the animals treated on
day 7, two days before death, survived.

So one conclusion from these studies is that
a significant improvement in survival in
demonstrable with CMX001 treatment at time points
that are at or passed several potential clinical
trigger points.

On to the monkey models, for reasons that
are both specific and general, we don't believe the
monkey models are ideal for development of CMX001.
Going from top to bottom, we're generally concerned
with the low and variable mortality of the variola
model, even when very high viral inocula are
injected into the monkeys, up to a billion
particles, and there are also significant facility
limitations with this model. The lack of
permissivity in general for monkeypox is also an
issue, again, based on just the high inoculum
required bypassing primary viremia.

We have a specific issue with CMX001 in that
the very high metabolism of CMX001 in the species results in very low exposures to the drug, as shown on the next slide. This slide compares PK parameters for healthy and sick humans, rabbits, mice, and monkeys. The most important part for this discussion is the highlighted column on the right, shown in gold, which shows dose normalized AUC. This is really just a handy way to compare exposures when different doses were given, and it's simply the absolute AUC divided by the dose.

Notably, there is no significant PK difference in healthy volunteers versus sick patients. These are statistically the same number, although numerically it's a little different between the 49 EIND patients shown under human patients, which appear to have a slightly higher exposure. However, rabbits had about a 35-fold lower exposure than human patients, mice had about a 57-fold lower exposure than human patients, and monkeys had about a 280-fold lower exposure than human patients.

Something that surprised me was that
apparently the significant differences in PK between monkeys and humans is not all that unusual. This slide shows a wide range of approved drugs that are metabolized much faster in monkeys than in humans, similar to what we see with CMX001. And so it's unlikely that these widely used drugs would be available if efficacy had been required in monkeys. However, unlike CMX001, cidofovir is effective in monkeys, and using cidofovir as a surrogate eliminates metabolism of the lipid side chain as a factor in our efficacy studies. And if I haven't already said it three times yet, CMX001 and cidofovir are anabolized to the exact same antiviral inside the cell.

This slide shows two studies run by Dr. John Huggins and colleagues at USAMRIID and the CDC in the monkey IV model, one with monkeypox and one with variola. In monkeypox, cidofovir showed a significant survival benefit with 88 percent of the treated animals surviving versus 12 percent of the untreated animals, and there was also a significant effect in lesion count in viremia.
In the variola model, there was no significant effect in mortality because the placebos almost all lived, two out of the three placebos survived. However, there was a significant effect on lesions and viremia in the variola model. And we feel the cidofovir work in monkeys is supportive of CMX001 efficacy in humans because they both work through the same antiviral.

Tenet 3 states that the animal study endpoint is clearly related to the desired benefit in humans. I think we would argue that clearly a desired benefit in humans is reduction to mortality. Our animal studies have showed a decreased mortality with treatment of CMX001 and, therefore, the animal study endpoint mirrors the desired benefit in humans, with the exception of the current variola model.

Tenet 4 states that PK/PD data in animal models can facilitate selection of an effective dose in humans. This slide shows exposure to CMX001 in mice and rabbits, receiving a dose of CMX0001 that is effective in prevention of
mortality and compares it to exposures in people, both healthy volunteers and sick patients. The adult fixed dose regimen being studied in phase 2 is 100 milligram twice a week. And those of you in the room can look up -- the tablet looks like this. I'm sure you can see this really well from the back, right? It's between my thumb and forefinger. So two of these a week is the dose that we believe would be appropriate going forward.

For an average person, this dose equates to 1.5 milligram per kilogram, which produces exposures that are two to threefold higher than the exposures in effectively treated animals. So again, if you compare either the human healthy or the human patient data on this slide, shown in the green at the bottom, to the rabbit and mouse exposures, they're at least two to threefold higher.

So we think that some of our data from ongoing clinical studies is quite relevant for smallpox. Our clinical experience we believe is relevant in a couple different ways. First, the
double-strand DNA viruses we are studying in phase 2 are widely disseminated in the case of adenovirus, perhaps an acute infection, and the viral target is the DNA polymerase which is essential for viral replication. We're not suggesting that these are surrogates for orthopoxviruses, but we believe these data could be supportive for the program. Second, safety and PK data in diverse populations, including sick children, immunosuppressed patients, and hepatic and renally impaired patients are supportive for use of CMX001 in a public health emergency.

This is a complicated slide that's trying to summarize our clinical trial data, at least some of our clinical trial data, in which we've dosed over 650 subjects with CMX001. Some of the trials listed here were healthy volunteers in dose escalation or food effect studies, shown at the top of the slide. Many were patients in our controlled phase 2 trial for CMV. That database is locked and the analysis for that trial has begun. And even more of the patients on this slide have
participated in open label or compassionate use protocols under EINDs or in Study 350. And the age and duration is highlighted in blue under EINDs. And you can see that we treated patients whose ages range from one month old to 79 years old, and dosing has gone out past six months. At the dose and duration we feel is needed for smallpox, we've treated about 200 patients, which includes about 40 children.

This last slide is our proposed CMX001 development plan for the smallpox indication. This slide is written in the past tense about data to date, much of which I've showed you. What we propose to do is to extend these findings to, one, demonstrated survival benefit in multiple animal models under GLP; two, show a reduction in lesions, in viral load in the variola model in monkeys using cidofovir; three, determine the safety profile in diverse human populations, along with efficacy data in other double-strand DNA virus infections. And as an aside, we plan to report the phase 2 CMV efficacy data in the first quarter of 2012. And
then, finally, we would use PK data from animals and humans to establish a dose that's likely to be effective for smallpox treatment. And in toto, we believe that efficacy in animal models combined with human clinical data does provide a development path forward for CMX001 for smallpox.

I'd like to end by thanking the FDA and BARDA for both timely and helpful feedback during this occasionally difficult process. And I would also like to thank the Chimerix team and many outside scientists who have made all this possible and many of whom would be happy to answer any questions that you may have now.

Thank you.

Clarifying Questions from the Committee

DR. CARGILL: Thank you for your presentation.

The clarifying questions from the committee for the sponsor, if you would please, again, raise your hand and Dr. Tran will acknowledge you.

Dr. Connick?

DR. CONNICK: Have you looked at metabolism
of CMX001 in other monkeys besides the cynomolgus monkey? And if not, is it reasonable to infer that metabolism in the cynomolgus monkeys will be the same as in others?

DR. LAWRENCE: I'm Lawrence Trost. I am employed by Chimerix as the executive director of toxicology and pharmacokinetics. The answer to that question is no. We have only looked at cynomolgus monkeys, and I'll leave it there.

DR. CARGILL: Dr. Goetz?

DR. GOETZ: Yes. Dr. Lanier, you presented the data looking at the AUC ratios in the various animal models and correlated that with a clinical responses.

Can you comment as to how confident you are that the AUC is the best measure of efficacy versus the troughs and, if so or if not, whether the troughs correlate across the animal models?

DR. LANIER: So this is obviously a nucleoside and the troughs actually are zero for all of these models. So we really can't correlate troughs. We have considered whether Cmax or AUC
are more appropriate. We get very similar ratios whether we compare Cmax or AUCs. So for the PK parameters that we can look at, we get very similar answers in that we get higher levels in humans than in the animal species.

DR. GOETZ: And just to follow-up, in terms of intracellular concentrations of the diphosphate, what about -- do the half-lives vary across the species?

DR. LANIER: We don't have the half-lives for all the species yet. Those are data that we're obtaining now. So we assume that they're going to be pretty similar, the data that we do have suggest they are. We don't have definitive data for all species yet.

DR. CARGILL: Thank you. Dr. Van Dyke?

DR. VAN DYKE: A couple quick questions. You mentioned pediatric studies. Is there a pediatric formulation of the drug?

DR. W. PAINTER: I'm Wendy Painter, I'm also an employee of Chimerix. Yes, we have a pediatric liquid formulation that we're using in the clinic,
specifically in our adenovirus study. Adenovirus is primarily a disease of children.

DR. GOETZ: Great. Thank you. And, also, in the poxvirus studies in animals, have you seen any evidence of viral resistance?

DR. LANIER: No. We have not seen any evidence of resistance either in our human -- the few human studies with vaccinia or in any of the animal studies to date.

DR. CARGILL: Thank you. Dr. Strader?

DR. STRADER: I wanted to ask a question about the AUC again. You show here that there's about a 30-fold increase in metabolism of the drug in rabbits as opposed to humans, but somehow that seems to be sufficient for you to consider that model a good one for your drug as opposed to the increased metabolism in monkeys. And it seems that the metabolism in rabbits and monkeys is closer than between rabbits and humans. So I just wanted to get a clarification.

DR. TROST: Right. I think that's an excellent observation and it's -- we do use a
higher dose in rabbits. I think that explains why we're using a dose of 20 mgs per kg in the rabbit model as opposed to the human doses that we're studying now that are more in the range of 1 to 2 mgs per kg. So there's a tenfold difference there, and part of that is to address that difference in systemic exposure.

I'll turn it over to my colleague, George, here.

DR. G. PAINTER: Hi. George Painter from Chimerix. Our experience has been that the lipid side chain is extensively oxidatively metabolized. It's omega hydroxylated and it undergoes beta oxidation. It's qualitatively the same across species, but varies significantly quantitatively. And in monkeys, the working hypothesis is that the rate of cleavage of the lipid carrier versus the rate of oxidative catabolism is slow. That results in catabolites that are water soluble and not subject to intracellular cleavage. So we've done in vitro and in vivo experiments, and it correlates with these levels. So to Lawrence's point, because
of varying degrees of oxidative catabolism, we have
to vary the dose.

    DR. STRADER: The difference between rabbits
and monkeys is statistically significant --

    DR. G. PAINTER: Yes, they are.

    DR. STRADER: -- but not between rabbits and
humans.

    DR. G. PAINTER: Well, it is different
between rabbits and humans. There is a
significantly higher level of a single oxidative
catabolite, and, again, that results in the varying
doses. So that our AUC is to the CMX001 in the
circulating volume.

    DR. CARGILL: I have a question in reference
to slides number 23 and 27. We're going to go back
again to the AUC question. On one slide I see that
we have human patients, on another slide you list
them as seriously ill. During the presentation, we
were told they have renal disease and other
illnesses.

    Is there either a slide that you have with
the ability to let us know exactly who these
seriously ill patients are and what are the ranges of diseases that they have? That would be very helpful.

DR. W. PAINTER: So Wendy Painter again. This slide refers to patients who were treated under a compassionate use program for serious or life-threatening diseases where they had no other alternative under FDA’s program. So the patients in there had varying degrees of problems. They were treated for all kinds of viruses ranging from adenovirus to CMV, HHV6, even JC virus associated PML, diseases like that. Many of them came to us having failed cidofovir and were in renal failure at the time, so we treated those types of patients. Many of them had hepatic involvement with disease, and so there's where the hepatic impairment came about.

In total -- not on this slide in particular for this PK, but in total we've treated 350 patients in our expanded access programs across all of these different types of viral diseases.

DR. CARGILL: Thank you. Dr. Bohm?
DR. BOHM: This is a follow-up to Dr. Strader's question. So if you modify the dose, milligram per kilogram dose given to nonhuman primates, why wouldn't that have the same effect as it does in the rabbit if there's metabolism?

DR. G. PAINTER: We've looked extensively at dosing, and we've dosed originally, or it appears that the first pass metabolism is, to a large degree, occurring at enterocytes as it crosses out of the gut, as well as in the liver. So we went around that, and we could give the drug IM and achieve reasonable plasma exposures.

But the key, even if we dosed high, was that the rate of cleavage of that lipid off versus oxidative catabolism was unchanging and more rapidly catabolized than the lipid was cleaved off intracellularly. So unless we could reach a point at where we saturated one of those pathways, we wouldn't achieve a different result, and indeed that's what happened.

DR. BOHM: And so that's not a desired effect to saturate that pathway.
DR. G. PAINTER: Right. We just couldn't saturate it. Monkeys are very active, catabolic metabolizers of fat.

DR. BOHM: Do you have data to show that?

DR. G. PAINTER: Yes, we do.

DR. CARGILL: Dr. Magill?

DR. MAGILL: I want to go back again to the PK parameter. I'm still not completely clear on this, and if I missed it, I apologize. But the survival in any animal model versus nonsurvivors, can that be directly related to a dose, a regimen, in any PK parameter such as Cmax or AUC and really relating that survival as the endpoint and does the PK -- is it predictive?

DR. TROST: At this point, we don't have pharmacokinetic data in sick animals. We have studies in sick rabbits planned. So the data that you're looking at are actually healthy animal studies. And so we have evaluated many different doses and regimens in our animal efficacy studies, but we don't have pharmacokinetic data for all those different doses and regimens. What you're
looking at is an optimized dosing regimen and then
correlating that to pharmacokinetic data in healthy
animals.

DR. CARGILL: Thank you. Dr. Bennett?

DR. BENNETT: In your clinical trials, have
you picked up any toxicity signals, things that,
for example, might not be picked up in animals such
as nausea, vomiting, headache, diarrhea? I realize
that in your compassionate trials, it's very
difficult to talk about toxicity.

DR. W. PAINTER: I'm sorry. I couldn't
quite hear all of that.

DR. BENNETT: Toxicity, clinical trials.

DR. W. PAINTER: Okay. So in our clinical
studies, in our phase 2 study in CMV, stem cell
transplant patients, that we just locked the
database. It was a dose escalation study. We went
through five cohorts. Our maximum tolerated dose
we hit at the highest. We did see an increase in
diarrhea in those patients. The dose that we're
using in the clinic now is a tolerable dose, and
the exposures in patients exceed what we
DR. CARGILL: Thank you. Mr. Raymond?

MR. RAYMOND: You described two studies that did not show you meeting the mortality endpoint for efficacy, the natural contact model for rabbitpox and the cidofovir monkey study with smallpox.

Do you have any hypotheses about why these models may or may not have been able to show efficacy with the treatment regimen you were using?

DR. LANIER: So correct me if I don't answer the right studies for you, but I think you were talking about the natural contact model with the rabbitpox, where 100 PFU did not show efficacy. But in that case it didn't because all of the animals survived, whether they were treated or untreated. So 100 PFU in the index animals, our hypothesis is that there just wasn't enough virus transmitted to the sentinels to kill them, period. So nobody died in that study.

Is that the one you were referring to? With 1,000 PFU --
MR. RAYMOND: I'm sorry. I'm looking at slide 18 where it says that six out of nine animals treated survived.

DR. LANIER: That's correct, yes. So 67 percent of the treated animals survived. And actually what that correlated to was one animal in each group, so it was a pilot study. We had three animals per group, and they were treated with one, two, or three doses of CMX001 at 20 mg per kg. And one animal in each of those groups did not survive, but two did.

So it's a pilot study. I think to get definitive answers to that, we would have to carry it further, but we feel like that is a good mortality benefit when all of the placebo-treated animals died. It's essentially 67 percent survival versus zero percent survival.

MR. ROBERTS: And the cidofovir smallpox study in monkeys?

DR. LANIER: Yes. It's a similar result to the 100 PFU in rabbits, where two out of the three placebo treated animals lived, and so did -- I
think it was seven of eight, if we go to that one. Anyway, all of the animals except for one in each group died. So there were more animals in the cidofovir treated group, eight of nine survived in the treated group versus two of three in the placebo group. So only one animal died in each of the groups. So you really can't make an efficacy conclusion on mortality based on that.

DR. CARGILL: Thank you very much.

We will now proceed with the second sponsor presentation. I would like to remind public observers at this meeting that while this meeting is open for public observation, public attendees may not participate except at the specific request of the panel.

