DEPARTMENT OF HEALTH AND HUMAN RESOURCES

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

Public Workshop on Animal Models and Correlates of Protection for Plague Vaccines

FDA's Center for Biologics and Evaluation
National Institute of Allergy
and Infectious Diseases, NIH
Office of Research Development
and Coordination,
DHHS Cosponsors

Thursday, October 14, 2004

8:30 a.m.

Marriott Gaithersburg Washingtonian Center 9751
Washingtonian Boulevard
Gaithersburg, Maryland

P R O C E E D I N G S

DR. BURNS: If everybody could take their seats, I think we'll get started.

I think we have a very interesting day ahead of us, with a lot of discussion. And we are starting--we have two talks in the first session, one by Ed Nuzum, who is heading up the effort to test the new anthrax vaccines in animals. And he and his team have been through quite a lot figuring out how to test these vaccines and then learning what problems are encountered along the way, what kinds of things really need to be thought about. And I think that all of us who are dealing with plague can learn a lot from what Ed and all of his colleagues have learned in going through testing the anthrax vaccines. And so, he's going to tell us about that.

And then that will be followed up by Karen Meysick, who is at CBER, who will present some considerations that we need to think about when we're looking at testing plague vaccines in animals. And then she will also present some

possible strategies. And this is really just a strawman and meant to be a starting point for discussion, nothing definitive. But simply a starting point from what we can take off from there.

So I think we'll begin now with Ed Nuzum, who will tell us about lessons learned from anthrax. Ed?

DR. NUZUM: Good morning. Can you hear me all right?

I feel like kind of the odd person out here, talking about anthrax in a plague workshop. But what I hope to do today is really talk more about the concepts that we've dealt with in relation to the Animal Rule as far as development of the anthrax vaccine. And to the extent those concepts overlap with any vaccine being developed under the Animal Rule, and certainly it applies to plague vaccine, I think. Hopefully, you'll find this useful.

So many of you are probably aware that in April of 2002 there was a workshop for rPA very

similar to this one. And from that workshop came a lot of decisions and guidance that's been absolutely indispensable to us in our efforts for working on rPA vaccine. So what I want to do today is talk about how we've gotten--or where we've gone since then, how we're integrating the Animal Rule, and talk about ongoing studies, planned studies to develop the concepts and meet the requirements to Animal Rule.

We'll talk about how it's being implemented. And I hope that you get the message that this is a very complex endeavor. More than once, I've heard Dr. Goldenthal say--Dr. Ed say that the Animal Rule does not make licensure of a vaccine easier. It simply makes it possible to license one that cannot be tested in large efficacy trials in the field. So it's a challenge. Very interesting, but challenging.

I think I need to give a little background about some of the things that evolved as this program was developed so that you understand why we're doing some of what we're doing. Initially,

there was going to be a down-select between one of the two contractors. After the first round of contracts, a decision was made not to do that. Both were awarded a second round of contracts to continue development of the rPA vaccines.

And part of that decision then, the government decided to sponsor the aerosol challenge studies, largely due to access to facility, gave us more control over the design and conduct of the studies. And in hindsight, I think it's been a lot of work for us, but I think it was the right decision.

Initially, the work was to develop a vaccine for typical use, a pre-exposure use. As the program developed, probably a year or so into it, we realized with a lot of guidance from ORDC, the department Operations Research Development Coordination office, that post-exposure emergency use indication would be a very high priority and is apt to be the first way that this vaccine would be used, in a post-exposure scenario. So that had a huge impact on the studies we had designed, and it

required rethinking of a lot of our timelines and specific studies.

Also within OBRA, within our Office of Biodefense Research Affairs at DMID, there's two other huge efforts. One is the IDIQ in vitro/in vivo testing contract that Judy Hewitt runs, and the other is the biodefense repository contract that Ken Cremer runs. Both of these have become instrumental in supporting the rPA vaccine development efforts. And these are totally complete subjects in themselves. I mean, we could have whole talks on those. The point is, is there's a lot of support required because this is such a complex program.

Most of you are aware probably that bioshield legislation has recently been signed. One of the new authorities granted in that to the FDA is the emergency use authorization authority, and that, combined with this perceived need for a post-exposure indication, means that all the work we're doing now, even though we're only at the Phase 1, Phase 2 stage, the quality of the work

we're doing now needs to be much higher than it normally would be at this stage.

Quality manufacturing, GMPs for manufacturing, GLPs for animal studies, documentation throughout is essential. So this is another important area because the vaccine could well be used in an emergency prior to licensure.

Bioshield also increased funding for and essentially validated large--provided mechanisms to obtain large stockpile requirements for the stockpile for the vaccines, especially for anthrax, which is what the one we're working on first.

Timelines. Anyone associated with this knows that the timeline has been very aggressive. And I can't say much more about that than it complicates things in that we're trying to--we're probably trying to push studies faster than we should from a purely scientific point of view and that we don't always get the complete analysis of current studies or finishing studies before we move on to the next one.

So the bottom line here is that, as in any

product development project, flexibility and focus is required, and I know all of you know what that means.

Well, this is the Animal Rule, and I'm not going to go through this in detail on this slide. I plan to go through each part of this and say how we've implemented it based on the guidance we received, based on our current studies. I want to talk about how we've implemented different parts of this. And Mark certainly did a nice job yesterday explaining Animal Rule.

One thing I'd like to point out on here, if you go through and count up, "human" is referred to five different times just in these phrases. So even though it's called the Animal Rule, it's really about people. We have to have data from clinical trials as soon as possible.

So the first element is knowledge of the pathophysiological mechanism. In other words, FDA wants us to know that whatever intervention we're developing, we can explain the rationale for how it works, why it works, and so forth.

So from the April 2002 workshop, and I'm not going to go through this in detail. I'm not here today to talk in detail about anthrax. My point is this was essentially cut and pasted from the minutes from the workshop. And so, the point is that a lot of discussion occurred considering histopathology, pathology, and the potential animal models and in relation to man. So just as we did yesterday, there's a lot of discussion about suitability of animal models and their relationship to man.

In the workshop, there were three different potential mechanisms for PA-induced protection that were proposed. I'm not aware really of any new information that supports stimulation of spore phagocytosis. I know there is now some additional information on antibody inhibition of spore germination. I think Sue Welkos has published on that. But really, this remains our main area of focus, the anti-PA neutralization antibody would be our main focus of the talk today, and it remains our main focus of

the actual studies that we're doing.

So just a quick recap to show that we do understand the mechanism of toxicity. Anthrax contains two plasmids. One produces the capsule. The XO1 plasmid produces three proteins. Those being the protective antigen, the edema factor, and the lethal factor. When PA combines with EF, it forms edema toxin, and with the lethal factor, it forms a lethal toxin.

And this is a cartoon that shows similar ideas. The PA-83 protein is cleved to form PA-63. This forms a heptamer and a pore, which internalizes the EF and LF, which results in edema or which is caused by edema factor. Lethal factor causes macrophage killing and lethality.

So, again, we're not going to become anthrax experts today. But the point is this is a very well-defined, well-accepted mechanism of how PA or anthrax toxin works and how intervention with anti-PA can provide protection.

The second and third parts to the Animal Rule deal with predictability for humans and then

also how that endpoint relates to the desired benefit in humans. Again, from the workshop, those areas were addressed. And again, I'm not going to go through all this in detail. The point is we discussed it in that workshop--well, I didn't. I wasn't there. But it was discussed. And conclusions were made that have been very important for development, essentially serve as the foundation for development of our program.

Note that the nonhuman primate was considered the model that best reproduces human disease, and it's a very important conclusion.

So, more recently, there's been a couple of important papers this year that deals with animal efficacy as well as relevance to humans. Little, et al., published--and I think Louise was on this--a paper, very nice paper on correlates of protection in rabbits. And Conrad and his group has produced or had a paper on immune responses in persons actually infected with anthrax. So this addresses--a recent paper that addresses efficacy in rabbits, this is a recent paper that addresses

relevance in humans.

Also very recently, CDC is conducting or they're currently conducting studies in the rat toxin model, where they give AIG, anthrax immune globulin, intravenously and now have proof of concept data that it does produce protection. So that's proof of concept based on a passive transfer model.

As far as relevance to humans, we know that we need to address the most likely route of exposure, and that's considered to be, from a bioterrorism, biodefense point of view, that is considered to be likely to be an aerosol exposure. And to get that data, we need aerosol. We need well-developed aerosol challenge models, using good laboratory practices, and it's very important that elements of those models that can be validated are validated.

And the model in general needs to be well developed, standardized, reproducible. Of course, that all goes to a validated model. Very difficult. And we have a lot of effort going on to

refine and improve the models that are in place.

So from the workshop, again going back to the workshop, the recommendations were that the whole immune response be investigated, not only the presence of antibody. Even though we know for anthrax, humoral antibody is important, FDA wants us to look at the whole immune response. Passive and active immunizations need to be evaluated. Comparison of kinetics of the immune response in animals versus humans. And this has become important because of the PEP/GUP indication.

For example, if we're developing just for a typical pre-exposure use, you want the highest response and the most durable response. However, if you want the vaccine to be used in a post-exposure emergency, you want the quickest response that provides protective level. So it totally changes the studies that you do to address those questions, and we're trying to deal with both.

And finally, very important, rabbit and NHP models would be utilized. This sounds very straightforward, very, you know, like maybe a no-brainer.

But, in fact, this recommendation is invaluable because it's allowed us to focus and concentrate on those two models.

It's hard enough just working these two models, getting them to where they need to be, developing new models for post exposure in two species, let alone if we had to actually go out and discover which species, which model best suits the Animal Rule. So this was very important. And hopefully, from this workshop, similar recommendations can be derived because it really is important for that foundation for going forward in the vaccine development program.

So the fourth part of the Animal Rule deals with kinetics and pharmacodynamics, and this gets us a little bit more to our current activities. And I can talk a little bit more now what we're doing currently.

Early on, we developed a working group, a governmental working group that involves CBER. Drusilla was very instrumental in this, Bruce Meade and others at CBER, Mark Abdy. DoD was involved.

Louise has been an active and very valuable participant.

We had discussions and developed some general guidelines on what studies needed to be done to address the Animal Rule with regard to the rPA vaccine. Those guidelines have been posted on the NIAID Web site, and I'm not going to go through this in detail. You've heard a lot of this before. Again, I'd reiterate the importance of early clinical trials for immunogenicity, and this only deals with GUP or the pre-exposure indication. There is another section for post-exposure indication. And in fact, most of our activity has dealt with post exposure because that's been the priority. For plague, I think we think--and that's going to be discussed later. I think we think that pre-exposure will be the main indication. So that's why I've concentrated on the GUP aspects today.

So from that guidance, we came up with a list of GUP studies that are either completed now, some of them are completed. Some of them are

ongoing. And others are designed and scheduled to be completed. So under correlates of protection, we had proof of concept studies. Those have now been completed in primates, where we gave two 50 microgram doses.

In rabbits, we did one relatively low-dose vaccine just to show what we called stressing the vaccine, just to show that it would--at a minimal dose, it would provide protection. We've had very good results in those studies. We've completed a dose-ranging study in rabbits. I'm going to show you a little data on that. We've not done that in nonhuman primates yet, but we definitely will.

Passive protection studies are planned. Right now we're in the process of getting human immune sera, either via plasmapheresis or other protocols to collect enough sera for passive protection. And actually, that's--I don't think I mentioned it yet. But another reason that's important is to get human reagent for assays, reference standards, control panels that we can send out that aid in assay validation.

Other studies that are planned include duration of efficacy, high-dose challenge, challenge with a non-Ames strain. And then when we get into the Phase 3 phase of the product development, a lot of this will be repeated. But it will be done as pivotal studies, completely GLP, validated models to the extent that that's possible, and so all the work we're doing now really is building up for these final studies in the Phase 3 timeframe.

So this is the one data slide I have. I hope you can appreciate that the government is not the IND holders for these products. So, really, we work closely with the companies to be sure we don't reveal anything they don't want us to reveal. Both companies, VaxGen and Avecia, graciously agreed for me to present this data today.

But I picked this data because I think it's a classical, well done with good results study that goes directly to the Animal Rule. And what we did was--and this is both vaccines. There is no effort here to differentiate between the vaccines.

That really isn't an issue for us at this point.

So these circles on the bottom are animals that died. The circles on the top are animals that survived. We gave 2 IM doses 4 weeks apart, ranging from .12 micrograms to 10 micrograms. Then challenged at 10 weeks, which is 6 weeks after the second vaccination. And then did ELISAs, which is on the X-axis here, the anti-PA in micrograms per mil. And this is the predictive--survival predictability on the Y-axis.

So you can see--well, you probably can't see. But this is 32 micrograms per mil, and so you can see any rabbits with titers above that survived. Another way to look at this statistically, if you want to predict that probable survival of 97 percent with a lower confidence interval of 89 percent survival, then you would need 100 micrograms per mil anti-PA.

So this study was very nice. It showed a clear dose response to the vaccine, and it showed--begins to develop a database that shows the minimum level of protection in animals. Because it will be

that information we'll want to be sure that we can show we could get that level of protection in humans when the vaccine is given.

So current issues. This kind of gets to the lessons learned portion of this, the experience we've gained. I mentioned this already, but clinical data is needed to develop and refine animal models so that the models will mimic the human response.

To me, I mean, with a toxicology background and so forth, I'm used to doing as much testing, and I think in drug development, you typically do as much testing as possible with animals before you go to people. For the Animal Rule, for vaccines, of course, you need to have sufficient safety so that you can go into people. But you need to get into the clinic as soon as possible so you can start generating that clinical data.

So animal studies are not designed to show how well the vaccine performs in animals. We know we can protect--early on, we knew--well, even

before this program started, we knew rPA protected animals very well. That's one reason it was selected for development. So it's not a point--it's not a goal to show how well it works in animals. What we want to do with the animal studies is design the study so we can delineate minimum protective responses, which is what I just showed in the slide previously. That way, we can then determine if those levels are attainable in humans.

I've already mentioned the need to do plasmapheresis early on in a Phase 1 trial. This is typically not something done in a Phase 1 trial. But I think it's imperative that you get that material as soon as possible for passive protection studies. You're going to develop your passive protection models, proof of concept, and you need it for reagent. It's critical for reagent for assays.

As you might imagine, there is a lot of variability in these models. The models themselves are variable. The animals are variable. The

assays are variable. So because of that variability, we're going to need large numbers of animals to attain the power we need. Probably not attainable for primates. That doesn't mean we're not going to do primate studies. We hope that we can use rabbits to get the power and statistical significance that we need, but then confirm those results in primates.

The goals of model refinement and improvement--I mean, development improvement and refinement in general should be a validated system, and I've already alluded to that. Very challenging. But by the time we get to Phase 3 trials, Phase 3 pivotal studies in animals, where we're testing Phase 3 consistency lot material, these models have to be nailed down. Part of that includes well-characterized challenge material, controlled dosing, and well-characterized animals. I'm going to come back to that in a little bit, actually.

Another huge issue is availability of GLP facilities and expertise. There aren't many places

that can do GLP, nonhuman primate BSL3 aerosol challenge studies, and that's one reason the government took over control of sponsoring these studies to help coordinate access to those facilities.

