DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

AND

CENTERS FOR DISEASE CONTROL AND

NATIONAL INSTITUTES OF HEALTH

STRATEGIES FOR DEVELOPING THERAPEUTICS THAT DIRECTLY TARGET ANTHRAX AND ITS TOXINS

Public Workshop

Thursday, June 10, 2004

8:00 a.m.

National Institutes of Health
Natcher Auditorium - Building 45
45 Center Drive
Bethesda, Maryland

PARTICIPANTS

ROY BARNEWALL, D.V.M., Ph.D.

SHUKAL BALA, Ph.D.
R. JOHN COLLIER, Ph.D.

DAVID FRUCHT, M.D.
JESSE L. GOODMAN, M.D., M.P.H.

SUE GORMAN, Pharm.D., DABAT
M. DAVID GREEN, Ph.D.

JUDY HEWITT, Ph.D.
NISHA JAIN, M.D.

BRAD LEISSA, M.D.
STEPHEN H. LEPPLA, Ph.D.

JULIE LOVCHIK, Ph.D.
ANTHONY MACALUSO, Ph.D.

JAMES McCORMACK, Ph.D.
KAREN MIDTHUN, M.D.

MARISSA A. MILLER, D.V.M., M.P.H.
CARL NIELSEN, Ph.D.

M. LOUISE M. PITT, Ph.D.
CONRAD QUINN, Ph.D.

JENNY MELLQUIST-RIEMENSCHNEIDER, Ph.D.
DAVID ROSS, M.D., Ph.D.

LEWIS SCHRAGER, M.D.
DAVID STEPHENS, M.D.

KEITH WEBBER, Ph.D.
KAREN WEISS, M.D.

ALEXANDRA WOROBEC, M.D.

C O N T E N T S

C O N T E N T S (Continued)

C O N T E N T S (Continued)

P R O C E E D I N G S

Welcome

DR. MACALUSO: Good morning, my name is Tony Macaluso. I'm a bio-defense project manager at the National Institute of Allergy and Infectious diseases. On behalf of the National Institutes of Health I welcome you to the NIH campus, and also to this workshop: Strategies for Developing Therapeutics that Directly Target Anthrax and Its Toxins.

This workshop will address issues related to product characterization, proof of concept and safety and efficacy testing in order to expedite the development of these products which are regulated by the FDA.

The Food and Drug Administration is the lead agency for organizing this workshop which is co-sponsored by the National Institutes of Health, the Centers for Disease Control and Department of Health and Human Services.

As the NIH point of contact it has been my pleasure to work with any people who helped make

this workshop a success. In particular, I'd like to take this opportunity to thank the lead organizers at FDA for doing an excellent job of putting this workshop together: Dr. Karen Weiss, Dr. Dale Slavin, and Ms. Melanie Whalen.

Before the workshop gets underway there are a few housekeeping issues that I've been asked to address. The first is that you're probably all aware--but just in case--the agenda for this workshop has been compressed into one day, so we will not--I repeat, not--be meeting tomorrow. This change from the original one-and-a-half agenda was necessary due to the closing of most government agencies as a mark of respect for former President Ronald Reagan.

I apologize for the inconvenience this may have had on your travel plans, but I think you'll all agree that this was an unforeseeable circumstance, and the alternative to postponing the meeting really wasn't a very palatable one.

The second item is the availability of slides and transcripts. The transcripts will be

available at this website, probably in about two weeks; the slides, probably within one or two days.

We will be having panel sessions after most of the sessions. I would encourage everyone to participate. There are microphones in both aisles. If you prefer to submit your questions in writing there will be some cards available from some of the staff and they will be picked up at the beginning of each discussion session, and also during the discussions sessions. And the moderators can ask the questions for you.

Regarding food: coffee and snacks will be provided during the breaks. Now, we realize that because of the compressed agenda, this meeting will last much longer than we had originally anticipated. To encourage you to stay through the last sessions--for which I happen to be the moderator--we've arranged for more than the usual coffee and cookies for the afternoon break. So I encourage you to chow down, if necessary, in the afternoon so that you don't feel the urge to leave early for dinner.

Also, we've reduced the time available for lunch. So you really will not have enough time to leave the campus for lunch and get back. So I encourage you to use the cafeteria, which is just one flight up.

The agenda for the last session wasn't given. I'd like to just go over that very briefly so you'll know what to expect. In addition to a presentation by Carl Nielsen of Challenges and Opportunities in Product Development, we'll also have short five- to 10-minute talks before the panel session. Those talks--the topics will be the FDA's Proactive Approach with Medical Countermeasures Development, Emergency Use Authorization, Information about the Strategic National Stockpile Program, DHHS Plans for Implementation of Project Bio-Shield, and Opportunities and Resources for Bio-Defense Countermeasures Research and Development that are available through the National Institute of Allergy and Infectious Diseases.

As a reminder--since we are on a

compressed schedule--it will be very important for everyone to try to stick to the schedule. I'll ask the moderators to try and push everything along to keep us on that schedule.

And then the last item is the location of restrooms and telephones. There are restrooms at either end of this hall on this floor, and also additional restrooms just one flight up. There's also telephones directly across from this auditorium, and also on the next floor.

At this point, I'd like to turn the podium over to Dr. Karen Weiss, from the center for Drug Development and Research at the FDA.

Karen?

DR. WEISS: Good morning to everyone. I am very gratified to see so many people here early in the morning. Like Tony, I extend my apologies for having to, at the last minute, rearrange the schedule and condense a day-and-a-half of what was going to be a fairly nice, leisurely workshop into a very whirlwind one-day workshop. But, we just have to kind of roll with the punches, and do the

best you can.

One other housekeeping rule to mention is that the conference center does not like people to bring food and drink into the conference. So those of you that have it there--just to let you know, that a during the breaks--whatever--finish everything up outside.

I also, like Tony, wanted to extend some deep appreciation to a couple people. This was a fairly--it was a very collaborative, collegial effort to put this workshop on, between four different government agencies: the FDA, the CDC, the NIH and Office of Emergency Preparedness under HHS. And we all worked for a long number of months, on numerous conference calls, to put this together. And, on behalf of the FDA I want to just extend my appreciation to all the participants in the working group.

But, in addition, I'd like to just highlight a couple of people and organizations, particularly: Dr. Dale Slavin, who's a project manager in my office, and volunteered to take on

this job in addition to her other full plate of activities, and did a great job just pulling together all the different participants for the various activities that have to be done. And I just owe her a great deal of appreciation for that; the Office of Communications, Training and Manufacturing Assistance--OCTMA--in the Center for Biologics Evaluation and Research--who worked on all the logistics of this conference; and last, but not least, Dr. Tony Macaluso, and the staff at NIH, for making this all possible by the funding through an interagency agreement, as well as being responsible for the food that's out there.

So, the carbs and the caffeine that are going to be with us for the day--which we're going to definitely need--are all due to his continually calling and nagging to get that accomplished. So I definitely appreciate that. It's very difficult to supply food when you're a government agency and doing something in a government facility. So it is a great effort.

And with that, then, I would like to just

go ahead and start the session by introducing our first individual who will provide a welcoming--a number of welcoming remarks. I'm very, very please to ask Jesse Goodman to come to the podium.

Jesse Goodman has been the Director of the Center for Biologics Evaluation and Research for a number of years. He's also an infectious disease doctor, and I appreciate him coming and making some remarks.

One quick thing is that Janet Woodcock, who was going to come, because of the compressed schedule can no longer be here this early in the morning, and asked me to convey her regrets. So, thank you.

Jesse?

DR. GOODMAN: Good morning, folks. I'll try to be, like, really quick because of the schedule and, if anything, let you get started ahead of time.

But, you know, if was more than two years ago, after the anthrax attacks that all of us in the Public Health Service and those of us who are

also clinicians realized there was a need for better therapeutic outcomes than were getting even from antibiotics, even those were better than expected. And some of the discussions that led to this meeting started then.

So there is a real need, you know, for the half or so patients who, despite aggressive treatment and supportive care don't make it.

And there's also a precedent for immune therapies working in acute severe infectious diseases; for example, in use of anti-serum early treatment of pneumococcal disease. So there is some reason to at least believe that there could be therapeutic gains from treatments directed at toxins.

I'd like to say, though, that the field obviously, as most of you know, is littered with failures, as well. And it's been very difficult, once there's disease, to intervene.

Excuse me--I got off a plane late last night here--so--

I think, therefore it's important that we

really have a good development program; animal studies that not only can prove success and promise of a therapy, but tell us if something isn't going to work.

In addition, you know, if we had looked at the experience after the anthrax attacks, let's say we had given everybody anthrax sera, and had observed a 50 percent survival rate, we would have said that anti-sera saved patients, because the expected survival rate, historically, was 10 or 20 percent. So we'd all be--probably not having this meeting, and be producing tons and tons of anti-sera, which may or may not work, as we know.

So I think while we're looking for better therapies, it's important we use the best methods to evaluate those therapies.

As part of that, I think it's also worth considering whether there's any possibility at all, if such therapies were ever to be used, of field evaluation of them. Even though it's unlikely, for example, that an intravenous immune globulin product would be harmful, it's remotely possible.

And certainly some of the chemical therapeutics--toxin inhibitors, etcetera--could potentially be harmful in humans with anthrax disease. So I think a very difficult thing to contemplate is the idea of controlled clinical trials in a disease with a high public profile and a high mortality rate, like inhalational anthrax. But it's something that I hope, in the discussions and the panels on the last day, that we'll look at. This is the last day, now.

[Laughter.]

You know, and again, just to emphasize, you know, I really do think if we had had immune globulin, for example, or an experimental therapeutic available for those few cases, and had used it, many, many people would be convinced that it had worked and it would become the standard of care. And, again, FDA is quite familiar with circumstances where this has happened--for example, autologous bone marrow transplantation for breast cancer, which appeared to give such excellent results that when subjected to clinical trials did

not hold up. And nobody would argue that that's something you want to put patients through unless they benefit from it.

So I think, again, the importance of really good animal models--which are an incredible challenge, especially if you are considering an animal model in a symptomatic disease state. Animal models are problematic enough, even when they're simple. And when you add things like disease and antibiotic therapy, the variability can become incredible.

So, I really look forward to hearing the results of this. There's a terrific group of people. And I know that the Department of Health and Human Services is very, very committed to improving therapy for anthrax, so this is a very important meeting.

So, with that, I guess we start the first session, so Karen will come back up. Thanks very much.

Part I - Introduction to and Pathogenesis of B. anthracis

DR. WEISS: Thank you very much, Jesse, for your opening remarks, and for setting the stage.

We're going to go ahead and get started right now with Session I: Introduction to the Pathogenesis of B. anthracis.

The objective of this session is to provide a critical background to facilitate and focus the workshop. And I have the pleasure of introducing two speakers who are going to be discussing things at this session.

There's no panel planned after the first two speakers, but we'll see what the time frame is like, and if there potentially is some time available after both speakers have completed their talks we might have a minute or so for some questions.

I'm going to go ahead and introduce them both at the same time so I don't have to keep popping up and down, and try to save a little bit of time.

So, the two speakers are, first, Dr.

Stephen Leppla, who is right here at the NIH campus. Dr. Leppla has done research on anthrax for well over 24 years and is eminently qualified to give one of these opening presentations.

And following Dr. Leppla, we'll hear from Dr. David Stephens from Emory University. Dr. Stephens is the head of infectious diseases at Emory, and he has worked in the Meningitis and Special Pathogens Group with CDC, and he was a clinical team leader with CDC for the 2001 anthrax outbreak. And he will directly follow Dr. Leppla's presentation.

Dr. Leppla?

Anthrax Toxin as a Target for Therapeutics-Overview

DR. LEPPLA: Thank you. Good morning, it's a pleasure to open the scientific aspects of this meeting. Since this is a very targeted meeting, and all of you, I think, have worked on anthrax, it's a little presumptuous, perhaps, to provide introduction about the basic properties of the organism. So forgive me if I say things which are too obvious to any of you.

So, the organism we're dealing with is bacillus anthracis. As you know, it's a Gram-positive spore former. It infects livestock. And we're here because it can also infect humans.

And it's virulence is sort of a classic in pathogenic microbiology. We think it's fairly simple, in that its virulence is determined by two virulence factors: the poly-glutamic acid capsule, which is anti-phagocytic, and the three-protein component anthrax toxin. And each of these is encoded by a large plasmid, PX01, and PX02.

I'm going to skip these two slides, in the interest of a shortened talk--except just to indicate, since you have this slide in your folders--that this was to indicate the difference between bacillus anthracis and its genomically very closely related neighbors, bacillus cereus and theragensis--but in spite of their very close genetic similarity, they have very different sets of virulence factors. Bacillus cereus having a set of secreted aggressins that are, in large part, transcriptionally controlled by a regulator called

PLCR. In contrast, anthracis has the two plasmids I mentioned--PLCR, which is the gene is there but it's been inactivated. So all of these aggressins are not produced by anthracis and, instead, you have the toxin and the poly-D-glutamic acid capsule.

So this is a pathogen that's evolved in a very particular way.

And the infection process as we understand it is that spores enter the body either through the skin, GI tract, or through the lungs. And those spores are engulfed by phagocytes--usually mentioned as macrophages, but I think other phagocytes are probably also involved. The spores are carried in those phagocytes to lymph notes where they geminate. The bacteria then escape from the phagocytes. Toxins are produced--both the capsule and the protein toxins--and these toxins have various activities which clearly suppress the host responses and allow the bacteria to grow to very high numbers in the blood of these infected animals.

The anthrax toxin accumulates as the bacteria grow to high numbers. But I think an interesting feature is that the toxin is not a hugely, highly cytotoxic and rapidly potent killer of cells or tissues. And that's really to the advantage o the bacteria, because its objective is to not only--parenthetically, to kill the host, but to produce a large crop of spores in the herbivores, which are the natural host of the disease, those spores would then be deposited in the soil, where they could remain for long times until another animal comes along and is exposed to them.

And the indications of the effectiveness of the pathogen are that in the best hosts--the large herbivores--the bacteria can grow to very high titres in the blood at the time of death. And, with any luck, the bacteria convert a lot of these into spores, and so you have a large infectious reservoir in the soil.

[Slide.]

Most of my talk, and most of this meeting,

is about the toxin, but I think I just wanted to remind people of recent work that--I guess I skipped a slide which is not showing up. That's okay--recent work from Tom Kozel's lab, indicating that the capsule plays a previously unrecognized role in immunity to anthrax. So what they did was that they showed that monoclonal antibodies produced to the poly-D-glutamic capsule were protective, in mice, against anthrax infection. And this had not previously been recognized, that antibodies played a potentially protective role.

So I think this is a newly identified target, and I hope and anticipate that there will be increased attention to this target of therapeutics.

[Slide.]

Now focusing more on the toxin, again, there are these three protein components--large proteins. The protective antigen is the central player, and its role is to deliver the two enzymatic moieties into the cytosolive cells. Adeolate cyclase, the edema factor, will raise

adeolate cyclase, or cyclic AMP levels. The lethal factor is a metalloprotease, which cleaves all of the MEKs.

Now, over the last decade or so, we've developed a detailed picture of how these toxin proteins interact to get inside cells and damage cells. So we now know that the protective antigen binds to cellular receptors. These were identified in the lab of John Young and Jon Collier. And they were first called "anthrax toxin receptor" It was then recognized that they are variants of a molecule called "tumor endothelial marker 8." And, more recently, another isoform capillary morphogenesis protein-2 has been identified.

So the toxin is bound to these receptors. It is then activated protealytically by cleavage with the cellular protease furin, with removal of a fragment. And removal of that fragment allowed the remaining portion of protective antigen to heptamorize into a very tightly associating heptamer. And on the newly exposed surface of this heptamer there are binding sites for the edema

factor and lethal factor components.

And John collier has shown that, actually, the binding site spans two of these monomers, and it follows--rather constraints that there's a maximum of three molecules of edema factor and lethal factor that can be bound onto the heptamer.

This heptamer is then internalized--presumably through lipid wrap and endocytosis to a vesicle which becomes acidified, and it then inserts in the lipid membrane and becomes a protein-conducting channel.

And through Dr. Collier's work in particular, that channel is probably the best understood protein-conducting channel now known, through extensive mutagenesis and biophysical and biochemical measurements.

So we believe that the edema factor and lethal factor proteins transduce--pass through the center of this heptamer channel to reach the cytosol. Again, then, the edema factor is an adenolate cyclase, and makes very high levels of cyclic AMP. LF cleaves all except one of the ME-

kinases, or MEKs, and the results of these two events--presumably in combination--cause the tissue damage and lethality which observe as the pathogenic effects of infection.

[Slide.]

This is genetic evidence that introduces the fact that the toxin--the two toxins are really the dominant virulence factors in bacillus anthracis. And I'm not going to go through the numbers here. This is work from Michelle Mock. But basically, it shows that if you knock out the capsule or the toxin, or individual toxic components, you greatly reduce the virulence of bacillus anthracis for mice. It's going from an LD 50 of 5 scores, up to, essentially, avirulent organisms.

So, to focus separately on the lethal factor and edema factor components of the toxin, we have for a long time thought that the lethal toxin is the major cause of pathogenesis. And the numbers from the previous slide showed that bacterial strains in which LF is genetically

inactivated are attenuated at least a thousand-fold. And, furthermore, there's a very large and growing body of evidence that the lethal toxin injected into animals duplicates the symptoms of bacterial infection. And, of course, an even larger body of evidence that antibodies to either--certainly to PA and increasing evidence that antibodies to LF protect against bacterial challenge. So all of this indicates the important role of the lethal toxin in pathogenesis.

[Slide.]

The established effects of the toxin in several system provide the ways to study the toxin, but also have find wide use in bioassays for characterizing the toxin and antibodies, as well. So, one of the early discoveries, by Art Friedlander, was that the macrophages from certain limited number of inbred strains of nice exposed to lethal toxin lysed in about 90 minutes.

But, again, this is restricted to certain strains of mice. And that's widely used as a bioassay, as well as a way to study the mechanisms

of action.

[Slide.]

And perhaps an even older--certainly an even older bioassay is based on the fact that in the Fischer 344 rat, intravenous injection of lethal toxin will kill them in as little as 38 minutes. Other rat strains are much less sensitive. So there's the unique feature of this rat. And this is widely used as a bioassay for protective agents that target the lethal toxin.

Mice are susceptible to the lethal toxin, but they die much more slowly. Typically, they take two to three days to die after lethal toxin administration.

[Slide.]

This is our data showing the sensitivity of two strains of inbred mice, the Balb/c being the more sensitive, and the C57 Black being somewhat more resistant. Mentioned here is the fact that though it has been the view that the susceptibility of mice is a function of the susceptibility of their macrophages, to lytic action of lethal toxin.

And so, indeed, the Balb/c mice, their macrophages, in vitro, are sensitive to the toxin, whereas the C57 Black macrophages are resistant in vitro. But we've looked at a large number of inbred mouse strains and, in fact, this correlation between sensitivity of macrophages and sensitivity of the whole animals is not a very good correlation. And it's certainly not true over a larger number of strains.

[Slide.]

Now, the neglected partner in this pathogen has been the edema toxin. And it's, I think, in part been neglected because the genetic evidence in the slide that I went over quickly, is that if you knock out edema factor from a bacillus anthracis strain, the virulence decreases tenfold. One should ignore tenfold, but in comparison to lethal factor, it seems like a minor player.

But we actually have now shown that the edema toxin injected into mice is, in fact, highly lethal to these mice, and that the edema toxin does produce some clinical signs that are similar to

those seen in bacterial infections.

[Slide.]

This is some unpublished evidence on the susceptibility of Balb/c mice to the injection--intravenous- injection-of the edema toxin PA and EF. And we find that the LD 50 is about 20 micrograms each of the combination of PA and EF. And at the higher doses, the animals die very quickly, with a wide--showing a wide variety of pathogenic responses, biochemical and histopathological changes--really a wide spectrum than is seen in the lethal toxin-injected mice.

So I'd like to suggest that the edema toxin has probably been ignored, and probably should receive more attention as a target of therapeutics.

[Slide.]

So, what kind of therapeutics are people developing? And you all know this very well. I'd like to, for the purpose of the discussion, divide them into these two groups: those which act extracellularly, and those which work inside cells.

And perhaps a unique feature of the anthrax toxin system is that lots of things are going on outside the cell, and therefore there are a number of steps which are accessible to macromolecular inhibitors; that includes, of course, antibodies, but also toxin fragments, receptor decoys and others.

And, in contrast, the agents that would block intracellular activities would be typically targeting the enzymatic activities of the adenolate cyclase and the protease. And this is more amenable to standard pharmaceutical approaches, where one looks for small molecule inhibitors that are cell-permeable.

[Slide.]

So, again, returning to the scheme of how the toxin gets into cells, let me highlight a number of targets that people are considering for therapeutic approaches. So these could include things like the receptor decoys. It's been shown that if you express--if you provide the extracellular domain of the receptor, it will interact with the toxin and act as a competitive

inhibitor and protect.

Similarly, fragments of protective antigen--perhaps Domain IV, or perhaps peptides--mimicking regions of Domain 4 that interact with the receptor could block that interaction and provide protection.

Furin inhibitors are a potential therapeutic. I'll give an example of one in a minute.

Antibodies to PA, which either bound to the receptor-recognition domain in PA, or, you could imagine, antibodies which bind onto the newly formed surface of the PA heptamer. Of course, antibodies to EF or LF have potential value; LF competitors. These could be fragments of LF peptides, and it's been demonstrated in the Collier lab, also, that these could be made more effective by increasing their valency through multimerizing them, because then you get an evidity enhancement.

Dominant negative protective antigen mutants. I think we'll hear more about those later from Dr. collier. And once we get inside the cell,

as mentioned, you have two enzymes which are susceptible to development of small-molecule drug inhibitors, either an adenolate cyclase inhibitor, or a protease inhibitor.

And then there's the large class of molecules that could be imagined as dealing with downstream consequences of the toxin action. And these are more in the class of supportive therapies. And as we know more about the pathogenic--the consequences of these two events, we could perhaps select a better set of supportive therapies.

[Slide.]

I'm going to give an example of a monoclonal antibody that's been developed. And here I'm just indicating again the potential targets for antibodies--targeting the extracellular steps. And we're guided in this work by work done in the middle-'80s in USAMRIID, where Steve Little and others developed a set of mouse monoclonal antibodies. And the ones that were neutralizing and are best characterized are 14B7, which binds to

Domain 4 and prevents toxin binding to receptor, and 1G3, which binds to the newly exposed surface on the PA heptamer and essentially competes with LF and EF binding. That's at this point.

[Slide.]

So, I was involved in a small way, and worked from the Lab of George Georgio, and what that group did was to take the 14B7 mouse monoclonal antibody and clone the genes, and produce a single chain antibody, based on the 14B7 sequences. They then went on, by phage display, to engineer a higher affinity variant of that antibody, and they were able to increase the affinity on a monovalent molecule about 40-fold. And so 1H was their improved single-chain antibody.

Then they resorted to the rat model, which I referred to earlier as a test of antitoxins. And again using the monovalent 14B7 they were able to recuse rats which were dying in the control group at 91 minutes--they rescued, in fact, 0 of them--none of them. It was perhaps a small delay in time to death.

But the affinity-enhanced variant of 14B7 saved three of the five rats and delayed the time to death of the other two.

[Slide.]

And this molecule--the enhanced 14B7--is actually being developed by Elusys, and they have humanized it, as you would want to do, and made it into a full-size antibody. In the interest of disclosure, I'd just say I have no financial interest in this, although I had a small hand in the early steps of its development.

So I think the antibodies have obviously attracted lots of interest as therapeutics. And that's, in part, because there's lots of expertise and skill in humanizing antibodies and producing them in large amounts. One could imagine that in a scenario where there's a mass exposure of a population one could administer a single dose and provide a number of weeks of protection against infection. It might not be practical to treat large numbers of symptomatic patients, but these antibody reagents could certainly have a potential

role in that aspect.

And there's always the concern about antibiotic-resistant strains. Again, these antibodies would remain resistant effective against those strains.

Less mentioned, I think, but deserving notice, is that an antibody product could provide immunity to infants, children, immunocompromised persons for whom vaccines might not be available or effective.

[Slide.]

So, I'm going to skip that--another example of an antibody which has been developed by a different company--Alexion--and it's based on the 1G3 molecule. And it has some unique properties which, I guess, are probably evidence in the slides that I provided.

[Slide.]

And here's an article from The Washington Post I thought was well written. This is where I get a lot of my information--

[Laughter.]

--about anthrax therapeutics. And this was from March, and it was pointing out the work from Elusys that I referred to and the perhaps even better known work from Human Genome Sciences. They also have a human antibody which seems to be highly protective in several models of infection.

[Slide.]

And so I highlight the quotes here: "So many companies have responded to the task that the government now confronts an embarrassment of riches."

And I think that's true. I think there are so many promising antibodies coming down the road--I think the public press lists at least five companies that have such products--that--and I think all of them have efficacy. I have every reason to expect that they would all be efficacious.

And so the director of my institute, Dr. Fauci, said we would completely break the bank if we committed to purchasing every one of them. So there is going to be a problem in choosing between these products.

[Slide.]

I'm now going to turn to a few examples with small-molecule inhibitors targeted to anthrax toxin. And, again, we have two enzymes here which are potential targets. This is a slide prepared for Dr. Fauci which I swiped from him, where he highlighted, in testifying downtown I believe, two drugs which have shown some promise--at least in very early studies.

And this one I had a small hand in. And this is an edema factor inhibitor. So the furin inhibitor--I pointed out furin is a potential target--the work here is from Iris Lindberg in Louisiana, and she has an inhibitor which hexa-D-arginine, which she had been developing as a furin inhibitor. And in this experiment she showed--again in the rat model--that the control rats were dying very promptly after just a few hours. Co-administration of this furin inhibitor did save half the rats. So this is a demonstration that furin inhibitors have potential.

[Slide.]

And the other inhibitor I want to just draw attention to is targeted to the adenylate cyclase. This is a model of the edema factor with calmudulin very tightly bound to it, and without. And Wei-Jen Tang, at the University of Chicago screened a number of compounds, and was able to identify adefovir as an inhibitor with nanomolar inhibitory activity against the enzymatic activity of the edema factor. And this shows, in cell culture models, that the adenylate cyclase production induced by edema factor--in the solid symbols--is blocked as you increase the adefovir concentration. And so he's--Wei-Jen Tang is trying to carry this forward as a first-generation, or at least a candidate lead compound from which other edema factor inhibitors might be developed.

[Slide.]

Lethal factor is perhaps has been a more popular target for inhibitors because it is a protease, and the pharmaceutical industry has comfort in dealing with--searching for protease inhibitors. The structure of lethal factor has

been solved, and a number of inhibitor candidates have been identified. And this is just a model of the active site of the lethal factor protease, in which three different--or I should say two protease inhibitors have been superimposed, along with the natural peptide substrate.

[Slide.]

Also published--already, now, two years ago--from Merck was a paper describing a peptide-based fluorescence assay which--it was clearly developed with the intent of screening this company's large family of protease inhibitors. And while we haven't heard anything more from them in public--to my knowledge--I do note that they're presenting this work at the Gordon Conference next month. So there's indication they're continuing to work on inhibitors to lethal factor.

[Slide.]

So, again, I've highlighted a number of targets at which one might expect to block the activity of anthrax toxin and thereby protect animals and humans who are infected with bacillus

anthracis. I'm impressed, in the short time that this has been--these targets have been under frontal attack by academic and pharmaceutical companies, I think tremendous progress has been made. And I think we can look forward to development of some effective products int he not too distant future.

So that's all I wanted to say. And now I'm going to pass the baton on to the next speaker.

[Applause.]

Clinical Aspects of Disease

DR. STEPHENS: Good morning, and I thank you very much for the invitation to be here.

Anthrax, from a clinical perspective, was of historical interest prior to 2001. In the 20th century, some 18 cases of inhalational or inhalation anthrax were reported. Most of those were in millworkers, associated with, in this country, goat hair importation. There were some cases of cutaneous anthrax occasionally in the midwest. But, certainly, 2001 was an important point in terms of our appreciation and

understanding of some of the clinical issues of bacillus anthracis.

[Slide.]

now, I think there are several lessons from that outbreak--and certainly lessons from my perspective. Those are the impact of surge; the clinical recognition issues and the differential diagnosis of anthrax. Some of the issues of management--and we're obviously focused today on anti-toxin approaches, but some of the issues of antimicrobial management were equally--and remain equally as important; issues of immune response; and certainly gaps, in terms of our ability to rapidly diagnose anthrax, its different clinical manifestations; issues of use of the vaccine; and issues of prophylaxis, in particularly, in terms of drugs.

[Slide.]

Now there were 23 cases of inhalational--or 23 cases total of anthrax in the outbreak; 11 inhalation and 12 cutaneous. I'm including one laboratory-acquired case that

occurred some months, due to handling of specimens from the outbreak.

However, there was at least a log higher of cases in which there was real concern about anthrax and the differential diagnosis of these cases remains--is, and has been, critical in terms of trying to rule out anthrax. So this required a lot of effort and a lot of involvement of the public health community, and as well as both the state and national level.

There were a lot of individuals evaluated--at least a log higher individuals evaluated for anthrax. 30,000 individuals were started on prophylaxis because of exposure in the areas Florida, Washington, D.C., New Jersey, New York. A number of people were obviously directly affected, and virtually the entire population was impacted.

[Slide.]

Now, we won't go into this. This is the--the point, really, of this slide is to emphasize the three clinical forms of disease:

cutaneous, inhalation and gastrointestinal.

[Slide.]

Most of you know this history well. The initial case report: a 63-year-old man from south Florida who had been on--he became ill on a vacation. He developed fever, myalgias, cough, headache, nausea and vomiting, and then he developed altered mental status and presented to a local hospital in Florida with a diagnosis of meningitis.

[Slide.]

His laboratory exam revealed a leukocytosis; LP was remarkable from a neutrophyllic pleicytosis, with large numbers of polys, but also--which is characteristic of anthrax meningitis--large numbers of red cells found in the spinal fluid. And this actually turns out to be a rye stain of the spinal fluid, showing large numbers of polys, and obviously large numbers of Gram-positive bacilli, which rapidly grew B. anthracis.

[Slide.]

The investigation: photographer for a tabloid newspaper. He was on a vacation when he became ill. Computer cultures yielded B. anthracis from the surface of the computer. Nasal swabs of a number of individuals in the building also were positive. Prophylaxis was ultimately given and the outbreak began.

[Slide.]

I think you're obviously very familiar with this kind of picture, in terms of inhalation anthrax. Incubation period is--from historical records--some 2 to 14 days, with a range of up to 60 days. And most of this has been covered by Steve in his talk.

Again, the importance, though, of mediastinal disease, edema, hemorrhagic mediastinitis, subsequent hemotogenous spread and meningitis should be emphasized.

[Slide.]

This is a diagram from a JAMA article looking at the inhalation anthrax in Sverdlovsk; the outbreak associated with a bioweapons plant,

looking at number of days after the accident and the onset of inhalational cases following the accident, up to one case at 43 days after exposure.

[Slide.]

There were two patients in the 2001 outbreak: one from New York City, and a second one from Connecticut, in which there was probably a longer length of incubation, although the exact time of exposure for those cases is not known.

[Slide.]

This is a summary from the article that was published by us in Emerging Infections in 2001. And the second are the 11 cases published in JAMA in 2002, looking at the clinical features of inhalation anthrax. Median age was 56. Most were males. Incubation period--which I guess is the important feature on this particular slide--was four days, in which the incubation period was known. And the median duration of symptoms prior to presentation was 3.5 days, with a range of one to seven days.

[Slide.]

Major features, in terms of symptoms were chills, fever, fatigue and malaise. Night sweats, in particular--or sweats in particular--drenching sweats--were also noted in a number of patients; a non-productive cough, nausea and vomiting, a dyspnea, chest discomfort--which was described in the older series, also occurred in these patients; rhinorhea and sore through--upper respiratory symptoms--were infrequent in these individuals.

[Slide.]

Fever, tachycardia was common. Very few of these patients were hypotensive on admission. Some subsequently develop hemodynamic instability. But hypotension as a presentation was uncommon. This is very different from, say, meningecoccal septicemia, for example, where hypotension and DIC are common presentations.

[Slide.]

The initial laboratory findings: the white count was high, but not excessively high; neutrophilia, however, was present, with greater than 70 percent neutrophils present on the initial

WBC. Transaminases, interestingly, were elevated in 10 of the 11 inhalation cases. And hypoxia, by some measure, was noted in 7 of the 11.

[Slide.]

The diagnosis was made by blood cultures in eight of the individuals who had not received antibiotics. Interesting, any antibiotic therapy rapidly sterilized the blood and the diagnosis in three patients was established by other, newer technologies: immunohistochemical staining of transbronchial biopsy specimens or pleural biopsy or pleural fluid, and detection of DNA by PCR in blood or pleural, and by the detection of immune response to PA.

[Slide.]

The initial radiographic findings in patients with anthrax: the chest x-ray was abnormal in all 11; mediastinal widening, infiltrates or pleural effusion was noted. Mediastinal widening-- considered to be the classic for inhalation anthrax--as not present in everyone. And, again, the chest x-ray findings, although

abnormal on admission, the findings were--could be subtle. And on a couple of occasions, the initial abnormalities were, in fact, missed.

And the chest x-ray abnormalities were noted within 48 hours of onset of presentation.

[Slide.]

This is Case 1--our index case from Florida. And I think you can appreciate that this individual did have mediastinal widening in this setting.

[Slide.]

However Case 2 from Florida was somewhat different in terms of its presentation: presented with infiltrates and a pleural effusion that persisted--and really never did have significant mediastinal adenopathy.

[Slide.]

And this is a CT scan showing the large pleural effusions, which were characteristics of these patients, and really complicated their clinical course.

[Slide.]

This is the case from the Washington area. There--and I think the findings were more subtle in this case. There is some mediastinal widening in this particular patient; maybe an early development of a pleural effusion on the left side, but findings can be, in fact subtle.

[Slide.]

CT was more sensitive, showing mediastinal adenopathy in this particular setting.

[Slide.]

So, in summary: profound seating and GI symptoms were notable; chest x-rays were uniformly abnormal, although with a variety of findings. And, again, the initial films could be--were subtle, and findings could be missed.

Blood cultures were positive early in the course, before antibiotics.

Pleural effusions were an important feature of the illness. Frequently it required drainage. And, certainly, some of the issues regarding improved survival have to do with aggressive attention to these pleural effusions,

and the alleviation of the respiratory compromise that was characteristic of these patients.

Pleural infiltrates were found in over 60 percent of patients. Survival was higher than the 15 percent previously reported. And, again, the emphasis on the newer diagnostic tests--IHC, PCR and serology--were very helpful in understanding the spectrum of this disease.

[Slide.]

This is a brief review. This is Jeanette Guarner and Sheriff Zaki at the CDC published this review of the pathology of inhalation anthrax on five fatal and three non-fatal cases--again, emphasizing these serosanguinous pleural effusions, the hemorrhagic mediastinitis, and the presence--in multiple organs, especially in the patients who died--of bacilli--of cell-wall or capsulary antigens in multiple organs. And, again, in this particular study, IHC was an indispensable tool.

[Slide.]

And just a couple of quick slides from that paper: hemorrhagic mediastinal lymph node;

lots of inflammation and hemorrhage in the mediastinum. And these--this is an IHC looking at antigen positivity in these specimens from patients who died.

[Slide.]

This is a series of pleural studies on patients during the outbreak of cell block, looking at pleural reaction. And these also include a pleural biopsy--again with lots of reaction at the pleura, with lots of B. anthracis antigen present in these specimens.

[Slide.]

Now, the differential diagnosis of inhalation anthrax includes influenza or a viral syndrome. Several of these patients were thought to have a viral syndrom; actually sought medical attention and then were sent home with that diagnosis.

The atypical causes of pneumonia--from mycoplasma through viral pneumonia, Q fever, psittacosis, Legionnella. And those conditions that are known to give you mediastinal are

endothoracic lymphadenopathy, histoplasmosis, coccidiodiomycosis, tuberculosis. And one patient was actually admitted and being worked up for a malignancy.

[Slide.]

This is--turning to the cutaneous cases, case one, from New York: a 38-year-old woman, assistant anchor, developed an erythematous papule on her chest; three days. She developed a vesicular, ulcerated, edematous lesion; had headaches, malaise, satellite vesicles; was started on Ciprofloxacin, and by 10/9 of '01 had developed a black eschar. IHC and serology was positive.

[Slide.]

And this is just the late features and manifestations of anthrax--cutaneous anthrax--in that particular patient.

[Slide.]

The only child in the outbreak was a cutaneous case: actually a seven-month-old child who, after visiting a network studio with his mother, his arm became swollen and he was given

augmentin but he remained febrile. He was ultimately admitted to the hospital and had edema and a large black eschar. Interestingly, he and one of the inhalational cases, late in their course, had evidence of hemolytic uremic-like syndrome, with hemolysis and thrombocytopenia. And in this particular patient, IHC and PCR was positive on blood for B. anthracis.

[Slide.]

And this is the ultimate course of his particular lesion in this child.

[Slide.]

And this just summarizes--the letters--some of the New York cases on onset. This was actually after this second--or the New York Post letter was identified, and resulted from handling of that particular letter.

[Slide.]

So, for cutaneous anthrax, the incubation period is 1 to 12 days. The papules are painless. Papules progress to vesicle or bullous formation with surrounding, nonpitting edema. The central

vesicle becomes ulcerated and necrotic, and surround--and becomes often surrounded by satellite vesicles, subsequently forming this black eschar, which is characteristically depressed and painless.

Fatigue, chills, fever, regional adenopathy may occur in these individuals.

[Slide.]

This is not from the outbreak, but just gives you a better sense of some of the progression of lesions from vesicle and papule formation, to eschar formation over a period of 7 to 10 days.

[Slide.]

Again, not from the outbreak, put showing you some of the differences--some of the clinical presentations of--and the earlier ulcerations of cutaneous anthrax.

[Slide.]

This actually is from the outbreak, showing an eschar--actually a debrided eschar--on a finger in one of the cutaneous lesions.

[Slide.]

And this is actually an early lesion

associated with the outbreak, showing you the initial vesicle formation.

[Slide.]

And this also is from the outbreak. And as you will--again, showing--this individual also had secondary staphylococcal bacterial infection complicating his anthrax, which were seen in a couple of individuals. This individual also had positive blood cultures for B. anthracis.

[Slide.]

So, the differential diagnosis is important. These kind of cases continue to occur. Although the outbreak is obviously over, the issues of differential diagnosis, the issues of unusual rashes and the concern about future cases continues. And there's a lot of--there's importance, obviously, in appreciating what are the most common differential diagnoses--what is the most common of the differential diagnoses of cutaneous anthrax.

Interestingly: spider bites. In a review of Sheriff Zaki, isolated lesions of varicella

Zoster were actually quite common in the differential diagnoses--from the New York area in particular, Rickettsial pox; herpes simplex type 1 also is in the differential diagnosis, as are the more traditional lesions associated with cutaneous anthrax: erythema gangrenosum or pyoderma gangrenosum, which also can present like cutaneous anthrax; tularemia, plague--and the importance, again, of the common, but sometimes presenting in an eschar kind of way, in particular staphylococcal infections.

[Slide.]

This is actually a case. Cases like this often occur on Saturday night. And this one occurred on Saturday night, and was a child of a laboratory worker, who was real concerned about what this was. It turned out to be a Brown Recluse spider bite in this particular child. But it gives you a sense of the differential diagnosis that is important to consider in these kind of individuals.

[Slide.]

Now, this is an article--Conrad Quinn's

going to be talking in a minute. There was an opportunity, obviously to examine the immune response in patients who were a part of the outbreak. And this is--the data I'll show you is an article that's in press in Journal of Infectious Diseases. Twenty-two patients comprised this group--this study. Serial serum samples were obtained; humoral response to PA and also LF is being looked at in these individuals, and also toxin neutralization.

[Slide.]

And I won't go into this assay. This is from Conrad's paper in Emerging Infectious Diseases, looking at the validated anti-PA IgG ELISA--originally developed to look at vaccine questions, but rapidly adapted in the outbreak to be very useful clinically with a very good sensitivity and a specificity which can be enhanced by a competitive ELISA.

[Slide.]

And I won't go into this.

[Slide.]

These are data from the outbreak looking at anti-PA IgG antibody in patients with inhalational anthrax, noting that in some individuals the response was quite high, approaching 1,500 mcg per ml. Most of the individuals--the lowest was around 150, as I recall--in terms of peak levels, the peaks generally occurred approximately 30 to 60 days after onset of symptomatology. And in following these patients out to a year, they continue to have levels of anti-PA antibody present.

[Slide.]

Cutaneous patients were quite different, however. They--only a couple of them mounted response greater than 100 mcg. Some of them were high early and came down quickly. In a couple of individuals the response was actually quite low, and there was a question of why this was the case. Was this antibiotic suppression? Many of these patients were started on antibiotics fairly quickly.

But the data would suggest a very

different picture between cutaneous disease. The one patient shown here, who mounted one of the higher antibody responses, was also a patient who was bacteremic.

[Slide.]