Again, both the Food and Drug Administration and the public believe in a transparent process for information gathering and decision making. To ensure such transparency at the advisory committee meeting, FDA believes that it is important to understand the context of an individual's presentation.
For this reason, FDA encourages all participants, including the sponsor's nonemployee presenters, to advise the committee of any financial relationships that they may have with the firm at issue, such as consulting fees, travel expenses, honoraria, and interests in the sponsor, including equity interests and those based upon the outcome of the meeting.

Likewise, FDA encourages you, at the beginning of your presentation, to advise the committee if you do not have any such financial relationships. If you choose not to address this issue of financial relationships at the beginning of your presentation, it will not preclude you from speaking.

Industry Presentation - Dennis Hruby

DR. HRUBY: Good afternoon. My name is Dennis Hruby. I'm the chief scientific officer of SIGA Technologies. SIGA would like to thank the FDA for the opportunity to be part of this important discussion on the identification of an appropriate animal model data package to
demonstrate efficacy and define the human dose for antiviral drug for smallpox.

Today we're here to consider the extremely difficult question of how do we develop a drug for a disease that's been eradicated. In the absence of a significant orthopoxvirus disease burden in the human population, traditional drug development, that includes demonstration of efficacy in infected patients, will not be possible. So to develop a smallpox antiviral drug, in consultation with our colleagues at the FDA, we've taken the following approach. We've proposed regulatory approval based on the high risk of morbidity and mortality if smallpox or smallpox-like virus were to be encountered; standard assessments of clinical and nonclinical safety, including a determination of the PK of drug exposure; and efficacy as demonstrated in appropriate animal models. Moreover, the animal models will need to be used to make the PK/PD link to determine the appropriate human dose.

In May of 2002, the FDA formalized what's
become known as the Animal Rule. The central tenet of this statute stated that FDA may grant marketing approval for new drug product for which safety has been established and for which the requirements are met based on adequate and well controlled animal studies when the results of those animal studies establish that the drug product is reasonably likely to produce clinical benefit in humans. In the next few minutes we'll review the data that has been obtained, which we believe meets and exceeds this standard.

Our drug candidate, which is called ST-246, was discovered by traditional, high throughput screening. ST-246 is a highly potent, nontoxic, and specific inhibitor of orthopoxvirus replication in vitro and in vivo. ST-246 is effective at preventing morbidity and mortality in more than 40 different animal model challenges from mouse to monkey against a number of orthopoxviruses, including variola virus. The drug is orally bioavailable, with predictable PK parameters. The drug has an open IND with fast-track status. Most
of the registration studies have been completed, and pending the outcome of your deliberations today, the remaining animal efficacy in human clinical trials are designed and ready to launch.

ST-246 is a potent and selective orthopoxvirus egress inhibitor. The target of this drug is a protein that is highly conserved amongst the orthopoxviruses, but absent in other organisms. Shown here is a replication cycle of orthopoxviruses, including variola virus. The viral replication cycle takes place entirely within the cytoplasm of infected cells and results in the formation of two forms of infectious virions: a single membrane, intracellular mature virus, IMV, and the double membrane extracellular enveloped virion, EEV, that is responsible for disseminating the infection within the infected individual, as well as between individuals.

The IMV to EEV conversion is catalyzed by a number of viral proteins, including a 37 kiloDalton palmitoyl protein, F13L in vaccinia virus, and C17L in variola virus. This protein is the target of
The amino acid sequence identity of this protein is greater than 98 percent conserved amongst all orthopoxviruses. No naturally occurring amino acid changes in the protein correspond to reduced susceptibility to ST-246, as demonstrated by nearly identical inhibitory drug concentrations for all orthopoxviruses. In the presence of the drug, the secondary development process is abolished. No EEV is formed, and the spread of the infection in vitro is halted. To demonstrate in vivo efficacy, we've turned to animal models.

We've had many discussions with the agency on the implementation and interpretation of the Animal Rule. At their request, we have completed and submitted a large number of small animal studies in which the animals were infected with several different orthopox virus pathogens. We have tested our drug in various strains of mice, rabbits, ground squirrels, and prairie dogs infected with lethal doses of vaccinia, cowpox,
ectromelia, rabbitpox, and monkeypox via number of routes. Drug was given before, concurrent with, or after infection.

The results have consistently demonstrated therapeutic benefit of our antiviral drug candidate. Although the time course of disease is greatly shortened in most of these models, with death occurring in seven to eight days post-infection, without exception, a single daily oral dose of ST-246 provided significant protection, even when the treatment was delayed up to 72 hours post-infection.

Small animal models are useful for proof of concept studies for smallpox antivirals, but they have a number of deficiencies for advanced drug development, including abbreviated time course of disease, modest lesional disease, the designated therapeutic trigger, and atypical disease pathology. Furthermore, as animal models will be used to support dose justification, depending on the drug candidate, this can be difficult with small animals given the differences in their
metabolism as compared to humans.

The ideal animal model will recapitulate human disease both in time course and with characteristic lesional disease. It will be reproducible, with defined quantifiable endpoints, including mortality, viremia, and the lesions, which also provide an obvious therapeutic trigger. There should be a correlation between clinical observations and outcomes. The metabolism of the candidate drug in the infected animal should be similar to the human to allow the appropriate dosage and frequency of treatment to be established.

Of the animal models currently available, the nonhuman primates best meet the criteria. Although several inoculation routes have been explored, only IV injection produces reproducible smallpox-like disease in nonhuman primates, and the three IV models are shown here.

There has been a concerted effort to develop a respiratory nonhuman primate model, but to date, these efforts have suffered due to a lack of
correlation between lesion number and disease outcome and difficulties in establishing reproducible mortality. The most well characterized models are the IV monkeypox model, and, to a lesser extent, the IV variola model.

IV inoculation, five-times-ten-to-the-seventh infectious monkeypox particles or one-times-ten-to-the-eight infectious variola particles into nonhuman primates accurately recapitulates the time course and pathology of human smallpox from the point of secondary viremia onward, including robust lesional disease. The major difference in the models is that the mortality in the variola model is quite variable, whereas this dose of monkeypox causes uniformly high mortality, as shown by the orange box.

It should be noted that these models set an incredibly high bar for therapeutic intervention, introducing 50 to 100 million infectious particles into susceptible animals, giving the virus a several day head start to establish and amplify the infection, and then trying to intervene in late
stage disease in an infected animal with an antiviral drug. Also, it should be noted that in the studies we've conducted with regard to ST-246, the nonhuman primate displays human-like PK with regard to bioavailability, half-life, and a positive food effect.

Based on this, we believe the IV monkeypox nonhuman primate model represents the best available model for smallpox antiviral development. However, to conduct a reality check, at the suggestion of the FDA in 2010, SIGA convened a panel of poxvirus experts to review the literature in our data package. In addition, a number of experts from the National Institutes of Health and BARDA were invited to participate.

The three-person panel consisted of the experts listed here. You've heard from Dr. Damon this morning. Dr. Goff is in attendance, as well. And Dr. McFadden is here with SIGA today and is available to answer any questions you may have.

After an open session, multiple presentations and an active discussion, the
advisory panel went into a closed session and produced an independent report. The highlights of the report included that the variola model as it currently stands is insufficient to support candidate smallpox antiviral development, and there's no scientific information to suggest improvements are possible or forthcoming; that the IV monkeypox model accurately recapitulates human smallpox; and, finally, that the equivalent in vitro and in vivo sensitivity of variola and monkeypox to ST-246, along with the conserved nature of the drug's target, supports the use of the IV monkeypox model.

Their summary conclusion reads as follows.

"The reproducibility of the monkeypox nonhuman primate model and its close similarity to the exanthemous phases of human monkeypox variola disease make it the best currently available nonhuman primate model for evaluation of potential antiviral efficacy of ST-246 for treatment of human smallpox."

To that end, to date, SIGA has conducted
nine IV monkeypox challenge studies. As a challenge virus, we have employed the monkeypox Zaire '79 strain. As you will note, this strain was isolated during an unusually serious human monkeypox outbreak that caused 27 percent death and 91 percent severe disease in those afflicted. This strain is fully virulent in the nonhuman primate, reproducibly killing 100 percent of infected monkeys when they are given five-times-ten-to-the-seventh PFU IV. Furthermore, this strain has been subjected to limited laboratory passage as the fully characterized and validated reference strain prepared by NIH and used as a standard for smallpox vaccine and antiviral evaluation at all their intramural labs, the Department of Defense labs, and at various contract research organizations.

The IV monkeypox studies have all had basically the same format, with animals receiving one-to-five-times-ten-to-the-fifth or ten-to-the-seventh PFUs of monkeypox IV on day 0. Under these conditions, the infected animals begin to elaborate lesions in three to four days, and typically
succumb to disease or are euthanized due to acute morbidity at 7 to 17 days post-infection.

The decision to euthanize an animal is based on prospectively defined objective and quantifiable clinical criteria that does not include virological data, as outlined in the study protocol. After blinded review of the clinical observations, final approval to euthanize an animal is given by the study director.

ST-246 at various times post-infection at various doses has been given once per day for 14 days, and the animals are evaluated for lesions, viremia, survival, and general clinical signs. After recovery and/or cessation of therapy, the animals are followed for a period of time to ensure no recrudescence of disease occurs. The initial experiments were conducted non-GLP, with most of the latter ones done under GLP. Protocols have been reviewed by the FDA as we have progressed with continual improvements to the protocols, assays, and euthanasia criteria evolving.

These first three experiments represent a
natural history, the first proof of concept challenge, and our initial dose-down experiment. As you'll see, we achieved complete protection even at a dose of 3 mgs per kg ST-246. The next three experiments sought to increase the number of test animals to improve statistical parameters, to validate that the proposed human dose was effective in late stage disease after lesion onset, and to determine the minimum protective dose. You can see that 10 mgs per kg was fully protected when given either four or five days post-infection, and that the minimum protective dose was confirmed to be 3 mgs per kg, as shown in the lower right of the slide.

These last three studies are quite recent, and were conducted at the request of the FDA. The first was designed primarily to provide robust PK sampling in infected and uninfected animals to obtain an appropriate data set for our dose justification modeling. However, you'll note the results confirm a dose of 3 mgs per kg or above is fully protective.
The second experiment, examining the minimum duration of dosing required, and, much to our surprise, clinical benefit was seen with as little as three days dosing, with complete benefit received by five or more days of dosing.

The final experiment tested how late in the disease course ST-246 could be given. Again, surprisingly, even six days after infection, when the animals were acutely ill, there was observable clinical benefit. These nine studies have been conducted by multiple investigators, different locations under BSL3 containment, but the results and endpoints have been remarkably consistent.

One point raised by the agency is that perhaps if the monkeypox virus was delivered via the respiratory route, that the infection would differ in character from an IV inoculum and that the drug would be less effective.

To test this premise, as Dr. Challberg described earlier, we have recently participated in an aerosol monkeypox nonhuman primate challenge sponsored by NIH. Although the data and results
are still being analyzed, the overall result of the experiment is shown here. Whereas only 25 percent of the control animals given one-times-ten-to-the-fifth monkeypox particles via aerosol survived, 100 percent of the animals given ST-246 at the proposed human dose, with therapy initiated at one, two, three, or four days post-infection, survived, and there was a marked reduction in lesional disease.

While variola studies may be useful to provide confirmation of antiviral drug efficacy, the obstacles to conducting studies in the variola-infected nonhuman primate, as well as the numerous limitations in the model itself, preclude its use for pivotal efficacy studies.

Recall that variola is a strictly human pathogen which allowed its eradication. Although numerous attempts had been made to introduce this virus into a variety of experimental animals by a variety of routes, only limited success with high dose IV inoculation into nonhuman primates has been achieved. Delivery of ten-to-the-eighth PFU of
variola IV results in lesional disease with a time
course similar to the monkeypox nonhuman primate
model, but with limited viral replication and
variable mortality. Delivery of ten-to-the-ninth
PFUs of variola IV results in high mortality, but
causes atypical hemorrhagic disease with a
shortened time course, and most animals die prior
to the formation of lesions, the designated
therapeutic trigger.

In addition to the shortcomings of the
variola nonhuman primate model itself, there are
some major logistical impediments to working with
the virus. Variola is known to be held at only two
high security labs, one of which is at CDC. Any
studies with variola virus must be conducted under
a protocol approved by the WHO smallpox research
advisory committee, an approval process with highly
stringent criteria. Also, the destruction of
existing viral stocks is under constant discussion.

Here in the U.S., only the USAMRIID group
has approval to execute variola nonhuman primate
experiments, and this has to be done with the
cooperation of their CDC colleagues, pending availability of the BSL4 suites at which activities and analyses are limited. However, even with these limitations, we have worked with our USAMRIID and CDC colleagues to execute three variola experiments, with a fourth in progress. These have included a national history observational experiment, a proof of concept experiment at a high dose of ST-246 that completely inhibited lesion formation, viremia and death, and a third experiment where infected monkeys received the proposed human equivalent dose after lesion eruption.

Underscoring the variability of the model, there were no deaths in the control group in this latter experiment, so mortality cannot be used as an endpoint. The drug did, however, provide clinical benefit, with a reduction in both viremia and lesions, even when given this late in the disease course.

To summarize some of the shortcomings of the variola model, here we compared the infected
placebo groups in two of our nonhuman primate studies, 26G and 1745. The blue line on the graph illustrates blood viremia levels and the red line represents lesion counts. We can see that after the eclipse period, monkeypox amplifies the infection by more than four logs, whereas the variola virus grows poorly with only a one-log increase. Peak monkeypox viremia occurs at 13 days post-infection versus seven days for variola virus, and monkeypox induces nearly three times as many lesions as variola. Finally, it should be noted that all of the monkeypox infected monkeys had died or were euthanized by day 17, whereas all of the variola monkeys recovered.

If a drug were delivered to variola infected monkeys at the designated therapeutic trigger, the best outcome one might expect to see is a one-log reduction in viral replication, a significant reduction in variola lesions, and no effect on mortality. This is exactly the result seen with ST-246 in this model.

So now let's look briefly at drug resistance
and the safety profile of ST-246. As expected, one can select for ST-246-resistant poxviruses in vitro, but mutation frequency is low, approximately 1.3-times-ten-the-minus-six. This rate of mutation is two orders of magnitude lower than single-stranded DNA viruses and three orders of magnitude lower than single-stranded RNA viruses. The mutations all map to a specific conserved region of the target protein. In vitro and in vivo analysis of mutant fitness indicates that they are reduced or equivalent to wild type, but not enhanced.

In all of our animal studies, we've evaluated the potential emergence of drug-resistant mutants. To date, no treatment failures have been seen due to the emergence of drug-resistant virus. In fact, no resistant variants were seen in immunodeficient mice even after 84 days of ST-246 treatment. Thus, we do not believe drug resistance will pose a problem for acute human use.

Going forward, we plan to continue monitoring emergence of drug-resistant viruses. Resistance mutations arise in the specific sub-
domain of the F13L gene. We have been developing primer sets and deep sequencing protocols to allow virus stocks to be quantitatively analyzed before, during and after challenges in the presence of the drug.

All of the IND and NDA enabling toxicological evaluations that have been conducted on ST-246 are listed here. All studies are complete. The study reports have been filed with the FDA, and we're currently drafting NDA module four for submission. Bottom line on these studies is that the drug is noncarcinogenic, it has a high therapeutic index when given either short-term or long-term to animals, including the nonhuman primate, at doses far higher than the anticipated human dose.