You need availability of product for testing. This has to do with where you are in the development process. If you're doing proof of concept studies, maybe some non-GMP pilot scale material is sufficient. If you're doing a "pivotal" study to substantiate to submit for emergency use approval, EUA approval, then you probably at least need GMP pilot scale material. And if you're in Phase 3, doing Phase 3 studies, you need your final scale, final product, large-scale CGMP materials.

So availability of product is, when you're on a fast-track project as we've been, it has been an issue. And of course, if you have a deal you want--you have to remember that that's a consideration.

I think the best way to address both of

these or one of the best way to address both of
these is to have a long-term plan, i.e., a timeline that facilitates the study design, resourcing, and execution. If you know what studies are coming down the road, you can plan your facilities. You can plan your personnel. You can plan your manufacturing to make sure that everything that needs to come together does come together.

Tech transfer and validation of assays. This has been another huge effort separate from the animal models themselves. We were very fortunate with rPA to have a lot of good work that preceded our efforts by Conrad Quinn at CDC, USAMRIID, DSTL in the UK. A lot of people had done a lot of assay work prior to us, both for the ELISA and the toxin neutralization assay. So we really did try to capitalize on that, and it was very beneficial.

Production and qualification of assay
reagents. This, in conjunction with assays themselves and reagents, I think is probably one of the areas that are most overlooked. You know, it's easy to make some stuff. It's easy to put it in

animals. It's easy to get some data. But the nitty-gritty of assays and quality reagents are problematic, and they take time to get and characterize. So these areas cannot be overlooked.

If you think you have a product that you want, are serious about advanced development, that's going to require the Animal Rule or you're going to transition it to someone for advanced development, you need to be addressing these areas as soon as possible.

Agreement on key parameters of the models, such as challenge dose and challenge interval.
Early on, we decided that we would use 200 LD 50s in our aerosol challenge studies. The challenge interval I've already mentioned was 10 weeks, 6 weeks after the second dose. Again, these are parameters that if you can come to decisions early on, it saves so much time because you don't have to address those issues in your model development.

Agreement on major questions to be addressed, which guides study sequence and design. That goes back to the timeline. And from our

working group, from our government working group early on, knowing what questions needed to be addressed, we were able to design studies, put them in a timeline, and it's been very beneficial as far as planning and overseeing the studies themselves.

Okay. So I'm running out of time. There's a lot of issues. I mentioned variability, and that comes down to various categories and challenge material itself, the apparatus, and then the in vivo activity of the challenge material, the animal health and immune status, and assay standardization.

I've already talked about assays. Going back to challenge material, you want to select the right strain. You want to produce it properly. You want to characterize it properly. If it's produced in multiple sites, used in multiple sites, you need--it needs to be characterized. You need a good, robust potency assay. Hopefully, that would predict different virulence in nonhuman primates because that's going to be used in your models.

As far as the apparatus itself, you need

good quality control documentations to help ensure that the hardware that you use is being used properly and consistently.

Spore kinetics. How many spores get into the lung? How many leave the lung and germinate? Those are issues we really have not addressed to date. You get a calculated dose, but you really don't know what happened to the spores after they go in the lungs.

There is some thought that some monkeys that come from China or produced in Texas--or raised in Texas, housed outside, may incur low-level exposures to bacillus-type organisms. And we have some concern there may be some pre-existing immunity. We're trying to address that with different assays.

So why do you need to reduce variability? It would allow fewer animals per experiment, better predictability, improve consistency, and potentially decrease numbers. Really, this is a resource and efficiency issue. The better your model is, the less variability, the better your

results are going to be and the less studies you're going to need to perform.

Finally, I just want to acknowledge all the people that have helped with this project. It has been very challenging, but also very rewarding. The cooperation that we at DMID have received from DoD, CBER, CDC, department--really, everyone--has been very rewarding to me.

The companies have both been very responsive, and we also use contract support, McKesson and EMMES. We have a very good consultant I don't have on here, and I should have mentioned--I meant to mention earlier. We also have a blue ribbon panel. Dr. Ferrieri is here today, and she's one of the members on our blue ribbon panel. It's an external group to help advise us.

Finally, I want to acknowledge all the help and expertise that Battelle provides. Several times I've alluded to the need for the proper facilities with GLP capability and expertise. Battelle's contributions to this, especially the Animal Rule portion of this, the animal study

portion of this, is invaluable, and they've done a good job--not that we don't have problems. All research programs have problems. But they've been very responsive, very professional, very helpful.

I'd also point out we're doing a lot of things besides just spraying animals with anthrax. We're trying to improve the model itself so that by the time we get to BL3, it will be validated to the extent possible. We're doing a lot on spore preparation and spore potency so that the challenge material is the best characterized consistent material we can use or we can get.

So that's all I have.

[Applause.]

DR. BURNS: Thanks, Ed. I think you did an excellent job showing that the Animal Rule is no shortcut to licensure.

Does anybody have any questions for Ed? And again, I remind you, please state your name and affiliation.

DR. NATHAN: --Nathan from Virginia Tech. My question was if there is an indication

that spore clearance and CMI is somehow involved, at least it looks like you have tried to address those issues, would this vaccine protect against potential biothreat using Sterne spore vaccine, which is devoid of capsule? Any other kind of challenge other than the Ames strain?

DR. NUZUM: We are going to do a non-Ames challenge. But I don't think I understood the question.

DR. NATHAN: The cattle vaccine is for Sterne. Would that protect against that type of challenge?

DR. BURNS: So I guess the question is if a strain doesn't make the capsule, would this vaccine protect against it? But--yes.

DR. NUZUM: Yes. Since it's anti-PA, I think it would for this vaccine.

DR. BURNS: Because those strains would make the toxin. Also those strains aren't very virulent. So I don't think they're a real biothreat.

DR. MIZEL: Steve Mizel, Wake Forest.

You mentioned that you're using 250 LD 50s, and I was very interested how you came to that because in the discussions yesterday, the challenge doses were all over the map. And so, maybe you could enlighten us how you came to that and what it was based on?

DR. NUZUM: How we came to using 200 LD 50? I don't think there's really a specific scientific rationale. I think, historically, that's been a challenge dose that was used, and it was just considered that if you got protection at that level, it should be adequate.

I don't know--Drusilla?

DR. BURNS: Maybe I could add just a little bit to that. It is known that the potential exposure in the situation that happened on Capitol Hill with the letters that were opened, the exposure may have actually been a lot greater than that if you were the person that opened the envelope.

But I think the thought was for most people exposed, 200 LD 50 pretty much captures what

they probably got. And then also technical feasibility comes in at this point. It's extremely difficult to do the 1,000 LD 50 challenges for routine challenges. But I think, Ed, some larger challenges are probably planned?

DR. NUZUM: Right. We do--and that's the reason there is a study that we will look at high-dose challenge to get some idea of what level of protection or how high a challenge can you protect against. And CBER is not saying that for the vaccine to be licensed, it has to protect at those levels. But it's largely information so that if someone is exposed to those high doses, we'd have some information how protective a vaccine might be.

DR. BURNS: I think that's an excellent question for the--as we go into the panel discussion, we're going to discuss what dose should be given. And I think we need to think about what are the most likely doses that people would be exposed to and then take into consideration technical considerations also.

Our next speaker is Karen Meysick, who

will talk about possible approaches to the development of correlates of protection for plague vaccines.

DR. MEYSICK: Can everybody hear me okay? If not, you know, wave a hand.

What I'd like to do today, since this is, I guess, the last official presentation of the workshop before we hit the panel discussion, is take a few minutes to talk about possible approaches to development of correlates of protection for plague vaccines.

And really, what I want to do with this presentation, the goal of this presentation is really to have people highlight points that I think we all need to consider, specifically for plague vaccines, when we start thinking about correlates of protection and also to suggest or offer a couple of strategies that could be applied in developing these correlates.

So, as you all know, we're here today because plague vaccines will fall under the Animal Rule, and the Animal Rule came into effect, it was

finalized in May 2002. Now you're probably--at
this point, probably tired of the Animal Rule. I know that Mark, initially, at the beginning of the workshop introduced everybody to it and went through it. What I'd like to do right now is revisit it and revisit it with the information that we got from yesterday in terms of plague pathogenesis and vaccines.

So, in general, the application of the rule states that the rule is applied when adequate, well-controlled clinical studies in humans can't be ethically conducted because the studies would involve administering a potentially lethal or permanently disabling toxic substance or, in our case, an organism to healthy human volunteers and field trials are not feasible.

So a couple points to think then in terms of plague vaccines, first off, there are obviously three forms of plague. But the two predominant forms are bubonic and pneumonic. Bubonic probably being more of the natural infection, but given the times and the nature that we live in today,

pneumonic plague is obviously the emphasis of this workshop.

That stems from the fact that there is the fear that Y. pestis could be used as an agent of bioterrorism, with the most likely form of delivery then being through aerosol dispersal, causing pneumonic plague. And as Dr. Perry mentioned earlier and so did Jacob Kool, pneumonic plague has a high fatality rate. And so, hence, the emphasis.

The other important point to remember is that the previously killed whole-cell vaccine that had been licensed before and is actually no longer being manufactured, except in, I think, Australia, has been shown to be ineffective in providing this protection against aerosol challenge in animal models. And that's both out of DSTL in the UK and USAMRIID. So right now, obviously, we need another, new generation plague vaccines that can protect against pneumonic disease.

So there are four requirements for the Animal rule, and the first requirement is that there is a reasonable, well-understood

pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product. And I think after listening to Dr. Perry and Dr. Bliska yesterday, with their really great talks, is that we do have a handle at least partially on plague pathogenesis or Y. pestis pathogenesis and those associated virulence factors or Yops that lead to the disruption of the innate immune response.

Not only that, but we also have a reasonable idea of the two antigens that are pretty much in the candidate vaccines right now, F1 and V. And I think after yesterday, we all know that there's pros and cons to both of them. But we know that F1 has some anti-phagocytic activity. And after listening to Dr. Bliska and then Dr. Straley's talk, we also know that there are multiple functions attributed to LcrV. Not only is LcrV obviously important for secretion of the Yops, but also it has this immunosuppressive effect, increasing the production of IL-10.

Rule two, or requirement number two is

that the effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans.

And if you know what we're discussing in the panel, the first questions actually all involve animals and what animal species are most appropriate. And again, the talks yesterday by Dr. Worsham and Dr. Pitt were, in my mind, awesome because I think they really addressed what we need to think about when we're trying to figure out what is the most appropriate animal model.

And those questions are really, first off, can the animal--whatever animal species you choose, can protection be measured? And this seems pretty much like a no-brainer, but I mean it's something you have to consider. So, in protection, we're

just looking basically at survival.

More important probably is the next point, which is does the clinical disease in those animals

look like that presented in humans? And I think, again, Dr. Worsham's and Dr. Pitt's talk pretty much showed, at least in my mind, that the mouse may be a very suitable small animal model in the nonhuman primates. But the whole idea is to really remember what Jacob Kool showed you and then try to relate that back to whatever animal you're looking at.

And finally, the last point is that the elicited immune response in whatever animal you choose resembles the human immune response, which again seems pretty obvious.

Requirement number three, the animal study endpoint is clearly related to the desired benefit in humans, which is generally the enhancement of survival or prevention of major morbidity. And again, because the emphasis is on pneumonic plague, we have to consider that Y. pestis could be a biothreat agent, with aerosolization the most likely route of dispersal. Therefore, animal studies should measure protection, in other words, survival, from an aerosol challenge.

And then we hit rule four, which is obviously the longest to type out. It's the data or information on the pharmacokinetics and pharmacodynamics of the product or other relevant data or information in animals and humans is sufficiently well understood to allow selection of an effective dose in humans, and it is therefore reasonable to expect the effectiveness of the product in animals to be, again, a reliable indicator of the effectiveness in humans.

And this requirement is obviously--gives us the greatest challenge. And there's a lot of points to be made within that requirement. But I think the two most important things are really at this point in time for plague, what are the protective immune responses, and how are they best demonstrated?

So in order to think about correlates or establishing correlates of protection, I think you have to really think about three different components. So the first component actually is evaluating immune responses in--and again, I say

the appropriate animal models. And really with these, you want to get data about the type of immune response and the magnitude of the immune response that is protective.

Another important component that sometimes you don't think about, especially if you're in basic research, is human immunogenicity data. That comes from clinical trials, and it's that data that can be used in terms of percent of folks that would respond in such a way that they would be protected.

These two are basically datasets then. You have to have a strategy in the middle that will allow for the estimation of the magnitude of that immune response that would protect humans. And whether that is quantity--that response or magnitude is measured as quantity of antibody or in other parameters is another question that we need to ask at the discussion.

So what I'd like to do now is just offer you a couple of suggestions as what we can use to evaluate efficacy in animal models. And the first possible way of doing this is by active

immunization studies. And what I'm going to do is use recombinant protective antigen in the anthrax situation as an example. And what I call a simple example, but I mean, I hope you realize that after Ed's talk, I mean, simple is--from one person to another can mean a very different thing.

I consider it simple because of the following. First off, we're looking at one antigen and one antigen only. Secondly, we know that neutralizing antibodies against rPA can confer protection. And we also have neutralization or functional assay that they can be used as a readout.

So for these active immunization studies, then the first stage would just be a straight immunization of whatever appropriate animal model or animal species you're using, and you use increasing doses of rPA. And what your readout is functional antibody levels. So you get a nice dose response curve.

Then you would repeat the experiment, and this time you would add challenge to it. And so,

what you would have is you would have a subset of these animals that--or get a certain amount of PA that would survive, and you can figure out from these survivors what their functional antibody levels are. So, basically, you have a threshold that you know what antibody levels that can protect.

Simple, but there's a lot of other points you need to consider for plague, specifically for plague. And the first off, obviously, is what type of immune response is elicited? And I think, again, the talks by Dr. Williamson yesterday and Steve Smiley, we know that protection appears to be principally mediated by antibody. But there also appears to be another arm of the immune response that's important, or the CMI response. And we need to do more work to figure out what the role of the CMI response is and how that response could correlate with protection.

A second point is that the immune response is elicited, needs to be considered for two antigens at this point. In next-generation plague

vaccines, who knows how many more antigens may be considered. But you have to think how can the relative protection levels be independently assessed for both F1 and V antigens?

Which, of course, then leads to the third point, and which involves assays. And really, the question is what types of assays are available or what need to be developed to demonstrate correlates of protection? And right now, I think from, again, Dr. Williamson's talk and Dr. Sue Welkos's talk, we have assays to measure antigen and antibody binding response. These are regular ELISAs and competitive inhibition ELISAs. But I think we also really need to focus and establish relevant functional assays.

Dr. Welkos and Dr. Williamson talked a little bit about macrophage cytotoxicity assays. These would be specific for V. But I think right now, at this point, there really aren't very many functional assays out there or even being developed for F1, and I think we need to go back and look at those.