There is a very good correlation--in work looking at the levels of anti-PA IgG with toxin neutralization, a good correlation between toxin neutralization and anti-PA antibody. In work done with Shane Croddy and Al Humed at Emory, we've been able to look at specific IgG memory cells in patients--the patients with inhalational disease versus cutaneous disease, and all of the six patients who were available, who survived, had evidence at six months and longer, of memory B cells that were present in individuals with inhalation disease, versus only one--and this was the bacteremic patient--who had evidence of memory B cells with cutaneous disease.

[Slide.]

And I won't go into that.

[Slide.]

So IgG and anti-PA antibodies in patients with inhalational anthrax were detectible 11 days after symptom onset. Anti-PA was a predictor of toxin neutralization and the development of specific PA memory B cells. And in the cutaneous anthrax patients, the magnitude of anti-PA-specific IgG and toxin neutralization and memory B cell response was less. And there really were two groups-those with a rapid rise and fall, and those with a very low and delayed response. And the reasons for that are not--at least in my mind--clear.

[Slide.]

I won't go in--because of the focus of this particular meeting--in terms of the antibiotic issues. I will point out that number of these patients did get protein inhibitors known to have anti-toxin effects; in particular, Clindamycin. And whether that was a component of the increased success, certainly, aggressive antimicrobial therapy, aggressive drainage of pleural effusions, aggressive supportive care were, in my view, key

instruments in terms of the success.

[Slide.]

There are lots of issues that I think are still out there regarding treatment--the best antimicrobial regimen; the treatment of meningitis, what are you using in the setting of meningitis, a pretty much uniformly fatal disease?

What about steroids? Steroids were used in several of the individuals--especially the individuals with extensive edema and cutaneous disease. Again, we're talking anecdote, in the sense of a limited number of patients, in terms of the data that we have in humans clinically.

Length of therapy, persistence of spores, and how long do you continue prophylaxis. There was, and still is some controversy about how long you should continue prophylaxis.

[Slide.]

And I won't go into this. This has to do with issues of long-term use of antibiotics.

[Slide.]

After the outbreak, there was a meeting to

discuss research priorities. And, obviously, this meeting is a continuation of issues of antitoxin immunotherapy and how to best approach that; also issues of antibiotic therapy, and the importance of animal models and establishing animal models that are reliable and predictors of human disease. As most of you know, there are lots of issues in that particular area.

I won't go into the anthrax vaccine, but in the interest of time I want to acknowledge the role of the CDC, the Meningitis and Special Pathogens Branch, the National Center for Infectious Diseases, the Clinical Team and State Teams, who were very instrumental in collecting a lot of the clinical data; obviously, Conrad Quinn and his laboratory at CDC; Sheriff Zaki and his laboratory; Patter Dull and Carolyn Greene, two former EAS officers who played a significant role in the evaluation of and obtaining specimens on the patients--surviving patients; John Hernigan at CDC and Emory; the local health departments; and individual physicians who were caring for these

very sick patients.

So, I appreciate your time.

[Applause.]

DR. FRUCHT: Thank you, Dr. Stephens.

PART II - In Vitro Characterization

DR. FRUCHT: It's my pleasure to introduce you to the In Vitro Characterization session of this workshop.

I'm David Frucht. I'm from the Division of Monoclonal Antibodies, and I'm happy to say that we've assembled an excellent group of speakers and panelists today.

I should say that our speakers will only be covering a subset of the bioassays that are used to characterize the large variety of potential anthrax therapeutics. However, with the group of experts that we'll have on the panel, I'm sure that we'll be able to answer any other questions, or discuss points that aren't covered in the talks.

Our first speaker today is Dr. Conrad Quinn. He's the Chief of the Microbial Pathogenesis and Immune Response Laboratory. Dr.

Quinn received his Ph.D. in microbiology in 1989 from the University of Wales, following which he did his post-doctoral training at the NIH. The current focus of his laboratory is the development of validated immunoassays for the diagnosis of anthrax in humans, and for quantitative evaluation of humoral immune responses to anthrax vaccines. In addition, his laboratory performs immunoassays for clinical trials in the CDC anthrax vaccine research program.

Dr. Quinn?

In Vitro Assays to Characterize Anti-toxin Based Therapies

DR. QUINN: Good morning, ladies and gentlemen.

Can I get the first slide?

[Slide.]

This morning I'd like to touch on some of the in vitro assays that we have been developing at the CDC for evaluation of toxin therapies. We will focus on one assay in particular--the toxin neutralization assay--because of its broad spectrum

application.

[Slide.]

Well, I'll start by firstly recapping on some of the things we've heard already this morning from Dr. Leppla and Dr. Stephens.

The causative organism of this disease is bacillus anthracis, which is a Gram-positive spore former. It's a large bacilli within the bacillus cereus group. It can be distinguished from its close cousins by its clear characteristics of the absence of motility; usually penicillin-sensitive, usually gamma-phage sensitive; and it is non hemolytic--which distinguishes it from bacillus cereus.

It also produces a tripartite protein toxin and a gamma-linked poly-D-glutamic acid capsule--which we heard about, again, this morning. And you can see it hear, stained with a McFadden stain on the outside of the organism, growing in serum or blood.

I'd like to focus on these two components here: the tripartite protein toxin, and the acid

capsule, because these are its major virulence determinants.

[Slide.]

Of course, the proteins can now be produced and purified to high levels of purity for analysis and antigen development and therapeutic molecule development. They are, interestingly, serologically distinct, which is illustrated nicely here by the rather old-fashioned but still very effective Ouchterlony double immunodiffusion technique. And, as we heard from Steve Leppla this morning, these toxins--these three proteins--act in binary combinations of PA and LF to generate lethal toxin: protective antigen and edema factor to generate the edema toxin. The effects of the lethal toxin are now known to be due to its anti-protease activity, which affects cleavage of kinases. It has also been shown to lyse certain macrophage cell lines in vitro; shown first by Dr. Friedlander at USAMRIID in the mid-'80s, and subsequently developed as a neutralization assay by Steve Little at USAMRIID.

We also know that it has an effect on cytokine modulation and perhaps immunosuppression in the early stages of infection. And some of the characteristics of its fatal effect in animals are hypoxic insult.

[Slide.]

The edema toxin is known to be an adenylate cyclase, converting ATP to cyclic AMP intracellularly. This has also been demonstrated to have some level of cytokine modulation, and the gross characteristic effects are the edema of the infection, characteristic in its diagnosis.

[Slide.]

Again, we saw this morning from Steve the mode of action--or the accepted mode of action from the toxin: as the protective antigen binds the cell receptor, gets cleaved by surface proteases such as furin; the 20-kilo-doltan fragment is lost, leaving this 63-kilo-doltan monomer which then heptamerizes.

This heptamer then complexes with edema factor or lethal factor to form a complex which is

internalized through septa-mediated endocytosis. And after acidification of the endosome, is translocated into the cytosol where the two different toxin enzymes exert their different effects.

[Slide.]

All three of the toxin proteins have been purified and crystalized. And we know that they have different demand structures--and these become relevant in terms of developing therapies, particularly for protective antigen, which undergrows this conformational shift and change when it forms the heptamer, exposing new sites, and perhaps hiding earlier epitopes.

Lethal factor also--which I'll focus on very briefly--crystalized and became structurally eluded. We see here the ainc atoms buried in the catalytic domain, in Domain 4. And the relevance of these structures, and our understanding of these structures and the conformational changes during intoxication, indicate that there are multiple sites of intervention for developing therapeutics;

be they blocking interaction with the receptor, blocking cleavage by the activating proteases such as furin; blocking heptamerization; complex formation; internalization and translocation; as well as the individual enzymatic activities of the proteins themselves.

[Slide.]

A very brief review of what's in the literature shows that the small-molecule inhibitors fall into three categories: inhibitors of edema factor themselves, as measured by reduction of adenylate cyclase activity, either intracellular or extracellular. And here we have just two relevant publications from Soleman and Shen.

The second group would be inhibitors of lethal factor, focusing on its endoprotease activity. And we have three representative literature citations here. These fall into aromatic pharmacophores, peptide inhibitors, and also polyphenol catechin.

The third group is innovation of protein interaction. And here I've put the furin

inhibitors, such as Hexa-D-arginine--which Steve Leppla referred to this morning; but also complex inhibition, such as the polyvalent protein decoys and dominant negative mutants which have been developed in John Collier's lab.

[Slide.]

in terms of immune products, these focus on ploy clonal or monoclonal antibodies--and these are, again, taken from the literature. In the late '90s Steve Little, et al., developed monoclonals from anti-AVA vaccinated mice, and also PA-specific and LF-specific monoclonals.

There are monoclonal anti-AVA, focusing on protective antigen. And, of course, monoclonals raised against recombinant proteins. And these all are featured prominently in the literature in the last few years.

[Slide.]

Steve Leppla also related to Tony Fauci's comment that we can't address or invest in every immune product that's out there. So at the outset of this year, with the CDC, we were mandated by HHS

to find out what was out there in terms of what candidates in immune-product development are available; what might be their stage of development and their availability for product development; and to do some sort of initial evaluation, using a uniform technology platform that would allow us to formulate a procurement strategy for later this year or next year.

[Slide.]

At the end of last year we put out a request for information, requesting responses by February of this year, in which we proposed to undertake an in vitro analysis of some of these products, using anti-PA analyses, binding assays, but, more importantly, the lethal toxin neutralization assay, which I'll focus on for the rest of this presentation.

This neutralization assay is a functional assay. It's essentially species-independent, and we intend to have this preliminary evaluation of responses to the RFI completed by the end of July this year.

[Slide.]

The features of the CDC assays that make it attractive in this context are that we have generated, as part of the anthrax vaccine research programs in the NIH RPA clinical trials, a series of qualified reference standards and reagents. We've also developed standardized technologies for these trials. And if we focus on the neutralization assay, which is lethal toxin-specific, containing both protective antigen lethal factor, we know that this assay is not species or molecule-dependent. We have modeled the response curves using the four parameter logistic log model. And this combination of science and mathematics allows us to generate calculatable endpoints with high precision and with high accuracy.

In some instances, where appropriate, we continue to use the ELISA--for example, for comparing polyclonal or monoclonal antibody products--human antibody products--to anthrax immunoglobulin which is being developed by CDC and

HHS as an emergency response measure.

[Slide.]

Using this assay, the sort of reportable values that we generate are effective concentrations, giving 50 percent neutralization, and the different ranges around this bioassay curve, such as concentrations giving 90, 95 or 99 percent neutralization. And at the low end of the curve, concentrations giving 1, 5 or 10 percent. And hopefully this will become clearer as I go through the presentation.

[Slide.]

Well, let me start first by briefly describing the way the assay works. We have a fixed concentration to protect lethal factor. The lethal factor here is in a stochiametric excess. We have a fixed concentration of cells per well in the bioassay plate, and we present varied dilutions of the test material. We record reporter signal as a surrogate measure of viability against dilution of the product in the plate.

[Slide.]

At the upper end of the curve we have our positive neutralization. Control. And, again, a zero neutralization control or 100 percent effective killing.

We then model our standards and our products, using a four-parameter fit sigmoidal curve to transform data. And this four-parameter model allows us to measure or determine the upper asymptotes of this curve, the lower asymptote, and the inflection point, as well as the gradient of this curve.

The inflection point of the four-parameter logistic log model we refer to as the 50 percent neutralization, or ED 50--effective dilution giving 50 percent protection in the cells.

[Slide.]

Because we use the four-parameter logistic log model, and developments thereof, we also can pick specifically, and with precision, different points in this curve which give us different measures, which we refer to as the quantitation titer and the threshold titer.

[Slide.]

These calculable or reportable value from these slopes are generated from a mathematical interpretation of the four-parameter curve; the first and second derivative. The first derivative measures the slope and the changes in the slope with that original function of the bioassay data. The second derivative measures the rate of change of slope in the original function.

[Slide.]

This is shown graphically here, where we have the bioassay curve with this four-parameter logistic log curve fit. And here we have the plot of the first derivative. At these intersections we have the threshold titer. And this is the first point in the curve which, after empirical evaluation, is shown to be statistically significantly above background--or the lower s-asymptote.

[Slide.]

Looking at the second derivative, based from the first derivative--so this is totally

data-driven--we have the minimum and the maximum points of the second derivative which define a linear or usable portion of this four-parameter logistic curve. And the lower of these we refer to as the quantitation titer. So this has got a higher level of robustness, mathematically, than the threshold titer, but it has lower sensitivity. That's why we opt to use both of them.

[Slide.]

So, taken together, then, we have these three reportable values from the neutralization assay: the ED 50, which is the inflection point of the four-p l fit; the threshold titer, which is the lowest point on that curve we report with acceptable precision; and the quantitation titer, which defines the usable portion of that curve.

[Slide.]

So if we have the standard curve fitted to each plate, and the test curve, giving a sigmoidal curve, things are fine and dandy. We can report either out our ED 50, and the CT and TT at the lower points of the curve.

More significant for serological responses or vaccine responses for which this assay was developed: the model fit also allows us to evaluate low but reactive sera responses or product responses by back-modeling but constraining to the positive controls. So we can develop theoretical ED 50 should the need arise.These together with the threshold titres are the reportable values for our products.

[Slide.]

The benefits of using QT, TT and the four-parameter logistic model fit are that it gives us higher precision of reproducibility than other available methods. The method itself has broad application to other assays; not just the TNA, but ELISA, for example.

And currently it's being developed in SAS. We call it the Taylor Method, after the statistician at CDC who's developing it. The four-PL model, together with the high through-put analysis that this system provides gives us the opportunity to apply consistent and objective QC

criteria to anything that we evaluate in this system.

[Slide.]

We also apply these assays and comparisons to a rigorous QC criteria. The standard curve provides this QC parameter, together with positive neutralization and negative neutralization controls. But the standard itself must return within an expected range and allowing a bracket of two standard deviations.

There must be a good relative fit of the standard stated to the model. We must have a good distribution of the data points across that model. We must have sufficient depth of curve, with a maximum OD and a minimum OD which are acceptable, showing good viability and good cell density.

And we also have low variability in the standards data, as well.

The negative serum control and the positive neutralization control define the upper and lower limits of the assay, indicating that it has succeeded.

So with these QC criteria, as well as the mathematical interpretation, this is the system which we are evaluating current responses to the RFI of February.

[Slide.]

If you look at the way this assay performs in terms of AVR414, which is our human standard reference serum, here we have a small subset of 96 plates. We see that the precision is high, with 7.7 percent CD. Intermediate precision of the assay is also acceptable and is good. We have four here: three test samples and the reference standard itself, all the CDs returning under 30 percent.

The mean goodness of fit of the data to the standards model is high. And the ED 50--the inflection point of this model for the AVA414 standards curve, is robust and reproducible--high precision here, as well.

The assay is sensitive--the assay system is sensitive, with a lower read on the standards curve of 41 nanograms per ml, and a quantification,

which is the range between the QT readings on the second derivative of .07 to .3 for this particular standard serum.

[Slide.]

We've evaluated this assay in a variety of species, including monoclonal antibodies--Murang developed at CDC. Polyclonal antivaccine antibodies in different species. And what we found is the performance characteristics of macaque, mice and rabbits on monoclonals are very, very similar.

[Slide.]

And this is illustrated here, just by giving the basic sigmoid curves from these three standard reagents in the same assay. The data points and error bars are left off for clarity.

But the point here is that the different species generate similar curves--different slopes in some instances, but essentially upper and lower asymptotes and inflection points characteristic of the human response.

[Slide.]

In this assay we know also that there is a

good correlation between the neutralization efficacy of serum antibody and the ELISA quantification of polyclonal serum antibody in both humans and also in macaques, who have a high correlation coefficient of .84.

[Slide.]

So when we put this assay to looking at immunotherapeutic testing, we actually flip the curve around, because we are no longer interested in just dilutions of product or serum, we now want to address concentrations. So we use the same parameters, the same set-up, the same mathematical evolution and development, but now we flip it around so that we convert dilutions to concentrations.

And this is what a typical standard response looks like. We have our sigmoidal curve with the four-parameter logistic log fit. We have our inflection point of the model which gives us 50 percent neutralization OF THE EC50. And we also report EC 1, 5, 95 and 99. And this becomes important where we have curves which are not

parallel.

[Slide.]

This is data generated--this is actual output from our SAS algorithm--again, based around 414--when the data has been transformed. We see a nice sigmoidal fit. And here we have the reportables at this point on our dilution scale of 1 percent, 5 percent, 50 percent neutralization--95 and 99 percent neutralization.

The untransformed data curve looks like this, and at this point when we map it from dilution back to concentration.

So for this particular polyclonal of human vaccine E serum, we see the EC50s here are 167 nanograms per mil. This is from a small subset of the recent evaluation data.

[Slide.]

So of outputs that we generate for each product, including the AVR414, the ED50 dilutional, QT and TT dilutional, and then the concentration values.

And here we can see that for each of these

reportable values, we have high precision, as reflected in low CV values here. And, as expected, the EC50--the inflection point of the curve--has the highest precision, manifest in the lower CV, and as we go down to the lower parts of the curve, the precision is lower, but still acceptable.

[Slide.]

Other outputs that we capture from this curve are the asymptotes and the slope around the inflection point. And there's a change here from the slide as to what is in the notes. There was a typo on the first copy.

[Slide.]

The reason for capturing all of these --as I alluded to--is that some curves will not be parallel. This assay does not address parallelism, but we want to be able to capture--for example, here--the standard; a nice curve which will report an ED50 or EC50 of a particular value.

But another product, or another serum, could have a similar or identical EC50, but very different slope characteristics. So, at this point

in the preliminary evaluation, it's important to capture the range from 1 to 99, or 5 to 95, as well as the EC50 and the slope of that curve.

We would expect that the upper and lower asymptotes of the curves are agreeable to similar.

[Slide.]

Here we can see shifts to the right for lower potency molecules, and to the left for higher potency molecules.

[Slide.]

We do recognize that at this stage in the assays, based on the rationale for which they were developed that there are limitations. The ELISA, for example, which we use extensively to analyze human response to vaccines and infection is restricted to human antibodies with Xc components; and that automatically excludes non-human antibodies, fabs, single-chain fraction variables, mimetics, and other small molecule inhibitors. So there are major limitations to using straightforward binding assays.

The neutralization assay, however, is our

broad spectrum application at this time. But it, too, has limitations.

Currently, as designed, it emphasizes the contribution of the PA83 molecule. And it is also possibly limited to PA and lethal factor or receptor-binding therapeutics, but that has to be countered with the fact that it can still be used in those arenas.

And we're currently contemplating modifications to broaden the scope of this assay so that it has more emphasis on anti-LF immuno products. We will be able to differentiate between pre- and post-receptor binding events. We'll be able to develop it for analysis of conformational-dependent events; and also for evaluation of small molecules. This is something we have not yet started in our labs at CDC.

[Slide.]

So, to conclude, then, our focus in immunsup product therapy, immune therapeutic product evaluation, is on the neutralization assay, which we have demonstrated to be accurate, precise

and robust.

We have a panel of standardized reagents and technologies which allow us a high level of quality control of the assay. And it has a flexible application in that it's species-independent and also quantifiable.

We do recognize the limitations currently, in that it primarily has a PA emphasis, but it can be optimized--and it is optimized--for antibodies other than small molecules.

An important next step in developing this, as well as broadening its scope of interpretation, is to relate what we see in vivo to what this assay tells us in vitro.

So, with that very brief overview of our work at CDC, I'll hand over to the next speaker.

[Applause.]

DR. FRUCHT: Our next speaker is Dr. Jennie Riemenschneider. She's a biologist in the Office of Blood Research and Review at CBER, FDA. She received her Ph.D. in molecular virology from Case Western Reserve University. Later, she was an

NRC fellow at the U.S. Army Medical Research Institute of Infectious Diseases where she worked on ebola and anthrax.

She joined the FDA in 2002, and has published work on sheep-derived anthrax antitoxins. She's currently studying a therapeutic role for both bovine-derived and human antitoxins.

Dr. Riemenschneider.

Development of Polyclonal Immunoglobulin Products

DR. RIEMENSCHNEIDER: Good morning. Today I have the pleasure of speaking to you about the unique and challenging issues that surround the manufacturing and testing of polyclonal antibody products.

As natural proteins of the immune system, antibodies make ideal drugs. And because of the inherent multi-valency of polyclonals, they have a unique place in the arsenal for the treatment of infectious diseases.

The Office of Blood at CBER has a unique perspective on these products, both from the regulatory and research points of view. As you

know, we are responsible for the regulation aspects of such products but, in addition, we also have active research laboratories studying polyclonal antibodies, including those agents of interest to counter-terrorism, such as vaccinia virus and anthrax.

[Slide.]

Immune globulins may very well be the first historically used plasma product, with Behring's work on diphtheria antitoxin, which is now over a century old. In 1893 he demonstrated that it was possible to treat diphtheria infection with serum. After additional research, Behring realized that antitoxin characteristic of blood was not found in the blood cells but in the cell-free serum. With his important discovery, Behring laid the foundations of modern immunology.

[Slide.]

Immune globulins can be used for a variety of different conditions. First, they can be used in the prevention of a variety of bacterial and viral diseases, and this is especially critical in

immune-deficient people.

Immune globulins are also critical treatment to those who have been exposed to certain pathogens, and this is the setting in which polyclonal Immune globulins are most likely to be used for counter-terrorism.

In addition, immune globulins have been used to prevent newborn hemolytic diseases, and also an immune modulation for patients with ITP.

Another critical indication for immune globulins is the role of antitoxins, which is especially important for this discussion. In this setting, polyclonal antibodies have been a critical treatment for diphtheria, as I mentioned, and also botulism and snake and spider invenomation.

Blood plasma contains a mixture of hundreds of different kinds of proteins, only a few of which are of therapeutic interest. To make plasma-derivative products, plasma can be treated in a variety of ways to separate the desirable products--in this case, immune globulin--from others. I'll go into a bit more detail about the

manufacturing in a few minutes, but I do want to point out early that the process of obtaining antibodies from plasma is very complex, and a variety of different methods can be used.

The economy of scale of manufacturing immune globulins and the need for a wide spectrum of specificities requires a large donor pool--typically, at least a thousand donors. However, for the manufacture of hyperimmune globulins this number may be less, mainly due to the availability of appropriate donors.

And because they're biological products derived from both humans and animals, there is a safety issue related to the transmission of viruses and other pathogens. And, in addition, adverse reactions can be encountered.

So, as I mentioned, there's different sources for polyclonal immune globulins, human and animals. And they both have their own set of issues that need to be addressed.

[Slide.]

For human-derived immune globulins, we

must be acutely aware of the potential to transmit diseases. In addition, there's the possibility of unwanted antibodies, such as anti-D or isoagglutinins.

A concern that is extremely relevant for counter-terrorism-related immune globulin is the fact that there may not be a large donor pool, or population for the collection of plasmas manufacture for the desired product. Certain issues need to be considered, such as who and when can be plasma-pheresed to obtain the desired hyper immune plasma? And will new individuals need to be immunized or re-stimulated in order to collect sufficient amounts of plasma?

Animals are the other source of plasma for immune globulin production, however there is a risk that the immune globulins themselves from animals may cause adverse events due to their cross-species immunogenicity. One way to address this is to remove the immunogenic region of the antibody--the Fc, with a method called "despeciation." Residual animal proteins can also be a potential source of

undesirable immune reactions, and because of this it is sometimes necessary to test for hypersensitivity and perform desensitization procedures prior to treatment.

And as with the human product, there is potential to transmit infectious agents--in this case, zoonotic agents, such as West Nile virus or rabies--although, to date, neither of these have occurred with plasma derivatives.

[Slide.]

So, because polyclonal immune products have been used for several decades, we have extensive experience with these types of products. And this experience even extends to bioterrorism countermeasures, such as vaccinia immune globulin derived from human, and botulism antitoxin derived from horses.

Because these products were licensed many years ago, modern efficacy studies were not performed, and licensure was based on literature and small case studies. And so, as we know, requirements for licensure have changed and will be

a major topic of discussion in today's workshop.

[Slide.]

So, first, I'd like to focus on the human-derived immune globulins. And as I mentioned already, they're already used for variety of indications. The bulk of these are in a prophylactic setting. This includes the use of IGIV in primary and secondary immune-deficient patients, and also in the prevention of diseases such as hep B, tetanus, CMV and RSV.

Human immune globulins can also be used for treatment, although this is a less common occurrence. Examples of this are in the treatment of infant botulism and tetanus. However, for the purposes of today's discussion, this is an important indication, since this is where hyperimmunes are likely to be used in the treatment of anthrax.

I'd also like to point out that immune globulins can be administered by IM or IV injection, and they're referred to as IG or IGIF, respectively.

And, also, there are already currently a variety of specific or hyperimmune products, several of wich are listed here.

[Slide.]

Now, obviously, human plasmas--the starting material for human immune globulins--and there's two classes of plasma recovered in source.

Recovered plasma is made by separating plasma in a donation of whole blood from other components, where source plasma involves the process of removing whole blood and separating red blood cells from plasma. The red cells are then returned to the donor and the plasma is retained for use and further manufacture.

For the manufacture of hyperimmune products, source plasma is often used. Plasma is often collected from those who have antibodies as a result of a prior immunization, such as with tetanus, or those who have antibody from earlier infection, such as with CMV or RSV, and in the case of Rh antibodies from Rh-negative women who have been exposed to Rh-positive pregnancies.

Source material can also be obtained from vaccinees in active immunization programs. And some examples include people vaccinated against rabies for the manufacture of RIG; men vaccinated with the Rh antigen to make anti-Rho-D immune globulin; HPV vaccinated people to make HVIG; and, of special interest is the SIP--or special immunization programs--for laboratory workers who have been a source to general vaccinia immune globulin--although I should point out for this product volunteers and military recruits were also a source of plasma. In fact, military donors are of utmost importance for the generation of polyclonal immune globulins that will be used in a counter-terrorism setting, since they are often immunized prior to active duty in endemic and/or high-risk areas.

[Slide.]

Before blood or plasma collection, there are several general issues that need to be addressed with a donor screening questionnaire. And the examples I've shown here were taken from

the new Uniform Donor Screening Questionnaire that was recently published on the FDA website. And the link is shown on the bottom of the slide.

And I do want to point out that I'm only showing a few examples of the type of questions that are asked of a donor. And these fall into several general categories, such as general health-related--"How are you feeling today?" on the day of donation; those questions related to viral risk, such as "Have you had a tattoo or piercing within the last year?"; and those more geographically related, such as "Time spent in the UK," which is related to TSE exposure.

[Slide.]

Now, for the collection of plasma for counter-terrorism products there are additional concerns that often arise in donors who are in the military or involved in special immunization programs. One of the biggest questions is whether or not the donors have been immunized with live vaccines to stimulate specific immunity, and whether there is potential for viremia at the time

of plasma donation for the manufacture of the product.

Also, we need to be aware of whether IND vaccines--either the vaccine given to elicit the desired immune globulin, or other INDs that may be administered around the time of plasma collection, and what is the donor deferral period for that particular vaccine? And then FDA assesses these situations on a case-by-case basis.

[Slide.]

In addition, the blood and plasma needs to be tested, and there are a set of specific requirements in the 29 CFR 610.40, which indicates testing must be done for HIV1, 2, HBV, HCV, HTLV1 and 2, and also syphilis. In addition to these agents, the agency may also recommend blood and blood components to be testing for additional agents, depending on the source of the materia; for example, West Nile virus.

[Slide.]

So, as I mentioned earlier, the manufacture

of immune globulins is a very complex process. And the story of IGIV really starts with the work of Dr. Cohn and his colleagues at Harvard in the early 1940s. The group developed numerous fractionation methods for the large-scale separation of plasma into its components. And the method that was favored involved the use of cold alcohol, sub-zero temperatures as a protein precipitant. John Oncley in Cohn's lab furthered this method to isolate IgG out of the plasma fraction 2+3.

Cohn's method is shown here. And rather than to delve into the specifics of the steps, I just want to highlight the complexity of the manufacturing process. And the arm that's shown in yellow is the part of the process that results in the fractionation of immune globulins, and that side of the process alone is quite complex, and also has many variations.

[Slide.]

Now, to emphasize this complexity even more, the Cohn-Oncley method is just one way to

isolate and purify immune globulins. The Kistler-Nischmann method is a modified version of Cohn's scheme--shown on the blue side--and was developed in the '60s as a simplified method to Cohn's method, which has fewer steps, but still maintains the basic ethanol precipitation chemistry.

And, also, column chromatography method, including ion exchange, gel filtration and affinity methods--in the absence of alcohol--are also used to isolate immune globulins.

[Slide.]

So, one of the most important parts, when you're dealing with human plasma, is to ensure that viral inactivation steps are done. There are several methods in the isolation process itself that help to partition viruses. And these include fractionation, precipitation steps, and column chromatography. There are also intentional viral clearance steps which are employed, which involve solvent detergent treatment, caprylate, heat treatment, nanofiltration, and treatment with low

pH.

Now, typically, FDA would recommend two orthogonal steps to clear each type of virus--and by "type of virus" I mean those that are enveloped versus non-enveloped, sensitive or resistant to different types of methods.

Validation studies are done and must show clearance of the actual virus when possible. However, when that's not possible, model viruses can be used.

[Slide.]

As I mentioned, adverse events can occur with these products. And this slide shows a list of both common and uncommon--and sometimes rare--adverse reactions that have been associated with immune globulins. And I just want to point out that the most common are mild to moderate headache, fatigue, chills, backache, nausea, low-grade fever.

And one thing that's interesting is some of these adverse reactions, over time, have been linked to certain measurable characteristics of the

products. And certain anaphylactoid reactions have happened, and have been associated with the presence of aggregated immune globulin, because this can cause an increase in Complement activation which is measured by a test, ACA, a typical lot-release test.

In addition, hypotensive reactions have occurred, and this has been linked, in some cases, to the presence of pre-kalochrine activator--or PKA-- and kalochrine, which are components of the contact activation system. So, PKA is also a common lot-release test that's performed on these products.

Now, there's CFR-required lot-release testing for human immune globulins, and these can be found in the 610s listed here. And I'm not going to go through them, but do want to point out that under 610.100, Subpart J, there are specific requirements for human immune globulin that are indicated, such as source material, heat stability and so on.

[Slide.]

So, in addition to the CFR-required lot-release testing, we often request other parameters to be measured, including tests for molecular distribution of the product into its fragments, monomers, dimers and aggregates; potency, if it's a hyperimmune, and the other tests shown here.

[Slide.]

For immune globulin, stability testing is performed to ensure integrity, safety and potency throughout the dating period of the product. An example--examples of typical testing parameters for that stability protocol are shown here, as is a typical testing schedule.

[Slide.]

So, as I mentioned at the beginning of my talk, humans are just one source of immune globulin, and animal species are also used to generate these types of products, with the most common today being horses, sheep and goats.

[Slide.]

So the considerations with animal-derived

immune globulins are somewhat different that those with humans. And, as mentioned, immunogenicity issues can arise because of the immune globulins themselves, or trace impurities that are animal proteins found in the final product.

Because of this, there is sometimes a need to test patients for hypersensitivity, as I mentioned, and perform desensitization procedures.

I'll talk in a minute about despeciation, and also like to point out again that zoonotic infections agents are of concern.

[Slide.]

And I put a few slides related to animal husbandry up here just because this is also an important part when you're dealing with animals for the source of immune globulins. And I just wanted to point out a few things. These are all located in the CFR 611 section.

For the laboratory and bleeding rooms for animals, they must be kept free of flies and vermin; and for animal quarters and stables, the same. Food storage area shall be of appropriate

construction, fly-proofed, adequately lighted and ventilated, and maintained in a clean, vermin-free and sanitary condition.

[Slide.]

The care of the animals need to be addressed. Again, the animal quarters and cages shall be kept sanitary, inspected daily; and competent veterinary care needs to be provided as needed.

There's a quarantine period for animals. Animals shall not be used in processing until they are kept under the appropriate quarantine period of time--here it states at least seven days. And horses and other animals susceptible to tetanus need to be immunized.

[Slide.]

Because these animals are going to be immunized against the antigen of choice so that you can develop your product, there's a statement that indicates toxins or other non-viable antigens administered in the immunization of animals shall be sterile, and viable antigens, when used, shall

be free of contaminants.

There's issues about blood withdrawals. And the CFR indicates that blood shall not be used if it was drawn within five days of injecting an animal with viable microorganisms. And the blood intended for use as a source for biological product needs to be collected in a clean, sterile vessel. And if it's intended for use as an injectable, it needs to be pyrogen free.

In addition, CBER needs to be notified if there are certain diseases that are suspected or determined to exist, such as foot and mouth disease and Glanders, and the others listed here.

[Slide.]

So, as I've mentioned a couple of times now, despeciation is one method that can be used to make the animal-derived product less immunogenic. And so what I'm showing here is digesting with pepsin, where you take an intact immune globulin and the result is an Fc fragment and an Fab prime 2.

And so what you'd want to do during manufacturing

is isolate this fraction of the molecule and remove these from the final product.

In addition, you could also digest with papain, and the result is 2 Fab molecules.

And when optimizing these procedures, the most important things to consider are time, temperature and the amount of enzyme in the process.

[Slide.]

So, with animal-derived immune globulins there's often been the need to test for hypersensitivity using a skin test, and looking for a weal and flare reaction. It's often also--or, not "often" but could also be necessary to do desensitization. And we have required this type of testing for animal-derived products in the past.

[Slide.]

So, standard lot-release testing for animal-derived immune globulins is shown here. It's not identical, but similar, to that for humans.

[Slide.]

And, again, stability testing is performed to ensure the safety, integrity and potency throughout the dating period.

[Slide.]

So, as we know, there's potential hurdles in the licensure, not just polyclonals, but all of these products for counter-terrorism. And efficacy studies cannot be performed in the absence of illness or prior to a bioterrorism event. So, alternative strategies need to be employed, which typically include Phase 4 study commitments.

Clinical safety studies with hyperimmune globulin can be performed, and are typically done in normal volunteers, which allows for common adverse events to be identified, and PK profiles to be investigated.

[Slide.]

So, because of the unique nature of CT products, current licensure strategies employ mechanisms such as the Animal Rule--which will be discussed later today--or Accelerated Approval designation, which is found in 21 CFR 601.40

through 46.

In these cases, licensure is based on surrogate markers for efficacy, but also comes with the need for Phase 4 study commitments to validate the surrogate marker.

[Slide.]

So, just as a quick summary, I'd like to conclude by saying polyclonal antibodies have the advantage of having multiple specificities against the particular antigen--anthrax, in this case. And a large amount of plasma for the manufacture of immune globulins can be easily obtained, and there are multiple sources, both human and animal.

Plasma fractionation is a well-studied process; has been employed for decades. However, it's important to keep in mind that transmissible agents are of utmost concern, and the manufacturing process must ensure that the viral inactivation steps are effective.

[Slide.]

So, I also just want to say thanks to the folks in the lab of Plasma Derivatives in the

Division of Hematology: Doug Frazier, Dot Scott, and Dov Golding.

Thanks.

[Applause.]

DR. FRUCHT: We're going to take a break now and reconvene at 10:15.

[Off the record.]

DR. FRUCHT: I should just mention that Dr. Collier's slides aren't available today, but they will be available on the WEB.

Also, if folks are looking for index cards, there will be extra index cards in the front, if you have written questions.

Well, Dr. Collier really needs no introduction, especially to a group like this. But I thought I'd highlight a few of his many accomplishments.

He's a pioneer in the field of diphtheria toxin research, an authored numerous landmark publications in this field. Fortunately for our field, he expanded his research investigations to other bacterial species, including bacillus

anthracis. In large part due to the accomplishments of his laboratory, we now have a much better understanding of the three-dimensional interactions of anthrax toxin with its target molecules in the cell. And this is the basis for developing potential therapeutics.

Among his many other honors, Dr. Collier is a member of the National Academy of Sciences. He is currently the Maude and Lillian Pressley Professor of Microbiology and Molecular Genetics at Harvard Medical School.

Dr. Collier.

Novel Inhibitors of Anthrax Toxin

DR. COLLIER: Thank you very much, David. It's really a pleasure to be here, and it's an honor to be invited to participate in this meeting.

I thought what I would do this morning is to expand on a couple of the inhibitors that Steve Leppla mentioned in his opening talk--inhibitors of the anthrax toxin that we've been involved with.

So--let's see here.

[Slide.]

So, just to remind you of the current model of the way the anthrax toxin components interact, assemble into the cell surface--assemble into toxic complexes at the cell surface--I won't go through this in detail, but I will tell you that the two types of inhibitors I'm going to focus on are, first of all, the dominant negative inhibitor, which is a mutant form of the protective antigen that acts to block the conversion of the heptameric pre-pore that's assembled from PA63 of the cell surface in the pore stage. And I'll detail that as we go along.

And the second type of inhibitor is a soluble form of one of the two receptors that Steve Leppla mentioned: the CMG2 soluble form created by genetically truncating the molecule and eliminating the transmembrane component of that part of that receptor, giving you a soluble form that can bind to PA.

[Slide.]

So, the concept of the dominant negative inhibitor is detailed here further. The idea is if

you have a form of PA that itself is unable to go to undergo the conversion from the pre-pore to the pore stage, you can envision subsets of mutants of that class that might be dominantly negative, in the sense that they would co-assemble with wild-type PA during the normal assembly process at the cell surface. And then getting down all the way here to the step of conversion of the pre-pore to the pore would dominantly inhibit the ability of the wild-type parts of that heptamer to undergo that conversion, and therefore would block the entry of both EF and LF into the cytosol, and therefore block all toxicity.

[Slide.]

So, the basic mechanism of pore formation--the current model is illustrated here. The pore-forming domain of PA is Domain 2, and I--sorry--I think a slide got left out here--Domain 2, that was, in any case, shown earlier, I think, in Steve's talk.

And during the crystallographic determination of the structure of PA, it was found

that there was a loop region up about halfway along the height of the Domain 2 that, first of all, was not seen in the heptameric structure--nor the monomer, for that matter. The loop seemed to have properties that suggested it might be able to form an amptipathic beta barrel that would span the membrane in this fashion--similar to what had been found by Eric Groh with the staphylococcal alpha toxin.

[Slide.]

And so this loop--according to the model, then, this loop region, in order to form the transmembrane beta barrel would have to be relocated down to the base of the heptamer, and that would imply a major conformational change in the pore-forming domain--Domain 2. And the way that is envisioned to happen is that Domain 2 is built in the form of a Greek key motif--as illustrated here--and one can imagine, then, that if you stripped out these two flanking beta strands--2-beta-2 and 2-beta-3--from the Domain 2, that would allow the loop to be relocated down in

this fashion.

[Slide.]

Now, one of the manifestations of the conversion of the pre-pore to the pore is illustrated here. In the pre-pore form, if one exposes the structure to the denaturing detergent SDS, the subunits will fall apart into--or the heptamer will fall apart into individual subunits. After pore formation occurs, the structure is resistant to SDS, and hence on SDS polyacrylamite gels one sees a very high molecular weight olicamer here formed that we believe corresponds to the pore form.

[Slide.]

In the course of studies a few years back, a post-doctoral fellow in the lab, Bret Sellman, was performing directed mutagenesis over here on the opposite side of Domain 2, from the pore-forming loop, in these loops here--and came across some sites which mutation absolutely blocked the activity of PA; and specifically blocked its ability to convert from the pre-pore to the pore

form. Three of these sites are shown here: this lysing 397, and this loop--aspartic acid 425, and phenylalanine 427 in this loop here.

It turned out that these two mutations--either of them--was dominantly negative.

[Slide.]

The K397 mutations--mutations of K397--blocked pore formation, but the mutations themselves were not dominant.

We've made several combinations of these--of mutations at these sites, and settled on, early on, a combination of D427K and K397D as being a double mutant that had very high dominant negative activity. And that's sort of become the working standard that we've carried forward.

[Slide.]

Along the lines of--in the theme of in vitro assays of this session, I just wanted to mention that sort of our standard bread-and-butter assay that we use is based upon--not upon using either EF or LF per se as the effector molecule but, rather, what we do, based in part on work that

was done in Steve Leppla's lab as well, is to take the N terminal domain of LF--and that we call "LFN." Catalytic machinery is "C terminal." We eliminate the catalytic machinery and replace it by the catalytic domain of diphtheria toxin. And, as most of you know, the catalytic domain of diphtheria toxin blocks protein synthesis in, essentially, every cell that it gets into but 80 pure eboscylating EF2. And it gives us a very nice effector molecule--at least nice for investigative purposes in the laboratory. We call that LFNDTA--diphtheria toxin A, chain-linked to LFN. And we look for the inhibition of protein synthesis as our standard bread-and-butter laboratory assay for translocation.

[Slide.]

So here is--this slide illustrates some of the properties of the negative mutants at these sites--at two of these sites. I mentioned the K397 and D425A. So we're looking at the K397 and D425A mutations.

And we see here, in this slide, that the

binding of these mutant forms of PA to cells is unaffected by the mutations.

[Slide.]

The translocation, however, is drastically affected. And here we're looking at an assay where we assembled the complexes at the cell surface, using a radio-labeled ligand--LFN radio-labeled ligand--and then acidify the medium and look at translocation across the plasma membrane, after pyridically degrading anything that's left at the cell surface. And so you can see that that step is drastically affected here--in fact, it's essentially completely dead, these molecules are.

[Slide.]

This panel shows that the ligamerization occurs normally, fostered by LFN, to compete off PA20 from nicked PA. And the central panel here shows the effect on LFNDTA, inhibition of protein synthesis. This is wild-type PA, and these are the two mutants. So either of these two mutations, then has strong effects on translocation, specifically.

[Slide.]