In addition to animal toxicology evaluations, we have evaluated drug safety in humans in three phase 1 studies that included an ascending single-dose study, a 21-day dosing study at supratherapeutic levels, and a PK crossover study comparing two polymorphic forms of ST-246.
We've also completed a phase 2 safety study with robust PK sampling. In all of the studies, there were no SAEs, no deaths, and adequate blood exposures were observed in all volunteers.

In addition, we have participated in four EINDs to treat patients, some with life-threatening complications from exposure to vaccinia virus. In each case, the infection was resolved and no drug toxicities were noted. Thus, the drug appears to be safe and effective in these cases.

We've also considered other ways to demonstrate smallpox antiviral efficacy in the human. We believe that human efficacy of a smallpox antiviral drug cannot be inferred by reference to antiviral efficacy against other unrelated viruses, such as herpes viruses, papovaviruses, or adenoviruses.

Relative to poxviruses, these viruses are genetically unrelated. They have different replication cycles, cause different diseases with different pathologies and kinetics. Poxviruses, such as variola virus, are uniquely successful
pathogens whose interaction with the acquired and
innate immune systems is an integral part of the
disease process. Luckily, there are insufficient
human orthopox infections to allow drug efficacy to
be demonstrated in controlled clinical studies.

Other than the occasional cowpox infection
in Europe or vaccinia infection in the U.S. or
Brazil, the only nidus of orthopox disease are
zoonotic monkeypox infections in Africa.
Unfortunately, besides the reluctance of local
health authorities and the WHO to participate in
clinical studies in this region, there are a number
of other obstacles to generating any meaningful
clinical data in this situation. These include
that overall disease burden is low and sporadic.
Disease occurs in a poorly accessible location.
Regional health care facilities are rudimentary,
making sampling and patient evaluation difficult.
Those primarily afflicted are children, median ages
9 to 10, and ST-246 has not been evaluated in this
population in the U.S. Patients are seen typically
only with late stage recovering disease. And,
finally, there's a high incidence of underlying
disease from other agents, varicella zoster, staph,
malaria and dengue, making diagnosis and antiviral
efficacy evaluations difficult in the field.

Now, let's look at dosing and
administration. In the event of a smallpox
outbreak, an important question is how
coadministration of ST-246 with either first or
second generation vaccines might interfere with
immunization. Remarkably, due to the unique
mechanism of ST-246's activity, the concurrent
administration of the drug and vaccine does not
diminish either the immune response or protective
immunity in either mouse or nonhuman primate
models. In addition, immunocompromised animals can
be safely and effectively vaccinated in the
presence of ST-246. Also, animal experiments have
demonstrated reduced shedding of live virus in the
presence of ST-246, and the mechanism of action of
the drug would predict a reduction in adverse
events caused by viral spread.

In addition to the demonstration of efficacy
and safety, the animal models are the basis of human dose justification. We have conducted a detailed analysis of the PK/PD parameters of ST-246 using the data obtained from the uninfected and infected monkeys using the IV monkeypox model and from our human clinical trials.

The bottom line is that the infection has no significant impact on drug exposure, and the modeling suggests that our proposed human clinical dose of 400 mgs per day is equivalent to 8 to 10 mgs per kg in a nonhuman primate, giving about a threefold efficacy margin over the minimum protective dose.

Finally, I'll share with you our development plan. Once we receive concurrence from the agency on the proposed human dose, we will proceed with a randomized phase 3 study in 400 healthy volunteers and a phase 1 thorough QTC evaluation, although our animal and human studies to date have given no cardiac safety signals.

As far as animal studies, we have a variola nonhuman primate challenge ongoing in which we are
looking at lesions and other clinical signs rather than mortality as the endpoint. We are replicating our previous experiment with drug intervention at four days post-infection, as well as treating a group at two days post-infection, which is a time point corresponding to more vigorous virus replication. We anticipate this exploratory study will confirm the activity of ST-246 against variola virus in the proposed clinical dose in an infected monkey.

In addition to the nine IV monkeypox nonhuman primate studies completed to date, we propose a final study using an updated protocol to be reviewed by the FDA. We anticipate this study will provide confirmation of efficacy of ST-246 in monkeypox-infected nonhuman primates at the proposed human equivalent dose of 10 mgs per kg. The primary endpoint would be mortality and the duration of the study would be two months. Sample size would be powered to demonstrate a statistically significant reduction in mortality. We propose that the successful completion of these
clinical and animal studies should be sufficient for an approvable package demonstrating efficacy and safety.

To summarize, in more than 40 animal trials using six species and six orthopoxvirus pathogens, ST-246 has consistently demonstrated protection of infected animals to morbidity and mortality. These include both small animal models challenged with low doses of natural pathogens and nonhuman primates, which more closely resemble human disease. In the best characterized IV monkeypox nonhuman primate model, ST-246 lowers viral load, reduces lesion formation, and protects from death even when therapy is delayed until after lesion formation in infected animals.

Similar results have been obtained in the monkeypox aerosol challenge, as well as the variola nonhuman primate model, despite its limitations. There have been no treatment failures due to the emergence of resistant variants in infected animals, including nonhuman primates. The minimum protective dose in the nonhuman primate has been
determined that suggests that proposed
400 milligram human dose will provide at least a
threefold efficacy margin. And, finally, the
proposed pivotal efficacy study for ST-246, we
think, should be in nonhuman primates with the IV
monkeypox model.

We and many experts strongly believe that
the monkeypox in nonhuman primate data best
characterize ST-246 clinical benefit in the event
of a smallpox outbreak. Using this model to
support the Animal Rule, we see evidence of
efficacy and can define dosing in humans, providing
us with sufficient data to conclude that ST-246 is
reasonably likely to show clinical benefit, thereby
allowing a regulatory pathway for this product.

SIGA would like to thank our federal
partners at NIH, DOD, USAMRIID, BARDA, and CDC,
whose support and cooperation has been essential to
the development of ST-246. Thank you for your
attention, and we look forward to answering your
questions. I'll be assisted by my colleagues from
SIGA, as well as two external experts, Drs. Grant
McFadden and Michael Corrado.

Clarifying Questions from the Committee

DR. CARGILL: Thank you. This presentation is now open for clarifying questions from the committee for the sponsor.

Dr. Henderson?

DR. HENDERSON: Thank you very much for the presentation. I guess what I'm concerned about would be what will the drug do between one and four days after lesion onset. If you think of this for treatment, the patients we're going to see are patients who have had fever and have a rash. And thinking through the pathophysiology of the viral infection, I'm curious about what the drug does after the first, second, third and fourth day into rash.

DR. HRUBY: We will continue to evaluate that. There are two points. First of all, in the monkey model itself, the IV monkey, we've been able to go out as late as six days post-lesion onset, or post-infection, and still see therapy. So that's about three to four days after we start to see
Secondly, the time course in humans is, if anything, slightly slower. We expect to see benefit. And, of course, the first sentinel cases are going to be recognized by lesion onset. Then I think with epidemiology tracing, some earlier stage patients may also be identified who would definitely receive benefit with this type of therapeutic.

DR. CARGILL: Dr. Strader?

DR. STRADER: You stated that ten-to-the-eighth PFU caused lesional disease in your nonhuman primate animals and that ten-to-the-ninth caused lethal disease. Is that correct?

What was the cause of death in those animals? Was it infection or was it pulmonary edema, was it hemorrhage? What was the cause of death, and does it approximate what we suspect as a cause of death in humans with smallpox?

The second question is when you are talking about resistance, you did your studies in vaccinia virus. Did you do those same studies in variola,
or if you had animals that were infected with variola virus, did you test -- continue treatment long enough to see whether resistance developed in those animals?

DR. HRUBY: I'll answer the first question and turn the first question over to one of my colleagues, Dr. Grosenbach. With regard to the second question, resistance, we have looked for the emergence of resistance in all of our challenge studies with vaccinia, ectromelia, cowpox, monkeypox, and variola. Typically, up to the cessation of therapy, we will monitor for at least two disease courses. In monkeys, this is out to 56 days now. And in all those cases and all those viruses, we have not seen any resistance.

Doug, would you like to comment on the similarities between the cause of death in the variola model versus the human?

DR. GROSENBACH: Right. So I'm Doug Grosenbach. I'm the associate director of pox virology at SIGA. There have been some recent papers published by Peter Jahrling's group looking
at the cause of death in smallpox-infected non-
human primates that have been infected with either
ten-to-the-eighth or ten-to-the-ninth PFUs of
variola.

So the lethal model for variola, that's
following infection with ten-to-the-ninth PFUs,
this is described as a hemorrhagic model. Many of
these animals die prior to the formation of lesions
by about day 4 post-infection, and you have
widespread hemorrhage and coagulopathy as probably
a cause of death there. Virus load is very high in
all the tissues regarding cause of death in humans.

So the hemorrhagic form of smallpox is very
rare. There is early hemorrhagic and late
hemorrhagic, and it accounts for less than 2 and a
half percent of all smallpox. So more typical
smallpox is considered the typical lesional
disease, and this occurs in a much higher
percentage, close to about 86 percent. But as far
as cause of death in hemorrhagic cases, it may be
similar to what you would see in the variola ten-
to-the-ninth challenge, a very small percentage of
patients, though.

DR. STRADER: What was the cause of death in
the animals that received the ten-to-the-eighth
PFU? That was lesional disease. So that wasn't
hemorrhagic disease. You said there was some
mortality there.

DR. HRUBY: That was one of the points I was
trying to make is that in our hands and the
challenges we've conducted in the variola model,
the mortality in that model has been nonexistent or
very low. In the one experiment I showed you, we
had nine experimental animals on placebo and none
of those died. So we can't really answer that
question.

DR. STRADER: So in the model that you used,
there's no mortality associated with --

DR. HRUBY: We've run it three times. One
time we saw one out of three mortality, the second
time we saw zero out of nine, and the third time,
the experiment I described is in progress and has
not been unblinded, so I can't answer that
question.
DR. CARGILL: Dr. Glenn?

DR. GLENN: I was just curious about the mechanism of action and I was wondering if you could tell me how the compound alters the interaction between this F13L protein and Rab9 and TIP47? And, also, have you tried it against other viruses that interact with those host proteins?

DR. HRUBY: To answer the second part of your question, to date, in the analyses we've conducted, ST-246 has been exquisitely specific for orthopoxviruses. It does not have any activity against other bacteria, fungi or mammalian cells. And as far as other viruses that use the same export pathway, it has no activity whatsoever. Now, with regard to the precise biochemical action of ST-246, we don't know. As you know, that path of the protein has both a conserved transmembrane domain, as well as a potential phospholipase domain. We have not shown biochemically interaction between ST-246 and the molecule to be able to track it. This molecule is fairly hydrophobic and hard to express in vitro. So
you're familiar with the literature that's been published, and that's the extent of our knowledge about the mechanism of interaction.

DR. GLENN: And so like positive strand RNA viruses, no activity.

DR. HRUBY: No activity.

DR. CARGILL: Dr. Van Dyke?

DR. VAN DYKE: I was interested in your comment that you could give the drug with vaccinia immunization and not inhibit the immune response of the vaccine. Surely this inhibits vaccinia replication. So how do you explain that? Is it the first cycle of replication of the virus?

DR. HRUBY: Well, I like to think of it as sort of like MVA, if you will. Basically, you're not inhibiting replication of the virus. You're inhibiting spread of the virus, and you basically set up a pro-inflammatory focus for the immune system.

We have done experiments in mice, we've done it in monkeys. We've used ACAM 2000. We've used MVA. And looking at immunological parameters, both
antibodies, T-cell parameters, and then subsequently challenge, we see no diminution of the immune response.

DR. VAN DYKE: Do you get a take to the vaccinia?

DR. HRUBY: You do. In monkeys, which is where we most closely evaluated it, when you deliver a vaccine, at least ACAM 2000, where you get a take, you get the same level of take. It's slightly smaller and it resolves slightly quicker. But you get an obvious take you could record.

DR. CARGILL: Dr. Murata?

DR. MURATA: This is similar to Dr. Glenn's question regarding the mechanism of action of this compound. I just want to clarify that there is a single target -- a single gene product that is targeted by the drug, and that is required for -- absolutely required for an orthopoxvirus replication.

DR. HRUBY: In our hands, in all of our attempts to deliberately select for resistance, they've all mapped within one sub-region of the
F13L gene in vaccinia. That has a homolog in all
the orthopoxviruses, as I mentioned. That's C17L;
in variola virus, C19L.

In all these viruses, we have -- in vaccinia
at least, we have tested what the phenotype is of
knocking out this gene product, and it's exactly
the same as in the presence of ST-246. You see
reduced viral spread. You see no plaqueing. And
when you put that virus into an animal model, as
Geoff indicated, in the absence of EEV, we see
about a 10,000-fold reduction in LD50.

DR. CARGILL: Dr. Van Dyke?

DR. VAN DYKE: I'm just curious what the
potential is for a pediatric formulation of this
drug.

DR. HRUBY: We're certainly very cognizant
of the need to protect the pediatric population.
We are in the process of developing a pediatric
suspension formulation to be used both in children
and the geriatric population, and we'll be bringing
that forward, as well as the oral capsule.

I would add that we have not tested directly
in the human pediatric population, but some of our studies have been done in juvenile monkeys, and the drug was protective.

DR. CARGILL: Thank you. Dr. Goetz?

DR. GOETZ: I want to go back to Dr. Strader's question and the response about the similarity of pathology in the monkeys that died with the high dose challenge of monkeypox versus human disease. You addressed quite clearly, at least if I heard properly, that the pathology resembled that of hemorrhagic smallpox. And yet, if I understand right, your plan is to go forward with further studies in the monkeypox model.

I wonder, how do you then extrapolate the effectiveness of the agent against the more common manifestations of smallpox that were described, where the lesions are not hemorrhagic in nature? There may have been an explanation there that I missed, but I'd appreciate hearing that again.

DR. HRUBY: Very good. The answer to the previous question was actually in response to the question about the variola model. With regard to
the monkeypox model, certainly, we're giving a dose high enough to achieve 100 percent mortality to allow mortality to be used as a quantifiable endpoint. However, we have secondary endpoints. Those are lesion count and viral load. And in both of those cases, we see effectiveness of the drug against the infection. And as you've heard today, those are manifestations that have been used previously to follow the course of both monkeypox and variola in humans.

DR. CARGILL: Dr. Magill?

DR. MAGILL: Two questions. One is I think on slide 38, you pointed out the proposed human dose of 400 milligrams, and yet all of your animal studies are in milligram per kilogram doses.

Comment?

DR. HRUBY: I will ask Dr. Leeds to tell you a little more details about our PK/PD modeling.

DR. LEEDS: So if I could have the slide up, this will just show you, in uninfected humans -- well, uninfected monkeys dosed with 10 milligram per kilogram at steady-state, the
plasma concentration time curve overlaid with our human safety study at 450 milligrams. We have then -- if you put the next slide up -- done a time course looking at the effective infection on the pharmacokinetics and it's the very mild effect.

We've developed population PK models in both the nonhuman primate, the monkey -- and humans. We've applied the effect, which is a small increase in clearance, to the human exposure, which is shown here on the next slide. And then we've done simulations of monkey exposure in infected monkeys over a range of doses, and then -- next slide, please -- we've shown which doses are equivalent to human. So we see both 8 and 10 mgs per kg are equivalent to exposure at 400 milligrams in the humans predicted for infection.

DR. CARGILL: Thank you. Dr. Camardo?

DR. CAMARDO: I have a question that's somewhat related to the clinical outcome and also to the dose. So I'll see if I can ask this clearly.