So for active immunization studies then,

again, you would take the immune response data that you have from your appropriate animal models, and that would basically give you an animal correlate of protection. You have your data from your clinical trials, where you have your human immunogenicity data. And then what you would do is basically compare these two datasets in terms of the quality of the immune response between the animals and humans. And that would then allow you to estimate the magnitude of that immune response that would be protective in humans and therefore give you the human correlate of protection.

But active immunization obviously isn't the only way. Passive immunization studies are also a method for looking at evaluation of efficacy. Now we know in the past, passive protection studies were obviously used to evaluate the killed whole-cell vaccine. And Dr. Titball talked about the mouse protective index. That was basically taking human sera into a mouse and then passively immunizing the mouse with human sera and then challenging.

So it works for killed whole cell, and we know with more recent data that it also works with anti-F1 and V sera. So passive immunization with that sera can also confer protection. So in passive immunization studies then, what could be done is that you just passively immunize the appropriate animal again with human antibodies. These come from clinical trials. And then determine the level of antibody that protects animals from challenge. From those studies, you can estimate the magnitude of the human immune response that would protect in humans.

The other thing that needs to be considered when you're evaluating efficacy of animal models is basically time course of the immune response and memory response. And again, what you could do is draw from information from active immunization studies and your human immunogenicity data from your clinical trials. And the kinds of questions you really need to ask or look at are, first off, obviously, are the immune responses elicited similar between these two?

And then from there, is the time course of that immune response similar after boost? And is the rate of antibody decline similar? And those are very important because what you want to do is really know in terms of efficacy how long you can extend your efficacy for or how long is it good for? Because really we're dealing with a threshold to start with in terms of protection.

So I'm going to wrap--well, not rap, but wrap up.

[Laughter.]

DR. MEYSICK: I could do that, too, but it would be really bad. Although it might be more entertaining at times.

Anyway, to establish these correlates of protection, I really think in a lot of ways more is better. Getting information from several different components or different studies can never hurt and is always better. So you could use the active immunization studies, passive immunization studies, and your human immunogenicity studies and combine all those datasets and spend a lot of time, you

know, homing over your computers and looking at the data to come up with the right or correct human correlates of protection and therefore human efficacy.

So just before I go, I just want to thank Drusilla Burns, Karen Elkins, and Mark Abdy, who've been a sounding board throughout this. And if you have any questions, now is the time.

[Applause.]

DR. FROTHINGHAM: Rich Frothingham, Duke University.

That's certainly a good summary of what we are facing, and I wanted to ask a couple of questions. First of all, the presentation we saw yesterday, very exciting in terms of the measures of different antibody subtypes, the competitive inhibition ELISA, et cetera. And there were correlates to an outcome, which is a dichotomous outcome, which is lethality.

And as we look at correlates of protection, we're generally comparing them to this lethality measure by a number of trademarks, either

survival time or survival percentage. Should we be using other standards? Is that single variable enough of a standard to correlate all of our immune correlates with? Or should we be looking at other measures of the whole host response?

And one of the questions to be raised along with that--it's a complicated question--is, is lethality in the mouse predictive of lethality in a human in terms of the mechanisms of that lethality? A complicated question.

DR. MEYSICK: At this point, in terms of lethality, I don't think we know enough to--and I think this is a good point for the discussion panel--to really hone in on anything else aside from, for lack of a better term, a feet-up, feet-down approach.

As to the second part, which was--sorry, again?

DR. FROTHINGHAM: Oh, do we--well, that was actually all one question. I'm sorry.

DR. MEYSICK: That's okay.

DR. FROTHINGHAM: The question of--there's

not much statistical power to this. So we're correlating all of our responses to that variable and if that variable is a legitimate variable.

DR. MEYSICK: Right.

DR. FROTHINGHAM: And yes, you've answered that question.

DR. MEYSICK: At this point, I don't think there's enough data on other aspects to really hone in for certain and say, yes, that's obvious. That would require more research in that aspect and knowing what to look at or for.

DR. FROTHINGHAM: The second question has to do with this gold standard challenge, which we are all in agreement that aerosol is the threat we are currently--is our current mandate, which is to protect against aerosol challenge.

DR. MEYSICK: Right.

DR. FROTHINGHAM: However, high-dose aerosol challenge is technically difficult, and it reaches limits that you cannot--for example, with anthrax, 100 LD 50 is the high as you can go efficiently.

The reason is that aerosol challenge is very inefficient. Most of the material either goes right through your apparatus and comes out, sticks to the animal's fur, goes to other places, gets licked up, et cetera. The stuff is everywhere, but very little of it is actually deposited in the alveoli.

So recognizing that aerosol is going to be our gold standard to develop vaccines against, is there a role for high-dose intranasal in the mouse or high-dose intratracheal in the larger animals to run alongside the aerosol to give us that 10,000 LD 50 as opposed to the 10 or 100 LD 50?

Thank you.

DR. MEYSICK: Yes, I guess, in a sense, what I think is at this point, one, we have to really figure out what kind of challenge doses we want to look at as to how much and whether in terms of the straight aerosol model, with the doses that are selected, if they're actually feasible.

If that's not feasible technically, then, yes, I think we need to go back and try either

intratracheal, intranasal responses. But again, I would like the panel at this point, after the break, to really kind of discuss those aspects, too.

DR. BURNS: No other questions? Okay We'll take a break. And we'll start promptly at 10:00, and we'll have our panel discussion and hopefully resolve all of these burning questions.

[Recess.]

DR. MEYSICK: I think we'll get going with the last session, which is actually I think probably the most important session, which is the panel discussion. And I'd like to introduce our moderator for the panel discussion, Dr. Pamela McInnes from NIAID.

DR. McINNES: Well, I think it's been a terrific day and a half so far. And I think this panel discussion is a very important element of the framework in how this workshop was established.

We have five panel members whom I will introduce to you. And I will start on my right with Dr. Louise Pitt from USAMRIID, then Dr. Pat

Ferrieri from the University of Minnesota, Dr. Robert Perry from the University of Kentucky, and Dr. Sam Katz from Duke, and then Dr. Rick Lyons from the University of New Mexico.

They represent a variety of expertise. Certainly, Louise is very, very well known in the areas of aerobiology and animal model evaluation and vaccine development arenas. Pat Ferrieri, I had the pleasure of meeting originally probably 15 years ago, when Pat was a grantee and I was her program officer, and has worked extensively in microbiology in general, also in animal model work, and in clinical diagnostics.

And then Dr. Robert Perry's background is in pathogenesis arena. Dr. Sam Katz to whom we look for all his wisdom and many years of experience in vaccine development. And then Dr. Rick Lyons, who brings a very broad expertise and very, I think, practical experience in animal model development.

Now does the collective wisdom on how to move forward on plague vaccines reside up here at

this table? By no means. I think what we need to understand is that nobody knows the answer. Nobody has the road map for how to move forward that will be unambiguous and clear.

The agency certainly doesn't have the answers, and we are collectively trying to move forward in a way that is logical and defensible

with data. I know that to some people the data-free zone is a very empowering thing. We try to

stay with the data and try to make some recommendations that we can substantiate based on the data.

So I would challenge everybody in this room. You have a responsibility to contribute to this panel discussion and the report that will get written from this panel discussion. If you have data that speak to a particular issue, if you feel strongly about an issue and you don't let us know that and you leave here feeling that people don't really understand, you have not exercised that responsibility. We need the collective wisdom. We need all the data on the table, and we need to know

about what you feel strongly about in order to make sense as we move forward.

Now it is early days with plague. Things may change. There may be lots of new data. We may have a revisit on this whole thing. But the way that government in particular works, the collective sharing of information from the experts and the wise people will be looked to for the blueprint on how we move forward. So I really would encourage everybody, please, you must contribute if you have something to add to this conversation.

I think we structured this really very much, with the help of Drusilla and Karen and Ed Nuzum and Judy, around a series of questions, and they can be broken down into their particular elements. And I think that's the way we're going to try to do it. It doesn't mean that later on if we finish discussing part E and a new thought comes up under part F, we can, of course, go back and look at that.

So I want to move to the first question, which is what are the most appropriate animal

species to use as models for either pneumonic or bubonic plague? And which models best resemble the clinical, histopathological, and immunological responses of plague infection in humans? What are the pros and cons of each model? And what information is necessary to validate those models?

My own view from yesterday is we had these beautiful presentations from Pat Worsham on small animal models and from Louise on the nonhuman primates. I think Pat laid out for us the background data on the common small models--the mouse, the guinea pig, the rat. And then some very interesting lesser known models, which I'd never heard of a multimammate mouse, and cats.

And the other part of our life, we work on H5 influenza, and cats are starting to become an important component in transmission of H5. So it was interesting that they came up here, too.

I think the conclusions in general, if I laid this out, was that--for the small model was that the mouse was probably the best established model and that other models seem to be less well

developed. And this very sobering comment that goes back really to 1936, which was the nature of the experimental animal was by far more essential to the results than the nature of the vaccine used. We have got to focus on characterizing and validating a small model and being very, very comfortable with it.

In turn, Louise provided a lot of background data on the nonhuman primates, describing studies in macaques and in the Cercopithecus aethiops. And I think the conclusion was that the African greens and the cynos were comparable with regard to susceptibility, similar pathologies, similar disease progression, and similar to man. And both seemed to be good. I think I was left not quite understanding the response to V in the Cercopithecus model.

And so, if I could turn perhaps to Louise to go to--if we can flip around and go to the nonhuman primate at this point? And I wondered if you would like to just summarize your data-driven feelings on these two models, what you are very

sure about, what you are not so sure about. And then we can open it up further.

DR. PITT: Can everybody hear me? I'll do my best.

We vaccinated both--the African greens we vaccinated with the recombinant F1-V fusion protein. It is clear that the response to F1 is much more consistent in the groups of animals that we have vaccinated than the V response.

Some of the responses to V have looked to us that the animals have been exposed to a V antigen previously. Some preliminary work has been done in that area looking at the different types of V from different organisms in the mouse. Antibody levels to existing V antibodies prior to vaccination have also been looked at in the animals, and occasionally, you do get that level above background. Whether that is leading to that variability, whether there is some immuno-suppression because of pre-existing V exposure is not clear.

The initial challenges that we did when we

vaccinated the African greens was with the C12 strain. That was done deliberately because that's an F1 negative strain, and we really wanted to see what the V antigen--we were more interested in how the V antigen was protecting at that point because we were interested in that V antibody response.

It appears to me, based on--again, it's very limited data that we've got, very small numbers of animals, very small numbers of study. But it appears to me that our challenges with the F1 negative strain was more successful than with the F1 positive strain. We get more protection with C12 than Colorado 92. And again, that's very--that's just my opinion based on very small numbers.

So I think we have a lot more work to do in understanding V and its response and how it's working. It's my impression that V is much more important in this vaccine than F1. We have never vaccinated with just V and done challenges to see what happens there.

DR. McINNES: And in any other parameters,

you--what is your feeling on the cynos versus the Cercopithecus?

DR. PITT: There is no doubt that the response to V in the cynomolgus is much more--I wouldn't say robust, but it's much more consistent. I think working on functional assays, it's crucial to understand what the antibodies to these antigens are doing, and I think that's a weakness so far is that we don't have robust functional assays in order to be able to really look at what is the difference between the response in the African green and the cyno, and why is one protected and the other not.

DR. McINNES: I'd like to ask anybody else on the panel whether they have any comments with regard to the nonhuman primate at this point as an animal model, the state of development, what more work should be done?

Pat, yes?

DR. FERRIERI: Well, I like some of the data that I saw that I would have to conclude that it's not sufficient for me to draw any firm notion

of whether the African green is superior to the cynomolgus. I think that the data, as I interpreted it, was that there were more inconsistent responses in the African green monkey. But again, we were exposed to a huge volume of data in the past day and a half. And so, I can't say that I remember it all.

But the basic issue is whether either of them mimics the anticipated and known pneumonic version of this disease in humans. And I would have to say, based on what I've seen, that the nonhuman primate mimics this quite well. Since I've never seen a patient with pneumonic plague, I feel more comfortable also talking about what happens to you if you get anthrax and develop severe edema and die than I do about what happens with your pneumonia due to plague.

And I anticipate that one would have acute respiratory distress syndrome develop and multi-organ dysfunction and collapse and an outpouring of various cytokines. So I haven't seen these types of information from autopsies to permit me to

understand fully what the pathway is once the process starts, except it's very fulminant, and death may ensue within 24 to 48 hours. So I take it as an article of faith that all of the other failure, organ failures will ensue. But I'm not absolutely sure of that.

But I like these animals, and I think we need a lot more data. I don't feel I know enough about an animal model for bubonic plague to respond to that part of the question. But it's clear that the--we have to have a smaller animal model as well because of the expense and the limitation in numbers for the nonhuman primates.

So I'll stop because I'm sure there are tons of things that others want to say.

DR. McINNES: Anybody? Yes, Sam?

DR. KATZ: I should preface anything I say by what I told Karen when she first called, and that is I know nothing about plague. All of my work has been with viruses, and my work with vaccines has been almost always with viruses.

However, I have worked with monkeys a good

bit. And I think that one of the advantages of the nonhuman primate, whether it's African green or Cercopithecus or macaque or whatever, is their size. And if you really want to look at pneumonic plague and its protective effect, the challenge can be a lot more effective if you put the organism via a bronchoscope down into the trachea and bronchi. Then you know what you're really challenging the animal with, with organisms that will get into the alveoli, which I assume is more relevant to what natural exposure and inhalation would be.

So that I agree with Pat, that when I look at some of the graphs that were shown with 50 animals on a curve, I shudder to think what that cost to have that many animals available to you. But if you can, Albert Osterhaus in Bilthoven in the Netherlands did very nice studies looking at challenges with measles virus, putting virus directly into the alveoli by bronchoscoping or putting tubes into monkeys, which you can do. Which perhaps somebody is clever enough to do with mice, but I don't know that I would want to be

challenged with that.

The mouse immunologic system is probably so much closer to man's than any other animal you could work with. But I think the monkey is still the gold standard for this.

DR. McINNES: Rick, I'm going to segue to you on going from nonhuman primate to moving over to discussing the smaller animal model. And your impressions from the data that were presented yesterday and this morning as regards to plague, and then your general experience in that where are you coming down on what animal model is currently supported by data as being useful for plague vaccine development?

DR. LYONS: Well, a couple of comments. One addressing, I think, what you raised. I think the pneumonic and bubonic issue, I think, is very important because, you know, I guess in the land of the flea in New Mexico, where I come from, we do see plague often. And it's interesting that patients come in, you know, typically with bubonic, they come in septic. I mean, you see the bugs in

their blood. But they don't die. They get better.

If you see bugs in the blood of somebody with pneumonic, they're probably going to die. And the impact of the lung seems to be playing a major effect on pneumonic, and I don't think that's surprising. But we don't understand it. And so, I think having both models is important, and that was an issue you raised.

I think we've looked at in primates a little bit into this more with tularemia than with plague in delivering things by bronchoscopy. And that is a very nice way of doing it. We do that for a lot of reasons. Because you can use other lobes of the same animal for controls to evaluate things. I mean, non-infected lobes versus infected lobes, these kind of things, which really are important when you're working with an outbred strain. And we know very little about the MHC of primates, and we just have no real baseline data there.