This is just--again, along the lines of illustrating some of the assays that we used for cell permeabilization by PA, the conversion of the pre-pore to the pore, we load cells with radioactive rubidium, and then look at the release of that into the medium upon acidification of the medium.

[Slide.]

And here's the illustration of the assay I just descried on using a radio-labeled ligand at the cell surface, looking at translocation across the plasma membrane in response to low PA.

[Slide.]

This is an assay showing the dominant negative character of the double mutant here, in comparison with a non-cleavable PA mutant that is a much weaker inhibitor of toxin action. So what we've done here is to set up a combination of wild-type PA and LFNDTA that would inhibit protein synthesis about 90 percent, and then titrate into that mixture the double mutant, or this non-cleavable mutant.

And as you can see here, by the time you get to a one-to-one ratio of the double to the wild-type PA, you've almost completely inhibited toxin action; whereas, the non-cleavable mutant, which will compete for the receptor, and inhibit toxin in that way, it's an extremely weak inhibitor.

[Slide.]

We've gone through--scanned through--the entire PA63 molecule in collaboration with Rod Tweeten and Jimmy Ballard, looking for other sites where mutation would create a defective PA. And we found a number of sites heavily concentrated in Domain 2, and the dominant negative ones--those that we found--we found a few more of those, besides the ones in this loop that are distributed in these two beta strands here.

How do the dominant negative mutants work?

[Slide.]

Well, these sites where the residues are mutated are in that pre-pore they're solvated. They're not in contact with any other part of the

PA63. But they've obviously got to be recognized at some point. And we think that what's happening is that if we envision these sites as composing one site that may be recognized by another site in PA63, and that we can envision some sort of a rotational model here where sites 1 and 2 in the pre-pore are not in contact with each other, but in response to pH, would come in contact--perhaps site 1 of one subunit with site 2 of the next--and that a dominant negative mutation would simply interrupt that link. And this has got to be a highly concerted process, the conversion of the pre-pore to the pore. And if all seven subunits do not work in concert, the whole thing won't work.

So, that's the basis--that's our current thinking, then of the way this whole thing works.

[Slide.]

Then the original studies on the Fischer 334 rat that we did a few years back. We combined 40 mcg of PA and 8 mcg of LF and the animal became moribund in about 90 minutes. And if you add as little as 10 mcg of either the double mutant or the

F427A mutant, the animals showed no symptoms and survive indefinitely.

[Slide.]

Recently--so I've done no more in animal work beyond this, but the company that's licensed this technology--Pharmathene--has conducted a spore-challenge model recently in rabbits that has given interesting results and favorable results to the whole thing.

And these are groups of six rabbits that were injected either with a high dose or a low dose and challenged with a whopping dose of spore--some 7,000 times the LF50. And as you can see here, with the high dose, then the animals--five our of six animals survived--well--indefinitely, through day 85 here.

If there are any questions regarding these data I'll refer you to Sol Layermand, who's in the audience.

So the dominant negative inhibitors them, in summary, combine with wild-type PA and dominantly inhibit pore formation and

translocation. We think that as little as one dominant negative PA molecule can inactivate, then, up to six molecules of wild-type PA, plus up to three molecules of LF and/or EF that are bound in that complex.

Interestingly, DNA--the dominant negative PA retains immunogenicity. We haven't seen any diminution in immunogenicity in the tests that we've done so far. And so potentially, therefore, the dominant negative PA represents a combination--potentially--of a therapeutic antitoxin and a vaccine in one molecule.

[Slide.]

I'm still struck by the fact that you can take a single mutation, or double mutation, and convert a toxin subunit into a potential inhibitor of toxin action, and potentially, a vaccine. There are many ligameric pore-forming toxins that assemble at the cell surface, or outside cells. And so potentially, this approach is generalizable to some other systems.

[Slide.]

Okay, finally, in the last five minutes I want to go one and tell you about one of the cellular receptors for PA. As Steve mentioned, there are two of them known: ATR artemate, and CMG2. And these are both single-pass membrane proteins that have a von Willebrand A domain; about 60 percent identity between the two examples here. And they both have a MIDAS motif, which is a metal ion-dependent adhesion site. That turns out to be important in the interaction with PA.

[Slide.]

Recently, Borden Lacy in my laboratory has determined the crystallographic structure of the extracellular von Willebrand domain of CMG2, which is illustrated here. I won't go into detail except to tell you that the MIDAS motif is up here, and there's a magnesium atom right there that we believe interacts with an aspartic acid in Domain 4 of PA to form part of the binding affinity.

The affinity is tight. The CMG2--soluble CMG2 binds in a one-to-one ratio with monomeric PA, and a seven-to-one ratio with the heptamer. So

there appears to be no stearic inhibition to the interaction of the CMG2 with each of the monomers of PA--heptameric PA.

[Slide.]

So, this summarizes what we know about the interaction. PA binds one CMG2 von Willebrand A domain at saturation. That's an AD molecule. The pre-pore binds seven at saturation--incredibly high affinity. KD is sub-nanomolar. It's roughly 200 picomolar. Whopping high affinity. The off rate--the rate of dissociation of the complex once it's formed is also extremely slow--on the order of a day. So once this CMG2 latches onto PA, basically you've locked it up for a very long time. So therefore it's a potential inhibitor of toxin action, in fact it has been shown to be so in in vitro systems.

[Slide.]

So, finally, I just thought I would mention a few other potential approaches in inhibiting anthrax toxin action. But these, basically, have all been mentioned and discussed in

greater detail. Steve mentioned the polyvalent inhibitors that we were involved in developing, and that work is being carried forward by a former post-doc in my lab, Jeremy Mogridge. We're not working on this anymore.

One thing that's not, I think, widely known is a project that's going on in collaboration with John Young--and, by the way, the CMG2, I should have mentioned--CMG2 was cloned in John Young's lab, and the ATR also was cloned in John Young's lab; the CMG2 by Heather Scobey. And we've been working collaboratively with John Young and his people for many years now.

And as an extension of that collaboration, we're involved in an NIAID-sponsored program project that's headed by Maryann Manchester, and involving John Young and Jack Johnson and a number of other people in southern California. And the idea is to take certain viruses--a plant viruses, Kalpi mosaic virus and an insect virus, flockhouse virus--very well characterized small viruses, and substitute certain peptides or even domains on the

capsid protein--one of the capsid proteins of these viruses, and perhaps creating what we call "molecular sponges" with bioactive peptides that might, for example, attach to PA and suck it out of the serum, or various other ways that one can envision that this technology might be applied to anthrax and many other infectious diseases.

[Slide.]

And then, finally, Steve Leppla already mentioned this--the hexa-D-arginine--and there are inhibitors of LF action that have been developed by Ben Turk and Lou Cantley's lab. And others--and there are a number of others, as well, that have come out--active site inhibitors of EF and LF that Steve already mentioned. And the one that's being developed at Merck by Jeff Hermes and his colleagues is also very exciting.

[Slide.]

So, finally, I'll conclude--and these are the people that have worked on anthrax toxin in my lab over the years. And I want to highlight the efforts of Bret Sellman, who isolated the first

dominant negative mutations, and Michael Mourez, who worked with him and has done--did the polyvalent inhibitor work, also; and Borden Lacy, who's done the crystallography in my lab. A number of other people have contributed to this: Jill Milne identified the heptameric form of the PA. And then a lot of this work is essentially--almost all the work I do is collaborative, because I love to work with lots of other people. And Bob Liddington did the original crystallography on PA and also on LF--and I won't take that further.

[Slide.]

And then finally, grant support: all my work has been supported by NIAID, basically. And I'm grateful to them for this. And, finally, I'm a co-founder of the company, Pharmathene. It's developed to--it was founded to develop the dominant negative inhibitor. And in case any of you are wondering, I don't want you to think that this company was formed and in response to the anthrax attack. It was actually formed in April of 2001 to try to develop, as I said, the dominant

negative inhibitors. And that work is ongoing in the company.

Thank you very much.

[Applause.]

DR. FRUCHT: Thanks very much, Dr. Collier.

I'd like to invite the panelists to come up and take their places please.

[Pause.]

Panel Discussion

DR. FRUCHT: First, I'd like to introduce some of the folks on the panel that weren't speakers and haven't been introduced yet: Dr. Keith Webber, Director, Division of Monoclonal Antibodies, and Acting Director of the Office of Biotechnology Products, CDER, FDA; and Dr. Shukal Bala, lead microbiologists, Office of Drug Evaluation 4, CDER, FDA.

And in the meantime, if people have questions that they've written down, we're collecting them now. Or if you'd like to give your question orally, we have microphones as well.

I guess I'm going to start off the panel with a question, and I'll send it to Keith Webber.

We've talked a lot about in vitro potency assays today. What is the FDA's expectations for the characteristics of potency assays, and what would you consider to be important milestones to reach during assay qualification--regarding assay qualification and validation as the product progresses through development?

DR. WEBBER: I think that's a great question, but I would have to say it depends, to a large extent, on what the actual therapeutic product is that is going to be used.

Generally, though, I think assays are developed early on in product development--usually with some idea of what the proposed mechanism--or potential mechanism of action is for the product; how it's going to work to combat anthrax. And so that's one of the main critical elements is that your potency assay should be relevant to what your potential mechanism of action is. Oftentimes you may not know exactly what the mechanism is in

reality, but it should be relevant to that.

Oftentimes people work with a cell-based assay which may or may not be as--ahh--well-behaved, let's say, as a binding assay. There's certainly nothing wrong with having two or more assays to evaluate your product, from the different aspects. So, relevance is one of the main critical areas.

Your assay, early on, should be reliable. What does "reliable" mean? Generally, you want to have an assay that--say, beginning in pre-clinical in Phase 1, it doesn't have to be fully validated, by any means, because that takes a great deal of work. But you should have confidence in your assay that it is giving reproducible results in the hands of the people running it. It should be sensitive to the therapeutic levels of the product that you're planning to use in the clinic or in your animal studies, so that you know that if it's--that the assay isn't giving you erroneous results, in that if you're assaying at a very low--say acetaldehyde--toxin concentration, or protective

antigen concentration, that it gives you great results, but actually what you would see in vivo is completely different and would not be particularly relevant to the clinical.

As you'd get further and further into clinical development, moving to Phase 2 and Phase 3, and on toward--hopefully--licensure approval, one wants to gain a higher level of validation of that assay, and determine its robustness in the hands of multiple analysts at different laboratories if necessary; and that if there are specific reagents that you're using--particularly, for example, a cell line or another protein that is part of that assay, that you have a reliable and reproducible source of those reagents, as well.

DR. FRUCHT: There's a written question for Dr. Quinn.

DR. QUINN: Okay. This question was from Martha Wilde of Alexion. And he [sic] asks that: "Antibody concentrations may vary relative to each other when using different quantitative assays such as OD, Virad or BCI-type assays. How do we

determine quantity for purposes of comparison with different antibodies?"

Currently what we do, we take the mass values as provided by IRAD or BCA, and we would take an agreement between the different assays--in terms of total protein and purified product.

In terms of assigning functional units to this, what we're planning to do is to define and apply specific activity determinations which will relate--for example--potency in vitro and, hopefully, in vivo to those mass values.

The connection between the mass value and specific activity in vitro and in vivo provides the essential link, I think.

I believe that--does that answer the question, Martha Wilder?

[No audible response.]

DR. FRUCHT: I think there's a question from the audience here.

DR. DRESCH: Yes, Stephen Dresch, Forensic Intelligence.

I'll ask the question here, because my

handwriting is so illegible.

In any event, the question is primarily director to Dr. Leppla, but I believe Dr. Collier might respond to it, as well. I'd hoped that Dr. Leppla would also be on the panel.

Anthrax strains differ significantly in--among other dimensions--their lethality. Do we know what explains those differences? Is that the efficiency of toxin production? The composition of the toxin produced by the various strains? And what are the implications of these differences for the development of toxin-targeting therapies?

DR. COLLIER: I don't know that I can say anything really meaningful about that, except that it's probably not the composition of the toxin. The toxins--there's very little variation in PA, for example, in strains. So that's probably not the case.

The rest--I don't think I can give you a really good answer. Maybe Steve can.

Do you want to come up, Steve?

DR. QUINN: Thanks, Steve.

[Laughter.]

DR. QUINN: I would agree, John--I think Steve would agree--that from what we know about the toxin and toxin-complex formation that the differences between the virulence of these strains probably does not reside there.

I think it's safe to say--and please correct me if I'm wrong--but from the sequencing of the genomes of these various bacillus anthracis, and also bacillus cereus isolates, we know that there are a range of other potential virulence factors in the genome. And it is possible that the in vivo transcription-translation of those gene products enhances the virulence of the different strains, but right now it would be speculating to go any further than that.

DR. FRUCHT: Would you like to follow up on that, Dr. Leppla?

DR. LEPPLA: [Off mike.] [Inaudible.]

DR. FRUCHT: Great. Thanks.

There's a written question for Dr. collier.

DR. COLLIER: It doesn't--there's no indication of who wrote this question, but--"The expression of PA, LF and EF in wild-type B. anthracis is known to be regulated at the level of transcription by ATX-a. Is anything known about the upstream regions of these genes and how positive transacting regulator affects upregulation? Is anyone working on a therapeutic against anti-regulation approach?"

Umm--well, certainly people are working on the mechanism of transacting regulation. I don't know that I know the latest on this, but Theresa Kohler at the University of Texas in Houston is actively working on this sort of thing. And Michelle Mock, at the Pasteur Institute. And I have no idea whether anybody's working on a therapeutic anti-regulator approach. I would suspect so, but I don't know.

DR. FRUCHT: Jenny was asked a question as well, during the break. She just notified me. And she's going to deal with that question now.

DR. RIEMENSCHNEIDER: Hi. Dr. Donlan from

HHS asked me a pretty interesting question during that break and asked me to address it here.

And that was--just to bring up the point that there are transgenic animals that are being developed to express human antibodies--polyclonal products. And we haven't had these products in the Office of Blood in the past, and so how we're going to handle these issues that arise, it's going to be an interesting story, I think.

But just from thinking about it, I don't see that there's going to be any differences with animal husbandry that need to be addressed specifically, unless they find that these animals are susceptible to particular types of diseases that we're not used to seeing. But, in terms of the product itself, it should be purified in a very similar way to human antibodies or animal antibodies from plasma that we're used to dealing with.

The one issue that we will have to address is the fact that these antibodies may not be fully humanized, or human, and they may still be

partially of the animal of origin. So if you want to call that a "contaminant," there may be that type of contaminant in the product. And whether or not there would be an immune reaction in the patients to that, we will have to address that at the time that those products come into the office.

Thanks.

DR. FRUCHT: It seems like we have a very shy audience today--unless this gentleman is coming up to ask a question.

Yes.

DR. HERMES: Jeff Hermes, from Merck.

I just wanted to ask John in the rabbit--the nice data you showed in the rabbit protection experiment, when was the dominant negative inhibitor dosed, relative to spore challenge?

DR. COLLIER: You know, I--

DR. HERMES: How long after?

DR. COLLIER: --I'm going to--I'm sorry--defer; push all these questions off onto other people. But I'm going to ask you to talk to

Sol Langermanns, because I was warned about doing promotional things.

So, if you'd contact him about that, on the details of that study.

I was not involved in that study directly.

DR. HERMES: Okay. I'll find him.

DR. FRUCHT: I have a question--and, actually, folks in the audience could answer this, as well. I'm going to direct it towards Dr. Quinn.

The currently available assays for anthrax lethal toxins depend on the effects of the toxin on Balb-C cells. And it's not clear if the effects that we see on Balb-C cells are the same effects that occur in humans, or even other mouse strains or in humans.

Do you think we have work to do on development of more informative bioassays? And, if so, have you heard of new assays being developed that might be more predictive of their function in humans in vivo?

DR. QUINN: Actually, I think there were several questions hidden in there.

DR. FRUCHT: Sorry about that.

DR. QUINN: Is there work to do? Yes, definitely. I think the important thing to do--the important focus in on function, and linking function in whatever in vitro assay we decide to implement or validate in our laboratories with function in vivo; administered pre-challenge at the same time as challenge; and, most importantly, as the infection develops. So, yes, there is more work to be done.

The assays we use currently are based on monocyte macrophage cell lines. We know that there are many cell lines that are not sensitive to the toxin. We know that from certain species--primary microphages are not particularly sensitive to the toxin. So it really is--the current assays are really surrogate markers for protection--blood function. And, in that case, they do have direct value. But the link is between the in vitro and the in vivo.

Am I aware of other assays that are out there? Yes, we are. There's some very elegant

assays being developed that look at the adenylate cyclase internalization; adenylate cyclase function. But, again, they all focus--the assays that I'm aware of, they all focus on the key steps of receptor binding, complex formation, internalization and translocation.

DR. FRUCHT: Thank you.

I'm surprised we haven't heard any questions about immunogenicity, because that's always a topic that comes up with large molecules. So I had a question I was going to send towards Keith Webber.

What does the FDA expect regarding assay qualification and validation of immunogenicity assays as product goes through development?

DR. WEBBER: The immunogenicity is generally a concern for most biotech products--proteins. I think for products such as these, one would need to include, I think, an immunogenicity evaluation in the clinical studies. And with regard to focusing primarily on both any potential for an adverse event due to

immunogenicity, also neutralization issues--those will probably not be as big a concern, I would think, in some of these therapies perhaps because, one, for the ones that are acute treatments, you may not be as concerned about neutralization because you would want--or you would certainly hope--that your product would have its effect before a specific immune response could be mounted.

DR. FRUCHT: Thank you.

Question from the audience, on the left here?

DR. BAKER: Yes, Phil Baker, from NIAID.

Would any of you on the panel, or perhaps someone in the audience, care to comment on the differences between children and adults in their susceptibility to anthrax toxins, or to the disease in general?

DR. FRUCHT: We might have to defer to one of the clinical folks here. Would someone with clinical experience like to comment on that question?

[Pause.]

Perhaps--is Dr. Stephens available?

[Pause.]

Okay. Well, we'll try to get back to you on the answer to that question. It seems to me that that's a clinical question that could be addressed by Dr. Stephens; perhaps by someone that cared for patients could get a feel for that.

Question on the right side?

DR. FRAZIER: Doug Frazier, FDA, CBER.

Regarding anthrax antitoxin--it's kind of a stopgap measure. You, you know, put whatever your immunogen is into animals, and it's not as sophisticated as some of these other methods, but it's quick. And you can use toxins, maybe, that you wouldn't want to use in human donors and so forth.

But what has been used is a Stearns strain, which lacks the poly-D-glutamic acid capsule--so you get antitoxins--anti-PA, LF, etcetera. Does anyone have any idea: would there be any incremental additional benefit to maybe having a separate animal herd immunized with a

strain that lacks the soluble toxins, but does have the capsule? If you had some additional anti-poly-D-glutamic acid in your antitoxin, might you get additional clinical benefit? It would be more complicated, because you'd need a separate assay for that component, unless you used like a--just a lethality challenge.

But does anyone have any idea, would that be worth attempting to do--or not?

[Pause.]

If not, we could do the experiment--

DR. FRUCHT: That seems like it's more of an animal-model question, and it's going to be a separate topic coming up. And I don't know if we're qualified--I'm not qualified to answer that, and the folks that will--well, we wouldn't want to steal their thunder, anyway. They should be coming up next. We're mainly dealing with bioassay issues here, and product development.

I'm sorry, but we'll be able to answer your question in the next session--or the second section after that.

Question here from the audience?

DR. GURELIC: Yes, Ken Gurelic, from Enzybiotics.

I was intrigued by the comments about DNI, that it may be acting both directly as an antitoxin and as a vaccine. And I'm interested in what kind of evaluation would be reviewed by the FDA to support that kind of a claim structure in the development phase?

DR. FRUCHT: I'm going to send that towards Keith Webber.

DR. WEBBER: With the evaluation--I think would have to--from a potency perspective, certainly one would want to have assessments that would focus on both of those aspects of the product, if those are proposed mechanisms of action.

Within the clinical trials--I don't want to expand too much on that--but certainly one would want to evaluate both a survival, but you may want also to look at the immune response to that product, if that was considered to be part of the

mechanism of action. You would want, certainly, to consider the follow-up time of taking samples from your patients in that regard to evaluate the--but, I'm not probably the right person to address the vaccine
issues, since our office really doesn't have any of the immunogens anymore.

DR. FRUCHT: Dr. Quinn has told me that he can shed some light on one of the questions that we deferred previously.

DR. QUINN: This comes back to the question about the anti-capsule antibodies. Early work indicated that the capsule materials is not particularly antigenic, in that it didn't generate good antibody responses. Subsequent work to that indicated that the Pasteur-type strains, which are non-toxin producing but capsulating, when used as vegetative cell or spore vaccines, were not protected. But I think there--I'm not sure if those experiments demonstrated that capsule was actually produced in vivo in those animals, and whether or not they contributed to protection, or

the absence of it.

As Steve Leppla alluded to this morning, some work by John Robbins' group, and some work done in mouse models have indicated that anti-capsium antibodies can contribute to protection. There is a slight caveat there, in that the mouse model is particularly susceptible to capsular materials or bacillus anthracis variants that are capsulating and non-toxigenic. So it may be a slightly skewed model.

But I think the bottom line is that any response to the virulence factors of bacillus anthracis that can be shown to be protective does have value. There's another slight caveat that, I believe in the mid-'90s, a Japanese group showed that the capsule degradation genes produced a low molecular weight material which may actually act as an antibody decoy. But the relevance of that to infection, I don't believe is clearly elucidated.

But I think, again, the bottom line is any antibody or cellular response to a known virulence factor is worth investigating.

DR. FRUCHT: There's a question from the audience.

DR. TAYLOR: Hi, my name is Kathy Taylor. I'm at NIAID. And my question is for Jenny.

It has to do with the use of Fab-prime 2 and Fab fragments--and maybe whole IgG molecules as well. We've heard that the affinity constants for, you know, some of the things that we want to inhibit for their natural receptors is in the sub-nanomolar range, with very low off-rates.

So how does that affect what our target antibodies need to bind with? And what's known about the half-life of the antibody-antigen complexes. And I guess my concern, is if you have--you know, how do you compete with those natural receptors if you have antibody-antigen that's circulating for a long period of time, and not being cleared because there aren't any Fc receptors, and it's then being competed for by its natural receptor?

DR. RIEMENSCHNEIDER: I'm not sure I can answer your question, Kathy.

You know, with the half-life of an Fab fragment is much shorter than that of Fab-prime 2, and that's much shorter than the intact molecule itself. I get the sense that that wasn't exactly what you were alluding to in your question.

I think perhaps the best thing to do is just talk after--during lunchtime--and see if we can flesh that out.

DR. FRUCHT: There's another question from audience over here.

DR. DAIQUITZ: This is Claire Daiquitz from CDC.

I just wanted to make a comment about the--or maybe take a stab at responding to the question about kids, and how anthrax behaves in kids. In my previous life I was a pediatrician, so this is of particular concern to me as I've been working at anthrax at CDC.

There are not a lot of data. In the historical literature on anthrax disease, there are some case reports of children as young as 10 and 11 acquiring what is described as primarily cutaneous

anthrax. These are kids who were working in the mills. And there are no data on disease in children, really, below that age; below the age of children who were working at that time.

And I think the youngest case that I'm aware of is the eight-month-old baby who was part of the 2001 cohort. And that started as a cutaneous infection. He developed a lot of edema, and then was sent to the ICU for further care.

So--not a lot of data.

DR. FRUCHT: Well, Thank you very much for that information.

Another question?

DR. GURWITZ: I'm Mark Gurwitz from Vaxgen.

Polyclonal antibodies probably aren't as important to anthrax as maybe it is to some other disease. But I just wondered if someone could comment on kind of the manufacturing quality issues in cell-based production of polyclonal, monoclonals--either putting a bunch of monoclonals together, or just making polyclonals directly?

DR. FRUCHT: That would be regulated through two different centers. So I can divide the wealth here.

Keith, I guess you can talk about monoclonal cocktails?

DR. WEBBER: Yes, the monoclonal cocktail is one--I mean, there have been proposals that we've heard with, you know, either making monoclonals separately, and then combining them, or having a mixture of cells that are producing a mixture of monoclonals--essentially a polyclonal mixture, but more defined.

The former is probably easier to control, from a manufacturing standpoint, because you can mix and match your products at your will. With a cellular mixture that's producing a polyclonal mixture of monoclonals, let's say, one will need to evaluate the consistency of that production that you're getting the same mixture lot-to-lot, and you'll have to have systems in place to do that.

In either case, you're working with what would be probably considered a type of a

combination product, or mixture of products, and depending upon the products you have, there may be need to evaluate, or justify why each of those components is part of your product. But that's separate from the manufacturing issues, per se.

DR. FRUCHT: Do you have anything you'd like to add to that, Jenny--regarding polyclonals?

DR. RIEMENSCHNEIDER: Just to say that all of the polyclonals that we regulate in blood are plasma-derived. And the manufacturing, I covered in my talk. And I think what the gentleman was asking was for the comparison to the cell-based derived products.

DR. FRUCHT: A question here on the left?

DR. WOROBEC: Yes, this is Alexandra Worobec, FDA, CDER.

I just wanted to, again, address the issue of disease susceptibility in pediatric population.

Based on what I've read on the Sverdlovsk--the attack or, rather, the explosion of the anthrax spores in Russia, I think the youngest person that had succumbed to anthrax was 24 years

old. And the age was usually in the 40s and 50s. Now we don't know--I don't know offhand how many children would have been exposed.

But there was a sense that children were less susceptible. And there was also a suggestion, in animal studies, that pediatric animals are less susceptible. So that is something that is--when we design these studies--and we'll probably be talking about that in the next session--something we do take into account: the age of the animals.

DR. FRUCHT: One last question.

DR. HERMES: Jeff Hermes, Merck.

It's known that there's different species sensitivity to lethal-toxin. And this is a question for the panel, or Steve Leppla, or anybody in the room.

Is there any in vitro assay that's predictive with cells from those animals, as to the different sensitivity to lethal-toxin? In other words, if you had something like a Lewis rat that's very sensitive, or a rabbit that's very sensitive, and then you have a species that's less sensitive,

is the particular cell type involved?

DR. FRUCHT: Conrad, would you like to start off with that one?

DR. QUINN: I think I'll defer. I'll take my choice to defer. Louise, would you care to answer that? You have extensive experience with different species.

I mean, there is a question here of the relationship between sensitivity to intoxication, and sensitivity to infection. And they are different things.

DR. HERMES: Right, and--

DR. QUINN: And it's my understanding that rats, for example, are very sensitive to intoxication but less sensitive to infection.

But I think Louise is one of the animal group experts.

DR. PITT: [Off mike.] [Inaudible.]

I believe you were talking about animal models for intoxication--

DR. HERMES: Right.

DR. PITT: --versus disease. And I think

we'll talk a little bit about that this afternoon.

But in terms of the literature, the suggestion is that animals that are resistant to toxin are extremely susceptible to infection, and vice versa. And then, across different strains, within each species you get the whole sort of spectrum as well.

So, I'm not quite sure what you're exactly looking for.

DR. HERMES: Well, what I was looking for was if there's different sensitivities in vivo to toxemia; just injecting toxin. Do you have to do those experiments at the level of whole animal, or is there a predictive cellular assay with cell types from those animals that would reflect that different sensitivity?

DR. PITT: Umm--I would come from the side of saying I don't know why you would do an intoxication in a whole animal when you're trying to protect against the disease, rather than the intoxication.

DR. HERMES: Thank you.

DR. QUINN: It's generally accepted that protecting against the toxin is a major advance toward protecting against the disease, because we do not believe there is disease in the absence of toxin.

There are also indications that low levels of toxin can suppress the immune system--the initial immune response, or priming the immune system to subsequent infection.

Is there a cell assay that's predictive of species sensitivity? I don't believe so. But I think the macrophage lysis assays are certainly predictive of function of a product--its ability to inhibit or interact with the toxin. So it's predictive to some extent.

DR. FRUCHT: I'm going to go ahead and conclude the discussion panel. Thank you very much, panelists, and thank you audience members for your helpful comments.

[Applause.]

Part III - Animal Studies

DR. WOROBEC: Good morning. We're going

to moving from the in vitro now to in vivo--the hotly debated topic of animal models.

My name is Dr. Alexandra Worobec, and I'm a medical officer in O6 of CDER at the FDA. And I've been in a review of these products, really, from the very beginning. And we do have a lot of them coming down the pike.

So I think we are going to have a very interesting discussion on a number of very complicated and actually quite controversial topics.

Before we start, though, I do need to make an announcement. We're going to have to make a little change in the schedule, and we will be taking lunch at 12 noon instead of 12:25, and we will resume at 12:45 with Dr. Julie Lovchik's presentation, followed by Dr. Louise Pitt, and then Roy Barnewall, and then we'll have our panel discussion after lunch.

So, turning back to what we will be talking about today, really our goals are going to be to talk about the application of the Animal

Rule, and trying to identify the most relevant animal models for evaluating therapeutics against anthrax; and how do we address optimal study design, especially given the caveats of different classes of agents that are now being developed? And, also, perhaps touch upon safety issues that we also might need to consider in our animal studies. After al, for full licensure we do need to look at other aspects besides proof of concept.

So we'll be trying to go through all of that today.

Now, I'd like to start with our first speaker, Dr. Lewis Schrager. Dr. Schrager truly is an expert on a lot of these issues, in terms of the application of the Animal Rule to the new therapies.

He is the Lead Medical Officer in the Division of Counterterrorism within the Center for Drug Evaluation and Research. He currently oversees a research portfolio that includes support for studies of antibiotic efficacy against pneumonic plague in African green monkey model;

studies of gentamicin efficacy against naturally occurring human plague; and the development of orally available drugs against smallpox. Furthermore, he has played a leading role in developing a national system for outcomes and adverse events surveillance following a terrorist event, in close collaboration with the CDC.

Without further ado, I welcome Dr. Schrager.

The Animal Rule Applied: Pyridostigmine for Nerve Gas Exposure and Gentamicin for Plague

DR. SCHRAGER: Thank you, Alexandra.

We in the Division of Counterterrorism at CDER, FDA, have become quite familiar with the Animal Rule, not only because we consult on IND submissions and new drug applications relevant to the rule, but because we actively design and support studies of selected drugs as potential interventions against terrorist agents.

This morning I look forward to sharing with you some of the lessons we have learned.

[Slide.]

Specifically what I'd like to do is to briefly review the criteria for drug approval under the Animal Rule, and then to turn to examples of issues that arise when applying the Animal Rule to the design and conduct of studies; specifically the pyridostigmine bromide approval for Soman exposure, and our studies of gentamicin for pneumonic plague.

[Slide.]

What has colloquially become known as "the Animal Rule" or "Animal Efficacy Rule" was promulgated in these two regulations: 21 CFR 314 Subpart I--for drugs, and 21 CFR 601 Subpart H, for biologics. The final rules were published in the Federal Register on May 31, 2002.

The rule allows the reliance--allows the FDA--for the first time--to rely on adequate and well-controlled animal studies as evidence of effectiveness, without having human efficacy data. The rule only applies when studies in humans are unethical or infeasible.

[Slide.]

The rationale for the rule was to further

the development of treatments to reduce or prevent the toxicity of chemical, biological, radiological or nuclear substances. And it does not apply if efficacy evaluations are feasible under any other FDA regulation.

[Slide.]

There are four scientific criteria that are needed for approval.

First the pathophysiology of the disease in question and the mechanism of action of the drug or biologic must be well understood.

Next, the therapeutic effect must be demonstrated in more than one animal species or in one sufficiently well-characterized animal model that would be expected to react with a response predictive for that in humans.

[Slide.]

Third, the animal study endpoint must be clearly related to the desired benefit in humans; most specifically, enhancement of survival or prevention of major morbidity.

And, finally, the pharmacokinetic and

pharmacodynamic data of the product in the animal models must permit the selection of an effective dose in humans.

[Slide.]

There are additional requirements. The research needs to be performed under GLP standards. Safety data needs to be obtained from humans. And there is a need for post-approval, or Phase 4, studies.

So I'd like to turn to our first example of pyridostigmine bromide.

[Slide.]

Pyridostigmine bromide is a cholinesterase inhibitor that has previously been approved for treatment of myasthenia gravis. It was approved as a pre-exposure antidote to the nerve agent Soman in February of 2003. And, to date, it is the only product to have an indication approved under the Animal. However, the approval is limited--"for military combat use only."

[Slide.]

There are two key points that I'd like to

illustrate regarding the Animal Rule that are illustrated by the pyridostigmine bromide approval process. First, the need to understand the pathophysiology of the toxic agent, and the importance of understanding the mechanism of the drug's activity against the agent. And, second, the need for using more than one animal species in studies that are expected to react with a response predictive for humans.

[Slide.]

Now, turning our attention to the pathophysiology of the nerve agents, and the mechanism pyridostigmine bromide's action: Soman and other nerve agents disrupt functioning of the neuromuscular junction, as well as other sites of cholinergic neurotransmission. Soman creates an irreversible inhibition of acetyl cholinesterase. As a result, excess acetylcholine builds up, and results in extreme overstimulation of cholinergic receptors. This overstimulation leads to--leads rapidly, in this case of Soman--to respiratory arrest due to failure of the respiratory muscles,

excessive respiratory secretions and bronchoconstriction, and central respiratory depression.

[Slide.]

The protective mechanism of pyridostigmine bromide is to reversibly bind acetyl cholinesterase--in essence, doing the same thing that the nerve agent does, only reversibly; and, in essence, temporarily shielding the enzyme from the nerve agent. So you've got two things going on at the same time with the pyridostigmine bromide protecting the enzyme temporarily. But, realizing this, it also brings up the need to have atropine and pralidoxime--or 2-PAM--to counter the effects of Soman, and to prevent pyridostigmine bromide's potentiation of the Soman effect.

How does this work? Well, atropine blocks acetylcholine receptors--except in the skeletal muscle--and 2-PAM, in essence, works as a kind of crowbar to pry the nerve agent off the acetyl cholinesterase, unless aging or covalent binding has occurred.

Now, for those of you whose neurophysiology is as distant--and sometimes fuzzy--as mine is, I'm going to review what I just said, pictorially--thanks to slides provided by the U.S. Army Medical Research Institute of Chemical Defense.

[Slide.]

Here we have the upstream neuron impulse coming down; acetylcholine in the presynaptic membrane.

[Slide.]

Impulse releases acetylcholine. Acetylcholine transits the synapse, and you get the promulgation of the downstream impulse.

[Slide.]

Acetyl cholinesterase comes in and, basically takes care of the acetylcholine, ending the impulse.

[Slide.]

With the nerve agent, the acetyl cholinesterases are bound up. You get this huge concentration of acetylcholine in the synapse,

creating massive downstream overstimulation.

[Slide.]

What atropine does is, in this case--an illustration from smooth muscle--is to block the downstream acetylcholine receptors, limiting the effect of this huge buildup of acetylcholine in the neuromuscular junction.

[Slide.]

The crowbar effect of 2-PAM--or pralidoxime--working to remove the nerve agent from the enzyme prior to the aging of the nerve agent on the enzyme.

[Slide.]

And, finally, this slide, that serves two purposes: number one, to show the aging or binding process, after which 2-PAM cannot remove the nerve agent, and this enzyme is permanently disabled. But it also serves to reemphasize what pyridostigmine bromide does. It does this. I mean, this is PB as well as Soman. Only PB doesn't do this. PB eventually disassociates.

[Slide.]

Now, there were early difficulties in assessing pyridostigmine bromide activity in animal models. As it turned out, the early studies in small animals--that is, mice and rates--revealed that pyridostigmine's effects were small and inconsistent. As it turned out, the effects of pyridostigmine in mice and rats were masked by high blood levels of carboxylesterase in these species. Carboxylesterase serves to inactive Soman in the blood, making the animals, in essence, highly resistant naturally to the effects of Soman.

[Slide.]

And this is a chart of the interspecies differences of carboxylesterase in the different species; plasma concentrations of carboxylesterase, as well as the resultant Soman LD 50.

You can see in the rat and the mouse much higher concentrations than in these other animals--guinea pig less, and in rhesus and humans no circulating plasma carboxylesterase. As a result, the LD50 is very high in these very resistant smaller animals, but then is almost

10-fold lower in rhesus macaques.

[Slide.]

Further studies revealed that the efficacy of pyridostigmine bromide plus atropine plus 2-PAM as prophylaxis against Soman was first demonstrated consistently in guinea pigs. And the critical PB efficacy study was performed in rhesus macaques. And in these critical rhesus macaque studies, PB plus atropine plus 2-PAM was shown to increase the Soman LD_{50} more than 40-fold over untreated monkeys.

And, additionally, PB plus atropine plus 2-PAM increased Soman LD_{50} more than 25-fold over monkeys treated only with atropine and 2-PAM.

[Slide.]

So, in summary, what the PB approval process demonstrated was that a precise understanding of the pathophysiology action of Soman and of PB's activity against the agent was critical the ultimate approval of the agent, and to developing instructions for use on the PB label, including these important cautions: that is, that PB is approved for use as pre-treatment for Soman,

and the actual efficacy is dependent upon the rapid use of atropine and pralidoxime after the Soman exposure occurs. Furthermore, because of its potentiation effect, pyridostigmine bromide taken immediately prior to or at the time of Soman exposure may actually exacerbate the effects of a sub-lethal Soman dose.

Additionally, these experiments and the approval process revealed the importance--that fundamental unanticipated biological differences between species--in this case, the presence or absence of carboxylesterase--result in differential activity of pyridostigmine bromide. And it illustrates the importance of understanding the pathophysiological mechanisms, and of using multiple species in testing these agents.

[Slide.]

Now I'd like to turn to the second example: testing Gentamicin for efficacy in pneumonic plague.

[Slide.]

The key points regarding the Animal Rule

that were illustrated by the gentamicin studies for plague were the importance of understanding the pathophysiology of the disease--much as in the case of the Soman-PB experience--and, additionally, the importance of the pharmacokinetic studies and PK bridging studies between animals and humans. It also illustrated the key role played by the requirement for GLP standards.

[Slide.]

Now, turning our attention to the pathophysiology of pneumonic plague, understanding plague pathophysiology was critical to our designing the actual timing of the gentamicin intervention. The reason for this is that we were seeking the indication for gentamicin for treatment of pneumonic plague, not for pre- or post-exposure prophylaxis. The approach that we took to address this question was to undertake a natural history study which was performed at USAMRIID under the direction of Dr. Louise Pitt and her staff, with the support of the NIAID.

[Slide.]

Again, the goal of the natural history study was to determine the timing for gentamicin intervention. In this study, six African green monkeys were exposed to aerosolize Yersinia pestis, strain CO92, with a gentamicin MIC of about 1 microgram per ml. The planned does was 100+ 50 LD_{50}s of Yersinia, delivered via the USAMRIID automated aerosol exposure platform.

[Slide.]

The results of the study were as follows: four of the six animals became bacteremic after aerosolized exposure to more than 20 LD_{50}s of Y. pestis. Insufficient exposure to viable organisms was likely responsible for the failure to develop disease in the other two animals.

All the bacteremic animals were blood-culture positive no later than 72 hours post exposure, and fever was the most consistent early clinical sign of disease.

[Slide.]

This table just shows the development of bacteremia in the four animals that did develop

bacteremia. And you can see, by 72 hours all of the animals that eventually were bacteremic--one animal actually developed bacteremia by 48 hours. The two animals that did not develop bacteremia were exposed to lower than anticipated levels of Y. pestis.

[Slide.]

And, just to show the development of fevers in the four animals that did develop bacteremia--and you can see a very nice correlation between the timing of the development of bacteremia and the timing of the development of consistent fevers.

[Slide.]

Well, the natural history study resulted in the following trial design regarding the timing of gentamicin intervention for a treatment indication. We decided to begin treatment 76 hours after exposure to Y. pestis or, with the development of consistent fever in the majority of the monkeys in each exposure cohort.

[Slide.]

So now turning our attention to the pharmacokinetic study, the goals of the pharmacokinetic study were to determine the gentamicin dose that would result in a peak serum gentamicin concentration 10 times the U. pestis MIC--or 10 microgram per ml, and then to determine the equivalent human gentamicin dose. This study was performed at SRI, under an NIAID contract.

[Slide.]

In this study, 12 African greens--six male and six female--were exposed to a single dose of gentamicin. Six received intramuscular injection, six received IV infusions. Three doses were chosen; 3 mg/k, 4.5 mg/k and 6 mg/k--with our target peak serum concentration of gentamicin, again, at 10 micrograms/ml.

[Slide.]

The results were that the lowest gentamicin dose from the PK study that achieved the target peak serum concentration was 3 mg/k. Now, the thing about this is that this once-daily dose left the serum gentamicin concentration below the

Y. pestis MIC--gentamicin MIC per pestis, of 1 microgram per ml for approximately 17 hours.

Now, in treating pneumonic plague, we didn't feel all that comfortable about that, and examined options that we might have to try to remedy that situation. And, clearly, dosing with gentamicin every 12 hours resulted in a greater time above the serum gentamicin MIC for Y. pestis.

[Slide.]

This is a chart of the pharmacokinetic data, briefly summarized. Here's the concentration of gentamicin time, and then this line here is the 1 microgram per ml MIC; three different dosing ranges. Just to focus mostly on this lowest range--the lowest dose, 3 mg/k, you can see a C max of about 17 micrograms per ml. But the key thing also is that the clearance ends up being about the same, and going below the one microgram per ml MIC at about seven hours.