Can you determine in the studies of
monkeypox in nonhuman primates what's happening to the animals who are not getting better because they got too low a dose, because if you extrapolate to treating people, you would be tempted to say this patient is not getting better, maybe they need a higher dose. It looks like it would be safe, but would it be effective?

So can you get that kind of clinical information in these kind of studies?

DR. HRUBY: Well, certainly in the monkey model, at 3 mgs per kg, we've had almost uniform survival when you look at mortality as the endpoint. If you look at other endpoints, such as lesion number or viral load, we can see intermediate effects versus, say, 10 mgs per kg. So we get some of that data out of the monkey model.

Now, Doug, I don't know if you can comment on what happens to 1 mg per kg to the animals that survive or succumb.

DR. GROSENBACH: Right. So the markers that we follow most commonly are viremia and lesion
counts, and as Dr. Hruby had said, a dose of 3 mgs per kg is efficacious, and we see a reduction, about a fourfold reduction in the amount of lesions at 3 mgs per kg. We also see a two-log reduction in viremia. But if you treat at doses lower than 3 mgs per kg -- for example, in our studies, we've used 1 mg per kg, .3 mgs per kg -- what was seen is that the viremia level and the lesion count are essentially unaffected relative to the placebo control. So there's a threshold right there between that 1 and 3 mgs per kg where you have nearly 100 percent efficacy versus almost zero percent efficacy.

DR. CARGILL: Dr. Strader?

DR. STRADER: I want to take another stab at this, because I'm having a hard time understanding it. So variola at ten-to-the-eighth PFU does cause lethal disease in nonhuman primates.

DR. HRUBY: Not reproducibly.

DR. STRADER: Not reproducibly. But high dose IV monkeypox sort of simulates what you think is happening with smallpox, albeit it gets rid of
the incubation period. So it starts from the point
of seeing clinical symptoms. And you think that
that model better replicates the variola model than
actual ten-to-the-eighth variola virus in the
nonhuman primate.

I'm having a hard time understanding if you
give it to the monkeys and you don't get lethal
disease, but we get lethal disease in humans, why
you think another virus that does cause lethal
disease is a better model than the actual virus
itself?

DR. HRUBY: I think you really have to view
both models in context. It's not either/or, it's
together. And I think we're proposing the IV
monkeypox model because, in fact, the time course
and pathology of symptoms and disease is really
identical between the two models, except for death.
That's a quantifiable endpoint that I think we can
get some statistically significant data around.

The other issue -- I promised myself I
wouldn't go here -- is the whole concept about this
being a high dose challenge, when, in fact, if you
look at the data that's coming out of the Congo, when they get a human monkeypox patient that is still in the active phase of disease, the level of virus that they have in their blood is actually quite similar to the monkey. So I would actually argue we're giving a very similar dose that you would have at the start of the prodrome area. And likewise, there are some papers in the literature, that you can go back historically, where they were enumerating the virus in the bloodstream with smallpox patients using the pock on the chorioallantoic membrane. And if you back-calculate, you come within an order of magnitude to the same level of virus that we're putting into these monkeys.

So it's high dose, but it might very well be equivalent to what the human is experiencing at that point in time.

DR. CARGILL: Mr. Raymond?

MR. RAYMOND: Thank you. I had a question about the monkeypox aerosol efficacy challenge. It looked like the protocol was vehicle-controlled on
day 1 post-inoculation and treatment, day 2, day 3, day 4. What would the corollary be with that in a human smallpox model? Would that still be the early incubation phase or would there be any symptomatology to trigger treatment?

DR. HRUBY: Well, I think there's really very little correlation. As you've heard today, the time course of human smallpox may be as long as 17 days before you actually see lesions and pathology. Here, by day, I believe, 2, if I remember Mark's data, you're starting to see fever, you're starting to see lesions. So it's a much more rapid onset of disease. What it does fall on is it says that whatever happens during the early stages of an infection prior to secondary viremia, the drug is also able to inhibit.

DR. CARGILL: Thank you. Dr. Magill?

DR. MAGILL: On your monkeypox nonhuman primate IV challenge model – and, again, I'm not quite sure I understand the lesion onset in one of your studies. The treatment was at lesion onset and then it was days post-infection.
What is the typical window in this model of lesion onset? And then in any treatment arms, have you treated past lesion onset, and specifically have you treated it -- essentially at the time you've met your surrogates for mortality, can you rescue at that point? So any treatments from lesion onset to death.

DR. HRUBY: In this model, as we have progressed, as you've heard, typically lesions start to be elaborated between three and four days post-inoculation. By four days, most of the animals have lesions. And so as we worked through these nine studies, we started marching out. And originally we did the three days and some animals were lesional, some were not. Then we went to four days, most were lesional, some were not. And actually the later protocols, what we've done is at lesion onset.

These monkeys are examined frequently, and we start treating when you see the first lesion. So they're all in the same boat, if you will.

Now, having done that, we can go out to six days post-infection, three days post a lesion
eruption and still see clinical benefit. So in that case, you're taking an animal that may only be one to two days away from crashing and being euthanized, you're able to give them drug and rescue them.

DR. CARGILL: Thank you. I'd like to come back to the question around the 3 mgs per kg dosing and the comment that was made, I believe, by one of your colleagues, that there is a level below which you did not see any difference in lesions or response. And my question is, while that was the case, did you see any evidence of resistance?

DR. HRUBY: We have not seen any evidence of resistance, even at suboptimal therapeutic levels in the survivor animals.

DR. CARGILL: Thank you.

We'll now go ahead and take our 15-minute break. I'm trying to read Paul's handwriting here.

Panel members, please remember there should be no discussion of the meeting topic during the break amongst yourselves or with any members of the audience. We will resume promptly at 3:20. Thank you.
(Whereupon, a recess was taken.)

DR. CARGILL: We will now proceed with our final presentation for today. I would like to remind public observers at this meeting that while this meeting is open for public observation, public attendees may not participate accept at the specific request of the panel.

Dr. Chan-Tack?

FDA Presentation - Kirk Chan-Tack

DR. CHAN-TACK: Good afternoon. This talk will highlight some of the challenges and issues in drug development for the treatment of human smallpox. To begin, the following will illustrate some examples of some desirable study design elements, and these include a primary endpoint of mortality, euthanasia based solely on clinical criteria, the ability to produce clinical manifestations of disease with consistent mortality after a low viral inoculum, and the exploration of higher inocula if needed to confirm the treatment effect.

It also includes that the routes of
inoculation be similar to that for human smallpox, that adequate dose exploration is undertaken, that the timing of treatment initiation relative to clinical disease manifestations are considered, that the optimal duration of treatment is evaluated as is an evaluation of a viral resistance, and that pharmacokinetic and pharmacodynamics information from animals and the PK information from humans are sufficient to establish an effective dose.

The drugs that are currently in active development for the treatment of human smallpox, you've heard from the sponsors' proceeding are CMX001 from Chimerix and ST-246 from SIGA.

CMX001 is a lipid pro drug that is a derivative of cidofovir. The oral formulations that have been developed include a tablet and solution. It targets viral DNA polymerase and prevents genome replication. Its antiviral activity is against orthopoxviruses and other double-stranded DNA viruses, such as herpes viruses and adenoviruses.

The available animal data for CMX001 is
highlighted below. The combinations of animal models and orthopoxviruses that have been evaluated included the mouse with ectromelia, vaccinia, and cowpox, dormice with monkeypox, rabbit with rabbitpox, and nonhuman primate, i.e., the cynomolgus macaque, with monkeypox. No planned efficacy studies with variola have been done or planned with CMX001. The sponsor, as you have heard, proposes to use the mouse ectromelia model and the rabbit rabbitpox model for its pivotal efficacy studies for establishment of efficacy under the Animal Rule.

Some issues have been identified to date, and these include that adequate CMX001 plasma exposure was not achieved in the cynomolgus macaque with oral dosing of CMX001, and this complicates efforts to develop a macaque model for the oral drug. Administration of CMX001 by intramuscular injection, which does achieve a higher plasma exposure, was explored in one monkeypox virus study in macaques.

So to present a highlight of the mouse
electromelias studies with CMX001, we've received the preliminary study reports and these are under review. These are notable for the use of low inoculum sizes, respiratory routes of inoculation. Dose ranging and treatment duration has been explored.

These studies were not conducted in accordance with good laboratory practices. They were non-blinded and randomization was not clearly defined. And as has been mentioned, which clinical manifestations in the mouse model that are most appropriate for treatment initiation needs further discussion.

The following two slides will summarize some of the recent rabbit rabbitpox studies with CMX001. Studies UF-010, 011 and 012 are randomized, blinded, and placebo-controlled. The route of inoculation is by intradermal challenge, a relatively low inoculum size of 100 PFU were used, with treatment begun at the time of skin lesion onset.

Mortality was based on clinical criteria for
euthanasia, insomuch that scheduled euthanasia of surviving animals occurred at the end of study, i.e., day 14 post-inoculation, whereas unscheduled euthanasia of moribund animals occurred at earlier study time points prior to the end of the study. And the dose that was explored was 20 milligrams per kilogram.

So I'll go through these studies. So as you'll note, they're all non-GLP, as was mentioned before. The dose in the first study was 20 milligrams per kilogram for three doses and begun at the time of lesion onset, which ranged between days 3 to 5 post-inoculation. The treated animals, the mortality in this group was 8 percent. The mortality in the placebo group was 83 percent.

In the second study, a single dose was used, again, initiated at the time of lesion onset, which was day 3 or 4 in this study post-inoculation. Mortality in the treated group was 42 percent versus 92 percent in the placebo animals.

The third study employed two doses, again, begun at the time of treatment -- begun at the time
of skin lesion onset, with mortality being 33 percent in the treated group versus 92 percent in the placebo group.

ST-246, an oral capsule has been developed. Its mechanism of action is it inhibits the replication of the orthopox extracellular envelope virus, but not the intracellular mature virus, and it has antiviral activity only against orthopox viruses. The current status of the available animal data is summarized on this slide. The combination of animal models in orthopoxvirus that have been evaluated include the mouse model with ectromelia, vaccinia, cowpox, and immunodeficient mice with vaccinia, the ground squirrel model with monkeypox, the rabbit with rabbitpox, the prairie dog with monkeypox, and the nonhuman primate, that is, the cynomolgus macaque model, with both variola and monkeypox. The sponsor proposes to use a nonhuman primate monkeypox model for pivotal efficacy studies to fulfill the Animal Rule criteria for treatment of human smallpox.

This slide highlights the nonhuman primate
variola data with ST-246. Study 1470, non-GLP, the
Harper strain was used, a high inoculum of ten-to-the-eighth PFU administered IV, randomized, non-
blinded, with a dose administered of 300 milligrams
per kilogram per day for 14 days. And this was
begun on either day 0 or day 1 post-inoculation
with the virus, and this was prior to skin lesion
onset in these animals. As you can see, the
mortality was zero percent in the treated group.
However, it's notable that the dose was high and
the treatment initiation was very early. The
placebo animals, there is two out of two mortality
that's noted. However, one of the placebo animals
was euthanized when non-moribund.

Study 1745, also non-GLP, also employing the
Harper strain, with the same ten-to-the-eighth PFU
IV administered inoculum, this was randomized and
double-blinded. The dose was 10 milligrams per
kilogram per day for 14 days and administered at
the time of skin lesion onset in these animals,
which was generally day 4, with a few animals
having lesions at day 3. One out of seven of the
treated animals died, and this animal died due to anesthesia-related complications and did not appear to die due to variola-related complications. There was no mortality in the placebo group.

So, at present, the available data with this model suggests that it is unlikely to be informative with regard to mortality given that these results are not reproducible in the placebo animals.

This figure shows -- the red marks are your ST-246, the blue, the placebo group, and this is the time course of the viral loads over time. Again, drug was started for most animals at day 4, a few at day 3, and ended 14 days later. And as you can see, the viral load curves overall generally followed the same path. And as noted, the viral load changes did not correlate with any mortality benefit.

So some issues and challenges that have been identified in this current model. The variola virus infection in the macaque seems to resemble human smallpox with respect to the mild rash
illness but does not appear to resemble human smallpox in other aspects. As mentioned, there is inconsistent mortality in the placebo animals with this current model. The clinical and statistical significance of the temporary differences observed in the mean virologic response is unclear.

There's potential for further exploration and refinement of the model, as you've heard earlier, and then there are the logistical and other issues that have been discussed earlier, including CDC being the only site in the U.S. for study, and the potential for destruction of all variola stocks in the future.

The following couple of slides will summarize some of the more recent nonhuman primates monkeypox virus studies with ST-246. These four studies were randomized, blinded, and placebo-controlled. The route of inoculation was via the intravenous challenge, with a high virus inoculum of five-by-ten-to-the-seventh PFUs used.

Treatment was initiated at four days post-inoculation in most studies, and this approximated
the time of lesion onset in these animals. Mortality was based on clinical criteria for euthanasia; that is, the scheduled euthanasia of surviving animals occurred at the end of study, which, depending upon your study, ranged from day 28, day 42, or day 56 post-inoculation. Unscheduled euthanasia of moribund animals occurred at earlier time points prior to the end of study, and the doses used you can see below, ranging from .3 to 20 milligrams per kilogram per day.

So this slide will start off with Study 09-26G, which was conducted under GLP. As you can see, it is a dose ranging study. The treatment duration is the same in all arms, 14 days. The timing of ST-246 initiation is the same, day 4 post-inoculation, i.e., at the same time of lesion onset. And one notices the mortality in the placebo group was 100 percent; in the .3 milligram per kilogram cohort, 80 percent; 100 percent in the 1 milligram per kilogram per day group; 20 percent in the 3 milligram per kilogram per day group; and, 20 percent in the 10 milligram per kilogram per day
Moving on to Study 10-037F, this was a non-GLP study. The dose and duration were fixed. The initiation time point for treatment with ST-246 differed: the first group, day 4, at the day of lesion onset; the second group, day 5, that's 24 hours after lesion onset; and, the third group, day 6, that's 48 hours after lesion onset.

The placebo group, you can see that the mortality was 100 percent. When initiated on the day of lesion onset, the mortality was 17 percent, as it was for 24 hours after lesion onset. And then for 48 hours after lesion onset, the mortality is 50 percent.

Study 10-038F, a non-GLP study, the dose is similar in all of the groups. The treatment duration differs, 3, 5, 7, 10 days, and the treatment initiation is the same for all the groups. Once more, you can see that the mortality in the placebo group is 75 percent; 50 percent when the drug is administered for a three-day period; zero for a five day period; zero for a seven-day
period; and, 20 percent for a 10-day treatment
duration period.

The final study on this slide, Study 10-087,
was conducted under GLP. It was a dose ranging
study with 3, 10 and 20 milligrams per kilogram per
day for 14 days, again, all with the same treatment
initiation time points of day 4 post-inoculation,
i.e., at the time of lesion onset. And as noted,
all of the treatment groups had zero percent
mortality, whereas the placebo group had
100 percent mortality.

Some examples of potential distinctions
between the historical human smallpox disease and
the various animal models that have been discussed
today include the following. The route of viral
exposure varies between models and often is not
similar to the human respiratory routes. Host
susceptibility varies; i.e., there is the need for
high viral inoculum in many models, as you've seen.
And there are differences that have been observed
in viral pathogenicity in different orthopoxvirus
host combinations.
Most models do not have incubation periods that are comparable to the historical human descriptions of smallpox. And the relationship between the time course of viremia and clinical manifestations may vary. There are many unknowns regarding the immunomodulatory properties of these different viral species, and there are many unknowns to the immune responses to different orthopoxviruses in different animal hosts.