For small models, I had several questions over the breaks because I've kind of given this

talk. But I think we use an intranasal plague model. We've done intratracheal, too. As Sam mentioned, that's not hard at all. That's been done. In the TB literature, legionella literature, it's out there, you know, quite a bit.

And I think one of the questions that people should ask themselves is why in a mouse, if you give a bug intranasally, do you get a routine pulmonary infection versus if you tried to do that in a primate or a human, it would not happen. And there is a real anatomical basis for that that's the reason why it works.

It's simply that in the human and primate, when you speak of "deep lung," you--there are multiple segments to get to deep lung in humans and primates. You go through several branches to get
to the alveolar spaces. And aerosol is a very important way of getting there in those animals, or by bronchoscopy. As Sam said, you have to go down deep to get there.

In the mouse, a mouse is totally different. The mouse is basically a model for

bronchial or lung. The whole mouse lung is almost the deep lung of a human, and there's only two to three segments to get to the deep lung, if you will, of a mouse. And so, that's why you can deliver bugs to the lung of a mouse very simply. I mean, you know, in the literature it's been done all different ways.

And so, you know, I think what Art said yesterday was very important. You need to define a model. You need to do the histopathology. You need to confirm your route as delivering to the lung. You need to do all those things.

But I'd hate to see science slowed down, you know, particularly when you're trying to evaluate multiple attenuated strains, multiple--multiple different vaccines for sake of having to deliver these very technology-oriented--to do it well, to do it in a very reproducible manner like they do at USAMRIID. To do it well is very difficult to do, particularly in the mouse. Particularly in the mouse.

Because the mouse turns out to be--or

rodents in general because of their turbinate structures, predominantly, they turn out to be the worst animal for trying to aerosol anything into. I mean, the efficiency in a mouse compared to a primate is extraordinarily different. You know, you can get up to 50 percent down to the alveolar space of a primate in an optimal situation, whereas in a mouse, you're doing really well if you get 5 to 7 percent. That's doing really well if you have a perfect particle, if you will. Those were done with perfect particles.

And in general, everybody that knows aerosol, you always get a bell-shaped curve, and you know those bigger droplets are going to end up up here, and the small droplets are going to end up in the lung. And some is going to end up in your dead space.

So, you know, I think my opinion is that I think the mouse is an excellent model for looking--for screening plague vaccines or pathogenesis. You can get in the lung a variety of ways. You just want to document that and make sure, you know, you

document and make sure it's going where you think. You may get some lymph node involvement. It was interesting that--you know, a good comparative study would be good.

I can't--I have to believe that whether it's delivered in any manner, you're probably going to be influencing lymph nodes all along the way down there, whether it's aerosol, intranasal. Probably if you really want to go in the lung, we like to do intratracheal because that bypasses everything, and you go right into the lung. Is it worth it? I don't know. I doubt it. I mean, we have data that says it's not. Whether we give it intranasally or intratracheal, it's the same thing.

So I think--I hope we don't get too hung up on this, particularly in the smaller model, because I think what the smaller model allows us to do is decide what we can take to larger animals, where we can really do things a little differently and better, and we want to move through the smaller models quickly and rapidly to get to the meat of the argument.

DR. McINNES: Thank you. I think you've touched very well on the models to say we've gone to the pros and cons, some of the pros and cons of those particular models.

And Drusilla, you have a question?

DR. BURNS: I just had--Drusilla Burns.

Rick, you said you could use the mouse for screening, which I think is a great way to use the mouse. But for the Animal Rule, we have to think about one other aspect, and that is we need probably two animal species. So for the experiments that you would do for the more pivotal studies in the second species, which would be perhaps the mouse, would you--

DR. LYONS: You mean nonhuman primate for a second species?

DR. BURNS: First would be nonhuman primates.

DR. LYONS: Okay. Okay.

DR. BURNS: But the second would probably be the mouse. And would you--for the pivotal studies where you're really evaluating a vaccine

for licensure, would you change the way you think at all in the route of delivery there? Or do you think intranasal or intratracheal or either one is just fine and reflects the disease in humans?

DR. LYONS: I think either one is just fine. Honestly. I think you can actually do something GLP in a mouse much better with an intratracheal or intranasal delivery than you can with aerosol in the way of multiplicity of infection, these kind of things. So I think you can. I mean, that's my opinion. I'm sure people would disagree with that. But--

DR. McINNES: Thank you.

Louise, you had a comment?

DR. PITT: Yes, I would like to comment about the aerosol versus intratracheal and intranasal. Being an aerobiologist, obviously, I have a bias toward aerosol. And in my opinion, intratracheal and intranasal are a poor man's aerosol. Yes, you deliver it to the lung. I understand that people are going to use intratracheal and intranasal because it is easier

to set up in your lab. It's a simpler process.
But I think we really need, particularly in the mouse models, we really need to try and standardize what is done.

I think the question of asking research questions about what's happening in the lung, et cetera, is very different from trying to develop a vaccine. I think we need to keep that in mind, and I think it's very important up front that we do some work looking at the aerosol versus the intratracheal versus the intranasal in terms of pathogenesis, pathology, same strain of organism, same techniques, same strains of mice in order to establish the parameters that you can use to understand your results when you use intratracheal versus intranasal and versus aerosol.

DR. McINNES: Thank you. Yes, sir?

DR. FROTHINGHAM: Rich Frothingham, Duke University.

I wanted to go back to something that Dr. Pitt mentioned quite early in this discussion, which was the irreproducibility of some of the

model--one of the primate models because of environmental exposure potentially or that issue. And as you're thinking about models, we have to ask what the purpose is of the model.

If we are doing development, when I'm developing something in my lab, I want a really uniform model because I want to be able to detect small differences in the vaccines because I want to optimize those. So for optimizing development, et cetera. But when I validate for licensure, I would like a model that resembles humans. And we're outbred for one thing, but our exposures are very heterogeneous. We live in the mountains. We live in the deserts. We live in the forests. We live in the cities. We live everywhere.

And so, a uniform primate model in my mind is not representative. And I would like to see us move to models that are varied, where there are
lots of dirty background exposures that will then replicate the human host that we're going to eventually try to test this in.

DR. LYONS: That might be difficult to do

in GLP. I don't know.

[Laughter.]

DR. McINNES: Dr. Perry, I wondered whether you would like to comment in terms--from your pathogenesis background in terms of some of these comments that are coming up about delivery route and whether we're going to have effectively inbred versus outbred and whether it's okay to have
very--try to get homogeneous during model--screening, but then you need to move to outbreds.

Would you like to comment on that?

DR. PERRY: Sure. I actually sort of agree that one of the first things that maybe needs to be done is to do a direct comparison of aerosol, intranasal, and all that--pathology, histopathology, pathogenesis. And let's see what kind of differences we're really talking about.

I mean, I think Pat Worsham did a good job yesterday of looking at all the different animal models, and she had a lot of old data about intranasal and maybe not all going down to the lung. But those--if you look at that, those are

one or two line descriptions of the protocol, and so what were they actually doing? How did they deliver it? That's going to make a big difference. What sort of anesthetics were used, if any at all?

And so, I think what, you know, the first thing here is to just let's get this out of the way and have some people look, make direct comparisons and decide if there's really significant differences.

The other thing I think, too, is that if you're worried about an aerosol attack, maybe we're a little too worried about simulating a direct injection or into the lungs. You know, if you're sprayed in the face with something, you're going to swallow some of it.

So maybe the model of not all of it goes to the lungs isn't necessarily bad. It may not be a pure pneumonic infection, but I think in most instances, you know, you're not going to have someone come up and cover your mouth and spray it into your nose. So there's that aspect.

I wanted to ask one other question about

the variability and the amount in response to V antigen. And I think maybe the DSTL people might have some interesting answers in a little while with their clinical trials. And that is, is the variability going to be something that we see in humans as well?

So, you know, this gets back to correlates. You know, is it going to be a good correlate to look at V, or are we going to see in humans a wide variation like you see in the African greens, or is it going to be more uniform? And I think that will be an interesting thing to look at, too.

What else did you ask me? I forgot.

DR. McINNES: I think that's very useful. So you're advocating we need to go back and characterize each of these variables so that we really understand.

DR. GOGUEN: I'm Jon Goguen, University of Massachusetts Medical School.

I wanted to echo several of the things that Rick and Bob had said and suggest what I think

would be an interesting experiment in terms of comparing and validating the aerosol versus intranasal models. I think the intranasal model has a lot of advantages in terms of control and something that would be very, very easy to do under GLP conditions.

As I said yesterday, we have some experience with this model and find it not only very convenient and very reproducible, but the disease that we see, we haven't characterized this in as much detail as we would have liked. We haven't characterized the pathology in as much detail as we might have liked, but at very low doses, these mice are dying with large numbers of organisms in the lungs in three to five days. So this looks a lot like pneumonic plague.

The experiment that I wanted to suggest is that we have these old vaccines that weren't protective against pneumonic plague, all right?
But did protect against bubonic quite effectively. And it would be interesting to see if in these models, both the aerosol and the intranasal model,

if these vaccines behave in the same way. That is, if the old--formalin fixed vaccines mimic this behavior.

That is, they failed to protect, as they failed to protect in primate experiments, against pneumonic challenge either by intranasal or aerosol but protect against the bubonic one.

DR. McINNES: Thank you. Yes?

DR. ADAMOVICZ: Hello. I'm Jeff Adamovicz from USAMRIID.

I actually have two questions for the panel. The first question goes to the issue of the relevance of the exposure method versus what we would expect to protect in humans. By that, I mean, for instance, we have this nice academic discussion going on about intranasal versus intratracheal versus aerosol, and I tend to agree with Dr. Pitt.

I think the aerosol is the more relevant one since we're talking about using animals as a surrogate for what we expect to be a case of human exposure, which would be a whole body aerosol

exposure. This includes routes of exposure other than just the lungs. It includes, as Bob mentioned, oral. It also includes conjunctival exposure. So I'd like to hear the panel's comments on that particular issue.

I would also like to hear the panel's comments on what they feel the relevance of this two animal rule versus bubonic plague. We've been talking about pneumonic plague. But as we've seen, there are places in the world that we could do clinical field trials for bubonic plague, and I'd like to hear how the panel would suggest we dissect out the requirements for a field trial, say, for instance, for bubonic plague versus two animal rule for pneumonic plague and how that would affect the vaccine, any vaccine.

DR. McINNES: All right. I think we are going to come to the question of what place field trials might have in this vaccine licensure path.

But I did want to see if anybody would like to address your first question, which was back to the I think somewhat expressed bias to the

aerosol, whole body aerosol approach and that it is not--that there will be oral intake, there will be conjunctival intake, and that that is really the way to look at it. And I wondered if anybody on the panel wanted to comment on that?

As I understood it, I think there was an expressed bias toward having an aerosol approach, that that is the way it would be delivered, and that it's not only going to be a respiratory route, but that there will be oral intake, and there will be conjunctival intake.

DR. LYONS: I'll just make a comment. I mean, I think that's--you know, I agree. It's probably more--the aerosol will be demonstrated to the host in a more similar fashion. Again, I guess you have to step back and say this is also a mouse with total different anatomical structure. So I don't even know--I mean, I know an aerosol does not behave in a mouse like it does in a human. That's been well documented by the toxicologists for years.

So you have to take that--all I'm saying

is you have to take the whole picture into account. You can't sort of isolate one thing about the whole--about the model. I mean, you have to kind of bring the whole picture together.

So, you know, I think I'm not saying aerosol is the wrong way. Don't get me wrong. That's great if you can do a good aerosol and everything else. I think it's fine. But I'm saying historically, at least for infectious diseases, the vaccines that have worked for aerosol, for intranasal, I have yet to see--and maybe Louise knows one. I have yet to see one that has fallen out as different, as being not protective in one, but protective in another. I just haven't seen that yet.

So I think the data suggests that getting in the lung, extremely important. The mechanism it gets there, maybe not has important for demonstrating protection. That's all I'm saying.

DR. McINNES: Well, I think that was a very eloquent statement and leaves room to explore the validity of that, and it may be equally valid.

I can take two brief questions, and then we will need to move on. Yes?

DR. LU: Okay. Thank you. Actually, I have three related questions or comment.

First, I think that while we are discussing mouse versus a primate--again, like Dr. Katz, I came from some nonbacterial or nonplague background. I think the plague has a unique situation here because a rodent is a natural host of plague. So that is really different from many other vaccine development. So this is natural host. So elevated value of a mouse versus a monkey, that's number one.

Number two, I really enjoyed the anatomical description by Dr. Lyons there. Being someone--I have been as a vaccine developer and also practically doing the trachea challenge model development myself for many vaccines, I don't know how many people know the trauma or the irregular variations of a trachea challenge. Of course, here, a bronchoscope is out of the question for a mouse. So from that point of view, I think a nasal

challenge is way more standardized if we talk about GLP or vaccine validation.

The third one, actually there's another concept in the last 10 years in vaccine research. The concept is to protect against disease versus protect against infection. Olden days, we saw every vaccine as generally [inaudible] immunity. So any pathogen coming we protect. Actually, this is not true. There are always a spectrum. Maybe some pathogen establishes early infection, but then the vaccine comes up and wipes them out.

So if that's true, then think about here whether we are looking at aerosol or nasal, the question is whether later the disease has been established by intranasal challenge. If that's the case, whether that vaccine can protect. So in that sense, I support Dr. Jon Goguen's observation. If mice is dying within three days, what type of infection that is by intranasal challenge? So I think that is valid.

DR. McINNES: Thank you for your comments. Yes?

MS. SCOTT: Leah Scott, DSTL.

Just a very quick point to address the issue about differentiating between whole body aerosol challenge and head-only aerosol challenge. I raise the point. I think it's important to remember.

DR. McINNES: All right. We're going to move on to the second big question, which has many subparts to it. Some of which we've already started working on.

What types of studies will be necessary to develop correlates of immunity for plague vaccines in humans? And specifically, question A, what immune responses should be examined in animals? What data are available or of interest to suggest a correlation between measures of cell-mediated immune response and protection?

And here, I think Diane Williamson and Sue Welkos gave us a wonderful start on this discussion, as did some of the presentations on virulence factors and host response. And we get into this eternal conversation, which I sometimes

feel we can--we set up this either/or philosophy. And to some, it's from the wars of HIV, where there is the antibody group, and there is the CMI group, and there's the TH1, and there's the TH2. And in fact, in looking at this, you know, anybody is good. Nobody is going to probably argue about the role of antibody here.

And I think we saw a fair amount of data that, in fact, CMI certainly contributes to clearance of infection. And so, I think we don't need to get into that we need the TH2 or the TH1. What we need to think about is, collectively here, what is it that we think is going to be important? What is it that we're going to be able to validate and characterize as being important? How are we going to measure them, and can we validate that measurement?

So all of those elements are going to be very important in discussing this. And again, I think it reminds us that we are in some ways going to be moving toward looking here not so much of a correlate, but as a surrogate. Can I measure B as

a substitute for A? And a correlate is not necessarily the same thing as a surrogate. So we have to keep that in mind as we move forward in this discussion.

