PUBLIC WORKSHOP

CURRENT STATE AND FURTHER DEVELOPMENT OF ANIMAL MODELS OF SERIOUS INFECTIONS CAUSED BY ACINETOBACTER BAUMANNII AND PSEUDOMONAS AERUGINOSA

March 1, 2017

DoubleTree by Hilton Hotel
Washington, D.C.-Silver Spring
Pinnacle Grand Ballroom, 2nd Floor
8727 Colesville Road
Silver Spring, MD 20910

Reported by: Michael Farkas
Capital Reporting Company

P A R T I C I P A N T S

Speakers and Panelists

Thushi Amini, Ph.D.
Associate Director for Research
Office of Antimicrobial Products (OAP)
CDER, FDA

David Andes, M.D.
Professor, Departments of Medicine, Medical Microbiology and Immunology
Head, Division of Infectious Diseases
University of Wisconsin

Robert Bonomo, M.D.
Professor of Medicine, Pharmacology, Molecular Biology and Microbiology
Chief, Medical Service, Louis Stokes Cleveland Department of Veteran Affairs Medical Center
Case Western Reserve University

P A R T I C I P A N T S

(Continued)

David Boucher, Ph.D.
Health Scientist Division of CBRN Countermeasures
Biomedical Advanced Research and Development Authority (BARDA)

Helen Boucher, M.D.
Director, Infectious Diseases Fellowship Program
Associate Professor of Medicine
Tufts University School of Medicine

Ed Cox, M.D., M.P.H.
Director, Office of Antimicrobial Products (OAP)
CDER, FDA

Binh Diep, Ph.D.
Associate Professor
Division of HIV, Infectious Diseases, and Global Medicine
University of California, San Francisco

P A R T I C I P A N T S

(Continued)

John Farley, M.D., M.P.H.
Deputy Director, Office of Antimicrobial Products (OAP)
CDER, FDA

Joanna Goldberg, Ph.D.
Professor, Department of Pediatrics, Division of
Pulmonary, Allergy, Cystic Fibrosis and Sleep
Emory University

Tina Guina, Ph.D.
Program Officer
NIH/National Institute of Allergy and Infectious
Diseases (NIAID)

Judith Hewitt, Ph.D.
Chief, Biodefense Research Resources Section
NIH/NIAID

P A R T I C I P A N T S

(Continued)

Julie Hutt, DVM, Ph.D., DACVP
Veterinary Pathologist
Lovelace Respiratory Research Institute

Robin Isaacs, M.D.
Chief Medical Officer
Entasis Therapeutics

Jane Knisely, Ph.D.
Program officer
NIH/NIAID

Matthew Lawrenz, Ph.D.
Associate Professor, Center for Predictive Medicine
Department of Microbiology and Immunology
University of Louisville School of Medicine

P A R T I C I P A N T S

(Continued)

Gianluigi Li Bassi, M.D., Ph.D.
Researcher, Department of Pulmonary and Critical Care Medicine
University of Barcelona, Spain

Gabriel Meister, Ph.D.
Research Leader
Battelle Biomedical Research Center

Samuel Miller, M.D.
Professor of Medicine, Microbiology and Genome Sciences
University of Washington

Sumathi Nambiar, M.D., M.P.H.
Director, Division of Anti-infective Products
FDA

P A R T I C I P A N T S

(Continued)

John Rex, M.D.
Chief Medical Officer and Director at F2G, Ltd.
Chief Strategy Officer at CARB-X - F2G, Ltd.

Andreas Wallnofer, Ph.D.
Interim Head of Development
Polyphor

Daniel Zurawski, Ph.D.
Senior Scientist, Principal Investigator, Division of Bacterial Diseases, Department of Wound Infections
Walter Reed Army Institute of Research (WRAIR)

Public Participants

Joseph Campbell, NIAID
Tom Dreier, BARDA
Carl Gelhaus, MRI Global
Jennifer Hoover, GlaxoSmithKlein

P A R T I C I P A N T S

(Continued)

Tomas Maira-Litran, Brigham and Women's Hospital in Harvard Medical School

Lynn Meisel, Eurofins Pharma Discovery Services

Craig Rayner, D3 Medicine

William Weiss, University of North Texas Health Sciences Center

C O N T E N T S

C O N T E N T S

(Continued)

C O N T E N T S

(Continued)

C O N T E N T S

(Continued)

C O N T E N T S

(Continued)

C O N T E N T S

(Continued)

P R O C E E D I N G S

Clinical and Scientific Challenges

Introductory Remarks and Panel Introduction

DR. NAMBIAR: So good morning, and welcome again to today's workshop. We are here to discuss the current state and further development of animal models of serious infections caused by Acinetobacter baumannii and Pseudomonas aeruginosa.

I'm Sumathi Nambiar, and I'm from the Division of Anti-Infective Products in the Center for Drug Evaluation Research at the FDA.

So in the last couple of years we've seen products come through that target a single bacterial species, and these bacteria are typically not very frequently identified at any one body site of infection. So the reason we are having this discussion today is that we recognize that there is potential clinical utility for such products, but we also recognize that there are many challenges and difficulties in developing such drugs.

As I already mentioned, it's not that infections due to Pseudomonas aeruginosa or

Acinetobacter baumannii are rare or infrequent; the issue here is that they occur infrequently in any one infection type, making it very difficult to enroll in a clinical trial. And in contrast to many other rare human diseases, acute bacterial infections pose additional unique challenges. These patients are sick, there's an urgent need to start effective therapy -- sorry. Why is it not showing up on the -- sorry, I didn't realize it wasn't coming up on the slides. Sorry. Okay, so here we go.