Some examples of issues for additional discussion are reflected on this slide, and these include standardization and/or consensus on clinical criteria used to determine the euthanasia criteria in a given animal model; how similar should the routes of inoculation be to that for human smallpox; further discussion about the timing of treatment initiation relative to clinical manifestations of disease; and, which clinical manifestations of disease should be used as a trigger for treatment initiation.

Of course, there should always be adequate exploration of dose and treatment duration and
evaluation of drug resistance; and then, lastly,
how much PK and PD information from animals and PK
information from humans is sufficient to establish
an effective dose.

In summary, both investigational drugs show
activity in the animal models in which they were
studied. There are challenges and issues with all
of the anti-orthopoxvirus animal models that have
been described, and we are looking forward to
further discussion and input from the advisory
committee as to which combination of models might
most predict, in a reasonable way, treatment
outcomes in human smallpox.

I'd be happy to answer any questions.

Clarifying Questions from the Committee

DR. CARGILL: Thank you. At this time, any
clarifying questions for Dr. Chan-Tack? Again, if
you would raise your hand and Mr. Tran can get your
name.

Dr. Lyons?

DR. LYONS: Thank you. One of the things
I'm struggling with with regard to the best model
is maybe FDA could expand a little bit on how they
think it's going to be used. The treatment of
smallpox, you must have received some briefing
about how a smallpox exposure would occur and when
the drug would presumably get to potentially
infected individuals. I mean, based on the
differences in the models, it would help, for me
anyway, to understand how you envision this
unfolding.

DR. COX: That may be a question answered by
other panel members or I don't know if any of the
guest speakers would like to comment on that topic.

DR. CARGILL: Dr. Khan?

DR. KHAN: Thank you. I showed a slide to
that effect during my presentation, and we can
bring that up again or you may be able to find it
within your materials. But there are a couple of
different ways that we could consider using an
antiviral in conjunction with other countermeasures
that are available to us during a smallpox release
in our community. And you do bring up a very
important question, which is the initial
recognition of disease and when would you recognize at least the first case. The subsequent cases are a little bit easier, but that first initial case is likely to be recognized at lesion onset, most likely. On the slide, you can see some of the various scenarios that we've laid out where we would consider using an antiviral.

DR. CARGILL: Dr. Goetz?

DR. GOETZ: So I can just follow-up on that. You say the first case will be recognized, sadly, after lesions come on. But it would seem to me that there would be -- if there is truly an event, a very hypothetical "if," that there will likely -- if it's really a large-scale event for which we truly need a large amount of medication, there will likely be other patients presenting late, as well. So we'll need both late and early throughout the considerations.

DR. KHAN: Correct. And it depends on the scenarios, and there are numerous models that examine this. Please also recognize that we don't have only antivirals -- potentially, we would not
only have antivirals available to us, we would also
have vaccines available to us, isolation,
quarantine, other public health measures that would
be available to us to try to get this outbreak
under control. So we wouldn't just be waiting for
another case to treat.

    DR. CARGILL: Dr. Magill?

    DR. MAGILL: Also, to follow-up on that, I
think once there was a confirmed release and a
confirmed outbreak of smallpox in a community or
perhaps even multiple communities. I think it's
being very optimistic and very naive to think that
there's going to be some sort of rules applied that
are going to stick. There's going to be a vast
number of worried well and a variety of folks who
are going to inundate all health care providers and
emergency rooms, and if there is known to be a
treatment, there's going to be a great demand for
it.

    So I'm sure this has been thought of and
considered by yourselves and many others. But this
concept that I think the drugs, if they're
available, or other interventions are going to be
given to people who are not infected, I think is
clear. And large numbers of people who are not
infected are probably going to receive some
intervention just because they're there.

I haven't heard any discussion of, quote,
"diagnostics." We've talked about clinical and
epidemiologic diagnostics, say, a classic
presentation, but what about virologic
confirmations either through classic means like EM
or some other measures? Is that going to be a
gateway to receive treatment and such?

DR. KHAN: Yes. That's a really good
question. The people wanting to be treated, I
think that will definitely be true as far as
vaccines are concerned, immediately. And this
comes up in our scenario planning, is if there is a
potential release of anthrax in multiple
communities, what is the trigger to essentially
start a nationwide vaccination campaign.

In addition to surveillance, I did put up
the fact that we do have diagnostic methods in
place. We have a laboratory response network in
the United States. There are 154 laboratories
across the United States that have the ability to
make a laboratory diagnosis of smallpox. That
would be required initially. Most clinicians,
fortunately, in this room don't know how to make a
clinical diagnosis of smallpox. There are a
handful that do. But once smallpox reestablished
itself, if we did see it again, people would be
able to make the clinical diagnosis again also.

   DR. CARGILL: Dr. Magill?

   DR. MAGILL: If I could just follow-up on
that. I was very much impressed in some of the
materials that were presented or prepared for us
and the presentations today; in a previous era, the
tremendous clinical diversity of responses. And
there is some sort of sense that there is a classic
or typical case, and that's certainly not what I
took away from my readings prior to this meeting.
And from what's sitting here today, I think there
could be a very dramatic diversity of responses.

   DR. KHAN: I think Dr. Inger presented that
quite well earlier this morning, that, correct, typical, I believe, presented 85 percent of cases, Dr. Inger? But there is likely to be a diversity of cases, and if we ever saw this disease again, I'm not sure those specific criteria -- set of conditions would last. I think now, with specific virologic diagnosis, we may be able to differentiate a lot better what these various symptom sets may look like.

DR. CARGILL: I would just point out to the panel that Dr. Khan will be with us only today, so if there are other questions that you specifically wish to target to him, this might be the time.

Dr. Reller?

DR. RELLER: Barth Reller, Duke University.

Dr. Khan or perhaps Dr. Henderson, could you refresh the relative importance of, in humans with variola, smallpox, the aerosolization versus droplet route of inoculation? And I ask the question because there are several possible ways that we might see smallpox again. One is reintroduction as a natural disease versus a
massive exposure, as bioterrorism, and there's
some, to me, logical implications of those routes
of spread.

DR. HENDERSON: Well, I must say since 9/11,
this has been a topic of a great deal of
discussion. And I think the question at the
moment -- and I think there's still -- what is it
that we have to be prepared for and what is likely,
and I think there's a division of views on this and
it depends on where you are. I think when you look
at it with a modest outbreak, let's say, of 50 or
100 cases, it will be a chaotic situation. We have
no diagnostic tools readily available.

The question is what are we likely to
see -- and based on some outbreaks we've had in the
past, what are we likely to see, and there is then
also the fear that this is an outbreak of smallpox.
I think we'll see a lot of people with fever coming
forward and wanting treatment.

What are we going to do on the vaccination?
I think until now, the thought has been that we
would do what we've done in the -- what we've laid
out beginning in 2002, what we call the containment procedure, and that is to isolate and contain, vaccinate the patient and those who have been in contact with them, hit the high at the hospitals and basically constrain the vaccination. So it's not a massive vaccination in which there are a lot of complications.

There are a lot of things to be worked out here. The one thing that many people don't realize is that this disease does not spread very easily. So the individual normally comes down with very high fever for a couple of days, and mostly they take to bed, they just feel so miserable. And then they don't begin to transmit it until the rash actually develops. And so it's three to four days after the beginning of the fever that the rash emerges, and, at that point, they're transmitting the disease.

But then we looked at the outbreaks in Europe from 1960s, '70s, the last in '72, and we found that half of all subsequent cases occurred in the hospital, as a result of hospital contact, and
another quarter of them were contacts within the household; so a quarter of them that actually were unaccounted for, primarily, but may have been community, but we're not sure about that. We did not see outbreaks in schools, for example. We did not see outbreaks in work sites, because the people are home when they're really infectious.

So there's a strategy that is necessary to control an outbreak. What needs to be settled really is a pretty firm strategy that can be well communicated at state and local levels so that everybody is sort of looking at this in a similar way, and we're able to communicate fully what needs to be done.

So there are a number of things that really need to be done, and Dr. Khan and his group are moving very rapidly on this now. But I think this is an important point, where would ST-246 come into play, which I think is important, and how much of a use would there be? And this is where I was worried about the rash, because the first day of rash in a patient is so minimal that it's almost
missed, the macular stage, and then you get into little vesicles, very tiny, and even these are often missed. It's really about the third day before the patient really -- it's clear that there's something more than just a few pimples.

So this is why I was concerned or thinking about suppose we have people showing up with fever and they may have a rash already, second or third day, and what effect would ST-246 or the other -- any antiviral, CMX, have at that time. So I think it governs in a way one's thinking about the studies that might be helpful here.

Then, of course, you're, in a way, thinking here really of some of these are being actually anticipatory, and it's more what we call pre-exposure protection; it's really what it is or virtually that pre-illness protection. And this is where, what is the disease? When does the disease begin? And the infection occurs, there's the interval of incubation, then the onset of the fever, then the onset of rash.

So I think there's a need to integrate the
thinking on this with the planning of what we would do and what we might expect to do based on previous outbreaks, which have occurred and reaction of people and try to anticipate how best to fit all this together. And it's very difficult to do, and I think, as anybody says, whatever plans you lay out, you'll find out afterwards they were wrong. It's not encouraging, but this is often the case. But I think we do have to do some very heavy planning at this point in time.

DR. CARGILL: Dr. Khan?

DR. KHAN: I don't really have anything to add to that. I think one of your earlier questions was about transmission, and, yes, we do know that variola is transmitted by small particle aerosols. So that is a potential route of transmission. It has been documented.

DR. CARGILL: Dr. Reller?

DR. RELLER: I started this train of thought because I wondered what the relative -- from a public health perspective, the relative importance of containing, if it were to happen, with the same
strategy that has proved to be so effective that
you led, Dr. Henderson, because of the relative
slowness of spread and the opportunity to encircle
and -- so is there -- what effect does early
vaccination have if you were there before you
obviously had the rash and there was a fear of
exposure as opposed to having confirmed that you
actually have the entity?

DR. HENDERSON: Vaccination would appear to
be reasonably effective through the first three to
four days of the infection. So that the first wave
of cases, so to speak, you're not going to be able
to stop a lot of cases. Then you've got a gap in
the -- the disease begins. Once the symptoms
begin, you're past the point that vaccination is
going to be of any value, and it'll be several days
before that even that it would not be of value, if
you see what I mean. It's at three to four days,
the individual is infected, and if you can
then -- you're vaccinating them at that time, he
doesn't know he's infected, and that is where your
containment comes in or where we've emphasized the
containment of those in contact with the patient, there you may be able to stop the spread at that point.

I think our belief is that based on some major epidemics that we dealt with in the past, that if we are well prepared and, indeed, we've got good communication to the public and there's an understanding of what's going on, I think we'd want to avoid a mass vaccination simply because there are a number of complications with the smallpox vaccine, and you'd just as soon not have that thrown in, as well.

So the point is the earlier we can get it, with the previous experience -- which we did model quite extensively in 2003-2004, that it looks like you could pretty well deal with an outbreak at least of moderate size. But if you're getting into hundreds and thousands of cases, things will break down.

The other question about diagnosis of cases, we can diagnose them, but we're not going to have sort of onsite diagnosis. At this point, we do not
have the capability of doing that. The patient appears in the office and you say is it or is it not smallpox; we don't have that capacity.

DR. CARGILL: I'd like to direct the panel to remember that we're at this point in time still looking at clarifying questions for our FDA presenter, and then we'll be able to continue this discussion.

[Pause.]

Open Public Hearing

DR. CARGILL: We're going to move to our next session.

Both the Food and Drug Administration and the public believe in a transparent process for information gathering and decision making. To ensure such transparency at the open public hearing session of the advisory committee meeting, FDA believes that it is important to understand the context of an individual's presentation.

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

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

The FDA and this committee place great importance in the open public hearing process. The insights and comments provided can help the agency and this committee in their consideration of the issues before them. That said, in many instances and for many topics, there will be a variety of opinions.

One of our goals today is for this open public hearing to be conducted in a fair and open
Dr. Knapp?

DR. KNAPP: My name is Kieren P. Knapp, DO, a fellow of the American College of Osteopathic Family Physicians. I'm affiliated with Jacobus Medical Center in Jacobus, Pennsylvania, and that's only because I'm the only doctor in the town.

I am an inadvertent investigator. I have no financial arrangements with anything. My only interest here was what is the outcome with my patient. I became an inadvertent investigator on or about May 11th. I received a telephone call from one of my patients, who was a 25-year-old Caucasian female, immunocompetent, who had been visiting her boyfriend in North Carolina.

About 10 days prior, she believed she had come in contact with his vaccination. This was on retrospect, because she had developed a large
pimple on her chin, although this did not look like any other pimple that she had had. Being very astute, she was a nursing student, had also looked it up, and when she described it, I immediately recognized it from my childhood as a possibility of vaccinia or vaccinosis, and put her immediately in quarantine at home and contacted the Pennsylvania State Department of Health.

After numerous hours of various negotiations, et cetera, for contacting the CDC, we were able to get an ability to have a culture and a PCR done, had it sent to the Pennsylvania Department of Health Laboratory Response Network, who did confirm that it was positive for the vaccinosis. The culture was negative for MRSA, which was our other concern.

The Pennsylvania Department of Health did put me in contact with the CDC, who did provide 6,000 units per kilogram of vaccinia-immune globulin intravenous, which was shipped to the area and was given to the patient.

That was on May 14th. By May 18th, after
the investigational bureaucracy was gone through
and we were able to get some of the experimental
medication, the patient had decreased in the amount
of fever, myalgias and other constitutional
symptoms, but had developed extra lesions on her
chin. These are the pictures of the progression of
this.

On May 19th, ST-246 at 400 milligrams per
day was started for 14 days. By this point, the
secondary lesions had erupted and the central
lesion had undergone its classic ulceration and
black centering.

By May 31st, which was treatment day 13,
PCRs were again obtained, sent to the State
Department of Health, and after holding them for
their appropriate amount of time until they were
starting to show contamination, all the lesions did
come up negative. There were a number of
serological tests that were done, which is not my
purview. My purview is only what happens to my
patient, which is my number one concern, not the
immunological response. But as you can see, we did
have nice progression to the end. And there should
be a second set of slides here -- is there or is
there not? I can't remember --

MR. TRAN: That's the only one.

DR. KNAPP: -- which should show the final
slide. No?

Anyway, we had a very good outcome. Growing
up and remembering a couple cases of disseminated
vaccinia from my childhood, my major concern was a
massive amount of scarring that was potential, and
it really wasn't. Unless she got caught in the
same traffic I did, I believe the patient is here
at this time, and, as far as I know, she is scar-
free.

So that concludes my presentation. I have
no affiliation with anybody. I paid for my own
trip, and I'm going to pay for my own meals.

[Laughter.]

DR. KNAPP: And if there's any questions,
you'll really have to forgive me, because there's
something in this room that's making my hearing aid
just go absolutely nuts.
DR. CARGILL: Well, first, I want to thank you, Dr. Knapp, for that and for coming down. And I'd like to direct the panel, if you have any questions for Dr. Knapp, to please indicate them to me and to Mr. Tran at this time.

Dr. Magill?

DR. MAGILL: I'm sorry. You may have said this, but what was the dose that was used in this patient and how did she tolerate the regimen?

DR. KNAPP: It was 400 milligrams per day. It was given in four divided doses with a fatty meal, and she seemed to tolerate it extremely well. And along with it, her symptoms did abate.

I hope I answered your question. Again, I only got part of it.

DR. CARGILL: Any other questions?

[No response.]

DR. CARGILL: If not, then thank you so very much. That was very illuminating.

DR. KNAPP: Thank you.