Now, just modeling a second dose of gentamicin at this dose level, you can see, you know, basically, duplicating this curve; more time

above MIC for that second gentamicin dose.

[Slide.]

Now, the thing then was this 3--to give 3 mg/k gentamicin every 12 hours in African greens actually mimics a daily human gentamicin dose of 10 mg/k. This raised a problem.

The problem was that the 10 mg/k daily gentamicin dose in humans is greater than the maximum recommended dose of 5 mg/k per day in humans with life-threatening infection. Doses over 5 mg/k per day significantly increase the risk of ototoxicity and nephrotoxicity.

So we had a decision to make. The decision was whether to just go ahead and use the high dose, with the idea of giving gentamicin the best chance of working, in the animal model; or whether to immediately scale down the dose that would make the experiments immediately relevant to the human condition.

[Slide.]

We decided on the former course: to use the higher dose in the African greens to achieve

proof of concept; basically, giving gentamicin the best chance of working against this very severe infection.

[Slide.]

However, we know this would have a consequence. And the consequence was to commit us to further studies to make the studies applicable to humans under the Animal Rule, withe the idea being if gentamicin efficacy was established against pneumonic plague at this higher dose, we'd need to test lower doses with an acceptable relative toxicity profile in humans.

And so we went ahead with the efficacy study.

[Slide.]

The goal of the efficacy study, again, was to determine the efficacy as treatment of pneumonic plague in this African green monkey model.

The design was a targeted inhaled dose of 100 + 50 LD50s. We used 10 treated African greens--five male, five female--at this 3 mg/k BID IV dose for 10 days. There were six untreated

controls.

[Slide.]

Well, among the controls, none survived. Among the treated African greens, 8 of 10 survived. We're currently studying the factors that possibly differentiated the survivors from the two treated African greens that died. Might it have been a different LD 50 exposure? Perhaps different gentamicin levels? Perhaps seeding of compartments that were otherwise unavailable to gentamicin therapy, such as the CNS?

Anyway, all these results are awaiting the results of pathology studies and laboratory studies.

[Slide.]

In the future, what we plan to do is to perform gentamicin efficacy studies with a lower dose of gentamicin that would correspond to an acceptable human dose, and then to repeat these efficacy studies for Cipro, levofloxacin, ceftriaxone and doxycycline.

[Slide.]

I'm going to skip the GLP slides--both in the interest of time, and because Dr. McCormack is immediately following me and will be addressing the GLP issues.

And so I'll go right to my conclusions.

[Slide.]

And that is that the Animal Rule creates new opportunity and new challenges for research mobilized to combat bioterrorism. There is a need for careful consideration and planning to address the four scientific criteria: the pathophysiological mechanisms; demonstrating the effect in animal species with response predictive for humans; studying the relationship of the study endpoint to the desired human benefit; and obtaining pharmacokinetic and pharmacodynamic data in animals that permit the selection of an effective human dose.

[Slide.]

Additionally, other requires must also be addressed, such as the GLP requirements, the need for human safety data, and the post-marketing

studies that we'll be discussing later in this meeting.

[Slide.]

And I'd just like to emphasize that in doing this, early communication with ourselves and the Division of Counterterrorism, as well as with the appropriate review divisions, are important in ensuring an efficient process.

I give you my phone number and the phone number of our division, as well as my e-mail there.

[Slide.]

And I'd like to quickly acknowledge just some of the folks who have played a key role in many of these studies: Mitch Mathis, from our division, who worked very closely with Russ Katz and the folks in OD 1 on the pyridostigmine bromide approval; Frank Pelsor, working with Phil Colangelo, on the pharmacokinetic studies; Tracy MacGill, our microbiologist; John Alexander, from OD 4, as is Phil Colangelo; from the NIAID, Judy Hewitt, and now Adeline Smith, as well; and from USAMRIID, Louise Pitt and her incredible team at

USAMRIID.

Thank you very much

[Applause.]

DR. WOROBEC: And now I'd like to turn our attention to the whole issue of GLP in animal efficacy studies.

And I'd like to introduce our next speaker, Dr. James McCormack. He's the Director of Nonclinical Laboratory Compliance in the Office of Enforcement within the Office of Regulatory Affairs of CDER. He is truly an expert on GLP issues.

Dr. McCormack has 15-plus years' experience in bioresearch monitoring at FDA; is the FDA's representative to the WHO health organization, and the Organization for Economic Development and Cooperation expert panels on GLP; and is the agency contact on International Memoranda of Understanding and Interagency Agreements pertaining to GLP.

Dr. McCormack is also responsible for organizing and conducting training of agency investigators in GLPs.

GLP Issues

DR. McCORMACK: Thank you, and Good morning. It must be either good planning or bad planning, they saved the regulatory guy until right before lunch when everybody's hungry, and body parts have become numb and things.

So I'll try to go through this quickly so you get on to lunch and a break.

Dr. Schrager covered a lot of the elements of the Animal Efficacy Rule. I'm going to skip through most of those slides and just get to one particular point that's of interest in the GLP area.

[Slide.]

I'll also cover what the basic objectives are of Good Laboratory Practice regulations. Some of you may not be that familiar with them. Some of you may. But I'll cover, basically, what the objectives of the GLPs are; talk about some of our experiences and questions that we've dealt with in dealing with the application of GLPs in biosecure facilities. And, lastly, I'll give you some

contact information that if you do have questions you can--if we don't get them answered in this afternoon's panel, that we'll be able to answer them later.

I'm going to skip through most of these slides. Dr. Schrager covered these.

[Slide.]

Essentially, the Animal Rule derived from Bioterrorism Response Act of 2001 which required FDA to develop a rule by which we could use animal data as evidence of effectiveness.

I'm going to skip through the basic requirements of the Animal Rule, or the conditions for the Animal Rule--get to the most important part, as far as GLPs are concerned--

[Slide.]

--and that is that the Animal Rule does require that all the studies are subject to GLPs and the Animal Welfare Act, which is administered by the USDA APHIS, not by FDA.

[Slide.]

There is a conforming amendment to GLP

regulations to accommodate that; to conform GLPs with the Animal Rule, to make sure that that is legally within the scope of GLPs. It will be published as a direct final rule, and it is currently in the agency clearance process.

[Slide.]

That being said, I'll go on to the basic objectives of GLPS. GLPs is a quality management system, and it's designed to ensure the quality and integrity of non-clinical laboratory studies--which, in this case, in the Animal Rule is the animal efficacy, as well as there may be safety studies as well.

And our inspectional process focuses on that quality management system; the test facility management, the quality assurance unit, and the study director, and how they interact. That is the quality management system that's described in GLPs, and that is the focus of our inspectional process.

[Slide.]

In studies conducted under the--well, actually in any studies, whether you're testing

bubble gum or counter-terrorism agents, the most important aspect of Good Laboratory Practice regulations is the involvement of management in that quality management system. It is, indeed, a quality management system, and any laboratory where the management of that laboratory is not actively engaged in the assurance of compliance and quality of the studies, we can pretty much guarantee that the studies are going to have flaws. It becomes problematic, obviously, for the review divisions.

[Slide.]

Management's role in that quality system actually may be heightened in biosecure facilities. There are a number of things--and I'll go through some of them--that management--additional considerations; it puts additional burden on testing facility management to ensure the quality and integrity of data.

[Slide.]

The objections of the GLPs is to permit reconstruction of the study events and verification of the final report, independent of the personnel

involved. You can easily say, "Well, I can recall what I did in the study. I don't need the document." But, really, the purpose is to make those studies independent of individuals' recollection; to be able to reconstruct it from documentation of the study, to know what's going on in that study.

I have a friend who is a quality assurance consultant, and she makes this point in many of her presentations. She used to use an example of asking study directors "What happens if you get hit by a bus?"--until, actually, she had a study director that did get hit by a bus, and she no longer uses that example.

[Laughter.]

But the important thing is to be able to reconstruct studies from the documentation, from the raw data, from the specimens that are collected during the study. In fact, some of these studies may float around for quite a bit of time. Just about a month ago I was contacted by the OECD--not part of the GLP aspects of OECD, but another

activity occurring within the OECD--and they were looking at some old data, and they wanted to know whether we had inspected that data, and what our opinion of laboratory that collected the data was. And for any of you that are familiar with GLPs, the data came from Industrial Biotest--which, if you are familiar, you realize that was one of the landmark criminal investigations, back in the mid-'70s that caused GLPs to come into existence. Basically, they had no trouble giving you a two-year chronic study on a two-month notice.

But that data is still floating around, and it was actually going to be used by the OECD for setting standards and evaluating toxicity of--I think it was pesticides in this case. And it was an interesting reply that--I said, basically, we didn't have any problem with the lab except for the four people that went to jail and all the data that was thrown out.

[Laughter.]

How do we accomplish this? Well, basically, we accomplish it through the inspection,

again, of raw data, specimens, records and other documentation in a study, and to see if we can reconstruct study events from that information. Basically, what we're trying to do is--the Pre-Clearance Review Divisions receive a final report. We're trying to deliver the answer to them that, indeed, what you are reading, what you are looking at, is indeed fact based; everything in that report is based on documented facts, and you make your decisions with confidence on that data. Or, if they have a specific question about an aspect of the study, we can go out and answer that question for them, looking at the actual raw data and specimens and things like that.

So that's the objectives of GLPs.

[Slide.]

Again the key factor is management responsibilities. And, again, it is heightened in the case of biosecure facilities. One of the basic requirements in any study is that management has to determine that the persons that are going to be involved in a study are, indeed, qualified and

trained to perform that study. They have to have the facilities that meet the requirements--the protocol requirements for conducting that study. And they have to have the equipment necessary to be able to conduct that study.

[Slide.]

This may, again, be heightened in biosecure facilities, where it may require specialized training. It may require certain changes to the facility operations and the physical facilities themselves. And we'll get into some of the additional burdens that may accompany biosecure facilities, as far as equipment is concerned.

[Slide.]

I've already talked about the training--specialized training that may occur in a biosecure facility. Again, management has to assure that test articles have been appropriately characterized. And I'm going to--I have a couple of slides on this later that aren't in your handout, but it's only two slides. I'll talk a little bit more about this.

But test articles and control articles, for that matter--you have to know exactly what those articles are, each batch of that. It has to be known so that you know what the exposure of the test system is to that article, and I'll talk more about that.

[Slide.]

In biosecure facilities, management may have to address additional and different demands on study directors, on personnel, and on the quality assurance unit. Because of the nature of a biosecure facility, you may need more personnel in a quality assurance unit, for example. Study directors may not be able to execute the number of studies that they have traditionally done outside a biosecure environment. So management has to accommodate for that; they have to account for that--that they may need additional study directors, they may need additional technicians, they may need additional quality assurance personnel.

They also must be able to accommodate that

internal quality assurance auditing, and external inspections of those facilities, and audits of those studies. And that may require, again, some preparation and some planning on management's responsibility to make sure that that can occur.

[Slide.]

Study director responsibilities: under the FLPs, the study director is the single point of study control, and that remains the same whether you're dealing in a biosecure facility or any other facility. It may stress--and it may make certain issues more important than they were in a nonsecure environment, but, essentially, communication becomes an issues.

How are you going to communicate? How is the study director going to communicate information--to the technicians that are actually performing the task? If there needs to be a change in procedure, and you need to deviate from a procedure, or deviate from the protocol, how is that going to be communicated? What challenges does a biosecure facility present for communication

amongst the staff? You can't necessarily call a quick five-minute staff meeting and talk about an issue. There may be individuals on the other side of a barrier that you can't get to readily. How are you going to do that? How are you going to anticipate that?

The standard operating procedures--to make sure that they are followed by the study personnel. And protocol amendments, or protocol deviations--again, if the need arises to deviate from procedures, or from the protocol, how is that going to be, again, communicated to the staff? How is it going to be documented--when, again, you're dealing with inside and outside of a barrier situation.

One of the big things is that a study director must assure that data are recorded accurately, and they are verified. Now, that presents a problem in a barrier facility because there obviously is an effect on the transference of information--if you even can--from within the barrier to outside the barrier. And it may

actually have deleterious effects, depending upon the method of reporting on that information.

The study director may need to plan whether they use traditional methods of recording data or look for non-traditional methods of recoding data; for example, the use of--well, I'll go through some of that later on--but, for example, paper. You may look to use types of paper that aren't destroyed in the decontamination process--nalgene notebooks, nalgene paper--for recording information, or other solutions to that problem.

Verification outside and within the barrier. Some of that information may be collected on one side and cannot be, for whatever reason--the methods just do not allow them to be transferred outside the barrier--for the quality assurance unit to do the verification. How are you going to handle that situation? How is the study director going to verify that information if they need to access that information readily?

[Slide.]

Corrective actions are one of the things that a study director really has to communicate to the staff. If there is an immediate problem--especially something that is brought to the attention of the study director--and, again, they need to make that change to take a corrective action that's been transmitted to them by the quality assurance unit. They may be doing an inspection and find a problem that they feel could affect the quality and integrity of that data, how are you going to quickly make that change when you're dealing in a biosecure facility?

And the overall responsibility of a study director is to ensure that all the GLP regulations are indeed followed. That still remains their responsibility.

[Slide.]

The quality assurance unit responsibilities: personnel must be qualified to conduct the inspections in secure areas. They may need to go through additional training and additional qualification in order to get behind

that barrier and to conduct their inspections and audits as necessary. And there may also need to be more than one person that's qualified to go within that barrier to do those inspections and audits, again, because you hope someone doesn't get hit by a bus.

The QA should explore the use of alternative methods to conducting inspections and audits. Again, if you have to go within a barrier, that may be time-consuming. It may put constraints on the resources. So you have to look for creative alternatives to doing inspections and audits of studies within the barrier and outside the barrier.

One of the things that really is incumbent upon the quality assurance unit is they need to make management aware, so management can address those issues, of what type of burdens a secure facility really puts on their operations. Again, you may have to have additional training of QA personnel so they can go within the barrier; and scheduling of those personnel. You may not be about to audits and inspections of other aspects of

facility operations because of the time it takes to do inspections of the studies that are conducted in a biosecure area.

[Slide.]

These are the two slides I put in here, because this question does come up on occasion, and that's test and control article characterization. In a lot of these cases we're talking about, the control article actually being the agent, and the test article being a marketed product, under the GLPs, a marketed product can be characterized by its labeling; in other words, it's already a marketed product, the labeling of that product is satisfactory for characterization.

But in these cases, oftentimes the control article is an agent, and needs to be characterized. Well, the regulations require that articles be characterized by their identity, strength, purity, composition, and "other characteristics." In these types of studies, it really is the "other characteristics" that really become the majority of identifying what that agent is, and to making sure,

from batch to batch it is the same agent, of the same potency.

[Slide.]

GLPs, themselves, do not describe what types of tests need to be performed. Basically--and especially with these types of studies--the people that are going to be reviewing the studies, the people who are performing the studies, they can agree on what is the necessary type of testing needed to really appropriately characterize that control article, or that test article. They may be different types of tests than are typically done; again, "other characteristics" that need to be defined about that. And that's really an issue that Dr. Schrager point that out, is a communication between the people that are going to be reviewing these studies and the people that are conducting these studies: what types of tests do you want to see that will prove to you that this article is indeed the same article from a previous batch and the next batch, and one study to another--what types of tests do you need to be

performed? That should be captured in the protocol.

As far as GLPs are concerned, we'll check to make sure that the type of testing that is necessary to characterize that article have been performed and accurately reported. But the exact type of testing that needs to be performed is really a scientific issue that is within the realm of authority of the review divisions that will be receiving that data.

So that's on test article.

[Slide.]

Raw data in secure facilities: raw data that's collected in a secure area, again, may be damaged as it is decontaminated. You may not be able to transfer that from within the barrier to outside the barrier. One of the things you should be cognizant of is that GLPs do permit the substitution of exact copies; that's a verbatim copy that's been verified accurate by a dated signature, for original source data.

So it may be that you may be recording raw

data on paper--the traditional manner--within the barrier. You cannot decontaminate that information and bring it outside because it destroys the records themselves. There are a number of different procedures that you could use to create an exact copy of that paper; for example, I've been in some biosecure facilities that just have a fax machine within the barrier. They fax out a copy. One person on the outside of the barrier looks at--they hold up to a glass door the original information. They do a quick check to make sure that the fax copy is, indeed, an accurate and exact copy of the original which is within the barrier. So that's one possible solution.

Again, because these require some non-traditional approaches, the training of the personnel--the technicians involved in the process--they need to understand exactly that verification process, and that it indeed will ensure that all the information is accurately and completely copied into that copy of the original records.

Again, some of the approaches that we've seen used are special materials--nalgene notebooks and paper and things; video and audio tapes can sometimes be used to supplement other types of documentation; and, of course, electronic data. If the data is recorded electronically at a terminal within the barrier, the servers are outside the barrier, so the information is accessible at both locations, within and outside the barrier.

The important part is also is to assure that whatever non-traditional method you're using, they permit records to be retained for the required period of time. That's an important consideration for some of the alternatives like video and audio tapes. Will they meet the retention requirements? And most of the time they probably will.

[Slide.]

Maintenance and calibration of equipment: management may need to expend additional resources. They may need to plan for additional resources to accommodate redundancy of equipment. You may have equipment that is held captive in one area of a

facility, and you may need it in another area. Management needs to know that they're going to need to buy three and four pieces of this equipment and not just one, because you're not going to be able to decontaminate it, or you're not going to be able to move it from one area of the facility to another area of the facility rapidly.

Another thing that study directors--and management and technical personnel, as well--need to be cognizant of is that for the standardization or calibration of equipment, you want to plan enough ahead of time to where you have some down time if the piece of equipment can be, indeed, decontaminated for calibration purposes, that that doesn't become a problem of conflicting schedules within a facility.

[Slide.]

Many biosecure facilities, by their nature--by how they become biosecure facilities--should already have in place a lot of--and be able to accommodate a lot of the requirements of GLPs; for example, the isolation of

projects, isolation of biohazardous materials. By the very nature of a biosecure facility, that is already accomplished. Quarantining of animals is generally a routine practice in biosecure facilities; and also, hopefully, preventing mix-ups of test and control articles. Most facilities that operate in a biosecure fashion are very cognizant of making sure that they don't mix up the anthrax with some thing else.

The bottom line is: GLPs do provide a significant amount of flexibility. You need knowledge of the regulation to understand where that flexibility is. We're more than willing to help if somebody--if you encounter a problem, "How do I comply, given this set of circumstances?"--and a lot of this is very unique--some of the facilities that I've been to--to a couple biosecure facilities, they've used very creative approaches that are still compliant with the regulation. They do require people to get off--or to move out of their traditional safe-zone of the way they normally do business, but still using practices

that are, indeed, compliant.

And we're more than willing to help you answer those questions.

This contact information is probably good for two more weeks, because my telephone number and address and things are going to be changing. There's always 10 percent of FDA that is in a moving van at any one time. In the next couple of weeks it's my turn.

My e-mail address will stay the same, so you can contact me there. And we'd be more than happy to get what your individual circumstances are, what are your concerns about compliance, and help you find ways to address those compliance issues, and to run these studies in compliance. And the end result is to deliver to the review divisions the confidence they need to make the critical decisions they have to make about the approval of these products.

So, hopefully, I haven't slowed you up from lunch too much. And I'd be glad to answer any questions during our panel discussion.

Thank you.

[Applause.]

DR. WOROBEC: We're now going to break for lunch. But I do need everyone to be back promptly at 12:45.

And for those of you who already questions for the panelists, you can pick up cards at the registration desk and write them down, and we'll be happy to try to answer them.

[Off the record.]

DR. WOROBEC: We're going to resume the afternoon session.

I want to make a post-lunch announcement, though. For anyone who may need a cab to the airport, just let the registration desk know and they'll arrange one for you.

And we are going to have to make some more changes.

Our first speaker is currently not here, and what we're going to do is have our second speaker, Dr. Louise Pitt, give her presentation. And we'll proceed from there. We may have a

shorter session, and therefore we may have some more time for discussion, which I think would actually be quite valuable for this particular session, since there is a lot to discuss.

So, I'm just going to now--I will now introduce Dr. Louise Pitt, who is very well known to any of you who have ever contemplated doing non-human primate studies.

She is the chief director of the Center for Aerobiological Sciences at USAMRIID at Fort Detrick, and has conducted research on animal models with anthrax and, in particular, on non-human primate models, since 1987. Prior to this time, she held the position of chief of the Department of Immunology and Microbiology at the National Center for Occupational Health in Johannesburg, South Africa.

And we are very pleased to welcome Dr. Louise Pitt to this workshop.

Animal Efficacy

DR. PITT: Good afternoon. The subject that I'm going to talk about is animal models for

bacillus anthracis infection, focusing on inhalational anthrax.

[Slide.]

This first slide is--I believe everybody knows this by now, but the reason for this is to point out that the capsule and the exotoxins--these virulence factors--are very important when discussing animal models, because different animal models are sensitive or resistant to these virulence factors to varying degrees. And as we go through the animal models this will probably become more apparent.

[Slide.]

Inhalational anthrax--I think everybody is aware now--is a generalized system disease in susceptible animals. And the end result is usually a septicemia.

The pathogenesis as we understand it today was actually based on work that was done in 1957 in guinea pigs. And this is the work that gave us that fact that when inhaled, these spores phagocytosed, carried to lymphatics--carried by the

lymphatics to the draining lymph nodes. The spores germinate. To date it is not quite clear where those spores germinate; whether they wait 'til they get to the lymph node, whether they do it along the pathway, or whether some actually do germinate in the lung. There is data for all of those, and it's probably a combination.

The bacilli then grow, spread to the mediastinal nodes, surrounding tissues, and then seed multiple organs. And we do know that--and this is based on, of course, on macaque data, that about 50 percent have meningitis.

[Slide.]

Just a little bit of historical dat. It was in about 1880 that inhalational anthrax was first reported as a disease. It was called woolsorter's disease. And then in 1886, a Beuchner, a scientist, actually recreated in animal models--rabbis, guinea pigs and mice--by exposing these animals to clouds of these spores by aerosol. He gave inhalational anthrax to these animals; described it; and was astonished to find that this

is not a lung disease, this was a generalized disease with very little evidence of the germination in the lungs and organisms in the lungs.

[Slide.]

So, moving on to animal models: the principal animal models that have been used in laboratories--of course the interest has been medical, and therefore these animal models that would represent the interest of the scientists: the mouse, rat, hamster guinea pig, rabbit; and then the non-human primates, of which the macaque has been both cynomolgus and the macaque mulatta and the rhesus have been the two models that have been historically used.

[Slide.]

Now, moving onto the mouse--and this is where it's very important to remember that those virulence factors in the mouse--the capsule is extremely important. Encapsulated non-toxin-producing strains of virulence to the mouse.

Again, mouse strains--because there are so many different inbred mouse strains--they differ significant in this innate susceptibility to lethal infection, both with the fully virulent and with the non-encapsulated, the Stern-type strain.

When you look at vaccination in these animals--and this is usually with a protein--chemical-derived vaccine--you can attain protection with an unencapsulated strain, but not with equally virulent strains.

[Slide.]

And this is just a little data emphasizing that, and looking at two different mouse strains and how they behave: with the A/J, when vaccinated with the licensed vaccine, you get an enormous anti-PA titer. But, again, this is just with a sub-cu challenge. No survival.

If you, however, give a life vaccine to the mouse, which produces PA--and this is B. subtilis in this case--again, an enormous titer in the A/J, but zero survival.

If you look at the CBA--with the ABA you

get a similar pattern. You get a huge titer, against the PA, and very little protection. But the CBA differs from the A/J in that with the live vaccine, you can get some protection.

So, mouse strains, we're looking--very, very different across susceptibility, although in general they are quite different from other species in how they behave with the capsule.

[Slide.]

As most of our efforts over the years have been on vaccines, we had done very little work with the mouse model, in terms of vaccines, but after 2001, the question arose was: we needed a model that would help us screen antibiotics. And that's when we thought, well, maybe we could go back and develop the mouse model, have an aerosol inhalational model, and would this help us to screen antibiotics, because we didn't want to do that in a non-human primates, and a triage model was what was required, and would this be useful?

So Hank Heine at USAMRIID honcho-ed this effort, and we looked at four different strains of

mice: A/J, the Swiss Webster, Balb/c and C57 black.

The A/J turns out to have the lowest LD 50; C57 black, Balb/c's are very similar, with Swiss Websters being sort of between A/J's and Balb/c's.

We are in the process of doing the pathology and looking at the pathology. It is not a complete picture yet. But as I talk about pathology of the other models I will mention where we think the mouse model will lie in terms of pathology.

[Slide.]

In choosing a mouse model to go ahead and look at this antibiotic therapy, we chose the Balb/c, again, because it was an inbred strain. It was intermediate sensitivity; and it was consistent with some sub-cu challenge data that had been collected by Welkas in '86. And then Hank Heine and his group have gone ahead used ciprofloxacin as the standard, and have established that 21 days of treatment gives you 100 protection against a round of 50 LD 50 challenge. This is all done with the

Ames strain of bacillus anthracis.

It was established that 14 was not sufficient. And based on this data, we feel this is an appropriate standard to maybe screen other antibiotic --newer antibiotics for efficacy.

[Slide.]

So, in general, we feel at this point that the Balb/c mouse may be a suitable model for inhalational anthrax, based on what we've seen right now on the pathology, and for screening antibiotic efficacy. And I want to emphasize: we built this model for antibiotic. We did not think it would be useful for immune therapy.

[Slide.]

Just moving on to the rat model, I think as every has heard today through different talks that the rat is very resistant to the establishment of an infection. And when I say "resistant" and "sensitive" in these models, this is based on sub-cu routes, not on inhalation routes.

Again, if you look at different rat strains, it is rat strain dependent, and it has

been shown that this resistance increases with age.

The rat, of course, is extremely sensitive to toxin--some strains much more sensitive than others; Fisher 344 versus NIH black rats, much more sensitive to toxin than the others.

And if rats are vaccinated with standard vaccine, this really--you don't really see a big improvement in protection because it changes the resistence very little.

I just put in a little bit about the hamster here. The hamster is extremely susceptible. It's very much like a mouse. It's just a bigger, uglier mouse, in fact.

[Laughter.]

Extremely susceptible, and is impossible to protect with acellular vaccines. I put that in there because the Russians used the hamster extensively, and all their publications, all their information on vaccine resistence, etcetera, usually came from the hamster model. So it can be taken with whatever that means.

[Slide.]

Just moving onto the guinea pig, now. The guinea pig has been used extensively, historically, for many, many years. It was used to elucidate pathogenesis with inhalational anthrax.

Guinea pigs are very susceptible to spore infections; very susceptible. When you hear people talking about different strains of bacillus anthracis spores versus virulence, they're frequently talking about virulence in the guinea pig.

Guinea pigs see differences in spore preparation in terms of virulence and susceptibility that other animal models like the rabbit and the non-human primates doe not see.

The guinea pig, on the other hand, is fairly resistant to toxin. And, as I said, it's been used extensively to characterize the pathogenesis of the disease and, in fact, to elucidate, as well, the role of toxin.

And when it comes to immunizing with the standard aluminum adjuvant of the vaccine, you get poor protection in the guinea pig against both

cutaneous and you get very, very little protection against an inhalational anthrax.

[Slide.]

So, moving on to the rabbit: again, the rabbit model used historically, way back, by the Russians, as well; it was used extensively in the U.S. and the U.K. until the guinea pig became popular--and cheaper, I would imagine.

It's--as I said--susceptible to infection. It's also sensitive to the toxin. When the rabbit is immunized with the aluminum adjuvant of the vaccines, you get complete protection against both a cutaneous anthrax and against an inhalational anthrax.

And we have shown that the vaccine efficacy is predictive of what occurs in the macaque.

[Slide.]

So, moving on to the macaque: the majority of the recent data has been collected in the Rhesus macaque. This was not necessarily true in the '50s, '60s and '70s. Rhesus macaques and

cynomolgus macaques were used fairly interchangeably. Talking to the older scientists to try and understand why that was--if it was a difference--it appears to be it was just a supply and demand. If they had cynos they used cynos, if they had rhesus they used rhesus. There was no real scientific reason. And the literature--the pathology at that time sort of bears that out.

The macaque is susceptible to anthrax and is sensitive to toxin, as were the rabbits. Everybody considers this the gold standard; the model of inhalational anthrax. We do have extensive pathology studies--which, as I said, range way back to the second World War. We understand this disease. It is the one that we all feel is the most close to the human disease, based on the pathology, the pathophysiology, and the immunization with the aluminum adjuvant of acellular vaccines--again, gives complete protection against both the cutaneous anthrax and inhalational anthrax.

[Slide.]

So I'm just going to present a little bit of infectivity data in the guinea pig, the rabbit, and the rhesus.

The rhesus--guinea pig, rabbit and monkey, here, having done with the same spores, in the same place, with the same equipment and everything--so are fairly comparable. And I think that's important to keep in mind.

We have 80,000 for guinea pig, 110,000 for rabbits, and 55,000 for rhesus macaques. Fairly similar.

Recently, Battelle did a cynomolgus macaque LD 50 that is published, along with the pathology. And they got around 62,000, with a different preparation and a different area. So, fairly consistent, in terms of LD 50s for the Ames strain.

[Slide.]

So, moving on to--looking at comparing the rabbit, the macaque and the human, a model is only ever just a model. So there are always similarities as well as differences to the human disease. And

the principal similarities are the hemorrhage, the edema, the necrosis, the limited cellular inflammatory responses. These same tissues are affected in the three animal models--I mean, the two animal models and the human. And the systemic findings are very similar.

We have not done any recent extensive pathology on the guinea pig, so do not--in the context of these studies--know how similar that is.

And, again, on to differences: as I said, a model is only a model. And so there are differences. And the lack of the leukocytic response in brain and meningeal lesions in the rabbit; relatively mild mediastinal lesions in both rabbits and macaques. And then you see a lower instance of pneumonia in rabbits and rabbits and macaques.

And this could, in many ways, be dependant on the fact that these animals die at different times post exposure. The rabbit dies, on an average, between two and three days post exposure. They die very rapidly.

The macaque--in general, it's between one and 10 days. However you do get outliers. They can go out to three, four weeks, where they normally die of meningitis at that time.

When we compared this to the human data that was available at that time, it was mainly based on the Sverdlovsk incident. And, based on that, the human data that we could collect, it looked like it compared to 4.7 days post-onset in those people. And the estimated days of post-exposure was round 18.5. However, based on the 2001 incidents, the timeline for the human infection was much closer to what we see in the mid-Atlantic that the Sverdlovsk incident gives you.

[Slide.]

So just to sort of go through what are the possible reasons of why we're seeing differences between rabbits, macaques and humans could be the host susceptibility issue, which is always there. Survival time is a very important issue, as the rabbits die very rapidly. And, in the mouse model,

we are seeing the exact same thing; the mice are dead by 72 hours. And, based on the pathology that's been looked at to date, it's very similar to the rabbit model, and could be based on that survival time post exposure.

And the macaque data indicates increased incidence of inflammatory, CNS, mediastinal, pulmonary and hepatic lesions as you get further and further out from your exposure time.

[Slide.]

I'm just going to go through a little bit about vaccination and passive transfer. I think, although this is very much a therapeutic-based discussion today, I think in terms of immune therapies, this is the foundation that the immune therapies are based on, and I think it's important to expand and understand it in terms of the animal models.

So this is just to show you some data on the guinea pig, the rabbit and the macaque--comparing them. This is with an RPA vaccine. This is showing that in the guinea pig,

as I mentioned, you get partial protection--at best. This is a fairly low LD50 challenge: 30 LD50s. And you can see, you do not get any gradation in response with the PA, going from .5 to 5 to 50, you still just get--you don't get any titration effect.

In the rabbit, you start to see a titration: 9 out of 10 at 5, and 6 out of 10 at .5. And then in the rhesus macaque, full protection, even down at the .5 microgram--well, 9 out of 10, which is pretty close--in the .5 microgram of PA.

The points I want to make about guinea pigs--because we did not use guinea pigs as a vaccine model when working with aluminum as an adjuvant. But if you use other adjuvants in the guinea pig, the guinea pig is actually quite a good model for vaccine efficacy. It's the aluminum adjuvant in the guinea pig that gives you very peculiar results; and, in fact, it is being used to study several different types of adjuvants, and it's very successful.

[Slide.]

So, as I said, in conclusion, we have focused on the rabbit and the rhesus as the animal models for vaccine efficacy, after looking at what we know of the human disease; what we understand about vaccination of humans, protection of humans with the vaccine. And it was based on the pathology, as I said, as well as the response to vaccination.

[Slide.]

And then, just a little bit of support for using antibodies. The in vitro correlate of immunity in a rabbit model was elucidated to show that antibodies to PA did correlate with survival. These are curves of animals that were vaccinated with different doses--this of the licensed vaccine. These studies have also been done with the RPA vaccine to show that antibodies to PA do correlate with survival.

[Slide.]

And then we went on and looked at passive transfer of antibodies. This was done at -24 at

zero time point. The animals were then challenged with around 200 LD50s, and we got survival in the animals with antibodies TO RPA.

[Slide.]

And this is just showing the levels that were obtained in the animals, and all the animals survived. So this helped to lay the basis for the fact that antibodies do play a role in protection of inhalational anthrax, and certainly would be useful in terms of immune therapies.

[Slide.]

And, with that, I will close with acknowledging some of the people who have contributed to this body of knowledge over the years: a lot of people at USAMRIID, and our colleagues at the top.

[Applause.]

DR. WOROBEC: Thank you, Louise.

I have the pleasure of introducing our next speaker, Dr. Roy Barnewall, who's going to talk about the practical aspects of doing these studies; some of the issues that arise in doing

aerosol challenge studies.

And Dr. Barnewall is a research leader of the Inhalation Systems Group at Battelle Memorial Institute in Columbus, Ohio. And I want to thank you for coming all this way to participate in this workshop.

Animal Efficacy

DR. BARNEWALL: You'll have to bear with me. We had a technology problem.

So, as Dr. Pitt just went through all the different models against inhalational anthrax, I'm going to give you an overview on how we do the actual challenge for the inhalation, in primarily the rabbit and the macaque model, so you can see what's involved in some of the technical issues that can arise.

Some of the topics I'll cover are the inhalation system and its components; the operating parameters--how we run it; the microbiology support, because once we collect the sample, it needs to be enumerated; then some of the factors that can influence the delivered dose to an

individual animal; and then a little bit on the exposure experience at Battelle in the years we've been doing it.

[Slide.]

So, the inhalation exposure components: there's the inhalation system. We also have subcomponents where we can capture the animal's respiratory parameters, such as tidal volume, net volume and respiratory rate. And we use the Buxco biosystem software. And then we also do particle sizing. We use an aerodynamic particular sizer made by PSI Instruments, and we do this because a function of where the spores will deposit in the respiratory tract is a function of the aerodynamic particle size. So we want a certain particle size, and this instrument will let us see that we've generated that size.

So, our inhalation system, the design and the operational parameters are modeled after USAMRIID's inhalation exposure system. And we did this so we can try and get results that are as comparable as possible because they've been the

benchmark for over 20 years in this type of research.

The system is housed within a Class III biological safety cabinet, of course, because we're working with dangerous pathogens. And, for the inhalation exposures, rabbits are done muzzle-only, and monkeys--macaques are done head-only.

And our system generates a particle size of one to two microns, which is the particle size you want to get deposited to the alveoli. And we generate our aerosol from a liquid suspension, as opposed to a powder.

[Slide.]

So the components of the system--we have a Colison nebulizer. This generates the liquid suspension of spores into the aerosol. It goes down a mixing tube to the exposure chamber, where the animal's head or muzzle is. Within this chamber we have a viable sampling for collecting samples of the spores to enumerate for viable counts, and we also have the particle-sizing port.

We also measure temperature and relative

humidity with a humidity probe. And we have a bubbler on the system that maintains our relative humidity between anywhere from 40 to 80 percent, depending on where we set it. And then we exhaust the system.

[Slide.]

And this is a schematic of the aerosol system. House air comes in, and it can be separated off into a continuous dilution flow of 8-1/2 liters. And then a bypass flow of 7-1/2 liters--or, if we turn on the Collison nebulizer--again, 7-1/2 liters of our liquid suspension of the bacillus anthracis is in the Collison. It is jetted out ports on the Collison; makes the aerosol. It goes out of the Collison, down the mixing tube to the exposure chamber, where an animal's head or muzzle would be, to be exposed. And this is also where we pull our AGI sample or our particle size sample, and then the remainder, underneath the animal's head is exhausted out and HEPA filtered.

We have a temperature and humidity probe,

and then we can expose an animal.

[Slide.]

Our house air pressure of the operational parameters to run the system--our house air pressure is about 30 PSI. Our Collison three-jet nebulizer runs about 25 to 30 PSI--this is Collison dependent, and we flow-check and verify these before use to get our 7-1/2 liters of flow. These typically generate a particle size of one to two microns of aerosol.

And then our dilution air is at 8-1/2 liters. We maintain the Collison air and the the dilution air with mass flow controllers. These are calibrated instruments. And you dial in the liters you want and it, by a restriction, it will let that amount of air pass.

So our total flow into the system is 16 liters. And within the exposure chamber itself, we maintain the pressure at slight negativity. We measure this with a magnahelic, and this helps maintain constant flow through the system so we don't get any stagnation.

[Slide.]

Our viable sample collection occurs with an all-glass impinger, AGI model 7541. This is based on a critical orifice concept. If we pull the sample at a certain pressure, we will get a sample rate of approximately 6 liters a minute. And before use of any AGI, we flow-check these with a calibrated instrument to make sure our Collisons are sampling at the rate they're supposed to. Because if you assume a wrong sample rate in the calculation, you could over or under-estimate the dose you deliver to an animal.

[Slide.]

To maintain our 6 liters a minute approximate sample rate, we have to pull that out of a vacuum of at least 17 inches of Hg. And then our particle size collection, with the APS, we sample at 1 liter per minute for 30 seconds, or 5 liters per minute, if we use a diluter, for 30 seconds. So we don't take a particle size sample throughout the entire run, and this has to do with you don't want to overload the APS. And so we take

a very short sample during the run.

[Slide.]

So, system checks--how do we know our system is operating properly? Our mass flow controllers are all flow calibrated. We send these to an instrument lab that calibrates our mass flow controllers. Our AGI--we flow check those before use, and we use a bubble meter manufactured by Buck, or another one called a gilligrator to flow check all our AGIs prior to use and make sure they're functioning properly.

Our temperature and relative humidity probe is calibrated. Again, our bubble meter that we check our AGIs with is calibrated.

We calibrate the Buxco system for the respiratory parameters prior to eery day's use. And then the APS--we particle size check. We use NISD-traceable polystyrene latex beads to make sure it's reading correctly.

And then, one o the most critical components is before any day's use, we balance the system. So , we balance the inflow with the

outflow. And if you had a leak or something not working properly, you wouldn't be able to balance your system.

[Slide.]

So this is just a photograph of a similar system that's in our Class III's, but on a bench top in one of our labs. So you can see, after all that big line diagram, they don't look all that too sophisticated.

[Slide.]

Our plethysmography, as I mentioned--we measure respiratory parameters such as the minute volume, tidal volume, and we do this real-time during the exposure. So the time while that animal is being exposed the spores is the time we're running the plethysmography so we can determine the actual dose that animal receives.

We use the BuxCo biosystem software. The animals go into a plethysmography box, which are basically just sealed chambers that then we can then attach a pneumotach and a transducer to, so as that animal breathes, as he inhales and exhales,

the change in pressure in the box from the inhalations and exhalations are picked up by the transducer; they're fed to the software, and it's converted to the data. And we validate the BuxCo plethysmography system. So we talk about GLP--you saw all the components that we have calibrated. And our plethysmography is validated.

Our particle sizing is also validated prior to use. Again, we use the APS made by PSI Incorporated. We do the particle sizing real-time during the exposure, but only for a small portion of the exposure. And we've done multiple samplings for various lengths of time to show that if I take a reading one minute into a run, or 10 minutes into a run, my particle size won't change. So it doesn't matter if I only take one small sample during a run. I know it won't change.

[Slide.]

And, again, this system is also validated prior to use.

Now--so we've done an exposure. Now the next key portion of this is to enumerate your

viable AGI sample, otherwise you can't determine the dose that animal received.

We enumerate via the spread plate technique. And, again, this is just serial dilutions to get into a countable range. We like to count between 25 and 250 colony-forming units per plate. And what do we enumerate? We enumerate our nebulizer--or our starting material, because that's critical to know, so you can base your length and animal exposure. And there's up to seven 1:10 dilutions to get the nebulizer material into the countable range, because we typically start with 10 9 or higher CFUs per ml material as a concentration. So we start with very highly concentrated material.

[Slide.]

And our AGI samples are diluted usually about five times at 10-fold dilutions. And then the last three dilutions are plated in quintuplicate to make sure we fall within the countable range.

[Slide.]

Now, any of you who have any microbiology experience know the spread plate technique can have up to 25 to 30 percent coefficient of variation. At Battelle, most of our microbiologists seem to be a little bit tighter, around 20 percent. But still, that's a fairly wide variation if you're targeting a certain dose, you can expect to already see a range in your delivered dose, just based on the enumeration of your viable sample.

So, that will lead right into the factors that can affect your delivered dose.

[Slide.]