For people on the phone, would you mind muting your phones? We're hearing a lot of background noise. So people calling into the meeting, would you please mind muting your phones? We do hear a lot of background noise.

So we've been discussing this topic internally, and again for the last couple of years, and last summer we had a 2-day workshop. On the first year of the workshop, we discussed how one can facilitate antibacterial drug development for patients who have an unmet need. And the second day was really focused on developing an antibacterial drug that targets a single

species, and we're hoping that today's workshop would be a continuation of the discussion that we started last year.

So on day one, it was clear that there are significant challenges in conducting a trial to show superiority in patients that have infections due to multidrug-resistant organism. We heard about the difficulties that Achaogen experienced in conducting their superiority trial with plazomicin.

There's a clear message that it is very important to understand how the drug behaves, the pharmacokinetics of the drug, in the target population in which the product is being studied.

On the second day, where we discussed drugs that act only against a single species that is identified infrequently, we had a lot of discussion about the practical difficulties in conducting such a trial. There was discussion about potential trial designs based on a hypothetical example. And all the options discussed had challenges and limitations, and you will hear more about this hypothetical case in the presentation from Dr. Rex this morning. And there was

also discussion at the workshop on potential roles for animal models of infection in studying such drugs.

So just to recap, what are some of the options for clinical development for such products that we have discussed? So one option certainly is to conduct a non-inferiority trial at any one body site of infection.

An example would be hospital-acquired bacterial pneumonia, ventilator-associated bacterial pneumonia. Such a trial is potentially feasible if you're willing to accept greater uncertainty, which translates to a wider non-inferiority margin than we would use in standard development programs. Such a trial will not need to limit enrollment to patients who have infections only due to specific resistance phenotypes, it could be an all-comer population.

Availability of a rapid diagnostic test would certainly help identify these patients but will not change the frequency with which these infections occur. In a non-inferiority trial, it will be very important to consider the potential for confounding by concomitant therapies which are used either to treat

other pathogens which are identified in polymicrobial infections, are often used empirically in these very sick patients.

Additionally, especially for a drug that treats Acinetobacter, the treatment effect for colistin-based comparator regimens may be difficult to assess.

Superiority trials are easy to interpret and assess efficacy compared to best available therapy, and such a trial will need to enroll patients with Pseudomonas or Acinetobacter, which are resistant to available therapies so then one can demonstrate superiority, but it is difficult to identify or enroll enough patients who have infection only due to the multidrug-resistant phenotype.

Such a trial could enroll patients with one or more body sites of infection, but it's important to keep in mind that demonstrating superiority over existing therapy can be difficult, and this opportunity is often time-limited and dependent on available therapy being suboptimal because once new therapies become available, their ability to demonstrate to

superiority becomes more difficult.

In the context of a drug that just targets Pseudomonas aeruginosa, one other option we had discussed was conducting a study in patients who have a great, higher, likelihood of having infections due to Pseudomonas, such as patients with cystic fibrosis, but it does raise the question of, how does one extrapolate from the CF population to non-CF population? whether that raises additional concerns.

The last option that was considered is the potential for approval under the Animal Rule where efficacy data is obtained from animal models of infection, and this might be a potential option if an informative efficacy trial in humans is not feasible. The animal efficacy data that will be obtained, should this approach be pursued, will certainly be supplemented with clinical data from patients who have a variety of infections caused by these organisms.

So I'll just give you a little bit of background on developing a product under the Animal Rule. It's in our Code of Federal Regulations, 314.600 to 650, and for Biologics, it's 601.90 to 95.

Approval under the Animal Rule applies to certain new products that have been studied for their safety and efficacy in ameliorating or preventing serious or life-threatening conditions which are caused by exposure to lethal or permanently disabling toxic, biologic, chemical, radiologic, or nuclear substance when definitive human efficacy studies cannot be conducted because it would be unethical to deliberately expose healthy volunteers to lethal or permanently disabling toxic substance, and field trials have not been feasible.

When we rely on evidence of effectiveness from animal studies, there are four criteria that need to be met: that the pathophysiologic mechanism is reasonably well understood; 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; the endpoints used in the animal studies is clearly related to the desired benefit in humans and is generally the enhancement of survival or prevention of major

morbidity; and, lastly, the data or information on the kinetics and pharmacodynamics of the product in animals and humans allows us to select an effective dose in humans.

There are some additional requirements for products approved under the Animal Rule. There is a need to do postmarket studies to provide evaluation of the safety and benefit if and when situations arise where the study is feasible and ethical. If needed, we could impose restrictions to ensure safe use of the product. And, lastly, labeling should include information to patients that, for ethical or feasibility reasons, the product was approved based on studies, efficacy studies, in animals alone.

So here are some examples of Animal Rule drug approvals in the infectious disease arena. Levofloxacin, ciprofloxacin, and moxifloxacin were approved for plague under the Animal Rule. And for inhalational anthrax, in addition to levofloxacin, we have two monoclonal antibodies that are approved. And then I've also listed some examples in the non-infectious diseases space.

So just to give you an idea of what kind of animal studies was done for levofloxacin -- and you will hear a lot more about the animal models for plague in Dr. Hewitt's presentation -- this was a placebo-controlled study in African green monkeys. Important to note, that levofloxacin was already approved for other indications based on clinical trial data, including respiratory infections such as community-acquired and nosocomial pneumonia.

So demonstration of efficacy in one species was considered adequate. The AGMs were exposed to Yersinia pestis, it was a specific strain, and they either received intravenous levofloxacin or placebo post-trigger, and the mortality in the levofloxacin arm was significantly less compared to that in the placebo arm.