So if we have on the table this first question, what are the types of studies in our quest to try to understand the immune response, the correlates, and hopefully the surrogates for protection, induced by vaccine, what should we be looking at? And what data are available to suggest a correlation between the measures of CMI, the contribution of the CMI response and protection?

And so, instead of assigning this, I will take volunteers from the panel, first off.

Pat? Yes.

DR. FERRIERI: I'd like to just initiate it by commenting on what I view is a very unique antigen, and that is--in its structure, and that is the V antigen, which I love because it has a coil-coil structure, and it's reminiscent for me of other coil-coil structures, such as M protein of group A strep.

But I consider the V antigen of Yersinia to be--to have many more functions and to be more of a challenge in understanding immune responses. And so, I use that as a kick-off. And then I will say that I am intrigued by the monoclonal antibodies that have been developed to certain epitopes and the utilization of this monoclone, whatever it was called, 7.3 in the competitive inhibition ELISA.

So I'm big on functional assays. So an ELISA reading doesn't cut it for me, nor a dilution titer. I need to know that what we're measuring has functional significance within the host that you're trying to protect and that it's great to have a titer that may be greater than 100,000, 200,000, but is it functional antibody?

And so, I heard data that suggests that there is a correlation between the CO ELISA, CI ELISA, and outcome. And so, I think that this is a great start as well as the other functional assay that someone else may wish to discuss as well, the macrophage cytotoxicity inhibition assay.

But the bottom line for me is that we have to have functional assessment of what this antibody will do in terms of protection.

DR. McINNES: So, Pat, to follow up on that, we clearly would like to have a workhorse assay for down the line vaccine development. We need to be sure that that workhorse assay has a very well-known relationship to the functional assay. And what I thought was I think an anti-V, a functional assay, and just much further behind in terms of having a functional assay for F.

And I wondered whether those folks who are involved in this functional assay development, the utility of the functional assay, the extrapolation of that to the ELISA or to the competitive ELISA, and how you and anybody else, how you feel that what point we're at now and what needs to be done to get to the point of having a functional assay that we think is meaningful and then translating that to a workhorse assay that is really what we've got to have for vaccine development.

Does anybody wish to comment on that on

the panel? Anyone from the floor? And the DSTL, USAMRIID folks, you've done a lot of work in this area. We'd like to hear.

DR. ADAMOVICZ: Yes. Jeff Adamovicz again from USAMRIID.

We, in fact, do have a functional competitive inhibition ELISA for the F1 antigen. You know, some folks don't consider competitive ELISA strictly functional. But I think it's a pretty good marker since we really can't come up with any function for the caps. Although there is nothing that's been described in the literature, as far as I can tell, that F1 does, per se, other than act as a target for opsonization or antibody binding on the surface of the organism.

So that's a tough one. The competitive ELISA may be as close as we can come, and we do have that assay.

DR. FERRIERI: May I ask why--this is my limitation. Why have we not developed a good phagocytosis assay for Y. pestis? If this were any other encapsulated organism, like the ones I work

with--Group E strep, pneumococcus--you don't just want an ELISA. We'd like to know whether you have internalization and kill.

Well, this organism isn't very well internalized. But apparently, early in the infection, neutrafils do ingest these organisms. And again, I'm open to correction, criticism. But I would view as the optimum this may be a surrogate, the CI ELISA, but I still think that it would be great to know the correlation of that with an opsonophagocytic assay. These antibodies need to be opsonic, in my opinion.

DR. ADAMOVICZ: I agree with that statement. I think that's important. But I think it's also important to remember that in terms of the pathogenesis of the organism, the F1 capsule is not expressed unless the organism has been artificially manipulated at the time of infection. And this is either via an insect vector or via aerosol from, say, a secondary exposure from an infected human.

So it's not--well, it's not likely. We

don't know that for a fact. I take that back. But clearly, in the insect, when a flea bites, there is not F1 capsule antigen expressed at the time of infection.

DR. FERRIERI: In establishing a lung infection, isn't there up-regulation of F1 if it's an F1 strain?

DR. ADAMOVICZ: That's correct. But generally, if the organism was not prepared at 37 degrees, if it was prepared at room temperature and/or stored, it's not expressing F1 capsule at the time of aerosol exposure. In fact, the studies that Dr. Pitt does, those organisms that are sprayed on our animals are not expressing F1 at the time of exposure.

DR. McINNES: Yes?

MR. : I think it's probably a mistake to think about F1 as you think traditionally about a capsule, and this whole antiphagocytic idea is somewhat suspect in the sense that the data are not strong and, you know, this is not a polysaccharide capsule. It's a peptide.

And in fact, it's very closely related to fimbriae, and it may be more properly thought of in the context of a loosely attached fimbrial protein rather than as a traditional capsule. And it's probably best to think that we really don't know what this does.

The problem of finding a functional correlate is complicated by the fact that we know that the mutant is fully virulent. So finding a functional correlate is maybe a hopeless quest, and I think probably the best that you're going to do perhaps is opsonic versus non-opsonic.

DR. McINNES: Could I just ask for clarification? When you said the data are not strong, you mean there are existing data and they don't support, or there aren't data?

MR. : There are data, but they're not strong.

[Laughter.]

MR. : They're old.

DR. McINNES: They're old. Old can be good.

MR.   : They're old. I think they come from--and maybe Sue can help me with this a little bit. I think they come from guinea pig experiments primarily? You know, it hasn't been looked at in a long time, and I--

DR. McINNES: They're not robust.

MR.   : They are not robust. There are lots of things that probably weren't considered at the time that could have affected phagocytosis other than F1.

DR. McINNES: Yes, Dr. Perry?

DR. PERRY: So he's right. A lot of the experiments are older, and I think not that old is bad. There are a lot of very good work done there. But in all the F1 negative strains that were used back then, we have no idea what the mutation was. And so, you don't know what you're really looking at in the F1 negative mutant.

And there were some data in vitro that it resisted phagocytosis and some stuff in animal models. And I can't remember which one that, you know, you got phagocytized early, but after they

were in the animal for a while, then that stopped. Again, that could be due to Yops as well as Fraction 1, which they didn't even know much about that at that time.

So it's not that the experiments were done poorly, but they didn't have genetically defined systems to really look at it as to what was happening.

MR. : And in those days, in fact, pestis was thought not to produce the Yops. There was a lot of confusion in those days.

DR. PERRY: Well, I think in those days, the only thing they made was V and the W antigen. Yes. So--

MR. : On the other hand, with V, I think there's really no problem to get good assays quickly.

DR. McINNES: Good. Thank you.

Diane, yes?

DR. WILLIAMSON: Diane Williamson, DSTL.

I just want to make a couple of comments about antibody and CMI. I mean, we have looked at

antibody across the species that we've vaccinated and seen a nice rise and very similar kinetics across the species, including in the small human trial that we--that I talked about yesterday.

I'm quite sure that functional assays are important. Functional assays of antibody are important. The difficulty, of course, with antibody is that it will rise and peak and then decline. So what kind of correlate are you going to look for when antibody is in decline? CMI has got to be important at that point and needs to be measured.

Just another comment on the variation in titer to F1 and V that we've seen. Now in the very relatively small human trial that we did, we did see variation in titer to both F1 and V. The--and that was probably expected. But it was to both antigens. It wasn't to V alone, as has been seen in the data that Louise reported yesterday. It was to both antigens.

And total titer of antibody actually correlated. Whilst it was on the increase, it

correlated with the readouts from the functional assays. So it's just a comment really.

DR. McINNES: Di, just to keep you there regarding your actual CMI readouts that you're using. As I recall, you had was it your in vitro proliferation assays?

DR. WILLIAMSON: Yes. In small animal model.

DR. McINNES: In your small animal model?

DR. WILLIAMSON: Yes.

DR. McINNES: Are there any other thoughts about other CMI readouts that people feel might have particular relevance? What data do you have to show the relationship of that to protection?

DR. WILLIAMSON: Yes, I mean, CMI is difficult. We have done some flow cytometry on our human subjects, too. But again, we saw a lot of variation in the markers that we looked at by flow. A lot of individual variation, which meant that there was no real trend, vaccine dose-related trend.

I think recall responses, CMI recall

responses, possibly ELISPOT assays on the proliferating lymphocytes are a possibility.

DR. McINNES: Good. Thank you.

Karen?

DR. MEYSICK: Hi. Karen Meysick, FDA. Just a point, and this is a personal opinion. It involves, again, the competitive inhibition ELISA. And my concern with that is the fact that at this point, we're only looking at one monoclonal antibody. And that to me is a rather--it may not cover the entire genre of what's going on, and I think--I mean, that in my small world, that in addition to a relative functional assay, would be a really great way of getting a better correlate than just sticking with the competitive. And that's my seven cents' worth.

DR. McINNES: Thank you.

DR. LU: Maybe I can just comment on one thing on the CMI part? I think it's important to look at the CMI. I personally do that and support it. But I think we should differentiate, especially if we are talking the regulatory scope

here. If some vaccine has to have CMI and can go through, say, a later phase of clinical trial or be licensed, I think this might be just too much because we don't know the contribution.

And also the assay for a lot of CMI are highly variable, such as the stimulation index. We know that can vary 10 times, 20 times. So I think it's very hard to use as the license, you know, requirement, I guess. So I think we should have different level of stringency to look at that.

DR. McINNES: The counterpart to that is it's hard to say we don't need the CMI readout on this particular vaccine. And I think the question is to try to identify an area that can be worked on with an assay that might give us some comfort zone about the way in which the vaccine is acting in terms of cell mediate, that whole arm of immune response. So I don't think we can say we're not going to look at that.

DR. FERRIERI: May I ask a question about the sequence of the V antigen, as I saw it there, and the antibody, the monoclone being used in the

inhibition assay is among a group that were studied. This was the most successful one.

But my concern is if there are any mutations that occur in the future, that we're basing all of this on one epitope, and do we know more specifically about sites within the sequence of V that we really understand what turns on--what really turns on the regulation and secretion of Yops then? And is that known? That didn't quite come across to me of the regulation from a genetic standpoint of Yops.

So as I understand this--and obviously I don't work with Yersinia--if we can turn off Yops, then we would be safe perhaps if we were assaulted with Yersinia. Is that a fair statement, and how do we target something that's more specific?

DR. McINNES: Go ahead.

DR. MIZEL: Steve Mizel, Wake Forest. Well, just a comment on that. Obviously, there's a lot of people interested in type 3 secretion inhibitors. But I don't think we're very far along in really getting good inhibitors.

But I wanted to come back to this question that you're focusing on, and that is that we're not dealing with these particular antigens, in my opinion, with inner molecules. They have biologic activity. So perhaps thinking about F1 and V is like thinking about LPS. LPS is an antigen, but it's a toll-like receptor agonist. And those two things produce very different responses and outcomes.

So one thing you have to separate is when you're using these molecules and you get a cell-mediated response as opposed to a humoral response, is that against that molecule or caused by that molecule? And those could be very different things. And one could be fooled into thinking that there's a cell-mediated component. I'm not saying there isn't. But you might be fooled into it because of that biologic activity.

So I think additional work is clearly needed to examine this in the way we've looked at LPS and other toll-like receptor agonists.

DR. McINNES: Very good comment. Thank

you.

Yes?

DR. STRALEY: Sue Straley. I'd just like to comment that we do need a lot more information about where the protective epitopes are in V. You saw yesterday that the region 2, which is strongly protective, is a huge region. Amino acids 135 to 275. And work at USAMRIID showed that this was a conformational epitope.

So linear peptides were not successful in identifying an epitope in more than one study. And some deletion analysis has been done, particularly in Hans Wolf-Watz's group with using pseudo TB as a model. And they could remove I think it was like four residues off the C terminus of V, and it was now not functional in delivering Yops. But whether that means that that's going to be an epitope is a totally different issue.

I mean, so what V is thought to do right now is to function as sort of a chaperone for the insertion of YopD. YopB/D are the ones that actually make the pore, and V does not make a pore,

although it originally was proposed to do so. But it's necessary for the pore. And there's another region that was shown you have to have this in order to--this subterminal region in order to make a pore.

So what we really need to know is where functional protective monoclonals are striking, and is there a large repertoire of effective monoclonals? And I would certainly support the--or echo the sentiment that a good competitive ELISA--I'm really excited about the assays that I saw yesterday, and I know that work is under way to make them robust. And one important aspect of that will be to have multiple monoclonals.

DR. FERRIERI: Thank you, Sue. That helped me a lot.

DR. McINNES: We're going to move on to the next. In fact, in part C, we kind of come back to some of these same themes. So we'll see if we've addressed them adequately or not.

So this second question here is should both active and passive immunization studies be

performed? And how should these studies be designed, and what challenge rate should be examined? And we're back to the theme of the challenge. So sort of rather like motherhood and apple pie. Should active and passive studies be done? Who would like to comment on that?

Yes, Sam? Thank you.

DR. KATZ: As one, again, with apologies, not working with plague, it seems to me the important question is in what context do you envision a vaccine being used? Are you going to give a vaccine to the whole population to protect them against an aerosol? You'll get a response like you got with smallpox vaccine, where only the Department of Defense members were immunized and a very tiny portion of the civilian population.

If I understood what I've seen, and Karen commented on this a little, the kinetics of immune response to these vaccines, the incubation period of the disease is much too rapid. So you're not going to give vaccine post exposure. And we can do this with rabies. We can do it with measles. We

can do it with smallpox because you have long incubation periods, and the vaccines work much more rapidly than the evolution of the pathogenesis of the natural infection. So this isn't going to be a post-exposure vaccine.

Fortunately, you have antibiotics, so you can treat recognized or known infections. So how are you going to use this vaccine from the point of view of active immunization other than selecting out populations you think might be exposed? And how you define them other than armed forces, military groups, I'm unable to say.

Passive immunization might be fine for the individual who's exposed and for some reason or other you're not going to give antibiotics. I was impressed with the data you showed on transfer of either antibodies developed in animals and mice or antibodies developed in other species. They work fine. But again, the pragmatism of how you would use passive immunization escapes me in the context of what you're worried about, which is a bioterrorism event.

DR. McINNES: Sam, thank you.

I think when we come to question 4, there's actually a somewhat pointed question. Is there any role for post exposure? And I think we want to bring this back up again in the context of how the vaccine might be implemented and what the indication might actually be.

In the context of studies to develop the correlates of immunity for plague vaccines in humans and looking at the animal studies and animal to human studies, what is the role of active and passive immunization studies, and should they be undertaken? I think we saw some very nice data on the passive protection studies.

I would like to pose whether there are any alternative thoughts on how these studies should be designed and, once again, to the challenge route, and if people have specific comments on active and passive immunization studies in the animals or between humans--vaccine stimulated antibodies and passive transfer to animals?

DR. SNOW: Hi. This is Doris Snow. I'm

from DVC.

And I actually have more of a general question. The panel's been focused really on pneumonic versus bubonic plague. But as a sponsor, we're going to have to have a very specific indication, and our Animal Rule studies are going to have to be designed to, you know, justify the use of our product for that indication.