Again, the spore enumeration variability we just talked about; spore lots--not all spore lots of the same strain aerosolize the same; and then spore lots of different strains also aerosolize differently. So, as we get different material in for different tests, we make sure we characterize those spores so we know how they will act in our system.

And some things that can affect spores in the spore lot are clumping and aggregations; how

sticky the spores are. And if there's a lot of debris in the sample or there's not.

They are notorious for adherence to glass and plastics. So spores are not your usual laboratory microbe.

The exposure time can affect your delivered dose. If you go--we used to go for timed runs, up to 10 minutes--we used to do 10-minute runs. And then, so based on a 10-minute run, if we had an animal breathing twice as much as another, theoretically that animal would get twice the dose as the other. So we don't do it that way any more.

And, again, your AGI sample enumeration.

[Slide.]

So here's an example of what can happen if you just look at the variability of your AGI sample.

If we assume this is the true sample concentration, and you convert that to an LD50 equivalent, and then somebody enumerates 25 percent lower than that number, you get 150 LD50s. And if the next person enumerates on the high side--say 25

percent--they enumerate out 150. So you could be targeting for 200, and right away you could except a range between 150 and 250 as your result.

And then the rest are just, you know, higher LD 50 and then higher percentage.

So, when we report out LD 50s, everybody wants to think, okay, I want to give 200, and I give 200 exactly. And that's never the case. We always give a range.

[Slide.]

Another thing that can affect the delivered dose is spore foaming. Some things that can cause the spores to foam in the Collison is if the pressure is too high. If I'm running higher than, say, 25 to 28, if I bump it up to 30 or 40, I can make my spores foam.

Different lots have more foaming than other lots, and that's one of the reasons you characterize it, so you can take that into account.

And also, the Collison tip might be too deep in the fluid level of the Collison jar. When you use a Collison nebulizer we like to keep the

tip around 2 to three mm below the depth of the fluid line. And if you put it in too deep you don't get recirculation of the material well, and so you don't aerosolize as many spores.

[Slide.]

This is an example of spore foaming. This is during a run. You can see--so what happens is, the pressure causes spores in the liquid to become sucked up through the bottom of this tube, and then there's little--there's three holes about at this level around this dip-tube, where they jet out. They impact upon the side of the jar. The one to two-micron spores become part of the aerosol and go up out of the jar down the mixing tube, and larger particles and clumps fall back into the Collison jar to repeat the process.

[Slide.]

And this looks like real thick milk here, but when you turn it off, you can see it looks like your latte foam on your coffee.

[Laughter.]

And what happens is when it foams like

this, it would go into the suspension to re-aerosolize and go back up out the jet to help aerosolize it. So it foams out of suspension, and it artificially lowers your concentration in your jar so you don't aerosolize as many as you thought you would have.

And so that's one of the factors you want to try and minimize or eliminate.

[Slide.]

And now this is just a little bit on our aerosol exposure experience. I've been at Battelle for five years, and in that time I've worked, with the help of Louise Pitt and the folks at USAMRIID, to get our aerosol up and running. So this is the data over that last five years--all summed up into two slides.

[Slide.]

This slide is for rabbits. It gives our target dose we were trying to hit during any particular study. The actual mean that we delivered during the experiment, with the standard deviation, and the high and the low, and the number

challenge.

And so here we were shooting a target of 20 LD50s. Our mean was 17.8; standard deviation of 7, with a low of a 5 and a high of 44. And we did 84 animals, overall.

At 50 LD50 target, we get 50 average, with a standard deviation of 32, a low of 28 and a high of 168, and only 19 animals done.

[Slide.]

And then, of course, at higher LD50s. And we've done many more animals at higher LD50s, because most of our studies have been vaccine efficacy studies, and we like to target 200 or more LD50s in most of those studies.

[Slide.]

So you can see we've done few number of rabbits in the last five years. And the system is very reproducible. Once you know how to use it, you can fairly closely hit the desired target you want, and with a fairly tight standard deviation. But you can see, with the tight standard deviation, we still have a fairly wide overall range that we

hit.

So you can expect to see some animals that get a lower than you target, and some that get a higher dose than you targeted. It's not like, you know, dissolving a toxin in a solution and then injecting it, where you can be very precise.

[Slide.]

And then this is our rhesus macaque data, and a little bit of cyno data.

Again, shooting for 20, we were pretty close--a little bit high; give 28, with the standard deviation of 12, in a fairly tight range.

We shot for 50 LD
50s; gave 49.5, with a standard deviation of 25. And we've done 30 that way.

And cynos--again, fairly close to what the target was, and a fairly moderate range.

And, again, you know, you can see we've done a fair number of rhesus or macaques in general; not quite as many rabbits. But, again, most people save the rhesus study for their pivotal study, and they're not cheap.

[Slide.]

And this is, again, a chart just like before, to show that we are as close to the mark as other institutions when doing aerosols. Again, the papers where these came from--these all came from the published literature. And here's a study where they didn't tell what they were targeting, but they gave a mean of 8 LD_{50}s with a standard deviation of 3. Again, no range--what you saw from ours--the range, the low and the high will be a little bit higher and lower on each end.

[Slide.]

Another one where they targeted 50--or, don't know the target. Most likely they were targeting 50, and they gave 50, with a standard deviation of 28--same as ours, practically.

And then here they gave 93, and with the standard deviation of 63. So they were probably shooting for 100 LD_{50}s.

[Slide.]

And here's another paper where no mean or individual animal. They just said the range was

from 255 to 760.

[Slide.]

And then a recent publication where they were targeting a certain dose. They gave another, with a fairly average standard deviation. So, again, to show we're just as reproducible as all the other labs. And, again, since our system is based off theirs, it's very reproducible.

[Slide.]

And that's it.

I'd like to thank our collaborators and acknowledge USAMRIID, and the folks at NIAID, and then others at Battelle that so graciously have been the study directors on these, and not me.

[Applause.]

Panel Discussion

DR. WOROBEC: What I'd like to do is now have the panelists proceed to the podium and take their seats. And I'm going to introduce one additional panel member who did not present: Dr. Martin David Green, who is the current Associate Director for Pharmacology and Toxicology for ODs 1

and 6 at CDER.

He was formerly the Branch Chief for Clinical Pharmacology and Toxicology in the Division of Clinical Trial Design and Analysis at CBER, and he has also done a lot of the research on pyridostigmine when he was in the military--and truly is an expert on animal efficacy studies for anthrax and other agents of counter-terrorism. So we're lucky to have him.

And I will actually now--I'm going to actually open this discussion session something that's really, I think, to me, at the heart of the topic that keeps coming up, with trying to design animal efficacy studies.

It is likely that antibiotic therapies or other types of therapies against anthrax will be used in conjunction with antibodies and perhaps other classes of agents. As we have seen, there are lots of new classes of agents being developed, and it's possible that what we will ultimately have are cocktails of agents that could be used--perhaps other additional immune-based or anti-toxin

therapies.

What I'd like to address to all panel members, and even members of the audience, are: what are the parameters--the critical parameters-that you feel--meaning the length pf protection or survival, whether there needs to be evaluation differences for dose challenges that would be critical for evaluating clinical effectiveness in animal efficacy studies? How would you envision designing such animal efficacy studies where you have more than one therapy being studied in order to identify the correct timing and dosing of your therapy for, a) the post-exposure prophylaxis indication and b) for the treatment indication.

And of those of you who do have experience in trying to design such studies, what types of problems have you encountered?

It's a huge question, but I think it really gets at the heart of the matter of the difficulty of designing these types of studies.

So what are sort of the minimal critical

parameters that need to be studied in designing animal efficacy studies?

Anybody?

[No response.]

No takers.

DR. PITT: I guess I'll start by saying there's no perfect study. And every--depending on--I think the first decision is what do you want the therapeutic to do--obviously--and then the best study design comes after that.

In terms of combinations, each individual component's going to have to be understood before you move on to combinations. And one would hope that by doing each of those individually, you would then have a better idea about timing for your combination protocol.

DR. WOROBEC: So you think that they would need to be studied first individually, then combined and then studied together as one product? Or would it be sufficient to study each individually, show that they're better than a control, for instance--

DR. PITT: I think that will depend on what you want your product to do.

DR. WOROBEC: Okay.

DR. SCHRAGER: Yes, I think--just seconding what Louise said--in terms of the indication, I think among the most common mistakes or problems that we see in submissions are submissions that state that the intervention being studied is being planned for one type--one specific indication--say treatment--and then seeing that the studies as designed actually, at best, address post-exposure prophylaxis, and in some cases really don't even get there, and are given at or jut before the time of exposing the animals to the agent, and so would best be classified as studies of pre-exposure prophylaxis. And that's simply not treatment.

So, you know, being as specific as you can--and realistic as you can--with your indication, and then matching your studies to that indication I think is really important.

The other thing is: in terms of basic

knowledge, you know, we've seen a lot regarding anthrax, but I mean--somebody correct me if I'm wrong--but I'm not aware of studies that, for example, will tell you when in the course of an anthrax exposure toxin starts to be produced by the organism; you know, particularly if we are getting into the realm of studying antitoxins, it would be nice to know whether or not we're giving those interventions before ore after we're expecting to have toxins being produced. And if those studies don't exist, it would seem that somebody ought to do them.

I guess another study--another kind of issue--and I' know it's come up in discussions of the issue of vaccination for anthrax, and vaccination and how a vaccination would potentially impact duration of antibiotic administration--which is a terribly important operational issue when planning for potential post-event interventions. How long do spores remain viable in the lung? How long do they remain viable--under normal situations, how long do they remain viable after

you start antibiotics and then withdraw them? Ho long might they remain viable after vaccination? We're wrestling with these issues now. But these are studies that need to be done to better understand, and better design studies.

DR. WOROBEC: Okay.

So, your take-home message to a certain extent that we don't have all the information really about pathogenesis that we'd really like to have to really in some ways design these types of studies at this point in time.

DR. SCHRAGER: Well, it gets back to what, you know, I talked about with the plague issue. You know, we didn't know what pneumonic plague actually looked like. We know it caused bad disease in the lung, but in terms of really targeting a point--designing a point for intervention, and saying that we are going for--not for post-exposure prophylaxis, but we are going for a treatment indication, we wanted to have a sense of what that meant in the animal.

So, Louise did the study. We found when

the animals went bacteremic, developed--you know, symptoms; how the symptoms correlated to the actual signs of disease, and used that information for, I think, a pretty accurate determination for our studies.

I don't think we can do that yet with anthrax.

DR. WOROBEC: Okay.

DR. GREEN: I think part of the issue that you're perhaps getting at is: how much rigor and how much careful consideration given to issues such as the performance of studies under GLP has to be given to answering what can be extremely complicated and resource-intense questions--

DR. WOROBEC: Mm-hmm.

DR. GREEN: --such as determining whether something is synergistic or additive when used in combination.

And I think, as was earlier mentioned, that it's important to understand in what therapeutic setting the intervention is going to be used, and basically reverse engineer the process.

That being said, I think that various types of studies should be--can be considered in terms of their nature, in terms of their criticality, in terms of the number of resources and types of studies that are done.

So, for example, a study which combines all the elements that approximates the human situation might be done under GLP, and other studies--maybe laboratory and not done under GLP--might providing supporting information which would minimize the resources, but provide a scientifically correct--or scientifically appropriate answer to further understand how these agents work together or singly.

So I think the way to look at this issue is to break it down into component parts to determine what aspect of the problem has to be resolved with what degree of resources, rather than having a comprehensive single solution to every question.

DR. SCHRAGER: And can I just--

DR. WOROBEC: Mm-hmm.

DR. SCHRAGER: --and I agree with that. But taking that one step further, I think, if I'm standing in the shoes of a drug developer, or a biologics developer, and I'm thinking of an intervention--particularly in the more complicated case of treatment, rather than post-exposure prophylaxis--and particularly if there is the potential of using it in combination rather than individually, I think that's a perfect example of the kind of question that you need to talk to the review divisions about before you start designing your studies--or as you're designing your studies. Because it's hard to answer. It's a very complicated question to answer here, you know, in a conference. But it is something that we in the FDA would very much look forward to interacting with you who are developing these interventions, and try to hash out, to make the process as efficient as possible.

DR. GREEN: Well, let me state something as a follow-on. I think that it's a wonderful practice to come to the agency and there are people

there in various parts of it who can help in various aspects of it.

But I think that it is a necessity that people who are beset situated to develop facts to help others like the FDA to develop an approach, have an obligation to provide data and an approach for which comments can be given. It is not going to be a terribly useful exercise to come, essentially, de novo to the agency. Therefore, meetings like this are really essential to people to anticipate what are questions, and to get the data, and then, many times, the interaction between the agency in terms of reviewing divisions, will be much more fruitful.

DR. WOROBEC: Okay.

All right, I have a question. This is coming from Claire Daiquitz in CDC--to Dr. Pitt--a question on the Animal Rule.

How many animal species should a product be tested in? What are the species? And do you have references that you can provide for the data that you presented?

DR. PITT: I think that first part is an FDA question.

DR. WOROBEC: I think it is, too.

DR. PITT: [Laughs.]

DR. GREEN: I was hoping for an answer.

[Laughter.]

Well, the answer is, I think people have to use--again, this is a question of looking at the science as we know it, understanding that that may change.

One of the--the provision of the Animal Rule that's critical is that something is being given, but something's also being asked. And what's being given is approval without the benefit of clinical studies. And that is the standard that one would expect--and understanding not having that standard essentially places an obligation to make up for the shortcomings of that.

The idea of testing in multiple species is that if it is true of multiple species, it is more likely to be true of human beings. And the smaller that database is made, the more endangered that

conclusion is--unless there are offsetting factors.

So, for example, if you know that human beings are responding to various virulence factors or pharmacokinetic relationships, and that is only met by one animal species, then it's safe to conclude that if it's true of one animal species it's likely to be true of human beings.

If animal species are imperfect, then a combination to overlap, or make up for deficiencies is important. As a rule of thumb--as the rule states--two species are normally thought to be appropriate. And this is, in part, based on the fact that, as a rule, for many toxicology studies, the predictability of findings made in non-laboratory and laboratory animals will be 80 percent--70 to 80 percent--as a minimum--well, 70 to 80 percent, oftentimes, in terms of their findings, in terms of how they translate into the clinic.

That's obviously not good enough, and therefore there may be a requirement for additional species beyond the two. Exactly the number should

be driven by the science and by the understanding of the various factors that relate in the situation.

So it's very difficult to come up was a standard rule.

DR. WOROBEC: Okay. I have a question that comes back--in the first panel, one of the panelists referred to Phase 2 and 3 studies. How do Phases 2 and 3 relate to a product being developed under the Animal Rule?

I think I could actually discuss some of that. I think what they were talking--they may be talking about clinical studies, which is a separate issue. But in terms of animal studies, what they're probably referring to are some of the more advanced animal studies such as establishing PK/PD relationships, and also what we call the "pivotal efficacy studies" that would be used for licensure--I think some of the early studies we like to refer to as "proof of concept" animal studies. And it's, to my knowledge, virtually impossible to go straight into a pivotal efficacy

study for these types of products, unless I'm missing something. There's too many unknowns, and trying to pick the doses, etcetera, and understanding the pharmacokinetics pretty much dictates that usually this type of drug development does have its own distinct phases--albeit, these studies are being conducted in animals.

DR. SCHRAGER: Yes, I think that--just echoing what you're saying--what the Animal Rule does is really create a different paradigm. You know, we're so used to talking about "pre-clinical" and "clinical" as synonymous--

DR. WOROBEC: [Inaudible.]

DR. SCHRAGER: --with "animal," "human." And then once we talk "human," then we can talk "Phase 1, Phase 2, Phase 3."

But in the Animal Rule, the preclinical is the clinical--at some point; preclinical becomes the clinical. Because that final key efficacy study is not going to be done in humans.

Now, where it does become relevant--the "Phase 1" terminology does become relevant, because

obviously you're going to need to get your safety data from humans. But otherwise, the language becomes a little bit different under the Rule.

DR. WOROBEC: I have a question for Dr. Pitt--actually, I have a question myself. It has to do with non-human primates models--picking between rhesus and cynomolgus monkey. This comes up with our discussion with sponsors--which should you study?

What are your opinions on this? As the preferred animal model, or non-human primates model that best mimics human inhalational anthrax?

DR. PITT: Cynos versus rhesus.

As I said in presentation, the most data that's been done in the last, say, 14 years, have been rhesus macaques--other than the recent experiments at Battelle with the cynomolgus macaques. But if people go back and read the literature, the early efficacy studies were done in cynomolgus macaques. Brachman's studies were cynomolgus macaques.

So, personally, I don't believe there's

much difference. But I have not seen a head-to-head study done recently--

DR. WOROBEC: Mm-hmm.

DR. PITT: --with cynomolgus and rhesus in a vaccine trial, or--I think there would need to be a sort of--some type of pivotal head-to-head study to--

DR. WOROBEC: Resolve the issue--

DR. PITT: --resolve the issue, because the macaques that were used 40 years ago had different health issues, different standards were applied. And that could probably be clarified in a head-to-head.

DR. WOROBEC: Okay.

DR. SCHRAGER: I'm going to go ahead and ask a question of one of our panel members. And I'd like to ask Dr. McCormack a question.

If I were now trying to develop an intervention for potential review under the Animal Rule, and I had not done GLP studies before--I'd come from a laboratory where that's not what we did. And it seems like a pretty intimidating

process. And I'm wondering how to do that.

Could you--you did bring it up in your talk, but I was wondering if you could potentially re-emphasize, or be more specific about what specifically would I do to get some of those kind of questions answered?

DR. McCORMACK: Cut me off by 6:00.

[Laughter.]

Assuming this lab has never done non-clinical laboratory studies for regulatory purposes; they have no infrastructure whatsoever. That is a task that will require some time.

First step is management has to make the determination that they want to be engaged in that type of work, and they are going to put forth the resources to do the training, to validate the equipment, to hire the quality assurance professionals that need to be there to do the internal monitoring of the laboratory.

How do people get started? That's a fairly frequent question. The agency doesn't have resources to do--we don't do "pre-inspections," for

example. We have to rely--because we don't have the resources, we recommend that people search and seek out a quality assurance GLP compliance consultant to work with them, to help them write their procedures; to guide them through the steps they need to develop the infrastructure, to become a compliant laboratory.

And many of these consultants are quite good. And oftentimes what they'll do is do a--essentially--mock inspection of the facility as FDA would. Our procedures are transparent. They're published. And people pretty much know how we conduct inspections. And they can go through the facility and evaluate the facility and its procedures, and its equipment, and its management organization to decide whether that facility can conduct a study to be compliant with the regulations.

But, again, it is a quality management system. So it's really the infrastructure of the laboratory that you have to organize and develop. And the whole concept behind GLP is that the agency

will never have the resources--I mean, these decisions were made in the '70s--to go out and audit ever study that's ever submitted in support of an application.

There are thousands of tox studies, and now there will be many studies done under Efficacy Rule. So we developed this regulation--developed a system of internal monitoring--that a quality assurance unit that conducts the internal monitoring of that quality management system that's the eyes and the ears of the testing facility management, to give them feedback on how the facility is operating.

Our role in the process is essentially one of monitoring the monitor. We go out and check whether that quality system is, indeed, functional. If the quality system is functional, then we can believe the product of that quality system--we don't have to audit every study that comes to us. If that quality system is functional, then we can believe that the data that is coming from that laboratory, that's coming from that quality system

is, indeed, high quality data, and has integrity. And we can also spot-check individual studies, and we do a number of--many, many audits. But it is a small percentage of the total number of studies.

DR. WOROBEC: Another quick question for Dr. McCormack: does FDA have any special requirement for the SOPs of GLP? And how and when should we contact FDA to check our facility's SOPs?

DR. McCORMACK: Okay--the first part of the question is are there any requirements on SOPs? The regulation does list certain areas where you have to have--I think it's about a dozen--areas where you have to have SOPs--standard operating procedures are required by regulations.

The actual construct of standard operating procedure is left up to the facility. And we have an inventory of roughly 250 to 300 laboratories. And how people manage their standard operating procedures is probably different in every laboratory. And it's really up to the, again, testing facility management to decide how they're going to write their SOPs, how they're going to

revise them, who's going to write them, what the content should be, what the level of detail should be. That is all left up to the testing facility management and the organization to decide that we'd look for--there are certain areas that they have to have SOPs, but it doesn't address the content.

And I think the latter part of the question was what I just touched on: is we do not do "qualifying" type inspections. Laboratories come into our inventory and our inspectional process when we receive data from that laboratory in a research or marketing application--or we become aware that that facility is doing work within the scope of the regulation by some other means. You know the laboratory sometimes calls us and says, "We're getting into the GLP business. We have a study we have contracted for x, and we're conducting that work ,and we want to be inspected because that helps us demonstrate our level of compliance." And laboratories enter our inventory in that manner, as well.

DR. WOROBEC: We have, actually two sets

of very excellent questions. I'll read the first one first. It comes from Joanna Clancy of Amtex. It's more for Dr. Barnewall.

In pulmonary infection studies, have USAMRIID and/or Battelle used other strains of bacillus anthracis other than Ames?

The second part of that is: do spore preps from the different strains have differences in aerosolation to animals?

And the third part is: how are the spores typically prepared and standardized for these models?

So the first one is: have other strains of bacillus anthracis been studied, other than Ames?

DR. BARNEWALL: At Battelle we've primarily used Ames in all our studies. We haven't used Volum or any others. I know they have that at USAMRIID.

DR. PITT: At USAMRIID we've used Volum 1-B and Ames for efficacy studies. We have also looked at geographically diverse strains, and

whether they protect--they are protective--I mean, the wrong way around--whether the vaccines are protective against these geographically diverse strains. And we have done that in guinea pigs, rabbits, and non-human primates.

DR. WOROBEC: Are the spore preps different when you--you know, from these different strains? Do you have any information on that, Louise?

DR. BARNEWALL: It would be too long to go into, but all our lots of Ames at Battelle we try to characterize. And it's a very long process, but we try to make every batch as similar to the last batch.

DR. WOROBEC: Okay.

DR. BARNEWALL: And we go through a process of characterizing those, where we look at debris, and virulence plasmids to see if they're all there to make sure that what we used at one time was the same as what we're using today, so that we're certain that the strain hasn't changed with time.

DR. WOROBEC: Okay.

The next question comes from Martha Wilde of Alexion.

With the high-law variation that you see in inhalational spore doses given, is there a correlation with results when protection is being examined? In other words, is it the high dose outlie that is responsible for one out of 10 animals' dying in what might be a protective dose? Or the low-end outliers surviving even in a control?

DR. BARNEWALL: No, there seems to be no correlation with survival--or death.

DR. WOROBEC: Okay.

I'm going to let the gentleman with the microphone ask a question.

DR. LOWEE: Hi. Thank you. Israel Lowee, from Meterex.

I'd actually like to follow up on that last question. But, in general, I have a couple of questions that, as a company trying to develop an agent for use we're really, I think--many of us are

seeking kind of clarity from the regulatory authorities as to what would be required.

So, in the service of that, one question for Dr. Barnewall: you presented a lot of data on animals' being exposed to a whole range of LD 50s, from 20 up to 200. And, presumably, many of those experiments involved just control animals that were not receiving any intervention.

It would be really helpful, I think, to sort of see what the curves of survival are across those kinds of LD 50s, because one question that comes up all the time is what LD 50 should one use? I mean, 200 LD50s is 200 times the lethal dose for 50 percent of the animals. And I've seen some things on the website of the NIAID saying, well, they think sometimes they'll need 1,000 LD 50s.

It's not clear to me why that differential would be required.

So, as a first step, it would be helpful to see what constitutes a reasonably acceptable standard LD 50 to apply in these studies.

A second question is: in terms of looking

at therapeutic studies, these questions about, you know, when--trying to determine when toxin is being produced. I think one can infer from the fact that one sees protection at a certain point with antibodies that target toxin, when that issue is becoming relevant. But in terms of trying to develop a therapeutic study, would it be just reasonable to go ahead with starting cohorts of animals that get treated at successively later times, rather than monitoring animals--you know, around the clock--and deciding when they have a fever. I don't think we have animal ICUs there where we can do that kind of intensive monitoring.

So those are the two questions.

DR. BARNEWALL: The first question, on the dose--

[Pause.]

--can you repeat your first question?

[Laughter.]

DR. LOWEE: The question was: you showed a wide range of lethal dose--or wide range of exposures, from 20 LD
50s--

DR. BARNEWALL: Oh, yes. We--

DR. LOWEE: --up to 200. So what's the experience, in terms of what it does to the course of disease? They're asking about what the natural history of the course of disease is. You've just--that's a very important piece of data that would be useful to be shown, and presumably it's from control animals; it's not giving any proprietary data.

DR. BARNEWALL: That data would be able to be shown. We'd have to get permission from whoever the sponsor was. Again, it's proprietary data.

But, information that we do have that shows, again, the disease course is the same at--if you give--in a naive animal, very low LD 50s versus very high LD 50s. And so--

DR. LOWEE: So what's the rationale for giving 200 versus 20?

DR. BARNEWALL: Historical. I'll let Louise handle that one.

[Laughter.]

DR. WOROBEC: I can interject on that.

Part of it had to do with the issue of variability. If you recall, for that 200 to 300 dose, some of the animals got 43. And we know, historically, in some of these studies, these animals can end up being survivors. And for this type of a study what you really want is a hundred percent--killing of all the--

DR. LOWEE: So you want a hundred percent of animals getting a minimum--

DR. WOROBEC: In the control groups.

DR. LOWEE: --LD 50 level--

DR. WOROBEC: Yeah.

DR. LOWEE: --of some value.

DR. WOROBEC: Yeah. And the other issue that comes up is a whole experience in 2001 with the anthrax--the mail scare--that it was determined that the amount of spores that were on those letters were in the thousands range. So there's discussion that, in the event that something like this could occur again, it's possible that the doses that would be administered would be very, very high, and there should be some sort of data

out there that gives us an understanding about protection or treatment in the very high dose range--realizing, though, that these types of studies are very difficult to do technically because of the whole clumping issue.

So we're sort of in a bind with than in trying to pick a dose that we think is sufficiently high--

DR. LOWEE: Or this--

DR. WOROBEC: --to kill all the animals, yet we can deliver more consistently spores in these types of studies. So there's--

DR. LOWEE: What about the therapeutic model experiment? Can you comment on that?

DR. GREEN: Well, I would just like to go along with what Alex said. I think looking at whether it's 200 or 1,000 in some ways is right on the point, but in some ways misses the issue. The issue is to have a reliable determination of the claim of preventing death. And as we saw that there is a range around all these LD50 estimates which can be wide. And, on top of that, there's

also a sampling problem, since you're not at one time--even if you're using it under one facility at one time in the same way that you would do a mouse or a rat LD 50 experiment, getting a group size with sufficient numbers of animals, all done at one time, to really have reliability.

So each one of those LD 50 estimates is, in a sense, taken from a population of LD 50 estimates, each of which has a range around it that might have occurred because of the sample.

So I think that those--wanting to make sure the decision is correct, and given that it is what is feasible to challenge the animals at a very high level and not endanger the conclusion, the most conservative and best way of approaching the answer of getting the correct answer is to challenge the system to the greatest extent possible.

And if I can just go on to the next question, again, as was mentioned earlier, it depends on what people are trying to prove. If they're trying to say an intervention should be

used upon evidence of fever, they have to monitor their animals and devise experiment contingent upon that, because the instructions for use will be based on that.

If they're making a claim in the end that it's going to protect under other circumstances, and it simply prevents or offsets death, then that's a different claim, and a different experimental design is necessary to support that.

DR. DRESCH: Stephen Dresch, Forensic Intelligence International.

Much of the discussion of--specifically of animal models has taken place more or less by analogy; that we have a number of animal-related studies that deal with such things as, for example, the efficacy of vaccines or of antibiotic treatment, but very few focused on the nominal subject of this workshop, and that is essentially toxin-oriented therapy.

Is it true that, in fact, we don't have prior experience in this area? Is there no experience we can draw on from, for example, the

Soviet bioweapons program?

Dr. Leppla--yes--established The Washington Post as a legitimate source to cite. According to The Washington Post, some very substantial work on antitoxins has been done at the Rodoplat research laboratory in South Africa by a veterinarian scientists, Dan Gusin.

Do we know anything at all about the work elsewhere in the world, some of which appears to actually have taken place, focused on precisely this topic. And is there anything we can draw from that as we try to go forward from it?

DR. GREEN: Let me start out and say I'm sure that there is experience and evidence that exists in the world in different ways. And that evidence and that experience is used in some ways to design new studies and to guide approaches to interventions.

I think that the question that is at issue, really, is: given that experience, can it be converted or used in a way to suit the purposes which are ultimately of interest here, which is to

provide therapies to the nation, and for situations that could possible occur due to bioterrorism.

And to do that, there is another standard, and that is a standard for proof of efficacy. And that proof of efficacy is a very high standard because it has got ramifications in many ways.

So I think the answer is: like a lot of situations faced for therapeutics, it depends upon the issue at hand. And where there's a need to circumvent what would be normally expected standards, there are ways of going about that.

One of the things we haven't talked at all here are the regulatory issues that allow therapeutic agents which are considered experimental to be used under INDs. And the agency is making its way to use emergency authorization provisions, as well as experimental INDs which allow these therapeutics to be used in a setting which legally and by society we think is appropriate; that is, with informed consent if possible, or waived if necessary. But with proper monitoring, which experience has indicated is a

very important component in not only treating individuals who need it, but also in the follow-up to those individuals, if we look at the experience of pyridostigmine, for example, it was used in various settings such as Kosovo and so forth, and the question of its implications in terms of Gulf War Syndrome and in other settings were also important.

And I think, as best as can be done, people in various federal agencies, as well as out in society in various aspects, try to use those experiences, as you've mentioned, in an appropriate way.

DR. GURELIC: Yes, Ken Gurelic, from Enzybiotics.

I'd like to ask a question about development of therapeutic agents for anthrax. I'm talking about agents that would be intended not at all for post-exposure prophylaxis but, rather, for people who would be hospitalized with symptomatic disease in the event of an attack.

The animal models that have been discussed

here have been thoroughly discussed in terms of the exposure, the spore, the system, the standardization. I haven't heard anything about the actual clinical manifestations occurring in animals.

In a clinical setting--for a human clinical trial it would be very obvious. You can do a sepsis trial, you can do a pneumonia trial, because these things are well established in humans. I haven't heard anything to talk about, or to help me design studies in animals that would help to treat symptomatic anthrax

I do know some of the models that have been discussed here, the animals are infected with the agent, and the next thing that's clinically apparent is death, which is a little late to treat.

So I'd like to hear some data about animal models and the way they could be used for the treatment of symptomatic anthrax infection.

[Applause.]

[Laughter.]

DR. SCHRAGER: Yes, I think you're

basically saying what I tried to say just a little bit ago: that that particular issue of treatment of anthrax in an animal model is one where there's a lot of information that's just not there--as far as I know, and as far as you know, as well.

And this gets into what the other gentleman had just asked a couple questions ago about, you know, what would be necessary for a developer? Does each developer, then, have to do natural history studies and monitor in animal ICUs, you know, the status of the animal?

And I think the answer is that we would all be better served if there could be natural history studies--again, drawing on the kind of study we did in African greens and plague. Well, maybe it's time for a study of natural history of anthrax in African greens, and in macaques, and in cynos.

And, you know, those data are published, and then they provide the template--that's the target. There's your information. And then you can design those studies around them.

Is that answering your question?

DR. GURELIC: I guess, you know, the thrust of my question comes to: you know, we've heard reference to, you know, one major pharmaceutical manufacturer that is working in this area, but most of the companies represented here that I've talked to are not large companies. I heard somebody quote the cost of a single cyno study on the order of two to three million dollars. I suspect the cost of a sufficiently large study to establish a natural history in monkeys is going to be beyond the resources of the vast majority of companies that are developing novel therapeutics and, frankly, if I were a large company and I were doing that study, I'd want that to be my proprietary information, since I'd invested the money in doing it.

I hear of a great deal of work being done at USAMRIID in a variety of things. I here that the NIH is now getting more actively involved. And I would wonder, then, if these data are not available, are there plans to conduct these studies

in a timeframe that would help us get drugs approved within the next two years?

DR. WOROBEC: Anybody from the NIH want to comment?

[Pause.]

DR. SCHRAGER: I think Judy Hewitt's going to be coming to the microphone.

DR. NEWSOME: I'm Ed Newsome from NIAID.

Very good questions. I mean, for treatment we don't have data, but we are doing a lot of post-exposure prophylaxis animal model development, which really is not complete yet, in both rabbits and monkeys. To the extent that will be made available--public--I'm not prepared to say right now. But we are making quite an investment in that area, and we hope that these models will be much better characterized--hopefully, by the end of the year is kind of the timeframe I'm looking at for post-exposure.

But that will also have overlap in general. I mean, just the improvement of these models will apply to everyone, whoever wants to use

facilities. I mean, at some point this information will be published, and I think it will become general knowledge. Quite frankly, right now, we don't have the final answers.

And then just another general point I wanted to make here--because we're kind of transitioning from animal models to human testing. And the basic underlying concept here has been danced all around, but I think I'll try to say it more directly: the Animal Rule is really about people. If you look at the major components, almost everything refers to people. So you have to know what's going on with your product in people.

So to me, that means you need to get it in Phase 1 as soon as possible. If it's an immune product, you need to be thinking about plasma-pheresis at Phase 1. If it's a small-molecule, a drug, you need to get extensive PK data.

So then--to answer the initial question from the panel here--what should the parameters in the animal models address? Many of those

parameters are driven by what you know about what happens in people. So I think that's an underlying concept. It's fairly straightforward, but I think it's one we need to keep in mind.

DR. WOROBEC: We have time for one last question.

DR. NAST: Dr. Merrill Nast, addressing these remarks to the FDA, and Dr. Schrager.

I think the Animal Rule needed to be written, and as written is excellent. But I think that the way FDA has applied it for pyridostigmine bromide licensure, for instance, is not proper because it was used in a vacuum.

Although you provided information that's been available for decades on the pathogenesis of nerve agents, and explained how this drug will work perfectly in the setting that you described, that in fact is not the setting in which it has been used in the military.

Military people used it for serin--not soman--where it may, in fact, potentiate the problem. People were aware that there was no soman

in Iraq. It was used in conjunction with other things, including pesticides, jet fuel, etcetera, which have been shown--in now approximately half a dozen different studies from several different institutions--that the use of these other things at the same time potentiated the negative effects.

And so, the licensure ignored that data. And, I mean, you're still the FDA, and you have to be clinical and use your head, and make sure that this product is going to be used in a way that's effective and safe.

For anthrax, the rabbit and the monkey models are excellent in many ways. But the rabbit and the monkey are both almost certainly more susceptible to getting the disease anthrax, and immunize better than the human. And therefore testing vaccines or drugs in those two animal models will probably make the efficacy of the drugs and vaccines look better than they will, in fact, be in the human model.

And I think that conundrum needs to be resolved before you go on to licensing using that

data.

DR. SCHRAGER: Okay. I can--I think--I'm certainly better prepared to answer the anthrax question than the pyridostigmine bromide question.

And, you know, for anthrax--I mean, clearly, what everyone wants are safe and efficacious treatments--interventions. That's what we want. Everybody wants it. Now, the question is how best to get there under the circumstances that we have.

For anthrax, you know, we've heard a number of presentations about the animal models. The approvals for cipro and doxy were important for inhalational exposure. And, you know, as--clearly, we'd like--if you believe that the animal models were problematic, that were used as the basis for those approvals, I guess we can draw some degree of comfort from the actual reality of the experience in the October 2001 mailings, where upwards of 10,000 people were deemed sufficiently exposed to require post exposure prophylaxis, who were obviously asymptomatic. And of those 10,000 or so

people, exactly zero got sick.

Now, some of them also got vaccine. But some of them possibly were exposed to hundreds, if not thousands, of LD
50 of anthrax when they opened the envelopes in the Senate office buildings.

So--you know, we don't know exactly know precisely how good it is, but it was pretty good. So I think we can all draw some comfort from that.

You know, for the pyridostigmine bromide experience, I'm just going to have to say: I wasn't here for the--I don't know the history of it. And I'm wondering--Brad, do you want to say a word or two about what--add a comment about the nature of the approval and the applicability of--the issues that were being raised?

DR. LEISSA: Brad Leissa, Center for Drugs, Division of Counterterrorism. When I finish with my statements please, if you have any as well.

All I can say to that is that there's a long history with pyridostigmine bromide, and that the way the product is labeled is that it was indicated specifically for its use in soman. I

can't address the issues that the person raised about its use with serin.

But with anything that is either approved through the accelerated approval regulation, or through the Animal Rule, there is the understanding that not everything is known about how a product works, from a safety and from an efficacy standpoint.

Therefore, what is incumbent on any product that goes through that, is that there needs to be a commitment for Phase 4 studies to better understand how this product is positioned and how it should be used.

So, there is never the belief that everything is answered when a significant amount of the data comes from either animals or from other surrogate markers. And one does the best they can with the type of data that they have, and where one has the authority, the regulatory ability to make those decisions.

So--David, do you want to say anything more?

DR. FRUCHT: With regard to serin, the 2-PAM and atropine are quite effective against serin intoxication. Soman is a particularly difficult threat agent to treat, because of the aging phenomenon, and that is specifically why pyridostigmine was developed: to address that particular aspect of that OP toxin.

With regard to any approval, it's always subject to the conditions that it was tested and labeled for. And as FDA knows, that even any regular approval based on clinical experience is limited once it is used in ways--in the general public, either because of the numbers of individuals or their conditions that might be unforeseen.

I think that your point about due diligence being incumbent in recognizing potential interactions is a good one. And I think to the best that people can, given that this is a moving target, that they will try to study the potential interactions to demonstrate whether there's a safety issue, or loss of efficacy issue, or some

relation to efficacy that is not fully appreciated.

In the case of pyridostigmine, there were many, many studies that were conducted, although in the absence of field exposure of threat agents, to try to understand potential interactions being pyridostigmine in a variety of military situations. And there were many physiological studies that were submitted to the file and are referenced in the open literature as well, that demonstrate to that fact.

And as Brad was indicating, recognizing that a story is never complete, there--inherent in the Animal Rule is a requirement to do post-approval studies under field conditions, and to make a good faith effort to collect data that might help better understand both safety and efficacy of it in real use.

DR. WOROBEC: I want to bring up just one question and point, that's something for the audience and also for panel members to think about.

We talked about efficacy, but the other flip side of this is evaluating safety, also in

animals.

Will reprotox, carcinogenicity studies be required for these types of products? Just something to think about.

DR. GREEN: Well, those are safety aspects. And they can be done. So--yes.

DR. WOROBEC: All right

DR. GREEN: But they would be done in the same way that any approval would be, given the population and their ration of use.

DR. WOROBEC: Okay.

All right, I think we're going to have to wrap up this session. And I want to thank all the panel members and the presenters for their excellent talks.

[Applause.]

DR. WEISS: I'm just going to have Dave Green stay here, because he's on the hot seat again, next.

That was a very, very lively discussion, this last session; raised a lot of questions and, I think, highlighted a lot those still yet to be

evaluated, and a lot that we don't know.

PART IV - Human Testing

DR. WEISS: We're going to be moving, then, into the next phase of this conference, and that is the section that deals with human testing that needs to be done, certainly in conjunction with the animal efficacy testing. And then, to kick off the session, after he gets replenished here with some water, is again, Dr. David Green. You already heard his introduction, so I won't do that again.

But, Dr. Green--please.

Clinical Pharmacology and the Development of Products for the Treatment of Anthrax

DR. GREEN: Thank you, Karen.

[Pause.]

I think I'm ready. Okay.

Well, good afternoon. Today I'm going to very briefly go over some of the aspects of clinical pharmacology as related to the Animal Rule, and hopefully I will give you some idea of the regulatory perspective that is incorporated in

the Animal Rule with regard to the need to conduct clinical pharmacology studies with an idea of understanding, basically, safety, and building the bridge between animal studies to the potential goals of getting approval for the clinical situation.

[Slide.]

So, basically, there's two parts to this presentation: one is that the clinical pharmacology aspects work within a framework which is the regulatory environment for approval, and they also provide a framework in terms of establishing relationships between the animal findings and potentially what will occur in the clinical situation.

[Slide.]

Now, in building the framework based on clinical pharmacology data, we have three basic issues that we need to consider, and they have an interrelationship between them, which you'll see in a diagram at the end. And that is that the drug aspects of it; the patient aspects of it; and the

infectious organism aspects of it.

Now, one advantage of working within an environment of clinical of clinical pharmacology aspects of it. Now, one advantage of working with an environment of clinical pharmacology is that although we demand a lot from it, we have the potential of getting a great deal of information out of it. And in some ways we need that information to study populations which will not be easily accessible, or in fact will just have to make the best faith estimates in terms of dosing and regimens that would provide them benefit, based upon other human--extrapolations to human populations; specifically, those which have impairments of renal or hepatic function, or pregnancy, or juvenile states, or geriatric populations. And, importantly, as we mentioned earlier, considerations for drug-drug interactions themselves, in regard to therapeutics will oftentimes be approximated through clinical pharmacology studies.

[Slide.]

Now what's that framework that clinical pharmacology works within? And that is basically the provisions for approval which--I think key words and well-controlled animal studies, as cited in the Animal Rule. And let's look at those aspects of "adequate and well controlled" to better understand what aspects of clinical pharmacology will be looked at.