So what do we hope to accomplish in today's workshop. We hope that discussions at today's meeting will help provide a better understanding of the relevant animal models of infection, the advantages and shortcomings of the currently available approaches, help us identify key areas for further work or

development, and the role that animal models might play in the development of such drugs so that we can get these products to patients.

And before I conclude, I just wanted to present a slide on the "Limited Population Pathway for Antibacterial and Antifungal Drugs" because that might come up during our discussion today and it's very relevant to products that would be developed using extremely small clinical trial datasets.

So as many of you are aware, the 21st Century Cures Act was signed into law on December 13th of last year, and Section 3042 of the Act covers the limited population pathway for antibacterial and antifungal drugs. And this pathway is for drugs that are intended to treat a serious or life-threatening infection in a limited population of patients who have unmet need.

Labeling for products approved under this pathway will include "Limited Population" in a prominent manner and also a statement that the drug is indicated for use in a limited and specific population of patients. And products approved under this pathway, there is a requirement for pre-submission of

promotional materials.

So we do have a fairly busy agenda for today. In the morning, we will talk about the clinical and scientific challenges in developing some products. We will hear examples both one from Polyphor for an anti-pseudomonal drug, and we'll hear from Entasis Therapeutics on an example of a drug that targets Acinetobacter. We have a session on "Lessons Learned and Considerations for Animal Model Development" followed by a session on "Pathogenesis" of Acinetobacter baumannii and Pseudomonas aeruginosa infections.

Following lunch, we'll talk about the "Approaches to Animal Model Development, Future Direction, and Next Steps." And we'll conclude with a panel discussion. There is opportunity for the audience to participate during the session, so we do encourage you to participate. Your comments and questions are certainly very, very valuable to us and help us formulate our thoughts moving forward.

So with that, I'll take the opportunity to thank all the panel members for participating and also

for introductions.

Dr. Guina, maybe we'll start with you.

DR. GUINA: Sure. Can you hear me? My name is Tina Guina. I'm a program officer in Drug Development Section in Office of Biodefense Research Resources and Translational Research in Division of Microbiology and Infectious Diseases at NIAID.

DR. BOUCHER: Hi. I'm David Boucher. I'm a project officer with BARDA's Division of CBRN Countermeasures.

DR. DIEP: Hi. Binh Diep, from the University of California, San Francisco.

DR. LAWRENZ: I'm Matt Lawrenz, from the University of Louisville.

DR. ANDES: David Andes, from the University of Wisconsin.

DR. GOLDBERG: Joanna Goldberg, Emory University.

DR. BONOMO: Robert Bonomo, Case Western Reserve and Cleveland VA Medical Center.

DR. MILLER: Samuel Miller, University of Washington.

DR. KNISELY: Jane Knisely, NIH/NIAID.

DR. COX: Good morning. Ed Cox, Director of the Office of Antimicrobial Products, CDER, FDA.

DR. FARLEY: Hi. John Farley, Deputy Director, Office of Antimicrobial Products, CDER, FDA.

DR. REX: John Rex, Chief Medical Officer at F2G and Chief Strategy Officer at CARB-X, a public-private partnership supporting antibacterial R&D.

DR. WALLNOFER: Andreas Wallnofer, Acting Head of Polyphor.

DR. ISAACS: Good morning. I'm Robin Isaacs, Chief Medical Officer of Entasis Therapeutics.

DR. HEWITT: Judy Hewitt, NIAID.

DR. MEISTER: Gabe Meister, Battelle Biomedical Research Center.

DR. HUTT: Julie Hutt, Lovelace Respiratory Research Institute.

DR. NAMBIAR: Two of our panel members unfortunately couldn't be here, so they'll be joining us by phone.

Dr. Li Bassi, would you like to introduce yourself? Hello, Dr. Li Bassi, are you on the line?

(No response.)

DR. NAMBIAR: All right. Dr. Boucher, are you on the phone?

DR. BOUCHER: Good morning. It's Helen Boucher, from Tufts Medical Center and Tufts University School of Medicine.

DR. NAMBIAR: All right. So Dr. Boucher is our first speaker for today, and will have to join us by phone, as she couldn't be here in person.

Dr. Boucher is the Director of Infectious Disease Fellowship Program at the Tufts Medical Center and Professor of Medicine at Tufts University School of Medicine. Dr. Boucher's clinical interests include infections in immunocompromised patients and Staph aureus infections. Her recent interests focus on Staph aureus and the development of new anti-infective agents.

Dr. Boucher, I'll pull up your slides in a minute.

A Clinician's Perspective

DR. BOUCHER: Thank you very much, Sumathi. And thank you for the kind invitation to be there

today. I'm really sorry not to be with you all in person. We actually have another regulatory body, the ACGME, here on campus for a visit, so I had to stay here for these days. But I'm certainly with you in spirit. I'm looking forward to learning a lot during the course of the day.

I've been asked to talk about a clinician's perspective on the topics of developing studies to study infections due to Acinetobacter and Pseudomonas. On the second slide are my disclosures.

And on slide three, I thought we could just sort of start our discussion of the day -- actually, if you can go back to slide 3, please -- just with sort of stating what's really well known to all of us who are here, but this notion that we have perhaps returned to the pre-antibiotic era. And I think observations like the findings of mcr-1 and -2, with transmissible colistin resistance and the sort of onslaught of truly multidrug-resistant Gram-negative organisms in the environment and our food chain and now unfortunately in our patients, it's frankly scary to all of us and something that we're contending with across the country

and around the world.

As a clinician, we have long been sort of forced to use the drugs that we have often with less than ideal data. So, for example, we've had to learn how to use inhaled and parenteral colistin, a lot of creative ways to use drugs like fosfomycin for ESBL and related Gram-negative infections, and tigecycline for multidrug-resistant infections despite known safety limitations. I think that's not even to mention the various combinations that we find ourselves using quite creatively in the clinic.