And I want to get an idea from the panel, do you assume that a pneumonic indication would actually be effective to use in a population of people that are being exposed to a threat which may not be pneumonic plague? It may be aerosol intoxication or a bomb or an event of some sort.

So does pneumonic plague indication equal aerosol exposure indication? Because I think that decision will really depend on which models we choose.

And then I think Dr. Pitt's discussion of the aerosol model is really the appropriate model. Because from our perspective, as a sponsor, if our indication is protection against an event, a biowarfare event, then the aerosol model is really

the appropriate choice. And that will lead to how we design and discuss challenge routes and model, you know, for those pivotal studies.

I think from an R&D perspective, you can have a lot of different models to screen potential candidates. And you have a lab set up, and you can screen and down-select candidates with a lot of different measures. But for really those pivotal studies, does pneumonic indication equal aerosol event?

DR. McINNES: Anyone on the panel wish to comment on that? Louise, do you want to comment?

DR. PITT: Well, I agree with Doris. I think if the indication is pneumonic plague, then aerosol is definitely the route for challenge.

In terms of the passive studies, I think passive transfer as correlates is extremely important. It's one of the ways where you can bridge species. You can get antibodies from active immunization in animals and humans, put them in a single animal model and start comparing activities, getting an idea of what the animal model versus the

human looks like. So I think they're extremely important.

In terms of the challenge route for those studies, I think that could be debated depending on the question you're asking of those passive transfer studies. And depending on that question, I could see potentially both a parenteral route and an aerosol route could give you different pieces of information and could both be important.

DR. McINNES: Yes?

DR. SMILEY: Steve Smiley, Trudeau Institute.

So in terms of this passive transfer, it's been well established in the mouse that that can protect. But in the primates, they seem to have high-titer antibodies, and yet they fail to be protected from pneumonic plague in some situations. So I think an interesting question is whether humoral immunity will suffice in the primates? And what I guess challenge people to do--I don't think it's been done--is can you passively protect primates?

If someone could show that, then we would know that antibodies would suffice in primates, and then we wouldn't have to necessarily develop assays for CMI in those primates. We'd be comfortable with assays for humoral immunity. I don't know if anybody from USAMRIID has tried that type of experiment. I know it's a difficult experiment.

DR. PITT: We would love to and hope to do that sometime in the future.

DR. FERRIERI: May I comment more on the passive immunity? I'm very enthusiastic about studies, doing passive transfer of antibody to understand a number of features here and what might be translated then to the human situation eventually.

And I can envision a situation where you might want to stockpile a plasma, for example, with antibody to the relevant virulence factors as we've defined them today because perhaps there would be situations where individuals receive different doses through a bioterrorist event. And they may have received, depending on your proximity to the

release of it, you might have had a smaller dose. It's conceivable that you might be a candidate, that children might be a candidate as well for such passive treatment with the equivalent of using intravenous immunoglobulin.

I was a big proponent of this on the IOM panel that I served on on anthrax, as were other members. And we had a very hard time convincing certain members of our wider community, public health community of the merits of doing passive experiments in animals, for example. And that has caught on now, and those studies are being done, as I understand it. But I think there is a role potentially for this. Not just for the sake of doing it, but because there may be a role eventually in application.

DR. McINNES: Thank you, Pat. Yes?

DR. LOCKMAN: Hank Lockman, Battelle Memorial Institute.

To build on that last comment, passive transfer may also serve to reduce the lethality post exposure, which I believe is based mostly on

the failure of antibiotic treatment. If antibiotics are not--if antibiotic treatment is not begun early enough, the disease is uniformly fatal. But passive transfer may rescue--may provide some rescue therapy that's not provided by antibiotics.

DR. McINNES: Thank you. Yes, sir?

MR. : So a comment and a question regarding the role of antibiotics. Just a comment. In terms of what to prepare for, I was thinking that we should assume that the aerosol strain would be resistant to all of the antibiotics that plague is susceptible to. Because the engineering of antibiotic resistance is so easy to do in a laboratory to all of the antibiotics we use.

But the question has to do with what study should we do for immune correlates? I've heard a lot about the acute response to vaccines. I've not heard anything about the long-term response and duration of response.

Now with other antibody-driven vaccines that are successful like hepatitis B, we know that

there's a certain titer that's protected. But you can fall below that, no problem, and you're still protected because you're going to boost. We don't know if that's the case for plague. We have no idea whether an anamnestic response plays any role whatsoever in protection from plague, and I just wonder if the panel can help us with that?

DR. McINNES: Yes, Bob?

DR. PERRY: Let me comment on aerosol delivery and multiple antibiotic resistant strains. You know, at a threat assessment meeting we were at several, maybe a year ago now, Luther? I mean, surprisingly, you know, everybody sort of decided that engineered strains were not going to be extremely likely. It was going to be using a natural strain in most cases.

Because while it's true that you can easily genetically engineer the bug if you've worked with for years and stuff, we're not talking about state-sponsored programs, and you're more likely to get somebody going someplace and picking up a dead prairie dog and isolating the organism

and distributing it. So we ended up thinking that highly engineered strains were a little less likely than I think all of us assumed when we first started talking about it.

And maybe the emphasis here is a little too much on an aerosol delivery. That was really sort of thought of as maybe the number-one ideal delivery route. But again, you might use more primitive methods. And so, there might be other methods that aren't that far down the list from aerosol as a delivery method.

Now at least I think that, you know, if you have something that's going to protect you from an aerosol delivery, it's going to protect you from any other route of delivery as well. So it's not maybe a huge mistake to focus a lot on aerosol. But I think we also need to do some testing along the way with other routes of delivery to make sure the vaccines that are developed work.

So like I say, I'm pretty sure that anything that protects against an aerosol is going to protect against any other form of

administration.

DR. MIZEL: Steve Mizel, Wake Forest.

One of the things we haven't talked about in this in terms of correlates is, are you correlating it with mortality? But what about correlating things with morbidity? So that you may be able to get protection where someone gets sick, but they have reduced morbidity. And so, we may throw the baby out with the wash if you ignore things that deal with morbidity as opposed to mortality.

So I'd be interested in the comments of the panel on that.

DR. McINNES: Thank you. Karen?

DR. ELKINS: Karen Elkins, CBER.

Bob, I want you to go back to that statement you just made about things that protect against pneumonic exposure would protect against other routes of exposure. Could the plagueologists, which are assembled here, comment a bit more about the data that speaks to that point and how strong it is?

DR. PERRY: Well, I think it's basically from the aspect that the pneumonic route is much more rapid, much more highly fatal, and there may be differences, especially with the lung pathology, in the two diseases as to what we see. But, you know, if you have a nonpneumonic route, you know, a lot of people survive on their own. The disease takes longer to develop.

And so, I think if you have something that's going to protect against this rapidly developing, fatal, you know, rapid bacteremia that progresses, it's likely to protect against the other types of disease as well. Not on any firm data on histopathology or things like that, just, you know, the level of lethality, fatality, and the time to death and incubation periods.

DR. LYONS: Yes, I think most of the data on that are sort of anecdotal experience with particularly two veterinarians that got pneumonic who were vaccinated with one that particularly protected them--would have theoretically protected them against bubonic, from historical data. But

they died from pulmonic. So it's pretty anecdotal.

DR. LU: For the passive immunization, I think there may be potential it has utility there as well pointed by several members. But I think to use passive immunization or antibody through IV, or whatever, as an immune or vaccine surrogate marker, I think we have to be careful.

This is basic immunology knowledge. Just think about that. When you have a pathogen come in, you have a passive antibody. A passive antibody cannot go higher. It just keep going down. But you have a memory response from active immunization, you will expand it quickly. It continues fighting, stimulate that. So I think that part is very important.

So if we use passive immunization, we can only use a secondary standard. Again, I'm only thinking about vaccine licensure or move from Phase 1 to Phase 3 or from animal to human study. So I think that we have to be cautious on that.

DR. McINNES: Yes, Pat?

DR. FERRIERI: Well, I'd like to respond

to that. I didn't mean to imply that the priority should be on passive. I view that as a secondary priority, and in no way do I view it as conferring long-lasting protection in any way. I view it as an emergency.

DR. LU: Oh, no, no. That part I agree 100 percent. I don't think there's a difference. But I think in the context of this discussion here, there are two definitions. One is as a surrogate and one as immune correlates, establishing the immune protection as a vaccine. That part I say we can use that. Actually, I think it's a great idea to use that. But it's not the same value as the active immunization.

DR. McINNES: And you're also making a plea for understanding kinetics of durability of antibody response and anamnestic response with waning antibody and exposure to antigen sometime remote from vaccination.

DR. LYONS: Pam? Just on active, you know, I notice something that's, to me, missing badly here. But--and I don't want to complicate

things. But it seems like we're basing all the information on one adjuvant that we probably know is not the best adjuvant in the world anymore. And you wouldn't want to miss the opportunity to incorporate studies with new adjuvants that--particularly for as we get into post exposure.

I mean, there are adjuvants that probably may enhance the immune response well enough to maybe benefit there. Right now we seem to be focused on alum. So or at least all the data I've seen has been using aluminum hydroxide of some base. And I know it complicates things, but you'd hate to miss the opportunity to take a look at that along the way. It just seems like such a ripe opportunity.

DR. McINNES: No. I agree. Certainly, I think in any R&D venture it is a ripe opportunity to look at alternative adjuvants. I think when one is--the counterpoint to that is trying to drive hard to a product--

DR. LYONS: Oh, absolutely.

DR. McINNES: --that can't be licensed in

interim, and you couple that with a novel adjuvant approach.

DR. LYONS: Sure.

DR. McINNES: But absolutely, we should use this opportunity to drive it.

Yes, Sam?

DR. KATZ: I think one aspect that relates to the question that the gentleman at the microphone asked previously is I don't think we have any vaccines that protect against infection. They protect against illness. And the shading between morbidity and mortality I think is in individual human response to a pathogen. But a successful vaccine will present--protect against morbidity as well as mortality.

However, what I haven't heard discussed, and maybe I missed it because I came a little late, is kinetics and duration. A, if antibody is the answer, how quickly can you detect effective antibody? And, B, how long does it persist? I agree with Dr. Frothingham that we've shown with some antigens, hepatitis B being the most cogent,

that you may have undetectable levels, but challenged, you will have an anamnestic response. Are you going to need boosters of this vaccine? Your primary immunization series, whatever it is, one, two, or three doses, 5 years later, 10 years later or what? Are you going to have to provide boosters if you really believe you're going to provide protection? And those sort of studies in animals could go on for years before you ever had a licensed vaccine.

DR. McINNES: Thank you, Sam. Yes?

DR. MIZEL: Steve Mizel, Wake Forest.

For those of us who stayed up last night and watched the debate, one of the pieces of data that we heard, in 2008, that the baby boomers will start to retire. And nowhere in our discussions yesterday or today have we talked about aging and the immune response in the aged, which is quite different. We know that very dramatically from the flu vaccines.

So one of the issues in these models and in these correlates is, at some point, we're going

to be looking at a population that, by and large, is not young monkeys or mice. It's old people. And we haven't discussed that. So somewhere in here we have to get to that issue of aged models and because that's a sizable part of the American population we should be thinking about protecting.

So I think that somewhere in our discussions this morning, that ought to come into play.

DR. McINNES: Thank you.

I think one would hope that if you go through the process of having a vaccine, going through your animal studies, developing an assay that's characterized, that's correlated with the functional assay, that some of that readout, in fact, would come from human clinical trials and looking at comparative immunogenicity. I think it's an interesting question on whether you need to have the equivalent counterpart in the animal world. I mean, traditionally, we have done that in human populations.

I'm going to move on because we're going

to loop back, with this particular question, to some of the issues we have already touched on, which is how can a correlate of protection in animals be translated to a correlate of protection in humans? And once again, the question about what functional assays need to be established and validated.

And I think if--I'm not sure if we've beaten that one as far as we're going to go on the functional assay. Drusilla, did you want some more on that one? Are you all right, Karen? So we could focus on the first part of this question, which is how can the correlate of protection in animals be translated to a correlate of protection in humans?

And I'm not picking on you, but I wondered whether someone from DSTL would like to comment about how they see this path of moving forward and the bridging that they would propose to be able to show? Sorry to do that to you, but did somebody want to--Di, thanks.

DR. WILLIAMSON: Well, I think the thing

is that one needs to be very certain of the assays of immune response in the animal models and then translate those assays to man, to the equivalent sera, peripheral blood mononuclear cells in man, and determine whether we're seeing the same kind of readouts. Simply that.

Also I think passive transfer of antibody from human to animal models is going to be vital. That's it. I mean, Karen's presentation this morning really summed up very nicely how to bridge from the animal models to human, I thought.

DR. McINNES: I agree. I thought it was very, very helpful. Are there any comments from the panel about this in terms of the strategy for bridging from positions of certainty? All right.

So any comments from anybody else from the floor, have any issues they want to share? All right. Yes? Yes, sir.

MR. HEATH: I just wanted to point out in the first--I'm Dave Heath from USAMRIID.

I just wanted to point out in the first presentation, there was a gentleman who gave a

presentation on the vaccines, the older vaccines. Jerry Andrews at USAMRIID looked at the Greer vaccine and found that it had plenty of F1, but it didn't have V. And hence, the protection against bubonic, but not pneumonic. So that's just an old historical perspective I wanted to throw in.

The other thing is about F1, on the actual F1 and V together, when you have F1 by itself, it does delay the time to death. So why is that important? From a clinical microbiologist's perspective, if you have, say, a person who was exposed and they're in the hospital. And you're giving that clinical microbiologist or the physicians a couple of days extra to discover the organism, to isolate it, to characterize it, that's really important.

And it really becomes more important when you see the variability in the V antigen. So F1 even becomes more important there. So I would posit that F1 is a very important aspect of the vaccine. So that's just all I wanted to comment on. Thank you.

DR. McINNES: Thank you very much.

DR. FERRIERI: May I ask a question about--

DR. McINNES: Yes, Pat.

DR. FERRIERI: Dr. Lyons, do you understand why people with bacteremia following a bubo, who become quite ill but die infrequently, versus the rapid pulmonary death in someone with pneumonic, and my question is what's happening within the lung, within the macrophages?

Is this the key that unlocks the difference for the fulminant downhill course from a pulmonary point of view? The cytotoxicity that everything's up-regulated within the macrophages, and they're not turned on in peripheral blood, or is there some other very simple explanation?

DR. LYONS: I doubt if there's a simple explanation. I don't know. That's why I think--I mean, that hopefully will fall out over the next few years as we study it more.

I think it happens with--I mean, we see it with a lot of--and Jon can jump in here, too. But

staph infections are the same way. I mean, when staph goes bacteremic from the lung, it's a horrible situation. And so, I think there's some damage to the lung that probably interferes with oxygenation, which we know is a big problem. And mechanical ventilation does not overcome that.

So not only now are you faced with classic sepsis, which, at least in our models, the sepsis developed by plague is different than the sepsis developed by classic Klebsiella, things like that. It's not as--once it starts, it's a real bad situation. But it doesn't kick in until--at least what we've seen, it doesn't kick in until the numbers are extraordinarily high, extraordinarily.