DR. CARGILL: The open public hearing portion of this meeting has now concluded, and we
The committee will now turn its attention to address the task at hand, the careful consideration of the data before the committee, as well as the public comments.

I would like to ask at this point that we continue with clarifying questions. I know a number of the panel members had some. So if you could please, again, indicate that you have questions, we can pose them to our presenters. I would submit to you all of our presenters today.

Clarifying Questions from the Committee (con't)

DR. CARGILL: Dr. Margolis?

DR. MARGOLIS: This is more of a comment and actually a question to the FDA, and it goes back to what would happen if we have a smallpox event. At least discussions from before, I think once you have the rash and it's diagnosed -- which I think there is great capacity at state laboratories -- then people I think are going to move back to the febrile illness. And as D.A. said, when you were in India in those days, yes,
you were down, but this is the U.S. today, and I think these people are going to show up in our hospitals and in our clinics, which is going to put tremendous pressure on utilization of antivirals, as well as all the vaccination issues.

So my question is we've talked about the efficacy around the animal model, but what about the safety side, safety profiles? I know this is something you all spent a lot of time on. But I don't know if somebody wants to make a comment about that. I mean, we've heard some safety data, but -- and the numbers, too. Again, there were earlier estimates of, what, 1.7 million full doses available. I could see that being overwhelmed rapidly as people come out for a febrile illness. And we're not going to be able to make the diagnosis of those. There it's going to be treat people who are suspect.

DR. CARGILL: I think I'm actually going to go back to our sponsors, and I'd like to start with SIGA.

DR. HRUBY: We're very concerned about
safety. And we agree, if this starts to be used, that's going to be of paramount importance.

What I can tell you that we didn't elaborate on during our presentation is that in all our complete toxicological evaluation of animals, we've seen no safety signals. We've had very high doses for three months in mice at 1,000 mgs per day or in monkeys at 300 mgs per day for three months and seen no ill effects whatsoever.

In our human experience to date, we've been in about 158 individuals, some of them at very high doses, as high as 2,000 mgs single dose, and we've seen no SAEs, very few AEs. They're typical of any clinical trial, the most common being a headache or nausea, and that's a very slight elevation over placebo, and it's not dose-related, in our experience. So to date, in the population we've studied, it's been a very safe drug.

DR. CARGILL: Could you stay there for one minute, please, because I have one other question for you, and will to our other sponsor as well.

But since you mentioned the 158 humans, have
you done any resistance data in those humans and what
did that look like, if you did?

DR. HRUBY: We've never seen -- well, none
of these people have been challenged, of course. The
one instance that we had a resistant variant came
from the case which I believe was in your briefing
book, the San Diego case, in which this was a very
ill young man who had progressive vaccinia and had
been treated to ablate his immune system.

In that long period of treatment, which was
more than 70 days, a resistant variant was isolated.
However, it should be noted that resistant variant
was isolated from the skin, never from the
bloodstream. And because of the extreme nature of
his illness, we were treating him with an
experimental topical formulation that had in no way
been optimized for use in animals, let alone in
humans. So that may, in fact, have led to the
elaboration of that resistant mutant. But it was not
found in the bloodstream, and its level of resistance
was such that the therapeutic levels of ST-246 were
still inhibited in vitro.
DR. CARGILL: Thank you. I saw a number of hands on the right side of the panel. Dr. Bennett?

DR. BENNETT: I'd like to return to the issue of safety, because ordinarily we have a phase 3 trial which will have hundreds or thousands of patients and we'll look at safety at that, as well as postmarketing indicators of safety. Here we will not have that. There will be no phase 3 trial.

So I wonder if it's unethical to give a 14-day course to several hundred normal volunteers. I don't know what an IRB would have to say about that, but this is a problem that I don't think the FDA has faced before, and I don't know what kind of guidance you can give to industry for what would be a reasonable safety population.

DR. CARGILL: I think I'd like FDA to respond. Then I'm going to ask both SIGA and Chimerix to respond.

DR. COX: Yes, Dr. Bennett. You're recognizing one of the difficult issues here. If the drug is not a drug that could be used for treatment
of other infections, infections that occur, then the
question is where does the safety database come from.
And, typically -- not typically, but in this
scenario, folks have looked to healthy, normal
patients. And, of course, in order to be able to do
that, the drug has to have a safety profile that
would make such a study ethical to do so.

IRBs, certainly, it's a central part of the
process to make sure that an IRB reviews the case, to
make sure that a field of this is an acceptable
study. It would be something that would be limited
to adults, given that this is not a situation, if
it's being given to healthy normals, there's not a
disease that you're treating. And, generally, the
size -- and the SIGA folks have pointed this out on
their slide on the size of the safety database that
they were talking about and the studies in the
neighborhood of about 300 patients who would be
exposed to the drug.

So it does get to be difficult to gather
safety data in the setting where the therapeutic
doesn't have another use. But in that setting, folks
have looked to healthy, normal adult populations who
consent to an IRB-approved study.

So any other further comments that the panel
has on that or other ideas or thoughts, it's a
difficult scenario.

DR. CARGILL: SIGA?

DR. CORRADO: Mike Corrado. My expenses and
honorary are paid to INC Research, the company for
which I work. Having been in clinical trial
development for a long time, this is a particularly
peculiar situation that we're in that you've just
described. But I want to kind of give you our
thinking in how we're looking at this.

So we look at this in terms of normal
volunteers; however, normal volunteers who have co-
morbid conditions, as well. In other words, to
represent what would be the typical American
population that's going to work every day. So
they're going to have hypertension, they're going to
have diabetes, they're going to be receiving other
pharmacology, as well. In addition to those who are
entirely well, they're going to have varying degrees
of renal competency, et cetera.

We're going to look at those, and one might look at this and say if I treat 100 such people, in theory, I can identify adverse events that occur at 1 percent. Well, that's not true, because it could occur in the 150th person. So if you go to 200, 300 and 400 people, you're getting a little bit better, not completely able, but better at defining adverse events that might occur at 1 percent.

Now, that, in and of itself, is meaningless until you realize the disease you're treating, because if we're treating acne, that's insufficient. But when we're treating a disease, as has been pointed out, that has significant morbidity and mortality as this, then we feel pretty good that that kind of population would give us a relative risk-benefit for treating those kinds of people.

When we get into healthy people who have a viral condition, other, and just have fever, that's a whole different problem. But the first problem is having a treatment for the people who really need it. So we look at it from that perspective. Then what we
do is we look for signals.

Now, to date, in the 100-and-some-odd people that we've treated for up to 21 days, I can tell you that the adverse events that we see look similar in frequency, the type of adverse events, the severity, and the seriousness as those we saw in the placebo group. It is only 100-and-some-odd people. So we take that where it is and we are going to continue to build on that.

What we're not going to have, we pray to God, is a post-approval ability to look at more data, because we hope we never use the drug. But if we do, for whatever conditions, we'll continue to add and we'll look for signals, as we always do.

DR. CARGILL: Before I have the Chimerix person speak, I want to make sure that we're evenhanded. So would you please, also, reiterate the issues around resistance and then go ahead into safety?

DR. W. PAINTER: Could you repeat the resistance question, please?

DR. CARGILL: Sure. What we had asked about
is in those humans that had ended up being treated
with the drug, what the resistance data looked like,
and then ask you to go ahead and address the safety
issue, as we had SIGA.

DR. W. PAINTER: So in terms of resistance,
we treated the same vaccinia patient that SIGA
treated, and we had no evidence of resistance in that
patient. We have treated many patients who have had
preexisting resistance to cidofovir and, as you would
expect, in some patients, we could not overcome that
resistance. In other patients, we were able to
overcome that resistance. And as Randall will
describe, there's a fitness effect, and he may want
to elaborate on that after I follow-up on the safety
questions.

In terms of how we look at the issue of
smallpox, were it to occur in the community, there
are lots of experts who know more than we do about
this, but we think about all of the patients that not
only would need access to drug in advance of the
vaccine being out there and in that time period
between which infection occurs and the recognition of
diseases there. And so we recognize that not only
will there be healthy adults, but there will be
children, there will be sick people, there will be
people with immune-compromising conditions of the
types that did not exist at the time that smallpox
was eradicated. There are many people on immune-
suppressing drugs, cancer therapies and things like
this, that really weren't complicated back in the
day.

So I know that if smallpox is there, there
are no contraindications to vaccine. However, we
don't have experience with those populations. We
also know that when you're immune-compromised, it can
take longer to clear infections. As you, for
example, get rid of the immune-suppressing medication
and allow the immune system to come back, that
coverage over time may be required. So we do think
that it's very important to get data across a lot of
populations, and that's what we've been trying to do
in our clinical program by going directly towards
patients who have significant and serious diseases.

We recognize that we will be developing a
safety database that includes lots of adverse events, because patients are sick and have lots of things happen. We're working closely in conjunction with FDA. We'll be looking with them at our phase 2 data when it comes out and looking at the placebo compared to the treated patients to see what that safety profile is to be able to write an appropriate label for the drug.

We also recognize that even healthy patients who develop smallpox are, by definition, going to be immune-compromised because that's what the virus does. So we believe that having data in those patients is a safety issue in terms of things like the development of resistance and like the ability to treat through when someone's immune system might not be fully functional, and the antiviral needs to have the extra umph to get you through that period.

Randall?

DR. CARGILL: Yes, please. Go ahead and target your remarks to the question.

DR. LANIER: Just a quick clarification on the resistance issue. The resistance that we have
seen from prior cidofovir use was for adenovirus or
with prior, again, acyclovir uses for CMV. We have
not seen resistance for the vaccinia cases, just to
clarify that.

DR. CARGILL: Thank you. Dr. Bohm?

DR. BOHM: I'm going to go in a little
different direction here, back to the animal model
question. Dr. Damon mentioned this morning that
prior to 1970, other species and nonhuman primates
were under model development for smallpox. And since
then, notably, the rhesus monkey has not been pursued
and the cyno has been.

I wonder, as the question to her and the
other speakers, since the rhesus monkey is, arguably,
better characterized in their immune response than
cyno, why do you think that that model has not been
pursued?

DR. DAMON: I think the reason the
committee, in its drafting of the current interim
recommendations, really recommended cynos was despite
the fact that quite a bit of work is done in rhesus
in other systems, there seemed to be more both
historic data and current data with cynos, and that's primarily the reason for moving forward with the cynomolgus at this point in time.

I think there are people within the group that felt that rhesus was equally -- it would be equally advantageous. I think historic studies with it have been more minimal. So it's one study done by Westwood in the mid '60s looking at aerosol exposure in rhesus which has been done previously. In that system, 109 animals were challenged. Two succumbed to disease. Disease manifestation, I think, had about a six to eight-day incubation period followed by fever and exanthemous rash. But I think it's certainly reasonable, with the work that has been done in genetic characterization of rhesus, that that's a good system, as well.

DR. CARGILL: Dr. Van Dyke?

DR. VAN DYKE: Just briefly to get back again to the issue of the safety evaluation of these drugs. I think particularly for the ST-246, where there's no other indication, I think the greatest challenge probably is going to be to get pediatric

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safety data and probably pregnant women safety data, because it's going to be very difficult to get that data from uninfected healthy people. Likewise, in those groups, I think getting PK data to decide what an appropriate dose would be I think is going to be very difficult. I don't know if anybody has any thoughts about that, but I see that as a real problem.

DR. ROBERTSON: Sarah Robertson, FDA. I guess addressing the PK question, you're absolutely correct and we anticipate having to use some physiologically-based modeling, extensive PK/PD modeling in those populations, which we've done previously when we've been unable to obtain PK data in those populations.

Safety, that'll be for somebody else to address.

DR. CARGILL: SIGA?

DR. CORRADO: You're right on target. The only thing that I will add to that in terms of the pediatric use, and we share the need for both of those populations, is one of the issues in pediatrics
is the particularly high renal function, glomerular filtration compared to body surface area and the size of the kidneys. This drug, for all intents and purposes, has no renal excretion. So we don't have that issue. We do may have an issue in very young children whose livers are not functioning properly yet, who are one or two weeks of age. But when we do have a formulation, we would intend to talk to the FDA about how to go forward with trying to elucidate this and trying to find the proper dosage in PK.

DR. CARGILL: I'm going to take the chair's prerogative and ask Chimerix, since you have a pediatric formulation, if you can address the PK issue, as well, in pediatric patients, and can you speak to the pregnant women issue?

DR. TROST: We do have a pediatric formulation. We have dosed approximately 150 pediatric patients as young as one month. And what we have observed -- our drug is also not cleared or not eliminated by the kidney. It is cleared by -- eliminated by -- excuse me. It's not excreted by the kidney. It's eliminated by metabolism, and
approximately 50 percent is excreted in feces and
50 percent in urine as metabolites. We have
extensive PK data in children, and we see slightly
increased clearance and we have -- using a liquid
formulation in pediatric patients, we have no issue
at all with PK and no material difference with adults.

DR. CARGILL: Thank you. For the record, could you please state your name again?

DR. TROST: Sure. Lawrence Trost, Chimerix.

DR. CARGILL: Thank you very much. And now, Dr. Bennett?

DR. BENNETT: I have two questions for Dr. Chan-Tack. I'll ask them sequentially. The first one, you made a point that none of the industry studies in animals were GLP. What significance do you attach to that?

DR. CHAN-TACK: Let's see. Well, Chimerix, there were two of their latter four that were GLP. The others for -- I'm sorry. For SIGA, two of the last four were GLP, and then for the remainder of the studies, I would imagine that those were primarily
done non-GLP, because they were pilots and exploratory in nature. With regard to the significance of GLP status for pivotal efficacy studies, I'm going to ask one of the pharmacology/toxicologists to speak to that in more detail.

DR. MYERS: Peyton Myers, FDA. Yes. For GLP, we expect GLP for all the safety studies for nonclinical safety. And for the pivotal efficacy studies, we would expect GLP studies to be performed, as well.

DR. BENNETT: So you attach no significance to a non-GLP study. Is that what I'm hearing?

DR. MYERS: I'm sorry?

DR. BENNETT: You don't attach as much significance to a non-GLP study. Is that what you're saying?

DR. MYERS: We accept non-GLP studies, but the significance of GLP -- the regulations for GLP were put in place to basically assure the agency that these studies were done very well. There's adequate documentation, adequate -- instrumentations are
adequately calibrated. It's basically a more thoroughly designed and performed study. So that's why we would like GLP for the more pivotal studies.

DR. BENNETT: Thank you. The second question is --

DR. COX: May I just add one comment, too, on the GLP issue? It really is to get at the issue of quality and reliability, and that's really the key. And then the question is if it's not a GLP study, are there other ways to further understand quality and reliability of the study, either by inspecting the study, inspecting the records, those sorts of things. So the goal is quality and reliability, and the question is if the study wasn't preplanned that way, what is the quality and reliability of the study and are there ways to figure that out.

DR. BENNETT: Thanks, Ed.

The second question has to do with the Chimerix study in animals and the application of the Animal Rule. If I understand your summary correctly, it was one route, one dose, one virus. What does it
take to fulfill the Animal Rule beyond what they've
done already?

DR. COX: So that's kind of getting to
tomorrow's questions. It really is.

[Laughter.]

DR. BENNETT: All right. Forget it.

DR. COX: We're wanting -- and that really
is. It really is getting to an opportunity to hear
from the companies about the information that they
have so far and then to get advice from the panel
about what they think about the available data that
we have so far and what additional work might help to
further understand the performance of the agents, and
then being able to make inferences about how the
drugs might perform in humans.

So it's a very good question, Dr. Bennett,
and I think we'll look forward to some more
discussions tomorrow on this.