Another sort of comment to make at the beginning of the day is that this discussion we're having about using and developing new drugs is part of a bigger strategy to combat antimicrobial resistance. And certainly our efforts in infection prevention stewardship and surveillance are very, very important, and efforts like the CARB initiative are helping us at least to make some strides forward in this regard.

On slide 4 is a list that's familiar to many of us. And if you could just advance one more time, please. This is a CDC list of pathogen threats from

2013, and, appropriately, the organisms that we're discussing today, Acinetobacter and Pseudomonas, are listed here as serious threats, along with a lot of other organisms of concern, including CRE, which were listed as urgent threats.

If you turn to slide 5, just this week, the WHO list of priority pathogens for which new antibiotics are urgently needed was released, and very importantly and timely for our discussions today, Acinetobacter and Pseudomonas have risen to critical priority, and clearly that's very much in line with what we see in the clinic and what's being observed around the world.

Other pathogens that we've spent a lot of efforts on -- (phone line skips) -- CRE are still quite important, as are other things more globally, such as Campylobacter and Salmonella and GC.

If we turn to the next slide, looking at where we've come in terms of (phone line skips) development progress, our IDSA 10 by '20 Initiative has made some progress with six antibiotics approved so far in this decade, including two that are active against Gram-

negatives, but clearly gaps remain and certainly there's more to be done, which brings us to our discussion today.

On slide 7, I thought it might be helpful to talk about a couple of actual patient cases that might demonstrate the importance of what we're undertaking.

So the first case I'll share is a 71-year-old lady who had laryngeal cancer, underwent surgery, chemotherapy, and radiation, and that was several years ago. She was cured of the cancer, but home on oxygen for her breathing chronic issues and had had some admissions to the hospital for tracheobronchitis, and had to go to rehabilitation, and came back to us from rehab with fever, flank pain, and breathing issues.

Next slide. She basically wasn't that sick, she had a cough, she had nasty sputum, but didn't have fever or other constitutional symptoms. Her evaluation did not show a virus or any other cause of infection. And ultimately her blood and sputum grew a Gram-negative that was identified as a multidrug-resistant Klebsiella with metallo-carbapenemase.

She did quite well that time, cleared her

blood cultures, and did not need to be intubated or put back on the breathing machine. We treated her with an interesting combination of tigecycline and colistin both inhaled and IV, and she tolerated a transition ultimately to minocycline.

On the next slide, her case progressed, and she came back to us again with pneumonia later in the winter, and then again in the spring, and ultimately she presented with respiratory failure and tracheobronchitis and urinary tract infection, again coming from rehab. She did well, got a 5-day course of levofloxacin, but cultures of sputum and urine came back with a CRE, a Klebsiella pneumoniae producing carbapenemase.

Now her respiratory status got worse, she's back in the emergency room, still sick, now febrile, needing more oxygen, and her urine grows greater than 100,000 Klebsiella, and we got the call that it was a multidrug-resistant organism. And so really what does that mean?

On the next slide, you can see the profile that came back from the laboratory showing resistance

to all of the antibiotics listed here. And, importantly, on the lower right-hand side, this organism was resistant to our two new agents, ceftolozane-tazobactam and ceftazidime-avibactam, in addition to our other kind of standard therapies, (inaudible) aminoglycosides.

So in the next slide, we discussed this with her family and the patient and the fact that there was going to be predictable renal, neurologic, and other toxicity if we attempted further therapy, and she elected to transition to hospice care and passed away. So this was a lady who was cured of cancer but ended up dying of a resistant infection.

If we turn to another type of case, this is a gentleman, a 46-year-old gentleman, who had heart failure along with the usual comorbidities of diabetes and obesity, and he came to us in cardiogenic shock and required placement of a heart pump, a left ventricular assist device. But before this, he was a healthy, or apparently healthy, young guy working full-time and was married with two young sons.

In the next slide, you see that a few months

later after placement of his ventricular assist device, he comes back to us with what started as some local irritation at the site, but got a little worse, as you can see in the photograph. That erythema and kind of purulent stuff was a local infection with a skin bug, Corynebacterium. Initially did okay with that, but came back a month later with worsening drainage, and now grew a Gram-negative organism, Enterobacter. Again, we managed this with local care and some oral antibiotics initially.

In the next slide, you can see a photo of his device. He had the HeartMate II device, on the left. And it's implanted into the heart and then into a pocket in his abdomen, just to give you a sense of the anatomy. He sort of progressed and made it for 2 more years actually.

In the next slide, he came back to us with a device complication where the device itself stopped working, so he had to have the pump exchanged, and so he went to the device on the right side of the slide we just saw, and had a bit of a rocky post-operative course, as patients do after that kind of a procedure.

But he went home and did well for a few months, but came back again with drainage at the driveline site like we saw in that earlier photo, this time with Staph aureus, another skin organism. It was MSSA, and he was treated quite kind of by the book with empirical vancomycin and then his therapy was narrowed down to cefazolin and then cephalexin.

In the next slide, we can see that he struggled and had a few (phone line skips) admissions with infection. And his last admission, he came in with pain and increased drainage, and now when we looked at a CAT scan, there was actually infection going deeper into the tissue of his abdomen. So he was put on intravenous antibiotics, and this time the cultures came back with Pseudomonas, and, indeed, his Pseudomonas was resistant to ciprofloxacin, aminoglycosides and -- oh, sorry, ciprofloxacin and meropenem, but susceptible to aminoglycosides.