And so, I think what's happened is you get some growth in the lung going, causing a lot of damage. And then, so now you have lung damage on top of sepsis, which is a real bad situation. But that's not the whole story, I'm sure. Jon?

DR. McINNES: Yes. Thank you.

DR. GOGUEN: I can offer--I don't know if it's correct, but I can offer a simple explanation.

And that is that in the bubonic case, there's a longer time for the host to develop a specific immune response. And so, they have this--you're looking at a race between the development of specific immunity and the increase in the bacterial population to the point where the host is not able to recover. In the pneumonic case, this happens much more quickly.

So it's simply that the host in the bubonic case has more time to develop an adequate response and has a chance of recovery. In an untreated bubonic plague, I think the recovery rate is something like 50 percent.

DR. LYONS: Right.

DR. GOGUEN: So you're right at the limit there. Pneumonic, it goes a little faster, and so there's--you just push it to the point where there's essentially no chance to develop an adequate specific immune response.

DR. McINNES: Sue?

DR. STRALEY: I'd just like to comment that we focus a great deal on the macrophage, and

it certainly is important because it's the cell on the spot. But I don't--I think we ought to not overlook PMNs because they're very numerous, and they make all of this--you know, a lot of cytokines that are important.

DR. McINNES: Thank you.

Just some very practical issues now regarding the strain that should be used in animal challenge studies. How should those strains be produced, characterized, and monitored for stability and virulence over time?

I think one of the rate-limiting steps that we really have identified in the rPA, and even in the MVA vaccine development efforts, has been challenge capacity, challenge dose, source of the challenge, characterization of the challenge, potency and stability testing on the challenge material, facilities in which to conduct the challenge. So I think we sort of added the second part to the question because I think it may not be terribly interesting, but it's terribly important in our being able to move forward.

And in terms of what I believe the two strains that I saw reported yesterday, the Colorado 92 and then the C12, which is F minus. Correct? Are there some--from people who have used those strains, are there some distinct pros and cons and specific utilities? Are there some things that should we be looking at alternatives? And then what are some comments from people who have been trying to go through challenge experiments in terms of accessed and what--should we be producing a standardized reference pool, for example, that has actually a stability and potency program established with it?

I'd like to hear some thoughts about that. So go ahead.

DR. PITT: Can I start off? The challenge strain with Yersinia pestis, I am fully aware of the problems that have been occurring with the anthrax rPA program. That one's extremely simple compared to Yersinia pestis because it's a vegetative bacteria. So every time you do a challenge you have to grow it up. You can't just

pull it out of the fridge, as you can a spore, and do your experiment.

So you have many, many more steps in where there is going to be variability. There will be much more variability. You can standardize your procedures. You can standardize your media. You can standardize everything, but you're going to have to change your lots of media at some point. You're going to have to change. So that's just something to keep in mind in terms of--

DR. McINNES: So you would, in terms of having I hate to say even an SOP process, but in terms of having some buy-in about how to produce each time, the media issues, you would see value to that to the community as a whole?

DR. PITT: I see great value in there being a standardized SOP because we have been through some very painful experiences where we've thought we had a certain concentration and we don't. Some of them are not as viable as you would want because they don't stand up to the aerosol procedure based on how long they've been incubated.

So standardizing the procedure is very, very important and sticking to that. The temperature the organisms are held at once they are prepared is extremely important. So, yes, a standardized procedure is invaluable. And a standardized stock that you then take out and just grow up for your challenge is incredibly important.

DR. McINNES: Correct. Any other comments?

Yes, Dr. Perry?

DR. PERRY: Louise is right. I mean, you have to standardize things. And it is going to be difficult from the standpoint of, you know, some of us have been trying to grow the organisms as we thought they might be from a natural infection or from a natural aerosol. And if you're worried about an artificial bioterrorism event, are these people going to grow them at 26 or 37, you're going to get very different profiles of what is made, the metabolism of the organism, and that. So that's going to be very different. There's really I don't think any way to predict that.

Another problem is if you want to store your strain, Yersinia pestis grows at refrigeration temperatures very slowly. So, once again, if you've grown the cells at 37, and now you stick them in the refrigerator over night, when you take them out the next morning, you know, they're not going to be 37-degree grown cells, they're going to have replicated a little bit or at least have adapted now to the cold. So you've got that problem.

With the issue of specific strains, if you look in the literature, you can see primarily three different strains have been used. The C12 strain is really a derivative. So I would say, you know, it's an isogenic strain of Colorado 92. So there's no big issue here. So Colorado 92 has been used extensively at USAMRIID and other places. We and others have used KIM.

So there's three biotypes of plague, and Colorado 92 is the orientalis, and KIM is the medievalis, and I don't know that an antiqua biotype has been used much in virulence testing or

animal tested at all. But if you go back to the old literature, there doesn't seem to be much difference in the level of virulence among the three biotypes.

I think DSTL had been using a strain called GB for a while. I'm not sure what biotype that is. So that's the third strain that's been used in some virulence testing, and I'm not sure there's a big difference in which strain you pick. I'm not sure there is a real issue of, well, we need to test more than one strain. I'm not sure you'll see a big difference.

The first two strains that were sequenced were Colorado 92 and the KIM strain. And you do see differences. The KIM strain has been in the laboratory longer, and so it's not clear whether there are some differences that have accumulated from growth in the laboratory. However, if you look at the degree of virulence, there's really not any significant difference between KIM and Colorado 92.

So I think we can pick one strain that can

be used. I'm not sure that we need to worry about different strains that we're looking at. But really, so the issue is how you're going to grow the strains, how you do this.

For most of our studies, when we have wanted to mimic bubonic plague, we have deliberately grown the bacteria at 26 degrees and have done it in the presence of either iron or hemin, since the flea is going to be probably a relatively iron-rich environment. So that's been our standpoint. But that's not really relevant for what we're considering today.

DR. McINNES: Thank you, Bob. Pat?

DR. FERRIERI: Well, I don't work with this bug, but I would make a case for great standardization and that you know the lineage of it and that everything in one lab. You're able to correlate with what is done in another lab.

And an anecdote. Years ago, one of my fellows wanted to work with HiB, haemophilus influenzae type B. So I called Arnie Smith, who gave me the strain that was used by Haddy Alexander

years ago. And so, we felt that we could talk turkey with everyone else in the field of HiB because that strain was not passage once a week, and you knew exactly where it came from and how it had been treated all these years.

So I mean, do you have something that's been lyophilized and it's been shared? It gets very complicated. And I don't understand. It's great that the genome has been established, I guess, for Y. pestis. But do we understanding its stability and the virulence factors, their stability? And what should we be doing so that--I mean, you're working with this bug. Well, we have a recombinant fusion protein vaccine, but we still need to have great standardization of the bug to do these critical in vivo animal studies and in vitro assays.

DR. McINNES: Anyone wish to comment on that?

MR. : I think standardization would be a critical thing to do, and probably CO92 would be the best strain at this point to choose as

the standard, I would think. It's the more recent isolate of the sequent strains.

I would also comment that I think at the end of the day, not necessarily during the testing, but at the end of the day before--I think we want to know if the vaccine is broadly able to protect against a variety of plague that's out there. And at some point, I think we'd like some recent isolates from different parts of the world to run against the final product.

DR. McINNES: Very good point. Bob?

DR. PERRY: I can't remember whether it was Pat Worsham or somebody else brought up the EV76 strain, the vaccine strain. And you know, everyone who works with plague has EV76 stored in their freezer, and I'll bet no two of them look exactly alike.

And I think we're even starting to see that with Colorado 92 because as it's been disseminated now, we're getting, you know, differences coming up in the laboratories where they've been grown. So, you know, the

standardization probably is sort of important.

DR. McINNES: Yes? One last comment.

DR. FERRIERI: There's a flea in the room that I hope didn't come from some animal experimental station. It was here yesterday, and it's circulating around the table today.

DR. McINNES: Go ahead. Yes?

MR. SCHRIEFER: Marty Schriefer at CDC in Fort Collins.

We are currently making available a panel of Y. pestis strains, eight of them, which include all three biotypes. And Colorado 92 will be made available through a subcontractor of ATTC.

I agree that standardization of protocol for growth and maintenance of any of these strains is critical to standardization of vaccine or other animal protocols and would be happy to participate in that. But would just like to let everyone know that within a few months, these strains that I just referred to should be available through this subcontractor, which is, I believe, BI, subcontractor of ATTC.

DR. McINNES: Thank you to CDC colleagues for that information and setting that up.

We're back to the challenge dose, for completeness sake, on E. And I think we heard--we heard a strong preference for aerosol challenge from some colleagues. I think we heard a little bit more pragmatic approach that alternative delivery routes might be valid. And I don't know whether we want to pin anybody down any further on that.

I think that the challenge by aerosol route has been the most compelling to this point, and I wondered whether we wanted to have just again some pragmatic thoughts about intranasal and intratracheal delivery. And Rick, do you want to just give us a summary again on how you feel about this and where it may play in some of these--which will become very important in the pivotal study.

DR. LYONS: Yes, I guess I believe in the mouse, particularly. I mean, the mouse just simply because the aerosol route is not efficient, and getting high doses is going to be difficult. I

mean, it's going up very high, and that was brought up today by someone else, and trying to do that.

I mean, that may be difficult even in--from practicality matters, even in the primate. But I couldn't say that with authority. But getting multiplicity of infections, moving logarithmically or however you want to do it, you know, that would be more straightforward to do by either intranasal or intratracheal routes.

And again, I just haven't seen any data to suggest that the behavior is different, no matter how it gets to the lung--yet. You know?

DR. McINNES: Thank you.

I wanted to go in terms of to the actual dose that we had some discussion about that, I believe yesterday it started, about what would actually constitute challenge dose. And I also want to pick up on here this issue of the readout of the feet-up and--the feet-up readout that we're currently dealing with, which is the mortality endpoint and the sort of theme that has been percolating around about some intermediate endpoint

that might measure disease conditions, some biomarker readout, some histopathological readout, some count readout.

And to toss out to people in the discussion with what should the challenge dose be, is something like lung infection for pneumonic plague a feasible endpoint readout? And I toss that out to--is it even feasible?

DR. PITT: Can I just comment on the nonhuman primates? I have never seen a nonhuman primate that gets pneumonic plague survive. If they get pneumonia, they die.

DR. McINNES: I think the point is can you, at an earlier point, instead of waiting for that, is there some readout at an earlier stage that you could think about, that you could--I'm looking at this puzzled look, and I'm thinking I'm not communicating properly.

DR. LYONS: I don't think I understood the question. I guess I agree with Louise. I don't think we have a correlate that we could rely on right now to say we should do this, if I'm

understanding your question. So like a morbid timepoint as opposed to--

DR. McINNES: Correct. A morbid timepoint as opposed to--

DR. LYONS: I mean, I think in mice anyway, if they get sick, they're going to die. I mean, that's pretty much what we see. But I think when you start looking at vaccines, I think Dr. Katz's point is well taken. I mean, we've seen some vaccine studies where they get very sick and they get better with time.

So you're really looking at a spectrum of illnesses when you look at vaccinated population versus in a pure naive population. So I think you want to be a little careful about calling your endpoint too short. That's all.

DR. PITT: Based on our IACUC animal requirements we already do, we do not allow our animals to go to death. So we are collecting some of those pieces of information as we do these studies. And I will say as soon as the animals have fluid on their lungs and they are audible

through your respiratory protection equipment that you are wearing, those animals are immediately euthanized because there is no way back from there.

DR. McINNES: Yes, Brad?

DR. LEISSA: Brad Leissa, Center for Drugs, FDA.

Since we're looking at correlates of protection through the Animal Rule, somewhat related to this. But interested if the panel, anyone here in the audience have thoughts about for the purposes of showing efficacy in correlates of protection, we're certainly--in the human trials that will be done, there will be women and men in that.

In the animal studies, do people have opinions on whether or not the nonhuman primates that will be tested, whether it should include both males and females or whether it really matters?

DR. PITT: We always use both. We mix them 50/50 whenever possible.

DR. McINNES: Thank you. Yes, Mark? DR. ABDY: Hi. Mark Abdy with CBER.

I want to get back to the challenge dose. I think I heard a discussion that sort of the group seems to be happy with going with Colorado 92. We had a discussion earlier this morning where we talked about plague, and we sort of settled on a 200 LD 50, and the question came up "why?" I think--

DR. PITT: About anthrax.

DR. ABDY: I'm sorry, anthrax. Sorry, anthrax. We had a 200 LD 50 target. And I say a target.

I want to preface this by coming back to the Animal Rule. We need to remember as we work on these studies that we don't want to set the bar low. We have to set the bar high because we will never have the ability to test this in human beings. So we need to be pretty conservative in how we do that.

Knowing that, is there a recommendation or any discussion on what our target challenge dose should be for Colorado 92 in a mouse model and in a nonhuman primate model? Because we obviously have

to talk to sponsors, and we have to recommend a dose or a target to go with, and I'm not a plague expert. But I want--I'd like to be able to recommend something.

DR. PITT: I can only tell you how we chose the range of challenge that we use. It was based on the probability curve, the lethality curve. We wanted to get above an LD 99 because we wanted all our controls to die. And so, an LD 99 is around 50 to 100 LD 50s. So 100 LD 50 is usually the target that we use.

Having said that, we usually get anything between 50 and 200, 250.

DR. McINNES: Yes?

DR. ABDY: What about the mouse?

DR. McINNES: The question is, what about the mouse?

DR. PITT: The mouse, we have taken that you can use multiple groups of mice so you can do multiple challenge levels. So we have not really done that academic exercise as to what is the most appropriate challenge dose for a mouse. You can

give 100, 1,000--10, 100, 1,000 LD 50s.

DR. McINNES: Your comment, yes?

MR. : At least in the case of plague, an LD 99 is going to be a lot less than 100 LD 50s by the intranasal route. I can assure you of that.

DR. PITT: Pardon? Could you repeat that?

MR. : I was just saying that in plague, an LD 99 is going to be a lot less than 100 LD 50s, at least by the intranasal route. That's been our experience.

I asked about this problem several times. That is what's the aerosol dose that one would expect in a terrorist exposure or whatever? And I never seem to be able to get a good answer. Somebody told me yesterday that the battlefield dose that they plan for--and maybe that fellow is still here. I don't know who it was--is 150 LD 50s, which turns out to be about the same dose that the DSTL people have been using, and that's about 100,000 organisms.

And it seems a reasonable level to shoot

for, and that's--

DR. PITT: You're talking about a mouse now?

MR. : In the mouse model, yes. And that's about the same level that we've used in our--in some of our challenge studies. So that would seem a reasonable target. But it would be nice to know what is expected, and I have no idea what that number is.

DR. PITT: I think that's an extremely difficult question because it depends on the scenario. I mean, you can sit down in a room and come up with multiple scenarios, all of which will have different exposure levels.

So the question is, do you want to go for the worst-case immediately, or do you want to pick a reasonable dose that's somewhere in the middle to establish your parameters, understand your vaccine and your models, and then go in and see how high can we protect against?

MR. : But we also know at this point that we can get protection with some of the

current vaccines at that sort of dose, 100,000 organisms or about 150 LD 50s.