One of those we've already mentioned: that is, looking at suitable subjects. And we've talked about the advantage of studying clinical pharmacology because we'll gain a great deal of information from studying, perhaps, a normal population and then perhaps extrapolating that data to other populations also of interest.

But in the next points--that is, minimizing the potential for bias, reducing confounding factors--those two aspects bespeak a scientific quality which become part of the underpinnings that clinical pharmacology studies will provide. And that is, there's a high scientific standard that's required for approval

under the rule--the Animal Rule. And the clinical pharmacology studies, in essence, validate that we do understand this appropriately.

One of the aspects of understanding that we have not had any bias introduced into the animal efficacy studies, and we've reduced confounding factors, is the appearance of a well-behaved experimental model. And that is a chief source of problems, and has been for pyridostigmine--and that is, when our expectations are not realized and animals that we expect to survive die, and animals that we expect to die don't die, due to reasons. And perhaps clinical pharmacology will provide an avenue to explain these things in a way that makes a consistent whole out of the data.

Another aspect under the definition of "adequate and well controlled" is it permits a quantitative evaluation. And clinical pharmacology comes to us in terms of providing dose-response relationships.

"Uncontrolled studies that are corroborative and supportive" essentially means

that all the studies that were done--in this instance, perhaps non-GLP--all point in the same direction; so that the literature, when analyzed as a whole, is consistent.

And as I mentioned, I think that the framework for effectiveness of the animal studies for clinical pharmacology is, in part, based on the mechanism of action; that is, the prevention of injury or death and the therapeutic effect. This mechanism of action, to the extent that it's know and understand--and that can be a simple to a complicated relationship--will become the vehicle, in terms of the collection of data, the type of data, that we will have to establish in the animals, and then use to establish that it also occurs in people; and also be proportionate in terms of response.

So, again, the clinical pharmacology endpoints provide that bridge between the animal species and human populations.

A problem with this, obviously is that it can be well understood in one model--one

species--and less well understood in a second model. Yet it's incumbent in the rule that we establish it in perhaps two or more cases, therefore increasing the challenge.

So the bridges that clinical pharmacology hopes to span are between laboratory animals and humans. But, also, within laboratory animals, in terms of cross species, and between groups of animals; between intra-subject, if you will, in those animal populations. And the discordances between these events--that is, one strain varying in terms of its responsiveness, or one animal not responding in the way that we expect, are significant impediments to making a conclusion that we understand fully that we do have an efficacious model, without exception, that can be applied to the clinical situation.

But besides these interrelationships and bridges, we expect that clinical pharmacology, if properly used, will also allow us to consolidate the data--no matter what the route of administration that was used, what the dose that

was used, and what the dosing regimen that was used.

There are two basic intellectual constructs that are instilled into the use of clinical pharmacology within the Animal Rule. And they are: the use of surrogate markers, as in accelerated approval, and therapeutic drug monitoring.

Now, clearly, the Animal Rule is not accelerated approval. And the use of clinical pharmacology end points is not a surrogate marker. Nevertheless, I think it's instructive to realize that there are elements, in terms of the scientific rigor, that are thought about in terms of a sum that are imbedded into the concept of using clinical pharmacology end points to create these bridges. And I think it's important, historically, to point out that pyridostigmine was originally considered under accelerated approval, using surrogate marker of acetyl cholinesterase inhibition. And when the Animal Rule was available, then converted to the animal approval,

which it is today.

Importantly, for the use of clinical pharmacology end points that has come from the surrogate marker literature is causation; that is, it is not the best standard to use a clinical pharmacology end point which is not necessarily involved. You should use one which is necessarily involved in the mechanism of action. That is the one to choose, in terms of using a bridge from the animal studies to the clinic.

And another aspect is proportionality; that is, dose response. And, historically, this is one of those backstops to making sure that we have a valid conclusion--proportionality. And in this case, dose response.

When things go awry, it is usually due to a dysjuncture in the theory. And that is, after all, what we're creating: a theory of what we believe will occur.

The other aspect is the therapeutic drug monitoring. And from this literature, into the clinical pharmacology bridging, we have an emphasis

on relationships and predictability with regard to efficacy and toxicity; an emphasis on analytical methodology and the importance of assays and sampling time; and mathematical characterization. And, as I mentioned, the relationship to be understood, both simple and complicated--the mathematical relationships may be simple to complicated.

Therapeutic drug monitoring also brings to us target levels, both in terms of using dose and dosing regimen, to achieve those target levels. In the case of infectious agents, oftentimes it's the floor effect that we're looking, in terms of the minimum inhibitory concentration, and we need to think about it in terms of whether it's concentration-dependent, or concentration-dependent mechanisms.

Oftentimes a ceiling for these approvals will be more related to the tolerance of human beings in the setting and the field setting that they'll be found in, and this will come from the safety data in relationship to the drug exposure.

[Slide.]

This idea of performance bands--that is, ceiling to floor in terms of its effect--again runs into trouble when we cannot--when we don't have a well behaved or consistent theory; that is, when we have treated animals which should have drug exposure, and they don't seem to survive, or conversely, we have challenged animals who should have died by don't die, or we can't demonstrate that they died for the reasons that we believe.

And a logical out of this situation is not to emphasize the individual, because those will range in terms of sensitivity both to the threat as well as to the therapeutic agent, but to emphasize the group response.

Nevertheless, there are additional aspects of clinical pharmacology that we shouldn't forget, and that is we'd like to know exactly what's going on at the site of pathology, which will be difficult to understand, but might be accessible through other studies, such as the PK-PD modeling, or by distribution studies. And, of course, we'd

like to know that it works in every instance that we think the challenge may arise, and that would be captured in terms of various isolates.

Now, I mentioned there were three basic factors that we can think about in terms of developing your concept to fit all our clinical pharmacology together. And those include patient factors, drug factors, and infectious organism factors.

Host factors we can simply lay out, and have been mentioned by earlier speakers. They include the route of infection, the ability and importance of various host defense mechanisms; pathophysiological pathways; signs and symptoms of illness; as well as pharmacokinetics.

Pharmakodynamics aspects might be the affinity and intrinsic activity of the therapeutic; mechanism of action, as well as the toxicity of the drug.

And, in terms of disease factors, or disease organism factors: virulence, the type of isolate, propensity to germinate, and expression of

virulence would all be factors.

[Slide.]

Here is the conceptual model that I'd like you to think about. And, luckily, I didn't have to develop this model. This model can be consulted and looked up in Applied Pharmacokinetic. Evans is the editor for it. And you'll find it in Chapter 15.

But I think as you think about creating designs and developing a drug development plan to develop these agents--these therapies=--using clinical pharmacology, I think this is a useful model to begin to put the pieces of the story together.

The other tasks that you should pay attention to include drug-drug interactions, including vaccines and hyper immune globulins, and importantly, is to consider whether we're talking about a fixed combination or something used in conjunction with other therapies, incompetent therapies, and whether we're asking, or we need to ask the question as to whether additivity,

synergism or antagonism is the issue.

In conclusion, this has been a brief introduction into the subject of clinical pharmacology. I think that you'll find that it's an important and integral part of any application using the Animal Rule. And I wish you good luck with your endeavors in that regard. [Laughs.]

[Laughter.]

[Applause.]

DR. WEISS: Thank you very much, Dave.

And now I would also like to introduce Dr. David Ross, ask him to come up to the podium, and give the second talk in this section. Dr. Ross is going to be talking about just issues in clinical safety testing in healthy volunteers.

Dr. Ross--I have the pleasure of saying--has now joined me in my office in the Center for Drugs as of April. He's an infectious disease doctor. He trained at Yale University. And I'm very happy that he's actually joined me and works with me very closely at the FDA, and has agreed to give this talk.

General Considerations for Safety Testing

in Healthy Volunteers

DR. ROSS: Thank you, Karen.

I am acutely aware that I am all that stands between this audience and a caffeine infusion. So I'm going to try to be concise, but also thorough. I'm certainly going to achieve the former. If I fall down somehow in the latter, then please ask me to amplify things during the panel discussion.

I'll give you an overview of how we think about clinical safety testing at FDA, and how we think about the design of clinical safety studies.

And I'm going to go through a number of aspects of these, ranging from effects, to how many people should be studied--or how we should think about how many people should be studied; how we should think about who should be studied; what we should study them for; how to pick doses and regimens; and then what sort of data we need to capture, analyze and present; and then, finally, talk about post-approval safety assessment.

[Slide.]

I'm going to be talking about a number of general principles, and I think it's important to remember why--like everything that we've been talking about today--this is really a dialogue between everyone involved in this endeavor. So it's not--this is certainly not the end destination. And I'd just like to echo Dr. Schrager and Dr. Green in terms of saying that we really, really want to work with the development community, and we're very anxious to have productive discussions. And we think that that will be one result of--or one important result of this conference.

So, the Key points that I want to bring out--and I'm trying to keep it shot and sweet, because I know--I certainly am suffering from caffeine deficiency right now.

[Laughter.]

Just to echo what people have said before: the Animal Rule requires clinical safety trials, in addition to the efficacy studies done on animals, in order for an application to be approved. And

the FDA review--and really, FDA thinking--focuses not so much on risk, but what is the equation between risk and benefit?

And then, finally, designing safety trials centers, a) on describing risk accurately; and who the agent is going to be used for, and what it's going to be used for.

[Slide.]

There's a lot of different audiences for safety data: patients, providers, public health community, various regulatory agencies, the development community and the public policy community. And they all bring their own conceptions and perceptions and, sometimes, misconceptions to what safety is.

Actually, if I could suddenly wave a magic wand, I might do away with the word "safety." Because I think really what we're talking about is "risk assessment." I think it's important to keep in mind, for example, that the most common cause of drug-induced liver failure in this country is an over-the-counter drug--acetaminophen. But is it

safe? Well, it depends what you mean by "safe." It certainly has risks associated with it. We consider it safe enough for over-the-counter use.

[Slide.]

So, really, the goals of "safety" evaluation are to describe risks; what sort of risks; what their incidence is--and I'm going to keep focusing on this issue of incidence and quantitative risk description, not in the sense, necessarily, of precise risk description, numerically pinpoint with precision, because that's not possible, but certainly quantitative.

And we get that from a number of sources: animal toxicology and structured clinical safety studies--which I'll talk about more in a minute.

[Slide.]

Risk-benefit assessment, which is really center. And I'll expand on that in a minute. Risk management--identifying risk factors for toxicity, and then ways to mitigate or minimize that risk. And then last but not least, communicating risk. And we think about this in terms of products, in

terms of the package insert, for example--black box warning; for investigational products, investigators' brochures; and for marketed products where new toxicities emerge, "Dear Doctor" letters and FDA advisories.

I want to emphasize that although I'm going to be focusing on safety and risk assessment insofar--talking about NDA and BLA review, these same sort of principles may very well be applicable to risk assessment in the IND phase, or under emergency-use circumstances.

[Slide.]

Okay. So what do we factor in, in terms of risk assessment and risk-benefit assessment? Well, first and foremost--to echo what's been said before--what is the agent going to be used for? We bring a much different perception to an agent for athlete's foot, versus one for invasive aspergillosis. What is our estimate--and, ideally, quantitative estimate--of the treatment benefit, either in terms of survival benefit, number needed to treat or prophylax, if we're talking about a

prophylactic indication?

Who's going to be exposed to this? In terms of numbers, and the makeup of the population. If we're talking about a population exposure that is on the order of thousands, our risk-benefit equation may be much different than if we're talking about, for example, an anti-microbial that may be prescribed for tens of millions of courses a year.

How adequate is the safety data-base that we have to describe risk in the real-world population? Anda this gets not just to the accuracy of safety and risk descriptions, but also the precision. How much uncertainty is there?

My brother's a business consultant, and he's told me that he hates uncertainty. Quantifiable risk he can deal with, but he hates uncertainty. And that's--I think in terms of safety assessment, is a good way to think about it. There's always risks, but we want to have as little uncertainty as possible about them.

What are the risks of other products for

the same disease? One indication, for example, for priority review is if you have an agent which is substantially safer in terms of an important toxicity than existing agents.

What are the risks of structurally similar compounds?

And, finally, how able are we to communicate risk via labeling?

[Slide.]

I think a central issue is that if there are greater risks for a product, then greater benefits are required to justify those risks. And, conversely, greater benefits justify greater risks

Amphoterecin carries a substantial risk of nephrotoxicity in any patient who gets it. But we tolerate that risk because it is live saving in invasive aspergillosis and other serious micoses.

It's important to remember that during the course of development or product life-cycle that the risks may change. You can see new risks in new populations. And then as you get to larger populations and larger exposures, rare risks

emerge. And these may change the benefit-risk equation. Or they may not. We may say the benefit's still there. The risks have emerged. But we still think things support use of this product.

Benefits may change. They may decrease in sicker patients, or in less sick patients. And it's important to remember that efficacy in trials that are carefully controlled and done under--I don't want to say "ideal" circumstances, but under less chaotic circumstances than the real world, is always greater than effectiveness in the real world.

Just as an example of this, I want to consider TNF receptor fusion protein, which was studied for septic shock some years ago. And people are probably familiar with this story, but this was a therapy that was effective in animal models. It was reasonably safe in healthy human volunteers. So this is really sort of the data base that we would be looking at for an Animal Rule approval in some respects.

And then it went into pivotal trials, with the theory being that as you give greater and greater doses, it would have a greater and greater effect on mortality from septic shock. And that was correct, but not quite the way people thought. It increased mortality in patients; that as you gave more of the agent, the patients were less likely to survive.

And the point of this is not that we shouldn't have the Animal Rule, or that we can't extrapolate. It just means that we need to have a health respect for the limits of extrapolation. And we need to always be open to acquiring new data about efficacy and safety.

So, how do we think about reviewing a safety data base at the FDA? What do we look for.

[Slide.]

And this is a very quick summary.

We first think: where's the data coming form? We look at controlled trials, uncontrolled trials, case reports--for example, from the MedWatch system. And these data may come from a

current application or from previous experience with the drug.

We try and characterize the safety population, in terms of its various characteristics. We look at the control group. We want to know what the extent of exposure was--both in terms of doses and, ideally, in terms of the pharmacokinetics.

We look at clinical adverse events, ranging from the most serious--death and serious AEs which are, of course, the major risks that may offset benefits--through discontinuations and then non-serious AES. And we try and characterize these quantitatively and also say "Is this something that is a chance even that just happened to be temporarily associated? Or is there a real causal connection?"

And in terms of seriousness, is this something reversible or irreversible.

Finally, is this something where the risk is spread evenly throughout the study population, or the potential intended population, or is it

something where it's restricted to a particular risk group?

We look at laboratory data, looking at measures of central tendency to compare groups, and look at outliers.

And depending on the class of drugs, or biologics, there are specific risks: immunogenicity, for macromolecules; hepatotoxicity and QT prolongation are examples of two possible toxicities for smaller molecules; and drug interactions.

[Slide.]

Under the Animal Rule safety has to be established for approval, by which we mean clinical safety. And this is established as for non-Animal Rule NDAs or BLAs. And no FDA talk would be complete without citation of the CFR. So those are the relevant sections.

It's also required for approvals under the Animal Rule that post-marketing safety and efficacy studies be done in patients with the disease, when they're ethical and feasible.

[Slide.]

So the sort of general questions that we think about when we're trying to advise sponsors about safety testing is: what should be done when? How many subjects should be studied? Who should be studied? Ewhat dose should you start at? How high should you go? What toxicities should be examined? And how should the data be analyzed?

So those are the goals; the question we want to answer.

[Slide.]

The problems are generally: making sure we describe risks accurately--and in a lot of ways this can be much more difficult than describing efficacy in a population; detecting rare but serious events; assessing causality; and then, finally, extrapolating to who's really going to get the drug out in the real world.

The specific challenges under the Animal Rule: at no point in the clinical safety testing program is there going to be any benefit to volunteers. And that's not completely unique to

the Animal Rule, as I'll show you in a minute. But it certainly is something that complicates things.

We won't have any data on drug-disease interactions. And as you saw from the data I just showed on TNFR fusion protein, you can get some nasty surprises in terms of those sort of interactions.

We won't have PK-PD data in ill patients, and we know, in a variety of venues, that pharmacokinetics and pharmakodynamics can be different in ill subjects compared to healthy subjects.

And, obviously, it's the increased uncertainty about the real risk-benefit balance is.

So what are the kind of factors that we need to take into account when we're thinking about designing studies in healthy volunteers?

[Slide.]

And I'm going to go over each of these areas: ethical issues, minimizing bias, sample size, who should be studied, in terms of study population; what kind of evaluation should be

planned? And picking the dose and regimen.

[Slide.]

Let me start with ethical considerations. It's important to remember at every stage that there's no benefit to healthy volunteers. And this is certainly front-and-center on sponsors' radar screen. Certainly, for traditional drug development programs, and certainly in Animal Rule programs. And it's important to remember that there are other sorts of development programs where you have health volunteers exposed to potentially toxic medications. Anti-infectives are one example. There certainly are others. Not every--I mean, we think of oncology, where we're not going to give very toxic drugs to healthy subjects, but there certainly are other areas--as I mentioned, anti-infectives, where we start out with healthy volunteers.

But because there's no benefit, risk minimization is critical. And written informed consent is central. And "informed" is really important here. It's important that investigator

brochures be as complete and accurate as possible so that investigators can obtain truly informed consent from subjects.

The investigators really have to know what they're doing and be committed to subject protection; be comfortable and experienced--or at least knowledgeable about good clinical practices; and understand what's required of them under GCP and protocol.

IRB approval is obviously a key part of this process. And, in some settings, a data monitoring committee may be helpful, especially if you have a blinded trial.

[Slide.]

In terms of making sure that we're getting an accurate risk description, it's important to try and minimize bias. There's a lot of potential mechanisms, Probably the most important is that you've got multiple adverse events that could occur that are going to be described differently by different investigators or patients.

There's uncertainty about whether an AE is

due, for example, to a particular agent. There are similarities between AES or different manifestations of the same AE. These all create problems in terms of saying, "What is the real risk? What is it's real frequency?"

There's a variety of measures that I think can be extremely helpful in terms of minimizing bias: having a concurrent placebo control group--in some settings, of course, an active control may be needed, but having a control group as a check on frequencies is important; randomization; blinding; pre-specifying safety definitions and evaluations; and following GCPs.

[Slide.]

Now, let me get to a central question. How many patients should be studied in a safety trial? And this is really a question for which there is no good general answer. But let me take a stab at some general principles that hopefully will be helpful.

I think the first question to ask--just to echo what Dr. Green said earlier--is: who's the

intended population? In other words, what's the intended use? Is this a prophylaxis population where you're going to be giving it to many health subjects? Are you going to be giving it in a mass casualty setting? Or do you anticipate relatively few sick patients? Because, as you remember from my risk-benefit slide, that's really going to drive how we assess risk and benefit.

For the intended use, and the intended population: what serious event rate is clinically acceptable? As I said earlier, a larger benefit may support a higher risk. Conversely, lower risks may be unacceptable if they outweigh the benefit. Anti-neoplastics are among the most toxic drugs we have, but they remain on the market because they have a huge benefit. On the other hand, there are plenty of drugs that have been yanked because they have toxicities that may be lower, but their benefit--certainly compared to other agents that were available--outweighed the risk. I'm sorry--the risk outweighed the benefit.

I think it's important to remember the

potential population exposure. Even if you have a very low mortality rate, if you're giving an agent--associated with an agent--if you're giving it to a lot of people, you can have a substantial number of deaths.

[Slide.]

And then finally, the acceptable risk may depend on comparison with other treatments. In terms of saying "what is that clinically acceptable risk? And what do we need to do to detect it?" the rule of three is helpful. And this is just a rule of thumb that many people are familiar with, saying that if you want to exclude an event occurring at a frequency of at least 1/N, with 95 percent confidence, you need to look at 3N patients. So if you want to find an event that occurs at a rate of 1 in 100, you need 300 patients. And I'll amplify on that in a minute.

And some of the caveats about this are: it assumes that you have a very low background event rate; you have a reasonably large sample size; and that the sample that you're looking at is

representative of the population that you're interested in.

So, for example, as I said, if you want to detect an event with 95 percent confidence, that occurs at a rate of 1 percent, you need about 300 patients--roughly. If you don't see any events in that 300-patient sample, you can be sure--confident, sorry--with 95 percent confidence that that event does not occur at a rate of 1 percent or more. And, similarly, for higher rates or lower rates.

Now, this is complicated because in real life there is a background even rate.

[Slide.]

This is taken from the package insert for Pavlizumab, which is an anti-RSV monoclonal antibody. You can see, if you look at the most common adverse events--which are not particularly serious, as these things go--we've got what look like very precise estimates for the biologic, but there's also substantial background rate. And, in fact, if you were to calculate a nominal

p-value--and I say "nominal," because with multiple comparisons like this, and lack of a pre-specified hypothesis, it's not really meaningful--there's really no difference.

If you, in fact--remember I said that a 1 percent detection rate requires about 300 people. Well, if you have a background--if you want to see if your drug is 2 percent versus 1 percent placebo, and you want to see if that's real, you actually need about 5,000 patients.

And I want to be very clear about this. I am not saying that we need 5,000 patients in a safety study, or 50,000. What I am saying is: we need to have a healthy respect for the limitations of data, if you have an underpowered safety study. And virtually all safety data bases are underpowered. That's been my experience in eight years at FDA, and it's simply a fact that you need very large numbers to detect rare events.

For example, if you look at ciprofloxacin--not at the common adverse events like dizziness or nausea--the things that we really

might be very worried about, like anaphylactoid reactions, they're very, very unusual. You're not going to see them until you've had a lot of patients. And again, I want to emphasize, we are not looking for a safety data base, before approval, of 250 million people. I don't want to get quoted in the Wall Street Journal tomorrow on that.

But I do think it's important to recognize that you're simply not going to see events at these rates in a typical development program.

It's also important to remember that even though you do see these rates, that does not mean the drug is unsafe. It means there's risks, but it means that we think those risks are justified by the benefits.

[Slide.]

In terms of who should be studied--in terms of the make up of the populations--remember, again: there's no benefit to subjects, so that even low risks need to be considered carefully, and informed consent is critical. Under healthy

volunteer studies, we normally see healthy adults, not pediatric, not geriatric, with balanced sex and racial distribution. That's actually a requirement in terms of application submission.

We generally do not see patients initially studied who have co-morbid conditions, because of increase in risk, as well as the fact that this may confound safety assessments. There may also be specific exclusions, depending on the product; for example, IG IV--patients who have thrombosis may not be good candidates initially.

Later studies are really going to depend on the intended use. Children, elderly and so on. Drug interaction studies--and I wrote "small molecules" here, but it's important to remember that there's potential interactions with large molecules. There's certainly literature showing that after influenza vaccination you can see changes in drug metabolism. And I think that has a bearing on the questions today about anthrax vaccination with concomitant therapies.

The elderly are a problematic group to

look at.

[Slide.]

This is data from the Harvard Medical Practice Study, just showing that as you get older, your chance of having an adverse drug event rate goes up. And there's a variety of reasons for that.

But you can get surprised. And that's why we want to see a well balanced distribution in terms of age and gender.

[Slide.]

This is from an approval for a recent ketalide--telithromycin--for respiratory tract infections. There's an increased risk of visual events, which are quite significant--such as blurring. Patients are actually advised not to drive when they get this drug. It's a very significant p-value--nominal p-value. But if you look at the risk, it's not the older individuals. It tends to be younger women where the bulk of the risk occurs.

[Slide.]

In terms of what sort of safety evaluations are important, clinically: structured clinical interview; looking at vital signs, physical exam; there may be product-specific evaluations--for example, infusion reactions for IG IV.

In the lab, under the Animal Rule, certainly pharmacokinetics are critical, both in terms of establishing efficacy in conjunction with the animal studies, as well as coming up with exposure toxicity relationships; A variety of lab parameters.

And then, finally, for large molecules, immunogenicity is a key concern. And I won't go into this in detail, but it's something definitely to have on the radar screen. And the reason for that--and I'll just go over this quickly--

[Slide.]

--this is an example of re-administration of abciximab, showing that he risk of thrombocytopenia is dramatically higher in those individuals who have pre-existing antibodies before

re-administration.

[Slide.]

In terms of what dose to start out, there's a couple of different strategies. And, again, there's no general rules, but things are driven by what the safety factor is that you derive from animal studies, and then there's a couple of different pathways, trying to define a maximally tolerated dose in humans; going back into the animal model and saying "What's the pharmacologically active dose?" And then saying, "Is the MTD greater than the human equivalent dose of the PAB?"

Or the sort of opposite tack--and it's going to depend on the exact development program, which sort of pathway makes the most sense.

In terms of escalation, there's a variety of factors that go into this, ranging from pre-clinical toxicology to human safety results at lower dose cohorts, to specific concerns from biologic products. I've talked about immunogenicity. Certainly, IG IV has a number of

toxicities. And Jenny Riemenschneider talked about this earlier, so I won't go into this in detail.

[Slide.]

So basically the schema for dose evaluation starts with pre-clinical studies, but it's important to recognize there's going to be--under the Animal Rule--a back and forth between animal studies and human studies.

[Slide.]

in terms of what sort of data--how data should be captured and reported, it's important to have structured case report form; to have good investigator training; to have pre-specified safety vocabulary, like MeDRA; standardized coding rules--and I'll talk about that in a minute; standardized severity scale that's designed for healthy subjects, not something for oncology patients, for example, who are willing to accept more toxicity; quality control and quality assurance; and then, finally, thinking about electronic data submission.

[Slide.]

In terms of how adverse events are captured, this is the ideal, where you've got related terms that map to a single, logical term.

In the real world, this is unfortunately what happens. This is certainly not what we want to see happen. You can see that this mapping is not ideal by any means.

So this is what we want to avoid. This is what we want to get.

[Slide.]

In terms of how clinical events are analyzed, we start out by looking at deaths, SAEs and discontinuations; try and analyze the frequency of these; look at the exact details and say, "This is really where we're going to detect a rare event that might not support the benefit." So we'd look at causality, looking at time relationships, trying to integrate pharmacokinetics, biological plausibility, and then move on to non-serious adverse events--same sort of paradigm.

This is actually, in terms of causality, where a placebo group can be quite helpful. You

would expect that if there's a causal relationship that you would not see as high an incidence in placebo-treated patients as you would in the active study drug.

In terms of laboratory event analysis, it's important to have pre-specified normal ranges, pre-specified significant changes. To summarize the data, in terms of descriptive statistics, what are the means for the study group and control group? How often do people in each group develop abnormal laboratory VALUES? What's the latency for developing an abnormality? And looking at a variety of subgroups, both demographic and--if you have more medically complicated patients, patients with co-morbid conditions.

And then, finally, looking at individual outliers and saying "Is there any information we can glean there?"

[Slide.]

In terms of post-marketing safety evaluation, the goals are really definitive evidence of safety and efficacy. We want to get

safety data from a broader population, focusing on patients with disease, as well as special populations, and populations receiving concomitant meds. Try, ideally, to get PK data from a broader population.

Some of the challenges that you're aware of are that there's unpredictable epidemiology of bioterrorism events. There's difficulties in terms of figuring out who has got a specific disease in a timely fashion; difficulties with follow-up; difficulties implementing a protocol; and difficulties collecting information.

[Slide.]

So, I think the important point here is that it's important to plan in advance; design protocols and CRFs--and I've listed a number of issues to think about, in your handout.

"Advance discussions with FDA and other public health agencies" is an understatement. And I think it's important to recognize that FDA, and CDC have a post-event surveillance working group for which I believe the lead unit within FDA is the

Office of Counterterrorism in Pediatric Drug Development. And you heard from Dr. Schrager about that already.

And then I just want to suggest that people look at some suggestions in another CT-related guidance on Developing Drugs to Mitigate Complications from Smallpox Vaccination, about possible guidance on this area.

[Slide.]

So let me summarize by saying that we really focus, in our review, on the risk-benefit ratio; designing a safety evaluation program as based on the pre-clinical toxicology; what the agent's going to be used for; and in whom it's going to be used.

And we really are urging early FDA consultation that is based on data and specific concepts about where want to go. And we'll be very, very happy to have a dialogue with sponsors in the development community.

[Slide.]

And, finally, I've just listed a number of

guidances that may be helpful to people in looking at this. And it's certainly not a complete list, but I think it's a good start.

So let me stop there.

[Applause.]

DR. WEISS: Thank you, Dave and Dave.

We're going to take another 20-minute break. I have 3:25, which means that we should be back in--if we can--at about--3:45. My math isn't so good.

Please get a lot of caffeine and carbs and things to get through the rest of the afternoon. We still have a panel to go, and then the last session. And we'll see you all back here in about 20 minutes.

And if you have questions that you can think about now, you want to write on the cards, they should be out on the registration desk.

Thank you.

[Off the record.]

DR. WEISS: I don't know if anybody out in the lobby can hear me, but if you can, please come

back in so we can start the panel.

They're coming?

We honestly had this timed really nicely if this was going to be a day-and-a-half workshop. I can tell you, there was going to be longer times for breaks, longer times for lunch.

[Pause.]

Panel Discussion

DR. WEISS: Haven't had enough caffeine. I was actually up at 3:30 in the morning because I got this bug bite, and I woke up at 3:30 with my eye swollen shut. So, in addition to being up at 3:30, I had a lot of benedryl, so I'm not very awake.

I'm going to go ahead and get Session IV rolling again. For the last part of Session IV we're going to have a panel discussion to address any of the questions, comments you may have regarding clinical testing--whether we call it "clinical pharmacokinetic testing," "clinical safety testing," potentially "clinical efficacy testing."

I'm going to go ahead and take my prerogative as a moderator by starting some questions while other people are finishing up their break outside and still straggling back in.

And let me ask my first question to Dr. Green--Dave Green. In the paradigm, we've all talked about the fact that this is going to be developing products under the Animal Rule requires a paradigm shift in the normal sequence of testing, putting products first in animals and getting single-dose and multiple doses, and your animal tox testing and your pharmacology testing, etcetera, and then you kind of venture into your humans with your Phase 1, and then on.

And it's a little bit of different paradigm because you need your human PK information to help, perhaps, design your animal efficacy study.

So, can you speak--I know, somewhat in generalities--but your view of how you might go about your developmental program with respect to when you would consider the PK studies. And

actually, it probably would also have some initial very, maybe small, proof of concept safety studies in humans, as well as the PK.

But can you address the timing of when you think those studies should be initiated and completed relative to, you know, the initial animal studies--the proof of concept, and then the definitive animal efficacy studies?

DR. GREEN: Well, I guess that if you had to look at something de novo, I would think that one way of going about it is, in some ways, to start out as if you would any other clinical study, and that is a Phase 1 study in an appropriate population, which might be healthy subjects, and look at the pharmacokinetics, and use that as the first level to make a decision about what animal models might best approximate PK and PD markers that might ultimately be used for efficacy.

So that, I think, is an essential component in doing the animal--to find out which is the most relevant animal model. However, that would certainly be benefitted by knowing from

various pharmacology--what would ordinarily be pharmacology studies which, in this case, are really tests of efficacy in animal models to know which of those animal models, if we don't have strong priors as to which is the best to select, is how divergent the population of response is, or how particular the response is; and then also study the clinical pharmacology PD and PK in those animals.

And taking the Phase 1 human experience, and comparing against that the animal experience would help us know whether we have a consistent data base, or discontinuities, or problems that will require explanation, as well as maybe being able to select down to two models that might provide adequate basis for concluding potential efficacy.

So I think, in some ways, that it looks like the regular development, in that you would do pharmacology studies. But in this case they would be efficacy-related studies to the Animal Rule. And then an initial Phase 1 study.

And because of funding, and submitting

INDs, I would think that you might--you would probably do the animal-related studies, which are not done under--for purposes of the Animal Rule, but would be minimal, in terms of resources, but gain enough information about PK-PD to make sense once the initial human studies have been collected.

DR. WEISS: And, follow-up to that, to Dave Ross, or to Lew or anybody--in terms of the safety program, and the somewhat larger exposure in the healthy volunteers, when would you anticipate those kinds of studies would be initiated relative to the animal studies that are--would you want those animal studies completed, for instance? Or--you know, when would be the optimal timing, I guess, for generating the human safety information?

DR. ROSS: I was afraid you were going to ask me that.

I think the--the evasive answer is that it's going to depend on the specific program. But I think that you--obviously, before going into humans, I think because these are healthy volunteers, I think you are going to want--or at

least his is my off-the-top-of-my-head thought, and it's certainly something that is, I think, is open for discussion--you want to have some justification for exposing normal volunteers to risk. And therefore I think you would want to have some indication that there is a basis to believe this might ultimately have a benefit.

And beyond that I don't know that it's possible to say a whole lot more.

Dave or Lew, do you want to tackle that one?

DR. SCHRAGER: Yes, I mean I can just tell you what we've seen. And that is that, you know, we've had developers come to us with that specific question, and the way we've handled it is just what you're saying.

If you have proof of concept--if you have proof of concept, and if you have, obviously, the appropriate toxicity data in the animals, at that point there's almost a dual-tracking that goes on. One is, you know: go ahead, get the IND and do your initial safety studies in humans. And at the

same time progress with your--quote-unquote--"pivotal efficacy studies" in animals.

DR. GREEN: Well, one question would be the dynamic range between doses that you might explore in a healthy population of volunteers, and how far could you go--could you go to maximum tolerated dose? That would be sort of, in a way--you would like to know both parts of the equation almost at the same time. And therein lies the difficulty.

So it's probably kind of reciprocal process of going back and forth. But I guess the question is: it's probably important not to do anything that looks like the Phase 3 study until you're fairly sure about either the levels that you want to study, or the doses that you want to study

DR. SCHRAGER: Absolutely. And this, again, gets back to emphasizing the importance of a dialogue between developers and the FDA, after you really do have the appropriate amount of data to engage in that dialogue.

But when it comes to planning these--you know, these kind of studies, these are difficult questions. And I promise you there's going to be a lot of thought put into a response to those questions, and a lot of interaction between developers and the FDA in trying to come up with the best approach under the circumstances.

But speaking generally, I think that, you know, the approach that we talked about is one generally that works.

DR. ROSS: As a follow up to that question--a pass on a question that Russ Pierce, from Office of Blood Research and Review asked me during the break--and I'm going to ask David this--is there--do you think there's any utility, in terms of trying to go above the--what you think you need in humans, as far as exposure. Would that give you perhaps greater power, as far--statistical power in terms of safety? Do you think that might be some utility to that kind of pharmacokinetic approach?

DR. GREEN: Using a dose that's beyond the

level that you think would be efficacious?

DR. ROSS: Yes.

DR. GREEN: Well, I think one--well, certainly, I think that, if nothing else, provides a safeguard on re-evaluation of the information such that other factors--for example, you talked about changes in blood flow, or changes due to the disease. And, for example, with OP intoxication there are profound differences in cardiovascular function. And there are other disease settings where that's known, as well.

And I think one of the problems with there area is it's developing so fast that new bits of information can have significant impact on an understanding and a decision. And so, again, I think it's an axiom that the more information that you have the better prepared you are for the uncertainties that may come about. And knowing what the maximal level is, or knowing what a greater than what you think would be appropriate, will probably safeguard the development and allow flexibility in terms of populations which may need

a variety of dosing.

So I think it's a very good thing to do, and I think it's the same consideration we have about what we think is a properly conducted Phase 2 study, going onto a Phase 3 study; that it's important to get a range of doses and understand their effects before going off to a Phase 3 study, instead of just verifying the dose that you think would be effective in a Phase 3 study.

DR. WEISS: I'm sorry--I also neglected to actually introduce Dr. Nisha Jain, who is joining the panel She's from the Office of Blood Research and Review in the Center for Biologics Evaluation and Research. And you're down at the end, so just wave your hand if you want to add in any comment.

DR. JAIN: Actually, I do have something to say in terms of biologicals. You know, I think you're generally talking in terms of drugs. But just to keep the biologicals, what the OBRI current thinking is, depending on the urgency and the imminent threat--you know, both the human PK safety and the animal study could be done concurrently

instead of in a sequential way.

DR. WEISS: Yes, from the floor?

DR. NAST: Thank you. Dr. Merrill Nast.

I feel that there's such a big disconnect in the room between what should be and what is. And what the speakers are saying I support wholeheartedly. But that is not how things are happening. That is, in fact, what you're encouraging private manufacturers to do, is follow the process, prove all these things--that they have a good and safe product--and that will be wonderful, and then we'll use it.

But in other situations--for instance, when the government wants a second-generation anthrax vaccine, we have a Phase 1 study that was done by VaxGen, where the--in a small number of patients. It went head-to-head with the BioPort vaccine, and the BioPort had an 18 percent systemic reaction rate, and the RPA102 had a 39 percent system reaction rate. And so what would any normal person do? We'd say we have to really look at this and see whether we want to continue with the Phase

2 and the Phase 3.

But what, in fact, just happened is that Health and Human Services--is the employer of probably everybody on the panel--said instead that what we want to do is buy 75 million doses of RPA102. And VaxGen said that will probably cost about $1.4 billion.

So the government asked for bids. The bids are in. And the government is now going to name a supplier in August--before a Phase 2 trial has been completed, with a drug that may be completely unlicensable. And I don't hear anybody at the FDA saying: "Stop!"

So let's talk about that elephant in the living room.

DR. WEISS: Thank you for your comment. It's a good one. I don't want to pass the buck, but we are going to be talking--on Section V, the next one that's coming up--a little bit about some of these issues about, you know, government, and funding, and acquisition, and the SNS, etcetera. That might be a better topic for that session.

I mean, part of the issues, as Dr. Jain has said, too, you're talking about--I mean, we're in a paradigm shift that's very, very different, because we don't know when, and if, and how bad a potential terrorist strike could be. And, of course, if there is something that's quite catastrophic, you know, people are going to want to employ, as best they can, the things that are available, or things that have been evaluated.

We realize that, you know--I think somebody said earlier on--we're never going to know enough, even at the time we do approvals for conventional products, for non-serious diseases, you tend to want to have a larger data base than you actually have available to you. And that's one of the reasons to get the extensive post-marketing information, and to continually evaluate things.

So, I don't know how--I don't know if I can actually--I know I can't answer your specific question, but I think it's an important one to keep in mind as one goes--Karen, do you want to make a comment now on that? That's Dr. Karen Midthun from

the Center for Biologics Evaluation and Research.

DR. MIDTHUN: I can speak from the FDA perspective--you know, what we are doing with regard to the development of the RPA vaccines, is that they are proceeding through the development just as has been described here. They are going from Phase 1 to Phase 2 studies. They will--if the data support, moving forward--go into Phase 3 studies to generate safety data. And certainly there will also be animal studies ongoing that will generate the efficacy data base that is needed.

So they will go through the development, you know, that is really analogous to the type of development that has been described here. And certainly, because vaccines are something that could be given to large numbers of healthy individuals, there will be a significant safety data base, just as there is for other kinds of vaccines, where there is also efficacy generated in human studies. Of course, here, the difference will be that the efficacy will rest primarily on the animal data, but with bridging of

immunogenicity between the animal and the human models.

And I don't know if there's someone, perhaps, from HHS who might want to comment. Or perhaps the next panel, as you suggested, Karen.

DR. WEISS: Does anybody else have any specific questions?

I have some, if nobody else wants to come to the floor. Thank you.

DR. GURELIC: Ken Gurelic from EnzyBiotics.

A question for Dr. Ross: in describing the safety package, it's very clear that under the Animal Rule the human experience is going to be entirely either pharmacokinetics, special population, or safety exposure.

Imagine that we have a protein therapeutic that's intended for the treatment of people who are clinically infected with anthrax. So we're not talking about a huge population at risk. When you gave your example of the numbers of patients to be studied--you know, you talked about a rule of

three; that if you wanted to exclude a 1 percent risk, you'd need 300 patients.

Is that the size of the total safety package that you would reasonably expect to see in an approvable MDA submission? Assuming that you had no signal in your animal safety studies and that, you know, things were progressing normally in Phase 1, 2 clinical development.

Second question is: do you expect to see double-blind randomized, placebo-controlled safety studies, or will open-label single-arm studies be sufficient?

DR. ROSS: I think in terms of --they're both very good questions. I think, in terms of the size of the safety data base, I think I would, I think, just underscore the risk-benefit equation.

Let me take the 2001 anthrax attacks, in which--we just focus on inhalational disease. We had a case fatality rate of 5/11, or roughly 45 percent. Now, the question is what level of serious adverse events is acceptable--clinically acceptable--in that kind of circumstance?

And I think that--what I tried to do in my presentation is not suggest a specific event rate in connection with that sort of case fatality rate, but just say these are the sort of parameters that we would look at in terms of making a decision of whether the risk-benefit ratio supported approval.

There's plenty of products--none of which I can discuss publically, of course--in which it's clear from the animal data--and I'm not talking about under Animal Rule, but traditional drug-development programs--where it's clear that the toxicities are going to be so substantial if you were to go into humans--there's one I dealt with some years ago where every single one of the animals getting the agent suffered, in the sponsor's words, "Acute sudden death."

[Laughter.]

You know--which I thought was a very nice turn of phrase.

[Laughter.]

But even if it had some therapeutic benefit, in a very sick patient population, that

"acute sudden death" problem seemed like it would outweigh the benefit.

[Laughter.]

So--more seriously--I mentioned amphotericin as an example. We know that if you have patients who have culture-proven invasive aspergillosis, that they have--untreated, they're--and I'm talking about neutropenic patients--they have a mortality rate that approaches 100 percent. If we're talking about a patient population for bacterial meningitis--again, untreated, similar sorts of mortality rates.