He didn't do well despite antibiotics. And he was not a candidate for aminoglycosides because of the ventricular assist device. You're not longer a candidate if you have renal failure. And he wasn't a

candidate for another pump exchange. And he wasn't a candidate for a transplant because they couldn't control his infection. So this 46-year-old man ultimately had to go to hospice, and he passed away as well.

So if you turn to the next slide, these cases I think -- and many others that my colleagues in the room I'm sure have seen -- show us that infections from resistant pathogens are serious and could happen to us and our families. Certainly having drugs that can target these pathogens would be useful to our patients. And the data that we have is often less than what we would hope. And Dr. Nambiar highlighted a lot of these issues in her earlier talk.

Certainly, we do often use data from infections at standard body sites, like urinary tract infections, for example, as the foundation from which we build. And we have to extrapolate, as clinicians, to treat our patients because of the various ways in which they present. And this allows -- we often rely on a variety of sources of information to do this.

On slide 18, we know that clinical trials in

these areas are very challenging, and Dr. Nambiar highlighted a lot of these issues. But I think there are really only a few areas where testing narrow-spectrum drugs in a clinic is really tractable, and that would be things like Staph aureus and skin infections, gonorrhea, and things like C. diff, and Pseudomonas in cystic fibrosis, as was highlighted earlier.

Certainly, we know that we need a path forward for infections like Pseudomonas and Acinetobacter, and we're going to hear about some candidates that are emerging, which is great.

Dr. Nambiar highlighted the issue of difficulty in enrolling in clinical trials because these types of patients, especially with one particulate site of infection, are rare, and, frankly, that's good from a clinical perspective, we don't want it to be otherwise. Dr. Nambiar also highlighted some of the complexities and really inadequacies in both non-inferiority and superiority approaches in this regard.

Diagnostic tests, and rapid diagnostic tests

in particular, probably won't fix this problem because they won't create the patients. They might help us identify them faster, which is good. And Dr. Rex will talk next about some of the other challenges and options that we discussed in the July workshop.

On slide 19, several ideas have emerged about ways we can address this challenge. Certainly, PK/PD-based dose selection and validation are very important.

Animal models, in particular, validated animal models, after the fashion of the Animal Rule, are going to be discussed.

Validated external controls perhaps with open-label data even with test agents might be useful.

And very small clinical datasets, as Dr. Nambiar mentioned, perhaps also with pooling of data from multiple body sites, may be options in terms of ways we can use clinical data.

As we think about overall development plans, I think a couple of themes deserve some attention. One is using a fully validated Animal Rule in the settings when we have no clinical efficacy data in the so-called Tier D development that Dr. Rex will discuss. And then

there is the option perhaps of using good animal models, maybe multiple different animal models, some of which will be discussed later today, along with limited clinical efficacy data as a path forward.

In slide 20, from a clinical perspective, I think as we look at developing drugs when we can't have fully powered clinical trials and when we're thinking about using animal studies in a bigger way, clinically, we hope that those infections will have some reasonable human correlates and that PK/PD can be optimized to understand and predict efficacy at a variety of body sites, such as the lungs, the bloodstream, and intra-abdominal infection, the worst clinical cases.

So in slide 21, pulling this all back together and kind of summarizing where we are from the clinical perspective on these narrow-spectrum indications, I think in 2017, we are forced to use drugs with limited data in the clinic in order to address the patients that we see. And looking ahead, I think the work in July, as highlighted earlier by Dr. Nambiar, really showed us that additional clinical development plans, non-inferiority or superiority studies just may not be

feasible.

When we look at small clinical studies, having high-quality data is very important. Having adequate safety data is very important. That's not necessarily the focus of today's discussion, but I wanted to make sure we highlighted that as part of a clinical (inaudible). And we really think that using clinical trial networks is a way to help to enhance the quality of the data that we do obtain. And we hope to see the inclusion of multiple body sites and infection types in order to help clinicians have useful data.

Next slide, please. In terms of animal studies, perhaps using the Animal Rule in addition to PK/PD studies we believe can provide a foundation for clinical development either in the scenario of the Animal Rule with no clinical data or in the scenario of using some animal models, good animal models, with some clinical data.

The LPAD mechanism, as Dr. Nambiar mentioned, does ensure that the use of these agents will be in a limited population with needed safeguards. We believe that ID physician-led stewardship will help ensure that

these patients are managed in the best way possible.

And, very importantly, just a couple of days ago, the WHO raised Acinetobacter and Pseudomonas, along with CRE, to the critical priority pathogens, and so they've moved from serious to critical in the past 3 years. So I think that further emphasizes the importance of our figuring out a way forward for studying these infections.

And, finally, just to bring us right back to the most important thing, our patients. The time to figure this out is now, and we're very gratified to be here today, and hopeful that we'll come up with some really good options forward.

And with that, I'll thank you very much for your attention.

DR. NAMBIAR: Thank you, Dr. Boucher. Thank you for agreeing to participate via phone. I know you have a busy day with other regulators onsite.

So with that, we'll move on to the next session, which is to talk about the "Challenges with Clinical Trial Design for a Drug Targeting a Single Species of Bacteria." So we have three speakers in

this session: John Rex, from CARB-X; Andreas Wallnofer, from Polyphor; and Robin Isaacs, from Entasis Therapeutics.

So I'll introduce Dr. Rex first, who is well known to many in the field as an ID physician and drug developer with 30 years of development and policy experience focused on antimicrobial drugs. Dr. Rex's experience includes moving compounds from early pre-clinical development through all the development phases in the context of various academic and non-academic positions.

Dr. Rex, thank you.

Challenges with Clinical Trial Design for a Drug Targeting a Single Species of Bacteria

DR. REX: Thank you, Dr. Nambiar.