DR. PITT: In a mouse.

MR. : In a mouse. So that's not unreasonable. I had something else, but I'll stop.

DR. McINNES: So just to our colleague who has made an appeal several times for a morbidity readout, I think I have a somewhat equivocal interest in that expressed from people in terms of looking, may have a place in terms of a challenge post vaccination, where you may be looking at amelioration of disease and some sort of morbidity readout. And I think that sort of summarizes how--correctly how people felt about that.

DR. FERRIERI: May I ask a question, Dr. McInnes?

DR. McINNES: Yes.

DR. FERRIERI: This is in response to Dr. McInnes's earlier point about earlier stages of morbidity. Can you attach, or maybe you do, a little oximetry device to a digit of the nonhuman primate so you know when they start to become

deoxygenated?

DR. PITT: By the time they're deoxygenated, they are really sick, and it's very obvious.

DR. FERRIERI: So you don't need that? DR. PITT:

No. The earliest--and that's why we use telemetry continuously in the nonhuman primates because that is the earliest notification that the animal is becoming sick. And the fever goes up before the animals show clinical signs. So--and I can tell you in our limited experience, if the animal's had a fever, the animal has died or has gone close to death.

DR. McINNES: Thank you.

Moving on, question 3 was regarding--I'm sorry. I'm going to have to continue. Oh, all right.

MR. : It's just an add-on comment for the small animal model for plague. Our experience with the mouse is that the only reliable parameter for morbidity is recumbence. When the animals become recumbent, they're going to die.

And we're talking about vaccinated animals. Every other parameter is not a useful indicator of outcome.

Now I'll also say that we did one study looking at hypothermia in the mouse, and that seemed to correlate with recumbence. But it's very difficult to do a large, you know, mouse study looking at temperature.

DR. McINNES: Very important. Thank you. DR.

PERRY: Can I make one comment? So in the subcutaneous mouse infections that we've done, if we look at--you know, we haven't done it real stringently. But we do see mouse that become moribund and nonresponsive, and a small fraction of those actually do recover, at least from a subcu infection.

Yes, you've seen the same thing? Yes, with a subcu. Now that's obviously different than aerosol. But you know, in looking at this, I think we want to be a little careful about assuming that everything's the same between aerosol and bubonic model.

DR. McINNES: Thank you.

Question 3. There are three more questions left, and I think they're sort of big picture, and we can deal with them in the time. We don't have a lot of time available.

The issues around the fact that there will be human safety and immunogenicity studies of candidate vaccines, and induced responses will be compared with those from the challenge experiments in animals to anticipate efficacy in humans. The question on the table is, in addition, should clinical field trials be considered to evaluate the efficacy against natural infection?

Sam, I'm going to, if you wouldn't mind, from your experience of thinking about the whole plethora of efficacy trials, the challenge of trying to do studies in endemic areas, the fact that being able to follow up on subjects and to do case ascertainment and to have medically appended illness in some of these endemic settings is really a challenge.

And seeing what you saw yesterday in terms

of the therapeutic intervention study and perhaps the site in Uganda and thinking about where you might have endemic disease, what are your thoughts on clinical field trials for vaccine efficacy in those settings and in those disease conditions? Yes? If you would be so kind?

DR. KATZ: I think that you have one advantage. That is that in the natural setting, you can treat with antibiotics if your vaccine fails. Whether you can organize a study of that sort in Uganda or Madagascar, where the disease is still occurring, I would ask Jacob Kool to answer that. He's been to these sites. I've been to Kampala, but I've not been out in the field in Uganda.

But it would seem if you were going to organize a study, it would have to be extraordinarily carefully monitored to see that if there were potential vaccine failures, you were onsite to treat promptly. You know, life has become much more complicated. I think some of us forget that in July of 1796, Jenner gave James

Phipps a little virila--or a little cowpox, excuse me, from Sarah Nelms. And seven weeks later, he challenged him with smallpox, and he resisted challenge. And that was an N of 1, and that proved that it worked.

We're not in that era anymore, and the other issue, of course, that comes up is doing studies abroad, international studies in populations who are resource poor and who may feel that they're sometimes used as guinea pigs. And I think you have to be very careful in that respect.

I go back to our own experience, and it's not--it's apples and oranges. But we were pleaded with by people in sub-Saharan Africa to come and do measles vaccine studies because they had a 10 percent mortality from measles. But we did not do them until we had done them in the United States and the vaccine had been licensed. And then we felt it was no longer a fact of using these children as guinea pigs but showing that you could or you couldn't protect in a population that was at high risk.

But I'd turn this one over to Jacob.

DR. McINNES: Jacob, yes.

DR. KOOL: Yes, thank you. I think you said it all. The best way to do it is to go back to the old days, and you guys should just inject yourselves.

[Laughter.]

DR. KOOL: If you're not willing to do that, I think it would be doable, logistically, to do a clinical trial of vaccines. I have to admit I have no experience with vaccine trials. But I would think that it would be easier than a treatment trial.

For the treatment trial, you have to capture the patient on the first day before he's gotten any antibiotic yet. For vaccines, you'll be vaccinating the population, if I understand correctly. And then you'll be waiting for cases to appear. So you don't have to be there prospectively. All you need is good communications so that you can get samples and confirm cases, if necessary.

What I think might be a problem is what--you mentioned that, too. You don't want to use these people as guinea pigs. And the only ethical way that you can do a vaccine trial is to be able to promise them that this will be in the advantage of the study population itself. So you have to be able to offer the vaccine afterwards to the country for an affordable price.

DR. PERRY: I guess there's one other question beyond the ethical issues, and that's the statistical issues. Since an N of 1 is no longer valid, how long is it going to take a study like this to accumulate enough data to be statistically reliable?

DR. KOOL: I can't tell you that. We are optimistic that we will get several hundred cases in our two-year study. Several hundred is all I can say. I will consider myself lucky if we get 400 cases in two countries in two years. But if we're not lucky--you know how it is with these plague seasons. Sometimes they have hardly any cases in a whole year. And other times, they have

huge numbers.

DR. McINNES: Right. Thank you, Jacob.

DR. LU: I think maybe FDA colleagues can provide more information on that. From my knowledge, including my present experience in the HIV field, even HIV is a very high incidence of disease.

Now the Phase 3 trial at a minimum is talking about 5,000 to 7,000 people, or several reasons the Phase 3 trial like from Merck, HPV, the requirement or the efficacy need 7,000, even go over 10,000 so for efficacy. So the vaccine trial requirement has become more and more complicated.

And also I agree with our colleague's comment that the ethical issue is not just providing vaccine. Later, when it becomes commercially available, actually now become ethical issue. You see, you have to go in with the public health education to reduce the incidence so they will not get infected.

So you cannot say I hope we have high incidence rate so we can see the efficacy of the

vaccine. You have to say public health education, including antibiotic treatment. So how do you pick a group treated, not treated becomes very complicated.

DR. McINNES: Thank you. Brad?

DR. KATZ: I think that one of the features that he's brought up that was tangential is at least Uganda, and I can't speak for Madagascar, does have a significant incidence of HIV infection. And whether efficacy of vaccine in an HIV-infected population can be extrapolated to a "normal healthy" population becomes another issue.

DR. McINNES: Right. Right.

DR. KATZ: And all the vaccines I think you're talking about are inactivated or nonreplicating. If you got into the business of an actively replicating organism or an attenuated live strain, I think that's out as far as any HIV population.

DR. McINNES: Valid point. Yes, Brad?

DR. LEISSA: Brad Leissa, CDER, FDA.
I assume that when we're talking about

natural infection, we're talking about bubonic plague?

DR. McINNES: Correct.

DR. LEISSA: Because naturally, pneumonic plague occurs as well, but from the statistical standpoint in deciphering primary from secondary pneumonic plague, you'd never do it. So to the bioterrorist threat, this wouldn't really suffice.

DR. McINNES: Just speaking from the perspective of really a lot of different field trials in some quite difficult diseases and in some really resource poor settings, the onus to be able to track and capture everybody involved in your study to thinking about the setting where you'd be implementing, the onus of, in fact, going through mapping your own trial site, knowing where everybody is, the fact that people are remote from health care settings, the fact that there will be deaths, the fact that you'll need to have some sorts of systems for validating cause of death, even if it's post mortem questionnaires, I think implementation of a prophylactic vaccine study in

such a setting is just an enormous challenge.

So that's outside of thinking about the numbers of cases that you would need to conduct a study. So I think one really cannot underestimate the infrastructure that would need to be established in order to conduct an efficacy trial in such a setting. I don't know if anybody wants to comment.

So moving on to really number 4, and I think, Sam, you had some discussion about this. Pat, you picked this up again in a sort of passive immunization from a therapeutic mode perhaps. I think we have all been talking about pre-exposure prophylactic use of the vaccine. And this is the situation that has come to really be an enormous challenge for us in terms of the anthrax rPA development program.

Are we thinking at all there will be any circumstances--knowing the disease, having the pathogenesis experts, all the animal model people here, are there circumstances under which vaccination should be considered in a post-exposure

situation of plague?

Yes. Dr. Perry?

DR. PERRY: I can't remember who gave the presentation, but there was one where they did a series of vaccinations, one, two, three, four, five, six days prior to infection. And it wasn't until you got out to six days before infection that there was any protection. And I think this is an aerosol model. It might be a little different for a bubonic model.

But I think that at least from what we see in mice, the disease is so rapid that you're not going to have time to develop much of an immune response, especially when we take into consideration not only is it post exposure, but you have to recognize there's been an exposure. So there's a couple more days after there's been the exposure. You've got your first people coming in sick.

And so, you know, you're really probably talking about three, four days, you know, after the event before it's going to be recognized enough, I

would think, and you'd be ready to give a vaccination. And for aerosol, certainly, it's already too late. You're already having deaths.

For another route, you're probably almost past the time when any sort of immune response is going to help before they reach that endpoint where they're going to get better or die.

DR. McINNES: Pat?

DR. KATZ: I tried to think hard, but the only post exposure I could imagine would be if you had a laboratory accident that you could time, and you then gave antibiotic, your immune globulin, and then also added after the immune globulin was catabolized, give your vaccine then.

DR. McINNES: Thank you. Pat?

DR. FERRIERI: Well, I think the priority in the vast majority of our resources for this whole project should be on pre-exposure. But I like this example that Dr. Katz gave. That laboratory person should have been vaccinated before, you know, in a perfect world. But there will be occasions when that hasn't happened.

And I thought I saw some kinetic data, and I can't find it, of course, that some antibody responses are as early as five to seven days? Would that be a true statement in the nonhuman primate, that you start to see a rise? This was--it's too late for--that's too late for a big dose that you've aerosoled--aerosolized into the lung.

But maybe not if you were in a subway situation or in a train and you had a low dose. And what about if this bioweapon, in the attempt to make it antibiotic resistant, also defanged the organism slightly. So maybe the organism doesn't have the potency, virulence that it would have, and maybe the illness would be dragged out and would not be as virulent and fast.

So I think we have to think out of the box about how else do you manipulate the bug and to make it maybe less virulent as an accident. Maybe the vaccine then would be relevant post exposure.

DR. McINNES: Mark?

DR. ABDY: Mark Abdy from CBER. I guess one scenario I'm trying to think

of, if it is a possible one with plague, I don't know what the antibiotic of choice would be and the duration of treatment. But if you wanted to shorten the duration of treatment because of compliance issues and then use a vaccine, a bit like what we're looking at for post exposure in anthrax. Is that a scenario that you could foresee in plague?

DR. POLEY: Gerald Poley from NIAID.

Post exposure for the plague vaccine, you're presuming just a single event. We have seen already that folks who want to do this may do it more than one time. So if one event does occur, that's your canary. And it will take quite a number of people, and it may be too late. But there presumably would be other folks who would demand protection and vaccination.

DR. McINNES: Thank you. Brad?

DR. PERRY: But I would say that's not post exposure for those people anymore. That's pre-exposure, you know? So--

DR. KATZ: It reminds me a little bit of

meningococcal disease, where with your initial
exposure, vaccine isn't any good. You give your antibiotic,
then you can give vaccine for subsequent exposure. But it's
not going to do any good for that immediate exposure.

DR. LEISSA: Mark Abdy raised the parallel with
regards to anthrax and post-exposure antibiotics, et cetera.
And I think they're very different in terms of not having a
spore, you know, the issue of 60 to 100 days of antibiotics.
But in
most settings, I think especially in a post-exposure setting
for plague, most people are
looking at seven days of duration. So I don't
think it's an issue.

I also don't think, from an indication
standpoint, that anyone would be comfortable with
just a vaccine for plague, that they would be giving
antibiotics and passive immunization as well.

DR. McINNES: Agreed. So I think what we--while I
don't think we got a resounding 100
percent agreement that there is absolutely no

indication ever, I think there was, Pat, I think you did support that the priorities should be focused on pre-exposure and that the animal studies and the vaccine development program should be focused on pre-exposure at this point.

Does that--the panel is now looking for data.

[Laughter.]

DR. FERRIERI: You stated it as we presented it, Dr. McInnes.

DR. McINNES: Thank you, Dr. Ferrieri.

We are, I do apologize, seven minutes late. But I want to thank the panel very, very much for your very thoughtful input, and my sincere appreciation to everybody at this meeting who contributed to this discussion and put their two cents and two dollars' worth in. Because you have to be part of the path that's being moved forward, and I thank you very much.

Drusilla and Karen?

DR. MEYSICK: There's one more slide. And actually, it's probably the most--but if I can--bear with

me.

It's the most important slide. First off, I would like to thank, personally and for the entire committee, all the invited speakers, moderators, and panel members. I think by the high quality of the presentations and the discussions that have gone on today that it's a testament to all the hard work they have put into this workshop. And for that, I'm very appreciative for all of them. Thank you. Thank you. Thank you.

Secondly, to the program committee, a lot of you see me up here, I'm like a figurehead. But there is a program committee that came about and helped really cement this entire workshop, and those people are from NIAID, Judy Hewitt, Tony Macaluso, Ed Nuzum, and Vicki Pierson.

From Department of Homeland Security, Captain Lauren Iacono-Connors and Luther Lindler. From HHS, Jerry Donlon. And to HHS, we owe extreme thanks for their generous funding of the workshop. And then from CBER, Mark Abdy, Drusilla Burns, Karen Elkins, and myself.

To Rob Watson and the staff at SAIC, who was around, thank you so much for your logistics and meeting support and help and your patience with me. Also to the transcribers, thank you very much for your patience.

And to the guys at the Marriott, who I think did a really nice job setting everything up.

Finally, and I guess also very more important, thank you to all you guys out there. Because it is all of us coming together and putting everything on the table and discussing things which is the best way to get around and to really figure out what we're going to need to do to fulfill the Animal Rule.

It's obviously not an easy task. There's a lot of questions. There are still things we haven't even talked about. But I think this is a great starting place, where we as the FDA can go back and sit down and have the most informative and current data to make decisions. And for that, I appreciate everybody sticking around for so long.

And that's it. Thank you. And have a

safe journey home.

[Applause.]

[Whereupon, at 12:10 p.m., the meeting was concluded.]