DR. CARGILL: Dr. Camardo?

DR. CAMARDO: Thank you. Since I'm from
industry, I'll ask an industry kind of question. I'm
not sure if it's for you, Dr. Cox, or maybe for
Dr. Khan. But, normally, we would be thinking about what the indication would be for the drug we're trying to get approved, and I'm bringing it up because I think I'm getting a little confused.

Do we have a drug for which we would like to prevent mortality in the absence of any other therapies, which is the situation for smallpox? And I ask that because, generally, a drug with a mortality benefit, we become somewhat permissive and we're pretty happy about even a small reduction in mortality, or are we looking for something that will contain the epidemic if it happens.

I'm not thinking we want that, because I'm not even sure this would do that. Are we looking for individuals who are at risk to die otherwise, because that would help me think about sort of what kind of expectations I might have for leaping from animals, where the drug is highly effective, into people, where it actually has a pretty good chance of being effective, but maybe not as effective as it is in animals. I don't know if that's clear, but you know what I mean.
DR. COX: I'm happy to try and offer some thoughts on that and then --

DR. CARGILL: Absolutely.

DR. COX: -- the companies may also want to do the same.

As we think about this, if you're talking about utilizing a drug for treatment of patients who are ill, very sick with smallpox, it seems that one of the outcomes of particular interest would be prevention of mortality in those who might succumb to disease if left untreated. So that is certainly an important outcome.

We've heard some discussion over the course of the day, too, about animal models and what the various different endpoints are in the animal models, mortality being one of them, some of the discussion that we'll look forward to tomorrow, some additional advice from the committee on particular endpoints for these animal studies. But, certainly, treatment of established disease and prevention of mortality would certainly be an important outcome.

You also asked about the potential to
prevent spread, and then the question is the types of
data and information that one might need to be able
to understand that further. And that may be
something, too, that is discussed further tomorrow.

I don't know if Dr. Khan would want to
express anything further. And then you may want the
companies, also, Dr. Cargill, your prerogative on
that, to describe their intentions, too.

DR. CARGILL: Dr. Khan, did you have
anything you wanted to add?

DR. KHAN: I think that was well said. We'd
like to save lives.

DR. CARGILL: I think we all could rally
around that.

[Laughter.]

DR. CARGILL: I think that's a pretty safe
statement.

I think that I'm going to table some more of
that discussion, because tomorrow is going to be a
pretty robust day, and ask -- Dr. Henderson, you had
something to contribute.

DR. HENDERSON: Yes. I think there's
something to be said in general to realize that with smallpox vaccine, we have a vaccine which we hope we will never use. And in dealing with a number of these agents that we have for the stockpile in case something happens, whether it's Ebola or anthrax or what have you, the question is how prepared should we be, because there's a limited amount of money.

If we, for example, wanted to replace our present smallpox stock with a vaccine that costs $10 a dose, which wouldn't be bad, it would be $3 billion. And this is a real problem. And one looks at all of the different possible drugs or, let's say, in this case, the antivirals for smallpox, the question is how far do we want to go, can we go in sort of working for clarifying everything on this drug when we do have a number of others, and it's sort of a balance we have to draw between a drug which we put in the stockpile and we hope we never use compared to one that we're going to use routinely, in which case you've got a lot of work to do with the infants and other susceptible populations. And I think we forget this at this
point in time, and I can say, spending some time on
the stockpile then, what we buy and don't buy. There
are very, very difficult questions, and we're still
wrestling with them. In a way, I think you're doing
some of that right here as to how much do we do to
put this drug -- characterize it in every possible
way. And in the course of this, if it's too
elaborate, we're going to be in trouble, because I
think we're going to discourage manufacturers
developing certain -- let's say, particular products
that would be helpful if, by remote chance, we have
something happen.

So I think there's a balance here that needs
to be kept, and think about this, because it is a
real problem.

DR. CARGILL: Thank you. Debra?

DR. BIRNKRANT: Getting back to the
indications for a product approved under the Animal
Rule, I think the labeling would have to reflect the
limited amount of knowledge that we have in human
subjects with the disease. So we have an indications
and usage section, and the indication would be for
treatment, but then we have a section that describes
the limitations of the indication. So we could use
wording such as "safety and efficacy have not been
established in certain populations," "in all
populations," that this was approved under the Animal
Rule, et cetera. So there are qualifying statements
that can go into labeling.

    DR. CARGILL: Thank you, Dr. Birnkrant.

    That's very helpful.

    Dr. Magill?

    DR. MAGILL: On the ST-246 presentation,
there was a bullet thrown in about a phase 1 thorough
QTC study, which sort of caught me by surprise,
because I didn't see anything prior to that. And I
just was wondering if this is based on already an in
vitro hERG and clamp type assessment and this has
already been discussed with FDA and it's clear that
this needs to be a thorough QTC, or what's the
background behind that?

    DR. CARGILL: I think we'll ask the sponsor
to respond to your question. Thank you.

    DR. LEEDS: Actually, there's been no signal
of QTC through in vitro studies, automated patch clamp. We did a three-month study in monkeys at 300 mgs per kg per day where they were evaluated for QTC prolongation at the beginning, middle and end, and a distinct study for 16 days at 300 mg per kg, where they were monitored each day.

But I could ask Dr. Corrado to discuss the rationale for doing a phase 1 study in humans.

DR. CARGILL: Before Dr. Corrado does that, could you please state your name for the record?

DR. LEEDS: I'm sorry. Janet Leeds from SIGA Technologies.

DR. CARGILL: Thank you.

DR. CORRADO: So an hERG channel is a screen. It's an in vitro screen. It's nice to know. It doesn't prove anything. The animal models are animal models. They're nice to know. But this is first in class. And so we always assume that a first-in-class drug has to go through the rigor of a thorough QTC trial, where multiple replicates of ECGs are done at peak plasma concentrations. So we would have those discussion with the agency, as well. But
at this time, as a first in class, we would
assume -- and that's why you saw it -- that we would
have to do one.

DR. CARGILL: Dr. Strader?

DR. STRADER: I'm not sure if this is
something best held for tomorrow, but I'm a little
bit nervous. I think we're moving very quickly to
talk about approving drugs for a disease we haven't
seen in many years and about which we know very
little. We are talking about putting together animal
models that somehow describe portions of a disease we
haven't seen and assuming that it is as virulent and
will cause as bad a disease as it did some 60 or
70 years ago.

I'm not certain that I'm comfortable with
the idea of moving that far forward without knowing,
does the -- how contagious is this disease? Is it
transmissible in the 17-day incubation period? How much
inoculum do I have to have sneezed on me before I get
sick? How many lesions do I have to have before a
drug will no longer work?

I mean, there are lots of questions that I
don't think that we have answered, and I'm not sure there is any way of answering them at this point. But I think that -- I heard the word "balance" mentioned before, and I think this is a very difficult situation to discuss. But before we race off to decide that we need to have drugs to treat a disease that we hope we'll never see, that we should know how this disease affects us and how aggressive we need to be about treatment options.

DR. CARGILL: Dr. Bennett?

DR. BENNETT: I have a question for Dr. Birnkrant. If the FDA thought it was in the public interest to approve a drug just to put into a stockpile for emergency use, do you have the regulatory authority to do that?

[Laughter.]

DR. BENNETT: I never heard of it. That's why I'm asking.

DR. BIRNKRANT: Our role is to make a regulatory decision when a marketing application is presented to us based on the safety and efficacy in that application.
[Laughter.]

DR. CARGILL: Dr. Goetz?

DR. GOETZ: Changing gears a little bit again. There are two questions that I have for Chimerix. First of all, in regard to the safety signal, one of the interesting things, of course, is that cidofovir's use has been limited by nephrotoxicity. And what's notable is that the intracellular concentrations of your compound are turned into cidofovir. I'd like to know if you could comment a little bit on what nephrotoxicity signal you have and if you don't have one, how do you explain that?

Then the other issue is a follow-up in parallel to the other compound, is there any interaction between administration of CMX001 and vaccine take?

DR. CARGILL: I think we'll ask the sponsors to respond to that, please.

DR. TROST: I'm going to answer the first part of your question, and then I'm going to ask my colleague, Dr. Lanier, to answer the second part.
about vaccine take.

DR. CARGILL: Excuse me. Could you please state your name for the record?

DR. TROST: Lawrence Trost, Chimerix. I apologize.

Obviously, the comparison to cidofovir existed from the time that we created the drug, and so that was something that was really incorporated in our development from the very beginning, a rigorous analysis of potential nephrotoxicity. And I have a good backup slide on this, but I think I'll handle it without.

As I mentioned before, CMX001 is eliminated by metabolism. It's excreted equally in feces and urine, but none of it comes out as CMX001 in humans. We do produce cidofovir as a metabolite and by oxidative catabolism, some other metabolites in which the lipid chain is shortened by two carbons, but -- I'm sorry. I lost my train of thought here. None of those are -- some are eliminated in urine. The point is that we have not seen any evidence of nephrotoxicity in any of our animal studies. In
fact, we're thinking now, in our 39-week study that we've just completed, we've administered a dose that's nearly 20-fold higher than what we are currently studying in the clinic, and we see no signs of nephrotoxicity.

DR. G. PAINTER: I can elaborate on that a little bit for you.

DR. CARGILL: Please state your name for the record.

DR. G. PAINTER: I'm sorry. George Painter, Chimerix. The drug is administered as this lipid conjugate, and because of its uptake in potency, we can eliminate -- we can administer it at a very low level. The toxicity associated with cidofovir is due to active secretion by an anion transporter that's in the basolateral membrane of the epithelial cells lining the lumen of the proximal tubules.

So when you administer IV cidofovir, you have to administer it at extremely high levels in order to get about 4 percent uptake into the target cells.

At the early stages of that administration,
you're many times, maybe two logs, above the KM for that pump. So in the absence of probenecid, you get very significant active secretion into these cells, which leads to the toxicity.

We can give many-fold lower levels of CMX001 because the uptake is facilitated by the lipid. So once the drug goes through the intracellular process, it's anabolized to the active antiviral, but ultimately eliminated as cidofovir. It's a log below the KM in the basolateral membrane. So it's not actively secreted. So we come in under the renal toxicity radar.

DR. CARGILL: Thank you.

DR. LANIER: Dr. Randall Lanier. I was just going to address the vaccine question. So we don't have data with CMX001 and vaccination, per se. We do have data with -- well, data has been published with cidofovir on vaccination. There is some attenuation of vaccination. It doesn't abrogate a response, but it does attenuate it.

However, we're going for treatment and so really I think that whether or not it's really
relevant for treatment, vaccination, as Dr. Henderson clarified earlier, would be useful for the first maybe four days following infection, well before any clinical signs and symptoms would show up. And we're looking at using this drug for treatment. So in that situation, vaccination would have already occurred by infection.

DR. CARGILL: Thank you. Dr. Reller?

DR. RELLER: A comment made by Dr. Henderson about the Animal Rule earlier this morning has stuck with me through the discussions, and I'm perplexed by how heterogeneous the handling of the different orthopoxviruses are by the different animals, and, also, that the disease that you get has so much to do with what the inoculum is, what route it's given. You get an entirely different picture. Dr. Chan-Tack emphasized that.

So is it possible that whatever is ultimately done with these potentially efficacious drugs would need to be addressed and decided upon without trying to go -- to make the basis for the Animal Rule if it's not possible to get there through
the Animal Rule, which relates to the genetic -- to me, at least, and maybe inappropriately so, the genetic map of the orthopoxviruses presented by Professor Smith.

So most of the virus across these has the same or very similar genetic material, and what makes it so heterogeneous, having to do with the conundrum, the difficulty in getting at the Animal Rule as it relates to human disease, if it does at all, has to do with a very small part.

So in any drug that's developed, how important is it to try to get an animal model that simulates the human disease if the drug is -- there's enough data for safety and it intrinsically halts replication of the virus?

Is the logic of that apparent?

DR. CARGILL: Dr. Cox, do you want to take that?

DR. COX: I'll say a few words. Again, I think we are starting to get into some of the discussion that we hope to have tomorrow. And I think, Dr. Reller, you're bringing up some of the
difficult issues that we face here. The biology of
the virus is the biology of the virus, and that's
what we're here to talk about and try and work
through. There are some very unique aspects to
smallpox. Obviously, the disease has been
eradicated. It presents some real challenges.

But I think inherent to that, are the risk-
benefit -- weighing risk-benefit as we think about
these issues and taking a risk-based approach as we
think about animal models, recognizing that there is
going to be some degree of uncertainty that we're
going to be faced with here, because the biology is
what it is, and then also thinking about are there
areas for flexibility as we approach this issue such
that we still can understand how the drug will
perform in humans, to the best of our ability. And I
think that's what we're wanting to hear more about
from folks tomorrow, and that's why this is
particularly challenging.

DR. CARGILL: Can I just add that I think
that we've heard a number of themes that will come
back to that, in addition to the Animal Rule and some
of the challenges in a disease we haven't seen in a long time. We've heard a very eloquent discussion of some of the balance issues, whether we're talking about cost or talking about data, talk about worried well, talk about populations we haven't seen, and I think that's what's going to make our discussions tomorrow that much more important, and, also, unfortunately, that much more challenging.

Dr. Magill?

Dr. Camardo?

Dr. Goetz? Someone is going to be a taker.

DR. GOETZ: I guess I'm back up again. And this may come up again tomorrow, but I'd like the agency to comment as to, we have two different agents which will have their own -- may have their own pathways toward approval. But how important is it to the agency that there be aspects of commonality in the approach that is taken in approval of agents for smallpox through the Animal Rule? And more particular, how important is it that there be commonality in the animal models that are used to go forward? In the proposals that we've had, the,
quote-unquote, "pivotal studies" seem to be in different animal models. It occurs, to me anyway, that some commonality would be useful, but I wonder if there's an agency perspective on that.

DR. COX: I'm assuming the key is to get a package of animal models that provide satisfactory information. The question is, is there only one route or are there other routes or different routes that somebody might take. So, again, we are starting to fall a little bit into -- and I understand why, because these are challenging questions that we'll be talking about tomorrow, but I wouldn't say that there's a single recipe, per se. But the question I think more gets to the package of data and what is a package of data that allows you to make the inferences about performance of the drug in humans. And if there are differences, that really is I think a discussion point, that there is no set single recipe, per se, if you will. But different, but satisfactory approaches could also be a way to look at this, too.

DR. GOETZ: Just to follow-up very briefly.
It seems that at some point, while we never want to have to use either agent, if we're in a situation where both wind up being approved and stockpiled, God help us, but if there be an episode where we need to use the drug, it would be -- another challenge would be knowing which drug to use and how. That's an editorial comment.

DR. CARGILL: And if necessary in sequence.

Dr. Magill?

DR. MAGILL: This may be a question to the folks who work in the animal models. But if death is an endpoint -- but yet it's not really, so it doesn't sound like death due to the disease is not really allowed in any of these models, it's usually some surrogate that's defined prospectively by an IACUC or some animal welfare consideration.

So I have two questions related to that. One, is there actually any bridge? At any time, has anyone in any one of these models really qualified that surrogate to death as the endpoint and how confident are you that those surrogates really work?

Then, secondly, why are they different? It
seems like the same models and the same -- that are used by different groups are having maybe different endpoints, and I'd be a little -- not necessarily concerned, but I would be questioning exactly how that moves forward.

DR. CARGILL: Dr. Roberts?

DR. BULLER: Mark Buller. I can comment on that, if you'd like.

DR. ROBERTS: Go ahead.