And let me remind everybody who is on the phone, please be on mute. We're hearing all of your papers rattle beautifully, which is a little difficult on our end. Thanks.

So thanks, Dr. Nambiar, for the opportunity to be here. My disclosures and affiliations are shown on this slide.

And our focus for today is narrow-spectrum drugs. Let me just make the observation that we're not here talking about narrow spectrum. Narrow spectrum has some values, there are some important potential microbiological advantages to being narrow, but really what we're saying is we need drugs for Pseudomonas and Acinetobacter. These are really hard bugs to find drugs for. And if you narrow your focus to a single genus, you sometimes have more luck finding a new agent.

And so I'm aware now of several candidates that they really are focused on Pseudomonas or Acinetobacter, and the molecule looks interesting, it just doesn't have other activities. So it would be delightful if it had those other activities, it would make it easier to develop, quite honestly, but I don't want to walk away from drugs that look like they might actually work here because I think we need a collection of things.

Pseudomonas and Acinetobacter are sort of the "Darth Vader" of bacteria, and we really need a bunch of tools. And so some of them are narrow, and we need

to find a way to develop them. So that's really kind of the setup for today.

With Andreas and Robin, we're going to do a little three-part act. We're going to give you three somewhat worked out examples of how you might develop a drug. And the theme that you're going to hear repeatedly is Helen has already told you about the unmet need, what you're going to hear from the three of us is that clinical trials can only get you so far with these drugs, but there are some things you can do to help yourself move forward. And there are three key ideas that I would like you to listen for as you go through the presentations today.

The first is the PK/PD theme that you've heard many times, is that MICs and drug exposures are pretty strong in this area; they're a little stronger than they are in other therapy areas. PK/PD gives you an independent proof of causality that reduces your need for empirical validation by clinical trials. Having said that, though, there have been exceptions. PK/PD is imperfect. And there will again be exceptions, I am sure.

And so my message is that we should always seek as much clinical data as possible, but we should be willing to lean more on PK/PD if required.

The second thing, idea, I want to be sure you're aware of is a bit of mental shorthand that is helpful in having these conversations. This is an idea that we introduced several years ago just as a way to help break down the conversation into useful blocks. And the idea goes under the rubric of the tiered mental model, which is to say that there are four broad categories of anti-infective development programs.

At Tier A, you're in the situation where you can do multiple Phase 3 trials. You can generate a lot of standard quality clinical data.

Tier D, the other end, is the Animal Rule situation where you can't generate any clinical data, you can demonstrate some safety data, but you just can't demonstrate, at least you hope you can't demonstrate, clinical data.

In between, we have Tier B, which is where it's possible to do a Phase 3 study, maybe once, it's hard, but it's possible.

And then Tier C is the situation where you can't even quite do the Phase 3 trial, I should say the standard dimension Phase 3 trial, a fully powered, full size, what you would expect, Phase 3 trial. And pretty clearly, we're here where we are today is talking about Tier C and Tier D. It's a helpful way to put yourself in the right mental box.

And Tier C, I think of it as animal models, plural, with clinical data. It's the animal model data are helping you validate the PK/PD relationship. You can generate some clinical data. You're looking for it to be consistent, but it will not be at the usual statistical strength. And these are taken together.

Tier D, as has been said, no clinical efficacy data possible. You can get the safety data. The animal data are actually -- they are the controlled trial until such time as you do a field trial. And here you really want the models to be even more close to being a mimic of the human disease if you can.

So it's a continuum. You do the best you can. Drug labeling should be suitably cautious as you go from A -- more and more cautious as you go from A to B

to C to D. And the LPAD language, and also there is some parallel language with EMA, that talks about how you might describe the limited datasets, should you have them in that fashion.

The third idea to be aware of is that superiority is not an escape hatch for us. I'm often asked, "Why don't we just show that the new drug is superior to the old drugs? Because wouldn't that be easier? The clinical trials would be smaller. It would be cleaner." And you're right, it would be cleaner, it would be smaller, but paradoxically, superiority is painful, indeed, I would say it's an ugly path for antibiotics.

We're not treating migraines. Inadequate therapy of serious infections leads to death. We must never knowingly randomize to ineffective therapy. Hence, if you want to routinely use superiority as the way you get to new drugs, you're going to have to let some things happen. One is you're going to have to let AMR progress such that highly resistant strains are sufficiently common that you can get them in a trial, and then the best available standard of care needs to

not really be very good. And then you would see superiority, but that superiority would be based on something kind of unpleasant, and this is not a hypothetical. Let me show you a real life example.

So Achaogen showed some data just before Christmas this past year about their new product, plazomicin, where they were studying it for culture-proven carbapenem-resistant Enterobacteriaceae infections. And in this study, they randomized to the best available care these days, which is a colistin-based standard of care. That is the best available therapy at the time they were doing the study.

And what you see in the graph is they've got these two groups, and the plazomicin group had 11 or 12 percent mortality, and the colistin group had a 40 percent mortality, and that's really quite different. And notice that the denominator is small. This is a small trial, but it actually suggests superiority of the plazomicin-based therapy.

Now, I point out, why did they see that? If you do the math, it means that about six people on the standard of care arm died due to the standard of care.

So I'm glad to have the clarity of the data on colistin's relative lack of efficacy and the value of the plazomicin control arm, but this is a steep price to pay on a routine basis. As colistin is displaced as standard of care, I hope that the next drug goes up against plazomicin, and it just becomes harder and harder to ever show something like this again.

So with that as my context, let me talk about Drug X-1, which is the first of the three drug examples where we're going to talk about how you might develop such a drug. X-1 was a drug that was invented as a hypothetical for a workshop last summer where we had about 100 people, and we spent a day talking about, "How would you develop this drug?"