So, if you have a benefit, then--you may have a substantial risk. And you'll notice I'm being careful not to give a specific sort of numeric risk-benefit ratio--but--if I can use another example, cloramphenicol is life-saving, or can be life-saving in typhoid fever, in Rocky Mountain spotted fever--even though it has predictable hematologic toxicity.

So, I don't think there's a specific event rate or sample size that I could point to. I think

that it's the sort of thing here, clinically, you have to say: "Is this benefit worth the risk?"

Having gone on at length, I've forgotten your second question. So I'm sorry--if you can repeat that? Would we want--go ahead.

DR. GURELIC: The question was: would you expect the safety studies to be double-blind, randomized, placebo-controlled. Or would you accept open-label, single-arm studies?

DR. ROSS: Let me answer in terms of statistical issues.

One of the difficulties in having an open-label study is that if you have, let's say, a 5 percent rate of a particular event, the question is: is that a lot or a little? It becomes even more important if it's a serious adverse event. Let's suppose you have IG IV, and you have a 5 percent rate of hives on infusion. And I don't know what the historic example is. Dr. Jain, I'll turn that over to you next.

But it becomes much easier to put that into context if you have a controlled study, in

which a patient population--or, volunteer population, is getting either study drug or placebo. Because then you can say this is a chance effect, or it's something related to the drug; and it's a lot or a little.

DR. GURELIC: Excuse me, I just want to clarify it, because, you know, your answers are absolutely what I would expect, you know, in a general discussion.

But we're talking about anthrax, which, you know, in a very small number, had a 50 percent mortality rate, which is within the 50 to 90 percent rates that are quoted. We're also talking about healthy volunteer studies.

Now, I'm assuming that there will be some sub-population studies in elderly, liver, etcetera. But a general population study so healthy adults, aged 20 to 65, you really don't expect them to have serious adverse events during a three-day infusion.

So, I guess I'm wondering--you know, it's the sponsor's risk issue, of course, but--

DR. ROSS: It's--well, it's difficult to

give a--well, first off, let me say this. A double-blind, randomized controlled study is obviously always better. It strengthens the package. At the same time, we're quite conscious that that's also--it involves more resources.

It's going to depend, in part, on the nature of the product. If you have a small molecule, for example, that causes--you think may cause QT prolongation, it may be very appropriate to have a controlled study in which you can say, you know, there's no QT prolongation, or there is some QT prolongation.

If, on the other hand, you have a safety study that's open-label, and uncontrolled, in which there are no adverse events with a reasonably sized patient population, then that also--I mean, then, you know, that may be easily interpretable information.

In terms of the risk of a development program, you run the risk, with the latter approach, that you may end up with a result that you're not sure how to interpret. That's really

the problem.

But I would never say "never." You know--I could, but I won't.

But I do think it's important to recognize that's there's risk--business risks and development risks to particular approaches.

Nisha, did you want to add anything in terms of--the experience with IG IV?

DR. JAIN: Well, if we take this particular example of anthrax immune globulin, let's say--in healthy volunteers, they are healthy, so--I mean, I don't see, you know, in general drug development, you know, doing a double-blind placebo is very good. It's always what we want. But for immune globulin, with an established--a sort of established safety profile, I mean I do not see, you know, with increment, if you do a double-blind placebo control, it increases the exposures of the--the sample size increases in order to get whatever adverse event rate we are looking at. I don't see, you know, the value of it.

And, you know, this is, again, my

thinking. It may be the FDA thinking. But, again, something which is a good point brought up, and we might have to talk about it.

DR. ROSS: Just one other point very quickly.

The other thing, I think, to keep in mind is that if you have multiple products targeting this indication--specifically, treatment--then assessment of the relative risks may become very important--not only for regulatory agencies, but for public policy makers, for individual health care providers. So there it may become--because you're not going to see a zero percent response rate. I mean, I just--I mean, my wife gets dizzy when she takes Tylenol. Of course she's sort of a dizzy person to begin with.

[Laughter.]

She's never going to know I said that.

But seriously, I think there it may also become important. But I think we also are--you know, it's not just rigid insistence on double-blind controlled trials, it's because they

are stronger, and they do provide useful information.

DR. WEISS: Go ahead.

AUDIENCE: yes, just a point of clarification. We're talking about the animal efficacy rule, where sort of proof of concept is done in animals and not in humans.

Where does "double-blind placebo-controlled trial" come in? Just for PK, PD and adverse effect profile? I mean--

DR. WEISS: The question was asking about the large safety trials, or whatever size safety trials that people are going to be doing, either after or in concert with the definitive animal efficacy trials. The question was do those trials need to be randomized and double-blind, or can they be, basically, large, open-label safety trials. And that was what the genesis of the discussion was about.

AUDIENCE: Okay. It just seems some products are sort of well proven to be safe, like hyper immune human IG IVs, and, to a lesser extent,

animal antitoxins that are pepsin treated, and so forth and so on. And there's some--you know, there's some volume of literature out there that, you know--it seems like we could maybe get them to market with less concern about risk.

And others--like, you know, small-molecule inhibitors, you have, you know--you don't want the stuff to run out the kidney before, you know, the disease is cured, or you don't want the liver to be chewed up or whatever.

It seems like for different classes of products we might need to fine tune, sort of, our level of concern.

DR. WEISS: Yes, I think that's a very good point and should be really emphasized; that, you know, if something is in a class--a well-recognized class-or, in the case of actually the development of purido and even cipro for animal--for counter-terrorism measures, there's already a large amount of data, albeit in another clinical setting, and perhaps with a different dose and schedule, but you had other information that

you can use to borrow from, and perhaps use.

And, you're right, if something is in a class--but if something is considered a little bit more of a risky kind of a product based on some of its manufacturing and other characteristics, you might want to see something larger.

I think that's some of the difficulties in trying to give, you know, real hard numbers, because each case is going to be somewhat specific. And, you know, you all want to hear some more specific advice on how to develop things, and it's a very difficult thing to do in an abstract type of setting.

DR. ROSS: I just want to reinforce that. I think--just to--I showed some adverse event data for ciprofloxacin, and quinalones, in general, have had very good safety record. But, of course, there are quinalones that have been withdrawn from the market because of safety concerns.

So, I think it's--there are class aspects of safety, but I think--even for a class that's as safe as cephalosporins, or betalactones--methcillin

is no longer used because the risk of toxicity is substantially greater than for other batalactones.

DR. SCHRAGER: I guess all I wanted to add was that, you know--it's been stated before, but the reality of the situation that we're dealing in is that--the reason we have an Animal Rule is that we're not going to be seeing disease in humans, and we really don't know what the safety, you know, might be in that situation, as well. And that's why the Phase 4, post-even follow-up is so important.

DR. WEISS: Yes, go ahead, please?

DR. ROBLE: Jim Robe, Hematech.

Is cipro treatment considered a standard of care for anthrax exposure? And how would you handle that in a safety trial?

DR. SCHRAGER: Well, in terms of standard of care, I mean we have--for an inhalational exposure, we have ciprofloxacin, we have doxycycline. You know, there's also penicillin that's been approved as well. But, really, we're focusing on ciprofloxacin and doxycycline.

The safety issue in a post-event situation is really--well, you get a sense of it when you look back at the 2001 anthrax mailings. The CDC, at the time--really, after the fact in this situation--engaged in a post-event telephone surveillance of the individuals who were recommended to receive the drug--any of the drugs. And from those data came the report on adverse events relating to taking the post-event prophylaxis. And that was published, actually, about a year, year-and-a-half ago, in Emerging Infectious Diseases.

In the future, the way we're going to approach it is through a joint effort between the CDC and the FDA, establishing a means by which the government would be able to assess--access and assess--post-event outcomes data and adverse events data; and also, in doing that, create a means by which the developers whose drugs would be in question would be able, to some degree or other still to be determined, participate in that process in accordance with the Phase 4 requirements.

DR. WEISS: We're actually about out of--do you have--are the comments quick?

DR. NAST: It will be real quick.

The Phase 4 is critical in the Animal Rule. However, you licensed small pox vaccine for the military. There was a study in civilians--that study is stopped; 39,000 people got it. There were too many problems. There's subclinical myopericarditis in one in 30 people getting the vaccine--it's been reported; totally sub-clinical. And the FDA has stopped some ongoing clinical trials from using the vaccine. Great.

But you haven't pulled it from the military. It's still licensed. 625,000 people have gotten it. You've got your Phase 4 trial, you haven't used the data.

DR. SCHRAGER: Karen, do you want to address that?

[Laughter.]

DR. MIDTHUN: Yes, the DryVax vaccine has been licensed for decades, and clearly there was large-scale use with the recent vaccination of many

individuals in the military, as well as certain individuals in the civilian sector. And those individuals were closely monitored.

And it is correct that there were new adverse events noted; namely, myopericarditis. And the label was changed to reflect that. And it is true that there was an ongoing study under investigation--new drug application--which is currently halted--that's public knowledge--while the data on sub-clinical myocarditis are looked at further to determine, you know, what the next best steps are.

But, in the meantime, also as part of that, that information will go to inform, also, the current use of the DryVax vaccine, and that's something that we're very actively engaged in at present.

DR. WEISS: So, Ross, I'm letting you get the final comment or question in this session, and then we're going to call this to a close so we can get to the last part.

DR. BRUCE: Thank you. Ross Bruce, FDA.

I just wanted to follow up on the question before last--or the answer before last.

Among the 10,000 individuals in the 2001 U.S. anthrax incident who were followed up by the CDC by telephone, how many of them said that while they were recommended to take the antimicrobial post-exposure prophylaxis they failed to do so. Because that would bear on our interpretation of the 100 percent success rate with doxycycline and ciprofloxacin in post-exposure prophylaxis.

DR. SCHRAGER: Yes. I don't remember the numbers off the top of my head. I mean, you know, the response rate wasn't great for the survey to begin with. I think you had about 9,300 people who were recommended to receive the post-exposure prophylaxis, and about 5,300 responded.

And I just can't--I can't tell you. I know that there were a lot of people who either didn't take the drug, or who were not deemed compliant with the--you know, with the full course of the prophylaxis. However, there were at least a few thousand who were.

And, you're right. You know, it's not 10,000 people we're talking about. It's probably three or four. But, again, it is three or four, which is better than the 10 monkeys that we had resulting in the ciprofloxacin approval. And of those 3,000 or 4,000--and some of them--you know, some of them--the nature of the exposure is not clear and may be theoretical. Some of them got it in the face. You know, some of them got a face full of powder. And none of them got disease.

So--you know, that's the best we can say. You don't want to say more than the data tells you. But, you know, there was some protection there.

DR. WEISS: I'm going to just call this session to a close because we have a time crunch, and still have another session to go through.

So I want to thank all the panelists and all the speakers and--

[Applause.]

--the audience for provocative questions.

And then I'm going to ask Tony Macaluso to come up and introduce.

Part V - Challenges and Opportunities in Product Development

DR. MACALUSO: This session on Challenges and Opportunities in Product Development is the final session in today's workshop. And I'm pleased to see that so many of you are still here.

This session will have a slightly different format compared to the previous sessions. We'll have one 25-minute presentation, followed by five short presentations, and then the panel discussion.

The presentations will in sort of diverse areas, but they will have the common theme of identifying mechanisms that either facilitate the development of anthrax therapeutics, or facilitate the access to anthrax therapeutics in the event of a national or military emergency.

I'd like to introduce our first speaker, Dr. Carl Nielsen. He's a consultant to DARPA and USAMRIID for new product development. The title of his talk: Challenges and Opportunities in Product Development.

Carl?

Challenges and Opportunities-Overview

DR. NIELSEN: Thank you very much.

What I'd like to do--we've talked about a lot of different aspects in this really short period of time, jamming everything into one day.

What I'd like to do is highlight some of the features that we've discussed so far, and try to reinforce some of those.

We have a lot of people represented here in the audience who are from various federal agencies. Hopefully those of you in private companies trying to develop products, or thinking about the same, will have a chance to interact with them.

I'd like to address my remarks to the people in the private companies, because those are the people who are doing the heavy lifting, in terms of coming up with a product. And the remarks I'm going to make are from the 30,000-foot level--as you can see here.

[Slide.]

And I have no data, so you don't have to worry about keeping your pencil handy. I'm just going to talk in general terms.

What I'd like to do is talk about how to come up with a developmental plan.

[Slide.]

And, as you've heard on a number of occasions, the first question you need to ask is: what is the indication for your product? How are you going to use it? That's the first question the FDA is going to throw at you.

And then the next question is: what is the safety profile of that product? And you need to support each of your answers to these questions with data. Clearly, that's been coming out throughout the day.

And you want to have at least a plausible story--supported by data--for what the mechanism of action of the product might be.

And then, if you're a small company, you need to ask the question: who's going to do the studies? Are you going to do them in-house? How

many of them are you going to do? Are you going to farm it out on a contract to somebody outside?

[Slide.]

Now, what about the animal studies?

We've talked a lot about animals, how to use them, the Animal Rule, different kinds of animals, different sorts of experiments to be done with them. Are you going to be looking at the mouse, the guinea pig, the rabbit? Which ones do you need to have before you go into non-human primates? And how are you going to do that?

And, oh, by the way, which non-human primates? We've kicked that around a little bit today. The Indian rhesus has been used in the past. You'll have a hard time finding any anymore. Chinese rhesus are somewhat available, but they're very expensive. There are some breeding colonies around the country.

Cynos--we have some data in. And, as Louise Pitt described for you, some of the older data is somewhat in question because of the conditions of animal husbandry.

There are some data available in African green--not a whole lot in anthrax. That seems to be more focused on plague.

There is some information coming out on the marmoset. There has been a DARPA contract to look at the marmoset as a potential model for use in the animal studies. It's a much smaller animal, requires much less in terms of housing and so on.

One of the big problems that we talked about in response to--or in consideration of animal models, is how well is the animal model characterized with respect to the disease process in man. We're trying to use something that mimics the condition in man.

So, as you put all these questions together you need to come up with a plan. And this is where we sort of bring everything together and look at something that might look like this.

[Slide.]

And the last panel just kind of provided an entre to exactly this, where we have pre-clinical safety taking place here, along with

some scale-up manufacturing, in order to be able to conduct animal studies to support a Phase 1 IND.

You conduct that study. And some point during that study, or some time thereafter, you would want to conduct your animal challenge study, or your pivotal study.

Exactly where that would go in terms of timing--most likely it would come rather late, in conjunction with your Phase 1 study, or after. But that's not necessarily the case. That's up to you, in terms of your plan, and how much risk you're willing to accept in development.

And then some time around the period of the animal challenge study, you would engage the possibility of starting up your expanded human studies for additional safety.

I put in this little piece right here, which I consider critical to any company's launching off into a product development plan. That's the pre-IND meeting. And I can't stress that enough--whether you're a small company or a large company.

If you want to get engaged in the Animal Rule studies that address the issues that we have in front of us, you need to get some input from the FDA. And you've heard several times today that the FDA is willing to provide you with advice and counsel in putting your plan together and telling you when you're way off base.

So I thoroughly recommend--heartily recommend--that you engage a pre-IND conference with the FDA as soon as you think you have a product that you want to develop. And, hopefully, you'll get successfully through it, and reach final approval with that information.

The information that you get from the pre-IND plan will provide you with a guide through the bottleneck of getting to your Phase 1 study. Once you get there, you want to continue your interaction with the FDA review team so that you don't fall astray and end up getting into expensive studies that you really don't need.

[Slide.]

And, speaking of those expensive studies,

there is a bottleneck. And I've been railing on this subject with a lot of people for a lot of time, and that is: how to get the studies done.

This is a difficult challenge. We've talked about a number of aspects of it, in terms of the various details that go into the study. We're looking at studies that must be done--in the case of anthrax--under BSL 3. And there are other bugs that we need to do under BSL 4 compliance.

That's a big project right there. Most small companies are not going to have that capability in their laboratories. They're going to have to go someplace else to get them done.

We're looking at aerosol exposure to non-human primates, and we've had a number of discussions about how difficult that is, and how careful you have to be in trying to put it together, and the impact of those errors on your study.

And then, number three, is the real catcher: full GLP documentation needs to be collected during the course of your pivotal study.

How are you going to do that?

[Slide.]

So, conducting the Animal Rule studies, we've got these very difficult challenges in front of us: the aerosol challenge, the non-human primates use. A lot of small companies do not have the capability of dealing with non-human primates. You have to do it in BSL-3.

And then here's the hard part. The NIAID has sponsored several--I think the number is five--centers of excellence around the country to do aerosol studies. And they do aerosol. They do non-human primates. And they're capable of doing BSL-3 and, in some cases, BSL-4.

I didn't say anything else. That's the problem. Where can you get this done? To my knowledge, there's only two places where you can come close to doing it. That's at USAMRIID and Battelle. And I've worked long and hard to try and get a third place, at Lovelace in Albuquerque. Hopefully they'll be up to speed in '05. But, in the meantime, we have two places where these

studies can be done.

And we've had a number of discussions about what full GLP is; what "very nearly GLP" is, and "almost GLP." "Spirit of GLP" is another phrase that comes out.

[Laughter.]

I think if you talk to Dr. McCormack you'll realize that there's either GLP or there isn't. There's GLP with exceptions.

And there are certain exceptions that have to be made in order to get these studies conducted, and we have to deal with them and try to improve on them as time goes on.

So that's a problem.And that takes care of the facility, primarily.

[Slide.]

What about the people to do these studies? Where are you going to get the folks to do them? Do they need to be vaccinated to conduct these studies? And, if so, which vaccinations? And what about people who don't take vaccinations well?

Do these people have to be cleared for use

of select agents, and how are you going to get them cleared? How much time does it take to do all this?

And then they have to be trained with non-human primates, with GLP documentation and BSL-3 techniques. You don't get these people off the street, next week. It takes a lot of effort to get these people up to where they're meeting all the necessary requirements to do this.

And probably the most difficult--again--is that GLP part. Universities do research. When you're talking about this kind of work you're talking about testing and evaluation. You have to take your head off, turn it around and put it back on again if you want to do GLP. It's a different kind of business. It's testing. It's not research.

It's very difficult to get a group of folks to turn their head around and be able to do these kinds of studies.

Okay, moving along in your development plan--

[Slide.]

--what about the clinical trials? Who's going to do them? And, worse, who's going to pay for them.

And that gets me off into an area that we haven't really touched on very much--and I put this slide in green so that it would bring you into the idea of money. Okay?

[Slide.]

Okay--grants and contracts. And we have to engage alphabet soup. There are more initials around this town than anyplace else on earth. And I'll try to run through a few of them so that you can become familiar with at least the concept of them.

Everybody's familiar with NIH and NIAID. Most of what comes out of this organization is original, innovative, basic research, but they're now getting into advanced development and clinical trials.

There's some concern about how we do peer review, particular for advanced development and

clinical trials. Those peer review panels need to be composed--at least in part--of people from industry who have experience in those areas. Sometimes difficult to get.

Another organization that funds research and development is DTRA--the Defense Threat Reduction Agency. They fund a lot of basic research and initial development on products, beyond a proof of concept. And they need to be "of military relevance." And, of course, anthrax is a prime example of something that's both of military relevance as well as civilian relevance.

[Slide.]

Another organization that has been funding research is the Army's Medical Research and Materiel Command at Ft. Detrick. Again, military relevant research; contracts; CRADAs with USAMRIID--USAMRIID comes underneath RMS.

Then here's another piece of alphabet soup: WRAIR--Walter Reed Army Institute of Research. There are some contracts for clinical trials that come out of MRMC, but most of that

activity is being focused on DTRA.

Another organization is DARPA, with whom I spend a lot of time, as well as with USAMRIID. This organization provides contracts for really innovative product discovery areas-focused, again, on military applications. DARPA is quite new to the field of biology. It's previously been a physics and engineering organization.

[Slide.]

BioShield is a new organization that's pretty nearly approved and funded. It's funded and almost completely approved. It's a DHHS agency, with the concept of fully burdened costs. And we'll hear some more details about that a little later when the panel gets up here.

[Slide.]

I've put this unusual slide in here to remind anybody who's in the business of product development that funding is like a lightbulb. And this is a carbon-filament lightbulb. You never know when it's going to blow out. You have to be real careful with it.

[Slide.]

One of the problems that we've had in this business over the years--particularly in the military--is there was very often a fair amount of initial funding to support the bright light of discovery, and now we're getting to some funding out here at marketing at government acquisition, we have a place to go. But there's this great chasm here, in between, that I call "The Valley of Product Death"--

[Laughter.]

--where you've got this great thing that can do something, and it looks wonderful in animals and in all your in vitro systems, but you can't get over here.

[Slide.]

So I've got another little slide that depicts this. This is the Valley of Product Death right here, and here's a little bridge that you want to construct over it. So we have a start-up agency that's going to get you your initial funding. And then we have, hopefully, government

acquisition from BioShield. And the real question is: how do you get across the rickety bridge over the Valley of Death?

And this is a concern that every company should have. You're going to have some outside funding? Are you going to have venture capitalists? How are you going to work that out?

Over here in the start, you've got NIH. You've got, perhaps, DARPA, maybe DTRA--depending on who you've been working with and how close you are to the military. And over here, maybe we've got BioShield. And, if you're lucky enough, you have an indication that you can market outside of the government requirements.

[Slide.]

One of the things that I try to get people to do with projects that I'm working with is to get on one indication that you find will be the most easily achieved; that you can collected data for and support. And don't try to cover the waterfront. Make it as narrow as you can and clearly support quickly.

Because what do you want to do? You want to get FDA approval as quickly as you can. Why? That offers you the opportunity to tell the world that, "Hey, we know how to do this. We can get an approval for a product. We can market this product"--hopefully either to the government or to outside interest. And then later on, what can we do? Label broadening; second indication, third indication--whatever you want to do from there--as long as you have the data to support it.

But you need, as a company--financially you need to get that first indication.

[Slide.]

We have some goals. The company has goals, and the interaction between various private companies and the federal government need to be brought together. What's the company goal? Make money--of course. If you lose track of that, we need to talk. And from my perspective, hopefully you're also in this business to help the country.

What's the government goal? We want to have the product available when we need it. And if

you are not sensible about the use of money--particularly the money that we give you--I say "we"--that's not we, that's this person over here. I'm a consultant. Anything I say does not bind the government.

The government wants to have the product available when needed. And the government wants to avoid having to develop the product internally. Our track record within the government for developing products--umm--is not the best.

[Slide.]

A couple critical questions you need to ask yourself: what is your incentive for getting in this business? Why do you want to do it? You want to make sure that you've got your incentives correct, and that you've got your goals on line.

Can you find an indication that will make you some money? Because if you can't, you're going to be dependent upon the government to make the acquisition. You would really like to have another indication--down the road, at least--that will allow you to market the product on the outside and

generate a revenue stream.

If you're going to be looking at the government for acquisition, what is the government restock rate going to be? That's going to depend, in part at least, on your shelf-life. And, of course, you'd like your shelf-life to be about two weeks--right?

[Laughter.]

[Slide.]

This question came up--or this concept came up a little while ago: how many products is the government willing to stockpile? There are several representatives of various companies in the room here. Those are just the ones that we happened to get today. So if we have four or five products for anthrax, and that's going to cost how many millions to buy to stockpile, and then we have to buy it again in two or three or four years--well, wait a minute, we've got plague, yet. We've got to have three or four for that. And what about all the other diseases? We've got smallpox. How many of these products is the government going

to be able to afford?

BioShield is now almost approved. How do you survive the "fully burdened cost" structure if that's the way business is going to be conducted--and we may get some changes in that in a few minutes. But if there's a fully burdened cost structure, which means that the cost of development will be included in the acquisition, how do you survive the Valley of Death when you have insufficient cash flow, until you get out here to the actual purchase?

And here's one that may kill your program: what is the indemnification profile of your product? You need to think about that carefully. Is the government going to indemnify you? Or are you going to have to carry the freight? How does it look? What's the long-term consequence of that product--particularly if you're going to be working it out in the open marketplace for other indications?

[Slide.]

Finally, where do you get help? Well,

each institution within the government has a staff and a website that can provide you with information to help you along. NIAID has one, DTRA has one, DARPA has one. Each one has its own style of doing business, and each one has its own concepts of the kinds of things it will do: early discovery, early development, later development and final acquisition.

And there will be--there should be a website already for BioShield.

The FDA website is an excellent place. There's a lot of information on there about how to do business. If you are not already familiar with it, before you do another thing, get familiar with it.

The pre-IND meetings, of course, are a very good source of information--one of the best--once you have a product identified and ready to go.

So that is what I have to offer you. Thanks very much for your attention.

[Applause.]

DR. MACALUSO: Our next speaker is Dr. Brad Leissa. He's Deputy Director, Division of Counterterrorism, Center for Drug Evaluation and Research at the FDA. The title of his talk is "The FDA's Proactive Approach with Medical Countermeasure Development."

Brian?

DR. LEISSA: The last group of us that's speaking now--we'll be relatively brief, because we'll be going into the panel discussion.

But, briefly, what I wanted to be able to go over to talk to this group about is where FDA's role has been with regard to facilitating, have a proactive approach, with regard to medical countermeasure development.

First of all, it's important to recognize that FDA is an integral part of the public health service. And it sees its role as critical with regard to product development. The counter-terrorism has been identified by the agency at the level of the Commissioner's office, as well as with both--with all the centers--product review

centers--as being a priority responsibility for the agency.

FDA also works closely with its sister public health service agencies, as well as with the Department of Health and Human Services in medical countermeasure development, and hopefully, that is somewhat evidenced by this meeting, where we have brought to this meeting so many within HHS who are involved in product development--as well as with the Department of Homeland Security, and the shared asset of the Strategic National Stockpile, and the Department of Defense.

CDER and CBER have many existing programs, as well as regulations, that are in place to facilitate medical countermeasure development. These include--and you've heard many speak about this--the very important role--where pre-IND, and we're sometimes referred to as "pre-pre-IND" meetings come to bear, as well as the opportunity for fast track designation. Once a product has shown that it has value in an unmet medical need, where FDA is able to designate a product for fast

track, which gives the opportunity for the studies that are being conducted under fast track designation to be reviewed--quote-unquote--"real time"--again, as a way to facilitate product development.

If a product gets to the point of having a licensing application approval submission placed, there's also the opportunity for a priority review. The typical review time for most products is somewhere between 10 and 12 months, but with a priority review, the agency makes a commitment to make a review--an initial review assessment within a six-month period of time.

As has already been discussed, there is also the opportunity for product development under the accelerated approval regulation; the surrogate marker regulation. That was the basis for the ciprofloxacin in inhalational anthrax post-exposure prophylaxis indication. And as well, as we've heard many times today, about the Animal Rule. The important thing, of course, with the Animal Rule is that it doesn't necessarily accelerate drug

development. What it does do is it provides an opportunity which otherwise did not exist prior to the rule, in that there was a --quote-unquote--"glass ceiling," where, if the studies that were needed to be able to be the basis for approval of a product from the perspective of efficacy were not studies that could be conducted because of concerns of it either being unethical or unfeasible, there would just--in that situation, product development was stagnated.

So that's an important point, of course, with regard to how the Animal Rule should be seen.

The Center for Drug Evaluation and Research also, though, has had precedents with regards to medical countermeasure development where there was no product developer. An example of this--as you've heard before--relates to the studies that are being conducted to date for pneumonic plague, and for gentamicin and other products. The issue here is that gentamicin, which has been on the market for many years, it is a generic product. So in a situation like that, who

is going to come forward to develop that product where there is no--quote-unquote--"market," nor is there any way, from an exclusivity perspective, to protect that market.

So, FDA--the Center for Drugs--has noted that this is a need, and has sponsored--working with NIAID and with the Department of Defense--to conduct the critical studies.

Another example is where, for new drugs--specifically in an area outside of infectious diseases--dealing with antidotes to radio nuclide exposure, where the FDA has placed out, in the Federal Register, notices of finding--of safety and efficacy--for two products--three products, indeed: Prussian blue, a radioguardase for removal of Cesium--radioactive cesium from the body, as well as Thallium; and then the calcium and zinc TTPAs.

In these situations, the FDA went to various type forms of data--clinical data--either which was published in the medical literature, or came from the ReAx, through Department of Energy,

to review such data. Because, again, it was not clear to FDA that this was something that a typical product developer would be able to do.

How this is applicable to anthrax and toxins are less clear, but the point is is that the agency is working, to the best of its ability, to try to bring products to market that may have life-saving capabilities.

The Center for Drugs and the Center for Biologics, as you know, now share responsibility with regard to immune-based therapeutics. And I want to assure you that CDER and CBER work very closely, are often in meetings together talking about product developments, so that the regulatory issues, the scientific issues that everyone is addressing these, considering them equally. And so even though the responsibility is shared between the two centers, it's the agency thinking through this, really, with one mind.

So, how does one come to the FDA? Well, these are some contact names. Within the Center for Biologics, the important thing is to go either

through the review division that's appropriate; for example, in the office of OBRR. But, if it's not clear who that is, Ms. Cynthia Kelley--she's the Senior Advisor for Counterterrorism and Medical Countermeasures within the Center for Biologics; her phone number and e-mail address are there.

Or, in the Center for Drugs--again, the review division--if it's unclear to you which review division is the most appropriate--there is also our division, the Division of Counterterrorism, who can help in terms of identifying where your product should go within the Center, and to try to get you the best advice that you can.

The key thing with products under the Animal Rule is to err on approaching FDA earlier than later, because since the animal studies that are being conducted are going to be so critical to product development, that's why people keep coming back to--coming to us during the pre-IND phase. Yet, at the same time--as Captain Green mentioned--it's important that we do have data.

Data is informative. It helps us to be able to guide better, with data.

And we at FDA spend a lot of time, obviously, with data. So whatever level of data that you're able to provide to us in those pre-IND meetings will only enhance the type of discussion and the type of guidance that we're able to provide.

And I can only reiterate the comment that's been made many times today, that it's very important, in terms of developing a product--even in the pre-IND stage--for us to hear from you what you see as the indication for that us. Because all of the design of the trials, how those are set up, will be very critical to the overall development program and plan.

Those are the conclusion of my remarks. So, hopefully, you have a sense about how the FDA and the Center for Drugs, the Center for Biologics, take this responsibility very seriously about developing medical countermeasures; but medical countermeasures that are both safe and effective.

Thank you.

[Applause.]

DR. MACALUSO: Dr. Karen Midthun is our next speaker. She's the Acting Deputy Director for Medicine, Center for Biologics Evaluation and Research at the FDA.

The title of her presentation is "Access to Investigational Products under Emergency Circumstances."

DR. MIDTHUN: Hello, and thank you. I'm sorry, I had thought it would be more sort of a panel discussion, so I didn't prepare any slides. But, hopefully, I can get through this and not stand in your way between now and dinner.

I'd just like to touch basically on means of accessing products that are still in the investigational phase of development. I think it's clear that the goal is for FDA to help facilitate an accrual of data that allows a determination that a product is safe and effective so that it can be licensed. But, obviously, we recognize that this takes time, and along that route there may be times

where there may be a need to use a product that is investigational, and that it may be appropriate to do so.

The main vehicle that we've had, up until now, had been use of the product under IND. And so we, for example--and I'm sure that Center for Drugs has done the same--that Center for Biologics work closely, for example, with CDC when CDC set up a contingency protocol that provided for use of investigational smallpox vaccine in the event of a smallpox emergency. And so, to that end, there was a lot of work to really come up with a protocol, and also consent form, that was streamlined, and that would allow for a large-scale use of this vaccine in the event that such an emergency were to arise.

I think it's clear that there are a lot of benefits to using a product under IND. For example, it's very clear to individuals who provide informed consent that this is an investigational product. But, having said that, we also recognize that this could potentially be a very cumbersome

process, to get informed consent, in the event that there were to be a need to use a product in a widespread manner in an emergency.

And, to that end, at the end of last year, in November of 2003, the National Defense Authorization Act was passed, and that provided for emergency use authorization of products in the event of a military emergency. As I'm sure you've heard in the newspaper--I also read The Washington Post--both Houses of Congress have now passed the BioShield bill, though I understand it has not yet been signed into law. Once that happens, that will also allow emergency use authorization for products that--where there is a national security emergency, or a public health emergency.

But let me focus right now on the one--the emergency use authorization--that is available for the military emergency, since that one has been enacted into law.

Under this circumstance, what would happen is that if the Secretary of the Department of Defense determined that there was a military

emergency, or a significant potential for a military emergency involving heightened risk to military forces of attack with a specific biological, chemical, radiological or nuclear agent, then the Secretary of Health and Human Services could declare an emergency justifying an emergency use authorization.

And there are conditions that would have to be met for such an authorization to go forth. And some of these include that the particular agent that this product is meant to treat, that the agent can cause a serious or life-threatening disease or condition; that the medical product may be effective in diagnosing, treating or preventing such disease or condition; and that the benefits of the product, as best they are understood, outweigh the risks; and that there is no adequate approved an available alternative that can be used.

And there are other conditions that also go along with the authorization--and I'll touch upon a few things--for an unapproved product. There's also a scenario for use of an approved

product for an unapproved indication. But I'll just say a little bit about the use of an unapproved product.

And in this circumstance, to the extent that it's practical, there would have to be conditions that would assure that the health care professional who is giving this product knows that it is an emergency use authorization; knows what the known or potential risks and benefits are; and also, you know, what alternatives there are--if any.

Likewise, there should also be provisions to allow those individuals to whom the product is being offered to know that it's an emergency use product; and, again, what is known about the potential benefits and risks; any alternatives; and that there is also an option to accept or refuse the product.

Clearly, other conditions also are important. For example, there should be in place a system to monitor for adverse events. There needs to be appropriate conditions for record keeping by

the manufacturer. And, also, there are other additional conditions that can be imposed; for example, obtaining data to address, you know, what clinical benefit there is from use of the product as it is used under that emergency use provision.

As I mentioned, this really, right now, is for the military emergency. But, you know, once the BioShield legislation is enacted into law, it would have a broader application for national security and public health emergencies, as well.

And that's basically what I have to say. So, thank you very much.

[Applause.

DR. MACALUSO: Dr. Sue Gorman is Associate Director for Science in the Strategic National Stockpile Program at the CDC. And she will tell us about the Strategic National Stockpile Program.

DR. GORMAN: Thank you. This is where the products are ultimately going to find their home, in the Strategic National Stockpile Program.

[Slide.]

Maybe. Okay.

The program started in 1999, and the focus of our program was mainly to stockpile countermeasures and medical supplies that could be used to respond to a terrorism even involving a Category A biological threat agent, or perhaps chemical nerve agents. And the mission of the program has expanded now to include radiation events, and burn-and-blast or trauma-type events, as well as other large-scale public health emergencies, such as a possible outbreak of pandemic influenza.

Our mission is very simple. It's mainly to deliver critical medical assets to the site of a national emergency.

[Slide.]

And we can respond in a number of different ways to one of these types of events. First of all, we can provide technical assistance through our Technical Advisory Response unit. And this is a group of personnel from the Stockpile program who accompany all of our large-scale deployments. They help with handing over the

assets to the affected area. They stay in the area for as long as is needed. And they help with reordering supplies that might have run out, or need other supplies.

We also can send what we call a "12-Hour Push Package." If there's a broad spectrum of support that's needed--for example, if we have no idea what kind of threat we're dealing with. An example of this would be on 9/11 we weren't quite sure what kind of threat we were dealing with, so we sent our 12-hour Push Package.

It's called a 12-Hour Push Package because it can arrive within 12 hours of the federal decision to deploy the assets--anywhere in the United States or the U.S. territories. And a Push Package--because the affected area does not need to ask for any specific items, rather we just push out a package that's comprised of 122 specialized cargo containers filled with over 130 different types of line items, hoping that some portion of that would be useful for the event in question.

When we do know what type of threat we're

dealing with, we can provide specific item support. An example would be with the anthrax attacks. We would not send a 12-Hour Push Package because a lot of that would go to waste. Once we sign these items over to the affected area we don't ask for them back. So if we know what type of even we're dealing with, we can specific items from either a Stockpile-managed inventory or a vendor-managed inventory.

And these things make up the large majority of our inventory: we have 12, 12-Hour Push Packages located around the United States, that makes up around 5 or 10 percent of our inventory; the rest is in Stockpile-managed inventory or vendor-managed inventory.

Everything that's found in a 12-Hour Push Package we also have in vendor-managed inventory or Stockpile-managed inventory, plus we have additional items that would not be found in a Push Package. And one example of this would be vaccines or antitoxins. We would only send these out--since there's in rare supply-we would only send them out

if we know that we were going to use them during an event. That way, we could ensure that they would not be wasted.

And if an item is needed that we don't have on our formulary, we can exercise our buying power through our contracting partner, which is the VA National Acquisition Center, to procure that item and send it to the affected area.

[Slide.]

As new products become available, either through BioShield or through other mechanisms, there is a lot of information that the Stockpile program would like to know as soon as possible so that we can continue to plan for being able to store and deploy these products when they're needed.

One of the first things that we're interested in is what type of storage condition the product is going to require; if it needs to be refrigerated or frozen. As you can imagine, space for storing large quantities of these types of items is at a premium, so we have to start planning

now for future procurements.

Also, we're interested in what the final dosage form is going to look like; for example, will it be a single-dose vial, a multi-dose vial or a pre-filled syringe? Were looking to find the best blend of cost-effectiveness, ease of use for the end user; the longest possible shelf-life and stability for a product; as well as whether or not it will require any ancillary supplies that would need to be married up with the product during transportation, or perhaps kit-ed with the product before it's transported.

Anything that requires ancillary supplies is going to require additional storage space, so that increases the amount of space that's needed, as well as the cost to store it.

We're also interested in knowing what kind of packaging you're looking at up front; not only what each unit is going to look like when packaged, but how many units you project to be in a case, and the case measurements, and how many cases are on a pallet--all so that we can budget and find the

appropriate storage locations for these items.

And we're also interested in knowing what kind of labeling is going to be placed on the product. Before things become licensed, the label would be an IND-type of label. And if it's possible to use a two-part tear-off label, one that can be used when the product is IND, which can be torn off once the product becomes licensed, that saves a lot of time and energy of sending everything back for re-labeling and going into all the storage locations and performing re-labeling.

So those are all points that are important to the Stockpile program, as soon as that information would be possible to know.

Also, if products are going to be stored at a location other than a government-owned stockpile facility, or a government-leased facility, we're very interested in knowing what kind of security is available at the non-government facility.

And, if the product is going to be stored at a Stockpile location, we're interested in having

a good working relationship with the manufacturer, because we need to ensure that everyone is happy with the storage conditions--temperature, humidity, etcetera--so that that information can be submitted to the FDA for the final biologic license application.

So these are some of the concerns that we're interested in in the Stockpile program. And if you're interested in knowing more about the Stockpile, we're linked to the CDC Bioterrorism website.

Thank you.

[Applause.]

DR. MACALUSO: Our next speaker is Dr. Marissa Miller. She's a Senior Advisor for Public Health and Emergency Preparedness at DHHS. The title of her presentation is "Acquisition of Medical Countermeasures for Biodefense."

DR. MILLER: Good afternoon. And good for you for hanging out so long. We appreciate your attention.

[Slide.]

You saw a slightly different version of the drug-development pipeline. And really, just to point out that the BioShield program is a procurement activity. So it's meant to pull products through the pipeline by providing funds at the end.

Now, this looks nice and continuous. And, as you've already heard, it's not a perfect pathway--as we know. But it is based upon the funding of basic research leading to the identification of targets, development of leads, and then the pre-clinical and clinical development of products. And this is meant to be a partnership among government and industry and academia.

[Slide.]

So the BioShield project was first announced by President Bush in his state of the union address back over a year ago. And this is a collaboration between Health and Human Services and the Department of Homeland Security.

Now, we've been working kind of on the promise of BioShield. A year ago, in July, the

House passed a version of the BioShield legislation. Just recently you may have notices that the Senate passed a new version. We are waiting for the House to reconcile with this new version, and for the legislation to be enacted into law when it is signed by the President.

Its purpose is to accelerate the process of development of medical countermeasures for biodefense, largely because there is no market for these products. In a normal situation, you would have prevalence or incidence of disease driving companies to develop new products. All we have at this time are the monies set aside in the BioShield program for the acquisition of these products.

Now, the underlying intent--and this is very important, because there have been questions and comments on this earlier--is to have a licensable product developed largely under the Animal Rule--but not exclusively under the Animal Rule--within eight years. So that is our goal in the Project BioShield.

[Slide.]

It is a three-pronged program. It--as I mentioned--established secure funding, a source of monies for the purchase of these critically needed biodefense countermeasures. It also provides increased authority to NIH--specifically NIAID--in order to expedite research and development. And then it also--as was mentioned by Karen Midthun--establishes an emergency use authorization of the product.

[Slide.]

So, the secure funding that has already--the appropriations bill was passed last October. So we, in fact, do have $5.6 billion set aside for the BioShield program. $890 million of these dollars were allocated for FY 2004. And we are in the process of spending those dollars now.

And, as was mentioned, the BioShield program, while the monies flow through the Department of Homeland Security, this program is administered through DHHS in the Office of the Assistant Secretary for Public Health Emergency Preparedness.

[Slide.]

Okay. I think I may have missed a few slides in there. But, skipping along, the acquisition of the recombinant protective antigen anthrax vaccine is our highest national priority right now. And this has been in process. And awards will be made before the end of the fiscal year.