DR. BULLER: We've used death as an endpoint in the ectromelia model for a number of years. So we have a lot of data that correlates death with weight loss. And we're now transitioning over to a weight loss criteria, but we have extensive data on that.

DR. CARGILL: Could you state your name, please, for the record? Thanks. Could you state your name, please, for the record?

DR. BULLER: I thought I did. Mark Buller.

DR. CARGILL: Thank you.

Dr. Roberts?

DR. ROBERTS: So you have touched upon an area that we have had great discussion on, because
with mortality as the most objective endpoint, feet up-feet down, one would think that that shouldn't be an issue, and there shouldn't be any kind of subjective bias put into it. But the reality is that IACUCs demand that your euthanasia criteria be defined, and you really cannot allow animals, especially as you get higher up in the species, to suffer or to experience pain. And so that's why these criteria are set up.

We have had discussions about the ability to -- is there a way to standardize them, but IACUCs approach this very differently. So it doesn't appear that we would be able to have standardized criteria that all IACUCs would agree to.

One of the areas that we have talked at various meetings about is the ability to have greater and more intensive observation of animals as they get closer to having symptomatology that indicates they're probably getting pretty sick. And laboratories -- for financial reasons and for reasons of employees, some laboratories don't observe through the night hours. So it's not like in a human where,
in the ICU, you would have 24/7 monitoring. They
don't have that capability. Sometimes it's for
security reasons. Even the investigators can't get
in after certain hours.

So we have initiated discussions, once we
found this out, that it's very difficult to do 24/7
monitoring to see are there ways that these animals
could be observed closer to the time, because we do
have concerns that they may be sacrificed because
there's concern they won't be there the next morning.
And if you sacrifice them, you're not going to know
if they're there the next morning, or if there was a
way to have somebody go in, if they can be monitored
and somebody to go in and check them through the
night. But it is a serious problem with this
mortality endpoint, we agree.

DR. BIRNKRANT: Maybe Dr. Goff can share his
experiences.

DR. CARGILL: Actually, I'm going to
call -- we're going to hear from the United States
Army as represented by Dr. Goff.

DR. GOFF: Thank you very much. So first
off, the question is dead-on, and that's part of the reason we're here today. I can also add to what Dr. Roberts stated and say that with the monkeypox model, we're very fortunate that the viremia and the lesion counts do actually correlate very well with expected outcome. There has almost never been an instant where animal was euthanized for humane reasons and the lesion counts and the viremia did not reflect that that animal was going to succumb on its own within a day or two. With a variola model, obviously, that's not the case. There needs to be some more refinement to get those correlates to be in line with euthanasia.

DR. CARGILL: Is this related to that?

Okay. Dr. Magill?

DR. MAGILL: Just a quick follow-up on that. That was certainly the impression I got. And certainly this is not my area, so I don't know the literature completely. But I've never seen this published, or has it been published, that it's very clear that I'm hearing from some of the veterinarians that work in this area that there are actually fairly
well established surrogates that do predict death.

Is that published in the peer-reviewed literature or is that lower amongst the folks who do the work?

DR. MOYER: Could I make a couple of comments on this issue? The issue of death is really a problem because many times what you actually find is you sacrifice animals who might actually survive. And the converse is also true, you let some survive that, quite frankly, die on you. That's the way it goes.

What we have found very useful, and I think others have, as well, is we have spent a lot of time compiling what we call clinical index parameters, and I mean we measure everything. Animals are chipped so their temperatures and whatever are monitored 24 hours a day. And after a while, what you really find yourself doing, even though you don't mean to do it, is you're really interested in the effects of this drug or that drug or that condition on the clinical score, which really is not the same as death, even though we know that's what's going to
happen.

So I don't know how others feel about this. We actually found the issue of death to be a real bummer when we were faced with the issue of finding surrogates. Now that we have good clinical parameters, we think are good clinical parameters, frankly, we don't find this so much of a problem.

DR. CARGILL: Thank you.

Dr. Bohm?

DR. BOHM: Just to add to this discussion. I think with respect to nonhuman primates, what typically happens is in the development of a model, early on in the development of a model, especially a lethal model like what we're talking about today, animals are allowed to go out a little bit longer than they would later on in the model once you establish these surrogates, and I think that's pretty consistent as you refine models across -- especially in infectious disease.

Speaking to the question about a paper, there was a paper recently published about a plague model in African Green monkeys, and they monitored
temperature using telemetry. And 100 percent of the
time they found, in early studies, when an animal
spikes and then drops by 2 degrees, they're going to
die 100 percent of the time.

So I think part of this discussion should be
how do we refine the models so that this
endpoint -- and we call it death as an endpoint, and
it's death because you're euthanizing the animal.
But what I think individuals are getting confused
about is that we're letting animals die in the cage,
and that's not the case, and we should avoid that.
But in some cases early on, it may be necessary to
establish the surrogates. You want to minimize the
number of animals that happens, and in order to do
that, you collect as much data as possible, and
that's the use of telemetry and observations.

As far as staffing and those type of
resources, those are things that you've got to take
into consideration. But I know that from the
standpoint of the IACUCs that I'm familiar with, you
can establish, again, very frequent collection of
that physical exam data early on in some studies,
establish what those parameters are going to be, and then later on you don't have the need for that. But you can modify staffing depending on that, and that's appropriate.

DR. CARGILL: Dr. Margolis?

DR. MARGOLIS: A slightly different direction. So making the assumption that we have one or two antivirals, that an event occurs, we're suddenly going to have the potential for kind of what I call a clinical trial on the run. So has the agency developed guidelines or discussions about, all right, so how are we going to collect data to really evaluate these therapeutics that were approved using the Animal Rule, assuming they're approved?

DR. BIRNKRANT: We have asked companies to have protocols in place under the IND in the event of an emergency, because that would be a situation where we could possibly get some data to be able to draw conclusions that perhaps we couldn't do with data just coming from animal models. So we view that as a very important piece of information that we would like to see, and, again, we've been in discussions
with companies to have something in place.

DR. CARGILL: Dr. Margolis?

DR. MARGOLIS: Kind of a follow-up to that.

Since it would be an IND in place, does that then imply that these patients would have to give informed consent in this kind of a situation?

DR. BIRNKRANT: I don't think all of that has been worked out yet, but I see your point. I think at the agency, we need to have further internal discussions.

DR. CARGILL: Dr. Van Dyke?

Sorry. Dr. Roberts?

DR. ROBERTS: I just wanted to make one comment. Under the Animal Rule, if a product is approved using that regulatory pathway, then the sponsor is to develop a protocol that could be used as a confirmatory trial if there should be an event; then, the question of IND.

There have been talks and there will continue to be talks about how to get data during an event, and it would need to be a very sort of simple protocol and not be done necessarily across the
entire body of people that were affected, but in smaller trials where you could attempt to get some informed consent and actually have some ability to follow those patients in that. But that's a real challenge to try to do, and you have to have all that in place prior to your event.

DR. BIRNKRANT: Right. But there would be a pre-approval IND study, and that's what I was sort of focusing my comments on. And, that is, we've asked at this point to have protocols in place in case there were an event, and that would be before any type of regulatory action such as an approval would take place. And then as Rosemary highlighted, within the Animal Rule, there are requirements to then be able to collect information, if an event were to occur, after an approval.

DR. CARGILL: Dr. Reller, does this relate to this? Okay.

DR. COX: May I throw in just one more point?

DR. CARGILL: Sure, Dr. Cox.

DR. COX: Since we're talking about the
Animal Rule.

DR. CARGILL: Sure.

DR. COX: So one other thing, and this has been hinted at in some of the discussions, too. But the Animal Rule also has a provision in there, too, about if it's necessary to ensure the safe use of the product, to have a restricted distribution mechanism in place. So that's something else that's in there and I just wanted to point it out. And that might be restricted to certain facilities or having certain procedures in place or certain recordkeeping, things of that nature.

So I'd just mention that, too, because I think we've come close to that in some of the points made so far, and I just wanted to bring that up since we were talking about the Animal Rule.

DR. CARGILL: Thank you.

Dr. Reller, does it relate to this?

DR. RELLER: Not about the Animal Rule. My question has to do with safety. And this is not necessarily now, but in tomorrow's discussion, were an event to occur, vaccination would be a very
important component of the total response. And I'm thinking in terms of collateral effect. So I would want to be reassured on the safety side that the two drugs under discussion or any drug would -- if it's possible to know that they do not have an unintended effect on vaccination.

I think about the drug pleconaril in rhinovirus infection. If my recollection serves me and if I recall correctly, the drug was efficacious, albeit a modest decrease in symptoms, and it was safe, but it had a collateral effect that I think led to its not being approved, and that is its interference with oral contraceptives.

So it was a relatively small benefit for a potentially big collateral effect that, on the surface of things, was not a safety issue for the drug itself, but the other effects. So this is something I'd like to have -- if its known or whatever data there are, that this would be a major concern for me.

DR. CARGILL: I think we can probably go back and revisit that -- we heard some of that today,
but revisit that tomorrow.

Dr. Van Dyke?

DR. VAN DYKE: My comment relates to the issue of endpoints again. I can understand the need for a clinical endpoint as the primary endpoint, despite the problems we've talked about with mortality being an endpoint. But there hasn't been much discussion today about virologic endpoints, quantifying DNA in the blood and probably, although perhaps, other tissues, and how important could that be and what's the role of that in the drug approval process. And it could be a nonlethal model in that case.

DR. CARGILL: Does anyone want to speak to that?

DR. BIRNKRANT: Well, the Animal Rule has wording in it such that the effect that we're looking for is related to survival or mortality, not a surrogate, that is, viral load. So for approval under the Animal Rule, I think we would need something more concrete and definitive, such as mortality or survival. Maybe Dr. Roberts can
elaborate a little more on that.

DR. ROBERTS: Debbie is correct that the Animal Rule is very clear on clinical benefit and how it's defined. There have been many people who have raised the question of couldn't we develop some other kind of a biomarker that we could use that would be reliable and quantitative, and so viral load would certainly be one that one could think about in this scenario. But in order for the agency to be able to accept viral load, somebody would have to be able to do studies to show that that viral load, if it reaches a certain amount, is going to lead to death. And then the agent would have to be shown that it impacts that viral load and prevents that from occurring, and that hasn't been done to date.

So it's a potential, but we'd have to have the studies and be able to look at them and be convinced that that actually is an appropriate surrogate.

DR. CARGILL: Dr. Magill?

DR. MAGILL: Sort of a follow-on to that point. I think a lot of the discussion I've heard
today has been what I call a typical sort of drug-\-bug
discussion. It's about, in this case, virus
endpoints and PK and PD. And this disease, like many
infections, seems to have a very significant host
response component to the illness. In some of the
forms, this is as bad as rabies, almost 100 percent
mortality. And yet, in a more typical form, it's
much less.

But what do we know about either of these
drugs and their ability to modify the host immune
response, either to downregulate immune responses or
such that -- and could that play a role in the
clinical benefit, which you might not see in the
typical virucidal endpoints?

DR. CARGILL: So your question is what role
do these agents play, if any, in altering or
affecting the host immune response. All right.

Well, I'm going to ask our sponsors, both
SIGA and Chimerix, to respond to that. I would ask
you to focus your response directly to that question.
Thank you. And to please state your name into the
record.
DR. GROSENBACH: My name is Doug Grosenbach. So in response to the question about the immune response in the presence of the drug, we have conducted a number of studies in mice and also the NIAID has sponsored studies in nonhuman primates. So what we've seen is -- well, the first question is what are the correlates of protective immunity.

So we know that from the smallpox eradication campaign, that a neutralizing antibody titer of 32 was thought to be protective. Also, we now that cellular immune responses are important in a naive host due to the fact that people with cellular immunity are more prone to adverse events from the vaccine. So we look at both humoral and cellular immunity.

What we've seen is that if we vaccinate animals with the standard human dose of the vaccine and treat with ST-246 for the 14 daily doses starting on the day of vaccination, that there is a very modest reduction in the humoral antibody response, but it is well above the protective neutralizing antibody titer.
Cellular immune responses actually appear to be slightly enhanced and the kinetics of that response are quicker and to a greater magnitude. We are seeing cellular responses, such as TNF alpha and interferon gamma, that are statistically significantly impacted by the drug in that they are higher.

Also, when we challenge animals, we see that animals are equally protected not only from high and low dose challenges, but that their protective immunity is good for short-term and long-term experiments. So that's in regards to mice.

We've actually seen very similar results in nonhuman primates in that the lesion forms -- you have a vaccine take, but the lesion is actually smaller. It resolves much more quickly. And based on our studies in mice, the shedding of the virus from that lesion site is greatly reduced, probably 3 logs reduction in the amount of virus shed from the lesion site, although the immune response is essentially the same.

Nonhuman primates that have been challenged
following vaccination and treatment with ST-246, you have equal protective immunity. Survival is 100 percent. Vaccine provides 100 percent protection either in the presence or the absence of the drug.

DR. CARGILL: Thank you. Chimerix?

DR. G. PAINTER: George Painter from Chimerix. We have not done similar animal studies. However, we have watched patients recover their immune response on drug therapy after removal of immune suppression, after engraftment and BMT.

DR. CARGILL: Thank you. Dr. Smith?

Professor Smith? Thank you.

DR. SMITH: Thank you. If I could just make a comment on the immune response in the presence of drug. A priori, you would expect that using drug ST-246, this is not going to stop the expression of any of the virus antigens during its life cycle. They're all made. What doesn't happen is that you don't get envelopment of the intracellular virus to make you the extracellular virus. But all the antigens are made.

So it's not surprising to me to hear that
vaccination at the same time that drug is administered still gives you a pretty decent immune response. The vaccine you administer is still going to be able to infect cells, make all the antigens during its normal life cycle, but it won't spread thereafter.

But it's rather different in the case of cidofovir or the acylated derivative, because there you are blocking the replication cycle. You are halting DNA replication, and, therefore, you're not going to express either the intermediate class or the late class of genes, and those will include the structural proteins of the virus against which you want to have an antibody response.

So I would expect that there will be a difference between the two drugs, and if a scenario was envisaged in which you wish to give both the live vaccine and one drug, then I would choose ST-246.

If I could make one other comment, it relates to the question of the genetic variation between the viruses that was raised a few minutes ago and the impact of that, the needing to have animal
models with many different viruses, and particularly variola virus.

Now, since the targets of both the drugs are highly conserved in all the orthopoxviruses, again, a priori, you would expect that these drugs would be efficacious at inhibiting either the replication, in the case of cidofovir, or the spread, in the case of ST-246, of all the orthopoxviruses, and all the animal model data that we have had today supports that view.

So because of that, my own perspective is that having variola virus protection data is less important than it would otherwise be if you had not got other viruses that are closely related of the same genus in which you can do parallel studies.

DR. CARGILL: Thank you. Go ahead.

DR. LANIER: Randall Lanier. So I'll just make a quick comment on that. We had mentioned before that there's data with cidofovir that shows that the immune response is attenuated, but not abrogated, as you would expect for an antiviral that stops replication entirely. So if you wanted to use
CMX001 and vaccinate simultaneously, you would need to use something more like MVA rather than the Dryvax or something like that that has long, multiple rounds of replication.

**Adjournment**

DR. CARGILL: Thank you. Well, I think it's fair to say that it's been a long and quite full day. I would ask the panel members to please leave your name tags here at your place. You will, however, I'm sure, want to take your materials with you, as I'm sure we will all be diligently studying before we meet back here tomorrow. I would also ask that you make sure that any belongings that you want to have, you also take those with you so that they will be here when you return.

With that, we are adjourned, and thank you.

(Whereupon, at 5:13 p.m., the meeting was adjourned.)