And we made up the case to really simplify the storyline. It was an activity limited just to Pseudomonas. We made up very clean pharmacology, a very clean Phase 1 program. The imaginary sponsor even did an imaginary study in Phase 2 in non-CF bronchiectasis and showed that their proposed dose knocked down organism burden in the lung. So the drug got into the lung and it had an effect on the bacteria.

It was not a study you can always do, but we made up the simplest possible case and said, all right, X-1 looks really useful. How in the world would you bring this to registration?

So we thought about it and we said, well, we could devise a study arm if you put X-1 with ertapenem, which is a carbapenem that lacks activity against Pseudomonas, but it hits all the Enterobacteriaceae, it's stable to ESBLs, it's indicated for complicated intra-ab, it's indicated for skin, it's indicated in the lung. So it's got all the body sites covered in terms of getting in there.

And the PK actually has been modeled, was modeled briefly last year, and then Paul Ambrose's group has modeled it in more detail, and we have to say that ertapenem looks like it should be just fine for ventilator-associated pneumonia. There will be an abstract on this at ASM Microbe this summer.

So if you put ertapenem with X-1, you've basically got a regimen, you can put it against the standard carbapenem, and anytime you treated a Pseudomonas, the X-1 would be the component that was

treating the Pseudomonas, and so you would have a clear demonstration of that.

There is a bit of an unreserved complexity around the need for the frequent wish for initial dual coverage that we need to resolve, get another debate in this area, but the point is it looks like you can develop a study arm.

The real issue is a mathematical one, which is the rate of cases of Pseudomonas is low. You typically have to enroll before the culture result becomes available. Typical rates of Pseudomonas and nosocomial pneumonia, intra-ab, and UTI are shown. And a diagnostic test, importantly, won't fix this problem. Diagnostics don't create the patients with the infection, they only help you find them. You still have to screen the number of patients required to find the patients with these infections.

And this leads to a trial problem, which is that if you just say, "I want a standard dimension Phase 3 study, endpoint 20 percent failure, non-inferiority margin 10 percent, power 90 percent," you need 672 evaluable cases, 336 per arm, and if you back

that up to evaluable equals culture-proven, you need either 3,000, 6,000, or 22,000 patients in your Phase 3 trial, which is clearly large enough for safety, but not feasible for actual development.

And I will observe that we have two examples recently that show that nosocomial pneumonia trials sort of enroll between 250 to 300 patients a year. So that 3,000-patient study is about a decade's worth of work.

So in designing options for X-1, we put some constraints on it. We said it's got to be common sense. We can't have a BFMI -- Brute Force, Massive Ignorance -- solution. We can't presume perfect diagnostics, instant susceptibility, instant knowledge, that only Pseudomonas is present. We're not going to have a superiority study because we're not going to have a perfectly timed MDR Pseudomonas aeruginosa outbreak. We've only got enough money for about 1,000 people.

And the money question is an interesting one. You might say, well, maybe we -- because a community could study 20,000, but if you commit 20,000 to

studying one drug, you can't study anything else. So it's not just money. The idea of studying an enormous program, it interferes with being able to do anything else.

Add-on therapy is unlikely to play out. Standard of care plus X-1 was unlikely to be better than standard of care because standard of care could only be done in settings of susceptible Pseudomonas. In short, we wanted to keep the miracle count less than 1. We would accept luck, but we wouldn't expect it.

The imaginary sponsor invented an imaginary screening device based on having found a monoclonal antibody in the Sigma Catalog to pyocyanin. And we thought maybe you could create a little lateral flow device that would get you up slightly in terms of your culture-positive rate. We have (inaudible) you can get up to 25 percent and 16.5 percent. It wasn't a clear test, but we thought, well, maybe you could do something like that to increase the rate of Pseudomonas.

So putting it together, and listening to Helen, Helen said, "I would like to see data in a

variety of body sites." You know, sort of we thought we would spread it out a little bit. So what we came up with was this design on this slide as sort of our base case: two trials that would generate data in three indications, and the hope was that if all three indications sort of demonstrated the same direction in terms of response, you would be convinced by putting it together.

So there was an RCT with separate sub-arms for nosocomial pneumonia and complicated intra-ab. And we tortured the data to create two subsets that were two tiny non-inferiority studies with absolutely the widest margins we could possibly justify based on any data we could find in the literature, 30 percent for nosocomial pneumonia and 25 percent for complicated intra-ab, and, yes, those are enormous margins. And we randomized 2:1, and there's the size there, 915 subjects. It just barely fits. But remember, if all of it lined up, you would have multiple bits of data pointing in the same direction.

We also said we would do an open-label study in patients with limited treatment options where we

just play "Go Fish," bring us your most difficult case and collect some data.

Is it feasible? Well, maybe. Hitting these numbers would be hard. It's just at the bounds of feasibility.

Is it credible? Maybe. You know, this really does -- we really have pushed the design limits very hard.

The discussion at the workshop was helpful because we had 100 people in the room and people were very engaged, and as we debated it, what we concluded was we hadn't overlooked some trick. There wasn't some clever thing that we could do instead of this. We were stuck. And they are going to be limited.

We also talked a little bit about what happened when we couldn't even squeak out these very, very difficult NI designs. And, again, no clever ideas came up at that time.

So, in short, our basic conclusion was there wasn't a trick, we weren't overlooking things. Sometimes clinical data would be very limited, and the tradeoffs were going to be required. And, by the way,

this has been written up, and there's a paper that I think is just about to be able to say it's in press at JID that describes these ideas.