[Slide.]

There are a couple of points that I wanted to make, and slides that apparently got deleted. And just to--in terms of the BioShield acquisition process, we are--yes, we are in a situation of critical need for medical countermeasures. And, in light of the fact that the government can't go and buy products off the shelf, we need this scenario to be able to have products developed.

And what we are attempting to do is to stimulate the development. We cannot fund R&D through BioShield. However, we are limited to the purchase of products--usable products--for placement in the Strategic National Stockpile.

However, as was mentioned before, development costs of these products can be folded into the price.

Now, the payment for these products--we are not buying products that are unproven or unsafe or not effective. What we are doing is setting up a situation where we can purchase these products. We will purchase them when they are ready to be used and placed in the Strategic National Stockpile. So our first payment for these products is made when the product can be used, through a contingency use IND, or when the authority comes about for emergency use authorization.

Additional payment--and then development of the products is continued. This is very important. This is critical. This is mandated in all of our contracts. So product development continues towards licensure. When licensure or approval is obtained, then additional payment is made on the product.

[Slide.]

And this is how it is structured. Again,

it relates to the critical need for medical countermeasures, the lack of products, and the need to be prepared in the best way possible, with the long-term goal of having fully licensed products.

[Slide.]

So, to underscore this need, we are now beginning our second acquisition of BioShield products. And this is for anthrax therapeutics.

We have out on the table--unfortunately, it didn't get put in your books--but on the table with additional handouts there is a pre-solicitation notice. This went up on FedBusOps as of yesterday. And what it does is it outlines a federal government, Department of Health and Human Services requirement, for an acquisition that's to follow, for a broad spectrum of anthrax therapeutic products.

Again--as was mentioned by a number of the participants earlier in this conference--we have a 45 percent mortality rate in the anthrax episode that occurred in 2001. This means we're not prepared, and we desperately want to become

prepared.

So we are initiating this first acquisition, which will be followed by a subsequent one, pending the availability of funds, in one year hence, and again in two years hence.

We are looking for products that will be BioShield ready. They must be licensable within eight years--as was mentioned earlier.

What's most important for the company representatives to know is that potential offerors are required to have submitted an IND application to FDA by the time of proposal submission. Now, what's sitting on the table is a pre-solicitation notice--meaning a solicitation will follow, followed by the collection of applications. And at that time you need to have filed an IND.

The other absolute criteria for evaluation is that you must have proof-of-concept data in small animals. So that is the entry criteria.

Again, this will be structured similarly to the other BioShield acquisitions, meaning that it's a fully burdened cost structure, so we do not

pay for research and development. We realize these products are in early stages--at various stages. Some may not be ready at this time even to apply. Hopefully they will be in the future--next year or the following year.

But the purchase will be of usable product. So that's contingent upon the contingency-use IND status, and/or licensure.

So, we hope--if you have any specific questions about this synopsis, or the requirement, please direct them to the contracting folks down at CDC.

And I thank you for your attention.

[Applause.]

DR. MACALUSO: our next speaker is Dr. Judy Hewitt. She's a Research Resources Program Officer in the Office of Biodefense Research Affairs at the National Institute of Allergy and Infectious Diseases. Her presentation is "NIAID Opportunities and Resources for Biodefense Countermeasures Research and Development."

DR. HEWITT: Well, thanks, everyone, for

staying to the bitter end, here.

I'm going to go--I have a lot of slides, but I'm going to go through them really quickly and try and bring up the high points for this crowd.

[Slide.]

Okay. The link for our biodefense website on the NIAID is here. And I would really encourage you to look there for information. It's constantly updated. And the three main topics that I hope to cover here are funding opportunities, resource awards that we've already made, and some other resources that you may tap into.

[Slide.]

This is a picture of the website. And I'd really like to draw your attention to the middle bar there, "For Researchers." The "Strategic Plan" has links for all of the Category A through C research agendas; the progress reports; sort of high levels documents like that.

The "Funding" link will take you to both current and expired opportunities. And I would also encourage you to pay attention to even the

expired opportunities, because we do sometimes recycle those--bring them back maybe in sort of a different form. But we do use them over.

"Resources" will take you to the web page that lists nine different resource awards that have been made that may be of use to you. And "Upcoming Meetings," also has links for meetings such as this one today.

[Slide.]

This is--real quick--just our standard investigator-initiated research grant application process. I'm not going to say anything more about that.

[Slide.]

We also have an SBIR program. The important thing to note about this is that the current notice goes through August of '05, which means there are four more receipt dates left on this before we'll make a decision about whether we want to reissue this notice or not. And the receipt dates are April, August and December.

[Slide.]

The Biodefense Challenge Grants--this is a closed initiative, but is one that is maybe of interest to this crowd, and perhaps also likely to be recycled in some manner. It's a three-year award--this also replaced the partnership awards.

These are three-year awards, and the important thing here is you have to have an identified candidate product. So if you're already into the development pathway, then this is a good opportunity for you if you have a good product identified.

[Slide.]

The Regional Centers of Excellence--we made awards in FY '03, but this initiative is back out again. And I would also encourage you to consider trying to partner with either a new Regional Center of Excellence application, or perhaps one of the awards that's already been made. The upcoming receipt date is in September. And I'll say something more about this when I get into the awards that we've actually made.

But, you know, this is a good opportunity

to think outside the box and partner with academia.

[Slide.]

We also have a notice out there that--a notice of two RFAs that will be coming out in FY '04. One is we are going to build additional Regional Biocontainment Laboratories; another is we're going to put money into alteration and renovation of existing space. So those are opportunities that will certainly help with research resources.

[Slide.]

Now, I'm going to switch to the resource awards that we made--in case you just crawled out from under a rock. This is where the RBL and NBL awards--this is the major construction that will increase our BSL-3 and BSL-4 capacity in the nation.

These are national and regional resources. And so once they're developed--and the timeframes are shown here--hey will support activities of not only academia, but there will also be government and industry work that can be done there. And the

exact processes will be determined. There will be user fees. But this is a possibility for companies to get work done, without committing to their own containment facilities.

[Slide.]

This map shows where the Regional Centers of Excellence are located. And, again, you can partner with some of these existing awards. And we hope to make a few more awards in this fiscal year as well.

[Slide.]

The thing to highlight about this slide is that the RCEs are supposed to interact both which pharmaceutical companies and biotech companies. So, you know, we're encouraging that. They may not be coming out and looking for companies to interact with, but certainly you're perfectly free to contact them if you see activities they're doing that could help you.

[Slide.]

And on this slide it gives the link for the RCE website--which will give you links for the

individual RCEs.

[Slide.]

This is another big award that we made: the Biodefense and Emerging Infection Research Resources Repository. The end of FY '03 this award was made, and this repository is getting off the ground. They have a website. And the main thing I would like to encourage you to do is to register if you would like to tap into any of the resources in this repository.

You can follow the links. There's a listing of materials. Right now, they're really in sort of a collection phase, and getting ready to distribute things.

[Slide.]

I'm not going to go over this. It's pretty self-evident.

[Slide.]

But, on this one, the thing that I'd like to highlight about this meeting in particular is that toxin peptides--proteins are available through this repository, and that's certainly something

that some of you have tapped into already, and would actually really support the development of antitoxin therapies.

[Slide.]

And, again, they have a website. And there are lots of links there. And you can follow that and get more information there.

[Slide.]

The Pathogen Functional Genomics Resource Center is another award that's a bit more mature. And their website has the request process, the forms--you know, a summary of the review process. And you can go there and get specific information about that.

[Slide.]

I'll just highlight a couple of accomplishments relating to anthrax. There has been a comprehensive genomic analysis of B. anthracis, and hee's the summary of that.

[Slide.]

There are microarrays that are available. And I highlighted bacillus anthracis as one of

those. So if microarrays are in your plans at all, then certainly this is a resource you can tap into.

In vitro and gateway clone sets are also now available.

[Slide.]

Another program is the in vitro and animal models program. This is the program area that I'm responsible for. And this is, in essence, a sort of a developmental pipeline. Three main parts: A and B are in vitro screening capabilities and clinical isolate panels; Parts C and D offer small and non-human primate models of efficacy or infection; and Parts E and F will provide us with a safety, toxicology and immunogenicity or pharmacology testing for the various therapeutics and vaccines that we hope to test.

I should point out that the in vitro capabilities under these contracts are very different than the in vitro capabilities that Conrad Quinn described this morning. This is really intended to give us some antimicrobial susceptibility testing to try and get more

antibiotics into the pipeline, because that's sort of been an underserved area in the pharmaceutical industry of late.

And I'd also like to stress, you know, the importance of parts E and F in developing the safety and pharmacology profiles for candidate therapeutics and vaccines.

[Slide.]

These--there were multiple awards made under these contracts, and they're issued task orders for specific pieces of work under that.

Our intent is to have a ready capacity to get services in any of those six areas at any time that we feel it's necessary. We made six awards in '03, and we're currently expanding that to try and increase our capabilities in all six areas.

[Slide.]

Importantly, the purpose of this program is to serve NIAID programmatic goals, as well as to bridge basic research discoveries from our very healthy investigator-initiated research platform. And those are sort of competing needs at the

moment. The capacity within this program has really sort of been taken over in the short term by our own programmatic goals. We need to support some of the advanced product development--particularly the vaccines that we've been supporting--to try and get them to licensure. But, along the way--as Ed stated earlier--as we develop these models, we will make them available to the community. And at the point when they are available, then there will be a website, and you will be able to go and find the process for getting into this pipeline.

In the meantime, I would say just contact me is the best way, until we have this--all the procedures set for how to access these resources.

[Slide.]

And this just sort of a summary of how we've been spending the money under these contracts. The blue piece of pie--44 percent--has been in small-animal efficacy models; the pink--48 percent--in non-human primates animal efficacy models; and then 8 percent support pharmacokinetics

and PK. And that's the money that we've spent to date.

[Slide.]

And also, then, by product category: we've spent the bulk of the money supporting our vaccine programs; about a quarter of the money in supporting antibiotic efficacy studies. And, lastly, a small piece of pie there for therapeutics. And that's really sort of a new activity as well.

[Slide.]

So, just quickly I'll go over a few other resources.

In December of 2003 we sponsored an Aerosol Challenge Technology and Applications Workshop. And the idea there was to really increase the field, to present all the technologies that were available. And the important emphasis, I think, that came out of that meeting is if you're talking about an early candidate, then you might consider some of the less technologically challenging kinds of challenge models, or aerosol

models, and pursue, you know, sort of full-blown GLP aerosol challenge models only as your product becomes more mature and is worthy of the resources involved in those kind of challenges.

And we may or not conduct another one of these kinds of workshops, and it may also turn into something of more of a general animal-model kind of workshop--now that we've already focused on the technology.

[Slide.]

Another big point that I'd like to make is that NIAID has invested heavily in a variety of sort of behind-the-scenes kinds of things as we've encountered different problems in our own programs. We've recognized the need to refine or further develop animal models, the challenge material; potency assays and acceptance criteria go into this as well; the challenge procedures.

Assays is another thing that's really important in getting all of these products to licensure, and making sure that the assays are robust enough to meet our needs for the long term.

And reference reagents--as we develop those, those will also be deposited in our repository and available.

The other thing I should point out about his is that NIH in general has a commitment to sharing models. That's going to become a condition of award in, I think, the beginning of FY 2005. So, you know, we're committed to--as we develop--get more information about these various animal models, we're committed to getting t hat information out there and making it available so that it's not necessarily tied to a particular product, particularly if it will help multiple products.

[Slide.]

This is just sort of a slide that kind of demonstrates the balance between meeting our own programmatic needs for the countermeasures that we've already committed to developing--balancing that against the wonderful ideas that are coming out of the investigator-initiated portfolio. And we need to make careful decisions about how we're

going to pursue all of these. And in the short term, I think, all of the benefits that will come out of our advanced project development activities will serve the earlier products that are coming out of the investigator-initiated platform.

[Slide.]

And then, just lastly, to end up with this slide.

Research Resources is an arrow sort of going along the bottom of this entire development pipeline. NIH has a huge history in conducting basic research. And we're really now having to focus more on goal-oriented activities--at the end of the day, having vaccines, therapeutics and diagnostics that are really moving through the pipeline; if not all the way, at least far enough along for BioShield to pick them up.

And the Research Resources--NIH has made a big commitment to those resources so that we can keep this pipeline moving, and keep things--as more discoveries are made in basic research, that they can move forward through the developmental

pipeline.

Thanks.

[Applause.]

DR. MACALUSO: Dr. Julie Lovchik from the University of New Mexico was scheduled to give a presentation in an earlier session, but she was delayed due to travel complications.

She is here now, and because the topic of her talk--"Animal Models for Testing Therapeutics"--is of great interest to this audience, we've asked her to give her presentation as the end talk in this session, and then to join us in the panel discussion.

Julie?

Animal Models for Testing Therapeutics

DR. LOVCHIK: Hello. I'm sorry to have disrupted your schedule today. I had a little difficulty getting here, and my luggage seems to have been touring the capital.

[Laughter.]

So, I definitely regret missing all the talks. I'm sure it's been an interesting day.

I work at the University of New Mexico Health Science Center. We have a BSL-3. And I was asked to share some of my experiences--our experiences in testing animal models--or using animal models for testing therapeutics.

The Center is under the direction of Dr. Rick Lyons, but he's out of the country. So I will try to fill you in.

[Slide.]

All right. So, the problem for investigators and biotech companies is having access to appropriate in vivo models to test their therapeutics. So, one of the goals in setting up the Center, in conjunction with DTRA, was to be able to take drugs from multiple companies and investigators that are tested in various ways, and to evaluate them through a standardized model in order to be able to better judge and compare the efficacy of different drugs.

[Slide.]

So we have a BSL-3 that we've installed in an SPF animal facility. And here are some of the

models--murine models that we've established so far in a standardized method. Include organisms of BW relevance such as plague, tuleremia, cowpox as a model for smallpox, and, of course, bacillus anthracis, which we'll focus on today.

[Slide.]

I think they put the wrong one in.

[Pause.]

Okay. Well.

So, the first thing--the first issue is the relevance of getting things into the--what is the beset method for getting things into the lung? And since humans are exposed to anthrax via aerosol, you would think that that is the most--naturally assume that that would be the best way to get it into mouse.

And this is a typical nose-only set-up for aerosol chamber. Mice are exposed via the nose only. And we've done a lot of work with Lovelace Respiratory Research Institute in New Mexico. And in collaboration with them, have learned that aerosol is a very complicated process. It

includes--there are many internal particle deposition mechanisms, including impaction, bifurcation, interception-diffusion. And these are controlled by various factors such as the characteristics of the inhaled particles themselves; the size, shape, density, electrical charge--and as well as the geometry of the respiratory tract and the branching patterns and the angles and the path length.

And also the ventilation, including the breathing pattern, the mode of the breathing, respiratory rate, and tidal volume.

In an aerosol chamber, the mice are awake--much rapid breathing, compared to being anesthetized, where their breathing rate is slowed down. And much deeper breathing. Sedimentation is actually decreased with increased rate of respiratory rate.

So--now on the one hand, the mouse lung is much less complicated than the human--the structure of the lung. Once you get past the trachea and the bronchi, you basically have a straight shot into

the conducting airways--compared to the highly segmented and branching structure of the human lung.

[Slide.]

And also the cells lining the majority of mouse airways are similar to the cells lining the terminal bronchioles of humans.

[Slide.]

However, mice also have a much more complex turbinate structure, which actually impedes optimal aerosolization.

[Slide.]

And this is just a schematic showing the efficiency of deposition among different species. This is from a book from LRI. And if you see--looking at the deposition into the alveolar region, in the particle range of--in the range of a spore, you can see that with humans, oral breathing, that you get good deposition into the oral--into the alveolar region. However, if you look at just the difference between even a human just nasal breathing, and the simplified turbinates

in the human, you can see that the deposition is much--has decreased.

Monkey is comparable--is very close to human. But as you can see, rodents have very low efficiency of deposition via aerosol.

So, it can be done, but--and there are advantages in that it can mimic particulates, and you give an even distribution. But the disadvantages are that it's technically challenges. It requires a large quantity of virus or bacteria.

The depositions are dependent on multiple factors, as I talked about--the rate of breathing when the mice are awake; environmental, such as humidity--which we have a lot of problem with--or lack thereof, in New Mexico.

Deposition efficiency in pulmonary region is very poor in rodents. A large portion goes into the nares, the upper respiratory tract and the gut--even with the nose-only chamber, you get a lot onto their fur, and they groom themselves and each other.

Special situations are difficult to adapt.

We work a lot with catheterized mice, and of course, it's variable transfer between institutions. So in comparison, intranasal and intratracheal delivery, some of the advantages are that it's technically easy to transfer among labs. Dosing is reproducible. Most of the dose is delivered to the lung. It's adaptable to modifications.

However, it does require a liquid and distribution may be multifocal, but not necessarily evenly distributed.

So, initially, we set up to look at intranasal versus intratracheal inoculation of spores into the lung. And --of mice--and you can see that both routes give you a dose response. We saw, however, that the intratracheal, rather, required much lower dose for a lethal infection than with the intranasal--again, probably jut because you're bypassing the nares and going directly into the lung.

[Slide.]

We tried to look to see what other

differences there were. We didn't see any histopathological differences. Also, the rate of germination initiated after either IT or IN delivery was similar.

[Slide.]

And this is just simply looking at the heat sensitivity of the organisms as an indicator that the germination process has begun. And you can see that after one hour, we see almost 95 percent have germination in both IT and IN.

[Slide.]

So, based on these studies, we standardized the anthrax model in Balb/C mice, using the Ames strain, with an intratracheal administration of 5-10,000 spores. The spores are grown according to your standard protocol developed by Terry Kohler at UT in Galveston; and titred and aliquoted in the freezer, such that each experiment, you simply have to take out an aliquot, dilute it to a known amount, and administer it to the animals.

Of course, you're dealing with live

organisms and live mice. In order to document the amount that's actually deposited into the lung, we always routinely take two to three animals, after 30 minutes of infection, and remove the lungs, homogenize and plate, so that we have an actual number that was deposited.

[Slide.]

We routinely see dissemination to the spleen between 20 and 48 hours, and death occurring a day or two later.

As I already said, we saw more reproducible endpoints with the intranasal; much more efficient--more efficient and reproducible results.

And just to compare to subcutaneous, it requires a much lower number of spores, you see dissemination to the spleen. The kinetics are a little bit longer--three to four days, and death in four to five days.

[Slide.]

And--I think I'm running late here, so--just quickly, that when we looked at the lymph

nodes at five hours, we could see organisms. We had to do this by giving more than the usual lethal dose--the minimum lethal dose of 5,000. It was difficult to detect in the lymph nodes at five hours. But at 50,000, you can see that they were detectible. And by 24 hours, it's very difficult to find the lymph nodes, suggesting that they have necrosed.

Dissemination--the spores, or the number of organisms tend to stay constant in the lung. About 20 hours or so you'd start to see dissemination into the spleen--I lost it--there.

And along with this systemic spread, you see some increase now of organisms into the lung, which is consistent with the hematogenous spread of the bacilli back to the lung.

[Slide.]

And I think I'll go through these quickly, because I'm short on time. But, just basically, that, remarkably, there's no inflammation seen with the primary inoculation into the lung. After 48 hours--you probably can't see this very well--but

there are some rods present in the capillaries and, to a lesser extent, in the pulmonary vessels.

The most significant, or characteristic, finding of the histological finding in the mice--similar to anthrax infection in other species--is the massive necrosis in the spleen. You get some congestion; fibrin deposits, and many bacilli present.

[Slide.]

This was just to try and show you the rods here. Sorry. I don't know if you can see that.

[Slide.]

So that's as much as I was going to go into of the model. There's a paper coming out in August, I think, in INI. It gives a little more detail.

But next I wanted to discuss just some of the strategies for looking at testing drugs. And we have several different ways of delivering--I'm sorry. Apologies. This is not the complete--it's the one I sent yesterday.

Oh, well.

I wanted to discuss some of the--initially, some of the advantages and disadvantages of other animal models. But--I apologize for that.

So, anyway, in the mice, the drug delivery--we have oral and intravenous, sub-cu and IP, and as well as we can now deliver continuous intravenous infusion.

[Pause.]

Okay.

[Slide.]

Well, and this is just an example of screening potential antibiotics using the pulmonary anthrax model. And one of the advantages of using mice, as compared to rabbits and guinea pigs, which are a very good model--the pathogenesis--is that they have much more sensitive GI tract, and are prone to antibiotic-induced gastroenteritis. With the mice we don't have that problem.

And, in all experiments, the standard control is ciprofloxacin, which the mice survive 100 percent.

[Slide.]

Another thing I wanted to point out was that the--although the death in the animals is due most likely to septicemia, regardless of the route of entry, that the route of infection is still important when you have to analyze certain drugs; analyzing different immunomodulators which act on the innate immune response.

We found that there was a significant enhancement in survival when the organism was given subcutaneously, but you did not see that with the pulmonary route of infection. And one of the likely reasons is simply because the fact that, well, if you look at the skin in the area of infection, with SQ, that you get a lot of inflammation compared to the lung, which you do not, and thus there's probably--the cells are there that the immunomodulators can act upon.

[Slide.]

Of course one of the limitations in the mouse model is that the main virulence factors--capsule, lethal toxin and edema toxin,

that the capsule plays a larger influence in the murine model than in other models. And the anticapsular monoclonal antibody seen here is protective. But the AVA vaccine, against a virulent strain of anthracis in Balb/C mice is not protective.

[Slide.]

However, we can get around this by utilizing the DVA-2 mice and the Stearns stain--a capsular strain which has a toxin, in order to screen various toxin inhibitors. And you see here that AVA vaccine protects these mice.

[Slide.]

And here's one example from a monoclonal antibody against toxin that showed fairly good efficacy.

[Slide.]

So, no model is perfect. B. anthracis, we have a lot of advantages and disadvantages. I had wanted to discuss more just simply cost is a huge factor, and also the amount of material needed from R&D companies to be able to quickly screen their

drug candidates; and that there's need to develop coverage of models.

So, for efficacy screening, we--you know, the mouse is a good model to quickly screen. And you can use statistically relevant numbers of animals. But then, of course, to refine--once you have a product that looks very promising, to actually look into a model such as the rabbit, and certainly validation in a non-human primates is important.

We are developing setting up rabbit models now, and with Lovelace Respiratory Research Institute, they are modifying a BSL-3 to now look at primates, in conjunction with them, for the final goal of finding a human product.

So, thank you very much.

[Applause.]

DR. MACALUSO: Okay, could I ask all of the speakers in this session to come up for the panel discussion?

Panel Discussion

DR. MACALUSO: Okay, I see we have a

question from the floor.

AUDIENCE: Back in 1999, when Executive Order 13139 was issued, which gave the President, the Secretary of Defense, and the Secretary of HHS the right to, together, decide to mandate the use of IND products to people in military service if there was a significant threat of biological or chemical attack, I pointed out that there weren't enough checks and balances in the system; that the President, the Secretary of Defense, the Secretary of HHS were usually not physicians, they may have no background in how to make the kind of risk-benefit assessment that's necessary. And, yes, there is some discussion with the head of FDA, who, of course, is under the head of HHS.

So, anyway, things happened, and FDA was starting to talk--back in 1999--about the fact that they would consider use of IND products, and get the safety data after the license was issued. That was even in one of the CBER annual reports--in 1999.

And what's happened in the meantime is all

that has taken place, and now we have legislation that will do the same thing for the civilian population. So theoretically, we could all be offered--and depending how that legislation is written, in some cases mandated, if we though we had an infectious problem and people needed vaccine--IND products.

And according to what I heard today, you could apply to get money--as of yesterday, this presolicitation notice--you could apply to get money for a product which requires only that you've just filed an IND; in other words, that you have no human data--safety data. And we were told that these INDs could be become part of the Stockpile; and that, in fact, the Stockpile has INDs in it which have labels, and maybe you tear of the IND part of the label when it becomes licensed.

So I feel, you know, things have really reached a turn where the potential exists that we could face some major threat; the country could be mandated to receive something like the Acambus vaccine which was essentially purchased by HHS

before it was even designed; it was purchased as concept that we will have a recombinant smallpox vaccine based on DryVax. And now we are about to purchase 75 million doses of an anthrax vaccine that looks lousy in its first human safety trial, and for which there's theoretical evidence back from the '60s that PA itself has significant toxicity. In fact, when it was injected into the cerebrospinal fluid of monkeys, it cause complete cessation of brain electrical activity for several minutes.

DR. MACALUSO: Okay--I think Dr. Midthun has addressed some of these issues, but perhaps, Marissa, you could make some comments from the DHHS point of view.

DR. MILLER: Yes, I'd be happy to.

Just to clarify--the pre-solicitation notice is a notification to the public and industry that HHS is interested in a future acquisition. What is stated in that presolicitation notice is that to be considered during the acquisition process, the two qualifications you must have met

are to have filed an IND, and to have proof-of-principle data in small animals.

Now, that does not mean we're ready to purchase a product at that stage. At the time of award, you must be found safe to proceed.

Now, the award is sort of a promise of future purchase of product that is deemed ready to be used in people. Again, we are under a threat of additional anthrax attacks. We know that that there's a 50 percent or so mortality, even in the face of the use of antibiotics--

AUDIENCE: Right--and doxycycline is 100 percent protective.

DR. MILLER: We need additional product. We're interested in seeing that product developed.

We will be purchasing the product when it can be used under a contingency use IND, or emergency use authorization when that becomes available. And that will be a partial payment for product.

The product will be held in the Strategic National Stockpile, and at which time it is

licensable, the labels will be changed, as was explained, and it will be used under approval or licensure, which is the ultimate intent.

That is the same case for the RPA vaccine. The contracts that potentially will be awarded in the RPA acquisition--anthrax vaccine acquisition--are an intent to buy usable product; usable and licensable product.

AUDIENCE: That's all very well. But I know that Acambus is already delivering--has been delivering smallpox vaccine. Somebody's paying for it. And it doesn't look like it's approvable.

So what I'm saying is: I don't think we're only paying for usable product.

DR. MILLER: I might suggest that you go back and you look at the BioShield legislation, because it is very clearly laid out in that legislation.

AUDIENCE: If you go back to the March Homeland Security Hearing, where Undersecretary Brown of FEMA was talking to the Appropriations Committee of the House, it was pointed out that a

lot of money has been allocated with the expectation that BioShield is going to be passed; that Vice President Cheney has been trying to get the Congress to allocate the entire $5.6 billion of BioShield to be spent in this fiscal year.

And I say to you: we don't have licensable products, but we're still spending the money.

DR. MACALUSO: I'm not sure we can go any further on this. Perhaps some discussion after this panel session might be in order.

We have another question from the floor?

DR. ROBLE: Yes, Jim Roble, Hematech.

I am curious about NIAID's effort for assay development. Are these assays to be used by industry? Are they to be used by industry? Are they to be developed and then transferred to industry?

What is the purpose, I guess, and will NIAID make the information available to industry?

DR. HEWITT: Certainly, the assays that are in development now are really to support our

current vaccine programs; both RPA and VMA, as well. But--I don't know--Ed, do you want to make a comment about transferring to other--

DR. ROBLE: Well, I was just curious. Are these for internal product development programs, or--

DR. HEWITT: Well, that's what's driving it right now--yes.

DR. ROBLE: So, for NIAID's vaccine development programs.

DR. HEWITT: That's certainly what's driving our, you know, putting effort into it.

But we do want to see products that we make a commitment to go down a pathway where--that's a bit more certain, rather than, you know, sort of tossing it out there, and tossing fate to the wind.

You know, there are a lot of issues associated with all of this. And it's clear that this is a new pathway, and that we need to support the contractors so that they have the resources they need to be able to carry these assays forward.

You know, if it's generating new reference reagents which then have to be qualified--you know, that's the extent to which we're supporting those.

DR. ROBLE: So NIAID has a full internal--what--vaccine and therapeutic development program that--is it essentially competitive with what is done with industry? Or what sort of program is it?

DR. HEWITT: These are basically the assays that support the vaccine contracts that we've awarded--you know, which have been initiatives that were out on the street, so they're widely known--the RPA and MVA contracts have already been awarded; MVA, the first phase.

There are also plague reviews being done now for a plague vaccine, and there may be other initiatives coming along, as well.

But as we put those initiatives out for advanced product development for vaccines, that sort of drives our investment into the animal models, the assays, the reference reagents that are required for those products to move forward.

Now, to the extent that those assays may be applicable to other kinds of products, I think we can explore transferring that to other places.

But Ed's really the one to comment on tech transfer.

DR. NEWSOME: I can expand a little bit. And, also, it kind of relates to the question earlier.

I mean, what we're doing with the models--the animal models and the assays is the same, in that what we're doing is in support of extramural contracts. So even though it's public money, we have extramural contracts with private companies that involve private products. So we're trying to walk the line between what we have to maintain, you know, private companies' sensitivities, and what we're trying to do for the public good.

Ultimately, as these assays are developed and models are refined, reagents are in place, all of this is in progress. It's really not ready for prime time yet. But when it is, I see that they

will be available for public use.

And just to clarify: the assays we're doing are being transferred from Conrad's lab at CDC--the ELISA and TNA--and he's been very forthcoming, as var as being willing to transfer his assays just directly from his lab to anyone that's interested. And you'd have to talk to Conrad about that. But we are--we do have a substantial effort in the assay development and validation and the tech transfer from his lab to our contractors.

AUDIENCE: Just a quick question.

When I heard the presentation about all of the new centers that NIH is putting up, and BSL-3 and 4 for animal studies, I saw a lot of connections between that and what Dr. Nielsen had put up about a critical need for BSL-3 and 4 facilities for animal testing.

The one thing I didn't see was he had a big red bull's-eye in the middle of his slide that said "GLP." And he also identified that as a bottleneck to development of drugs for bioterror

weapons.

Now, I'm very sensitive to, you know, not wanting to take the bread out of the mouths of, you know, our excellent colleagues at Battelle who are, you know, working diligently in this area. But I suspect, from Dr. Nielsen's comment, that they may be overloaded.

Is there any possibility that some of these BSL-3 and 4 sites will be able to implement GLP? And I say that knowing that it would probably take--you know, two years--conservatively--to take a center from zero to GLP.

DR. HEWITT: Certainly, in the RFP for the RBLs we've encouraged them to do that. Now, we can't mandate that they become GLP.

The downside of the RBL awards--which are the BSL-3s, compared to the NBL awards, the BSL-4s--there will be some operation support contracts for the NBLs, but not for the RBLs. So, in fact, it's going to be very difficult for them to do GLP.

Having said that, I think that certain

places recognize the need for GLP studies, and I think some of them may sort of step up to the plate and make a commitment to becoming GLP--but not all of them, by any means. And whether there's enough work that can come their way to really support the maintenance of a GLP capability, I think remains to be seen.

AUDIENCE: One more comment to that--and that's that this whole biodefense construct is artificial. It's an industry that has no market, and it has nothing to treat. It is very much a government initiative--very appropriately so--to protect the nation against a potential threat.

And I don't think there's a person in this room that would want to make a lot of money out of this, you know, by seeing the threat arrive. But if, God forbid, it ever does, we'd better be prepared. And I think that there's great credit to be given to the government for having seen this and taking some action.

I suspect that maybe there's a need to throw some money away; in other words to--just as

we may be throwing money away creating stockpiles of drugs that will never be used, maybe we should throw some more away creating GLP sites that won't need to be used after three or four or five or six years but, you know, should be supported during that period of doing so.

DR. HEWITT: There is some level of support in these facilities. You know, he put up--in Dr. Nielsen's slide it talked about aerosol challenge, and BSL-3, and GLP and non-human primates. And so there are--in our portfolio, there are contractors who may have had several of those pieces, but may have been missing another piece. They might have been GLP, but didn't have aerosol capability. And so if that's the capability they need to build, then we are supporting that.

It's a lot easier to support building aerosol capability than it is to support GLP infrastructure.

But, yes, we recognize that need.

DR. MACALUSO: I think one question has

been waiting here first.

AUDIENCE: I just wanted to raise an issue in childhood vaccines has occurred recently, for those in the future that are going to maintain the stockpiles of either vaccines or perishable biological therapeutics. And that is that when you first--if you keep the stockpile on your own premises, as a manufacturer, and if you rotate product for some of those products that might be used for first responders or, you know, military, I think you're going to run into the same problem with this new Securities Exchange Act revenue recognition guidelines. And that is you won't be able to declare that on your books as revenue until it expires and you throw it out, and you--even if you get paid by the government for it.

So the childhood vaccine manufacturers--even though they've been paid for these vaccines--in order to prevent waste, they store them and they rotate them for the government on a one-for-one basis. Then even if they keep them separate, they're deemed by the Securities

Exchange and the Price Waterhouse, and all these auditors, as not being revenue.

So, for a company that's starting up, it's going to be a little difficult when you have to set aside revenue and you can't use it, after you've already received it, until the product expires, or someone uses it, and you actually draw down your stockpile.

That's going to be an issue that's going to be--it's unique to vaccines and perishable stockpiles. It's a new issue. It's never been a problem until these new guidelines are out, and there's going to be a need for either the Securities Exchange Commission and HHS to kind of figure out how to do this, or to go to Congress, you know, for--otherwise people might not be as interested in going into this business.

AUDIENCE: Can I just make a comment on some of the things that have been mentioned in several different veins. One is--I'm Jim Easter from Battelle, and we do a lot of GLP work. We've done about 50 GLP studies in animal models over the

last three years.

And it is something--if you talk about GLP, the issue is not whether you can or cannot--you have to do it constantly, every moment of every second of the day. It's not something you turn on and off, and it's not something you can say, "We're going to do a few GLP studies here, and then, you know, a couple months from now if we decide to do it, we'll go back and do GLP."

It's a constant effort to do GLP.

It's not impossible to do. And if you make an effort and you have quality assurance program in place you can keep up with that.

But when you get into the arena of doing containment research, animal models, aerosol exposures, and qualifying all your reagents--and your actual challenge material is a critical reagent. When you pull all those pieces together, it is extremely complex to pull off a good study. And it is a real challenge.

And so when you're looking at all of the resources that are being put out into the United

States in the form of these RBLs and NBLs, their real strength is not going to be doing product development, last part GLP studies to push things into the market. It's really exploratory development, bringing new items forward. That's their real strength.

There isn't going to be a huge need for facilities--every facility to do GLP. I don't think that's necessary, I don't think it's anywhere close to what the nation needs. And I don't see many of them going there.

And as far as capacity. It is very difficult to project what the capacity is going to be required to conduct the GLP studies for the types of products that are going to be coming forward. In a facility like I have--which is undoubtedly--currently--one of the largest in the United States to be able to conduct GLP studies, against aerosol challenges in a variety of animal models, to include--up through primates--we are very heavily work-loaded. But we are not at capacity.

What happens is when you get a person who wants--or a company, an organization, who wants a study done, in many cases--I'd be quite honest--the product isn't ready for GLP studies. Many of them are exploratory. There are a whole bunch of stages of formulation. There's a whole bunch of issues that have to be put in place before you would say "this is a pivotal study" to take a product to license.

And until you have those kind of components--and the assays--we've worked extensively to validate methods and assays. And until you can pull those pieces together, with a product that you have in a good formulation that you would want to take to humans, it really doesn't--in my personal opinion--it's not ready for GLP studies.

But, it is ready for high fidelity in the methodology and the research. But many cases, people are ready. They want to go forward. There is a big rush. There's a tremendous push to get something out. But until it's ready, you're going

to wind up re-doing that work anyway.

So sometimes it's better to take a little more time, be a little more thought provoking in how you design your studies. And then whenever you come and are ready to do the studies--quite honestly, I have capacity today, as do a few other places around the United States that are either into or getting into the GLP arena.

We don't have enough products to put in that market yet to saturate it, in spite of the fact that I'm very heavily work-loaded. If there's a good product, and it's ready, we can put it in the queue today.

And I think that's--the projection of how much capacity is going to be needed is not easily definable, and has not been defined, in my mind, by anyone today or in the past.

DR. MACALUSO: Okay. Are there any more questions from the audience?

Gerry?

AUDIENCE: First of all, I want to thank Julie for making the effort to come and make her

presentation, because I think it was a very informative and critical question on the animal models.

And my question--maybe for Julie, or Brad, or Karen, basically--in anthraxes, one of your models was looking at the intranasal installation of Ames strain in mice, and looking for antibiotic --screening of antibiotics, which was targeting the bacteremia of the anthrax.

But there's also a toxemia to the disease, and some products are targeting the toxemia of anthrax.

So my question is this: I mean, are there really two models that you need to develop here? One for the bacteremia and one for the toxemia, such that there should be an animal model for--toxin challenge model that would basically establish the efficacy of an antitoxin?

Julie? Brad? Karen?

DR. MIDTHUN: I don't know enough about the particular models that Dr. Lovchik was presenting to really be able to comment, but I

think that it would depend, in terms of what model you choose, how it really could relate to the human condition. And I think that was really something that, you know, Dave Green spent quite some time discussing earlier today.

I mean, I guess I would just like to say that I think that with all of these different efforts it's really, really important to come and talk with us at FDA. I think coming in for a pre-IND meeting, even a pre-pre-IND in some cases, we are happy to do that also, because we would really like to have a good flow of information and communication to try to facilitate the process.

One other thing I'd just like to add that I omitted to say when I was talking earlier was that FDA is actively working on drafting some guidance for emergency use authorization. And so we would hope to have that out in the relatively near future, also. Thanks.

DR. LOVCHIK: Yes, I think that it's important to have a model that you can look at, depending on the drug; whether it's against a toxin

or the bacteremia. And that's why we have--we essentially have with the mouse, kind of separated.

However, you certainly have to decide if that drug would work against a full-fledged infection, and in an animal that is susceptible to both. So, regardless of what happened in the mouse, you would certainly need to go into a rabbit or a non-human primates and see if that drug had efficacy against a whole infection.

I think it's a little dangerous to look at just toxin injection, because--I mean, it really isn't clear what are the levels of toxin in human, as well as in the monkey or the rabbit.

And during a real live infection, and which of those are actually the most critical, as far as lethality. And, in addition, I also I had meant to mention that we also are working with Terry Kohler, and she has developed several mutants of anthracis that have either a lethal toxin--or lethal factor or edema factor mutated, such that we can even go further and look at which toxin, and what their role is in the pathogenesis, as well.

And targeting drugs for those particular toxins, as well. If that answers your question.

DR. LEWIS: Richard Lewis, from Access Bioconsulting.

I had a question for Dr. Midthun--actually, two quick questions--on the emergency use authorization.

The conditions sound an awful lot like a treatment IND. And a treatment IND, there's an assumption of cost recovery. And you didn't mention cost recovery as part of emergency use. And I wondered if that is a component of it.

And my second question: you mentioned that for emergency use of the product, there is an assumption of effectiveness, and that there's a risk-benefit determination that's made.

Both of those conditions sound like there's an expectation of data. And I wondered if a sponsor for one of these products should be thinking about the data that might support an emergency use authorization. And how forward-looking should a sponsor be in putting that

together--prospectively, before there's an emergency event.

DR. MIDTHUN: Good question.

First off, I can't comment exactly on how the ultimate emergency use authorization will pan out for the BioShield, because that hasn't yet been reconciled and signed into law.

But as far as the emergency use authorization that was enacted for the military emergency, there is nothing that I recall about cost recovery there. Certainly, you are right that there is information that is really important to have, because we can't make an assessment that the benefit exceeds the risks unless we have a certain body of data. And so I think that, you know, for such products where there is, you know, a good likelihood that that might be something that would be of great utility in an emergency--then, yes, I think it is important to be forward looking.

And, again, I think the guidance that we are working on really speaks to a lot of these issues, Richard. And so we're really hoping that

that can really be more specific in addressing these issues.

But you're absolutely right. I think, certainly for investigational products that are under development, you know the mechanism there would be that for those products, they will be developed under IND, so information will be accruing for those products that would inform these different issues and allow and assessment to be made.

DR. MACALUSO: Okay, I think we're starting to run overtime. And I think it's time to probably wrap up this session.

I'd like to thank the members of this particular session.

Before you leave, though, I think Dr. Karen Weiss would like to make a few final comments.

Wrap Up/Adjourn

DR. WEISS: Just to thank those who have stayed to the bitter end. We know it's a marathon day. Again, it was not what we had planned when

we started this six months ago. But these things happen. And I do appreciate everybody staying; everybody's input.

I appreciate all the speakers' coming and providing their expertise.

I think this whole area of development of therapeutics for anthracis can be summed up in the title of your last session: the challenges and opportunities. I think we've heard--I've heard--a lot about all the challenges yet to come, and that are facing all of us, in terms of developing these products. There are great opportunities, and clearly there's a critical medical need for these things.

And I think this conference highlights, again, all the issues; the collaborative spirit with the government and other groups to try to work together and come forward with plans and actual data.

Our hope--like Dr. Midthun said--is that we're going to be using information from this workshop and others to try to develop guidance that

the FDA can put out to, I think help people; and to try to address the kinds of studies of various kinds, and the kinds of data that we'd be looking for as we go forward; whether it's for emergency use or, ultimately, for an approval under the Animal Rule.

So, with that, I would just like to give a round of applause for the panel; for their diligence--to all of you. And wish you good luck getting out of the D.C. area in this unusual circumstance that we're facing.

Thank you.

[Applause.]

[Whereupon, at 6:32 p.m., the conference was adjourned.]

- - -