So this is my last slide, which is to say that narrow-spectrum drugs, it isn't just that it's narrow, it's just that for Pseudomonas and Acinetobacter, these are often going to be narrow, and if you want these drugs, we have to facilitate these pathways.

I've given you an example that we cooked up last summer as a possible way. You're about to hear two more examples. And I'll just say again that all three of the examples you're going to hear have clear limitations, but if you want to see any progress here, we're going to have to accept these tradeoffs. And my last comment is that lack of action would be action that has consequences.

So thank you very much.

DR. NAMBIAR: Thank you, Dr. Rex.

So our next speaker in this session is Dr. Wallnofer, and he will discuss with us their experience in developing POL7080. Dr. Wallnofer is a clinical pharmacologist with additional expertise in

pharmaceutical medicine and business management.

Dr. Wallnofer spent more than 20 years at Hoffman-La Roche, where he was a member of the R&D Leadership Team and Head of Clinical Research and Exploratory Development. And in his current role, he is at Polyphor as Interim Head of Development since 2016.

Thank you, Dr. Wallnofer.

DR. WALLNOFER: Thank you for the introduction. And let me first thank the organizers for the kind invitation to this workshop.

So my talk is on behalf of Polyphor. Polyphor is a Swiss biotech company that has developed a novel class antibiotic against Pseudomonas aeruginosa. And nosocomial pneumonia is the leading cause of death of nosocomial infections in the U.S., and Pseudomonas is, as we just discussed, one of the most frequent pathogens in infections with Pseudomonas, have adverse outcome, and particularly patients that are infected with MDR strains, they have increased risk of mortality. So we really think it's important to develop such new medicines for patients' needs and to

the community in times of increased resistance development.

So I'm acting as the Head of Clinical Development at Polyphor, and I'm consulting with the company on this program and on others.

The molecule that we are talking about is murepavadin, which is a first (inaudible) of novel class antibiotics that target specifically proteins at the outer membrane of Gram-negative pathogens. This specific mechanism of action allows to target specific pathogens and makes the mechanism of action specific to targeted therapies.

The molecule is highly effective against Pseudomonas, including resistant strains. It's a cyclopeptide, as you can see in the right corner there. It has very favorable pharmacokinetics, distributes well to the lung. Distribution is similar to the free plasma fraction. And it has a half-life elimination kinetics of 6 to 8 hours at an acceptable safety profile.

Now, the drug has been, of course, extensively studied preclinically. As you can see here, this slide

reflects the pathogen's specificity of the compound with high potency against the different Pseudomonas specimen.

Strains from all geographies of the world have been assessed. And as is shown here, murepavadin is active against Pseudomonas for non-resistant and resistant strains. It's a similar comparison. And that's also shown here. And the drug was investigated in a neutropenic mouse model, that you see that the drug was efficacious even in models where polymyxin was hardly active. There are further data that show that murepavadin can overcome colistin resistance that indicates the importance of this novel antibiotic.

The drug is eliminated, cleared, renally, and, hence, we adjust the dosing according to renal function. There is a very substantial PK/PD effort done for the development of this program, and all this modeling, integrating the clinical and preclinical data, we have indicate that at the proposed dosing regimen, we would cover 400 percent at the MIC of 0.25 and up to 80 to 90 percent at 0.5.

So this is just a quick screen of the profile

of the molecule, and should actually illustrate that you really have here a very potent anti-pseudomonal agent that is active also against resistant strains. Murepavadin has the potential to become a precision medicine. It is suited for guided therapy and stewardship as a tailored medicine for patients at risk of MDR infection.

And we also feel for the data that we have that the drug has a limited risk of resistance development of cross-resistance with other commonly used antibiotics. And obviously given the mechanism of action, it doesn't put pressure on the microbiome, and therefore has a lower risk of secondary infections.

The drug has been already studied in clinical studies. Eight studies have been conducted. There was one study in the target population that was a small study focused on PK and safety. Nevertheless, some early exploratory efficacy data were collected there. There was a low mortality, and that case was actually reinfection after discontinuation of the therapy. There were several cases of MDRs in that trial. And all of these cases resolved. Obviously, you cannot say

too much from such a small trial, and many of the reports are rather anecdotal. And the true question is, of course, how can we study this systematically to really prove the efficacy and the benefit-risk of this medication?

Here are some comments about the challenges now from a company perspective. I mean, it's in a way reiterating what has been said before by Professor Boucher and John Rex and Sumathi in the introduction. It is difficult to apply traditional drug development or clinical trial as scheduled to such a drug. Superiority trials are almost impossible.

And the conduct of the trials is limited, of course, by the availability of the patients, by the logistics to include these patients within a short time window and get informed consent from the relatives, as well as by co-medication that may affect the scientific evaluation and conclusiveness of these trials. So many factors have to be managed in order to get to a valid scientific experiment.

We have done some initial feasibility evaluations of the protocol under consideration, and

they go into the direction that they probably would need 10 centers to recruit one patient per month. So it is challenging.

The other challenge that we have faced is that the different regulatory agencies in the U.S. and Europe, they put emphasis on different aspects. So the guidance from the European partners was more about demonstrating efficacy in the resistant strains, whereas the FDA gave us a very clear message that actually what really would help the evaluation of the drug is to demonstrate efficacy against Pseudomonas as monotherapy. Obviously, both points are valid, and they would form together a comprehensive picture of the value and effectiveness of the drug.

Now, on the very good side is that we have really -- and I must really also thank the FDA colleagues for the very constructive discussions that we have because everybody tries to get their head around, how can we do this in a correct, scientifically correct, medically correct, way? And we reached conclusions with European and FDA regulators about how we could do this.

So basically the conclusion from several discussions we had with the agency was that we really would actually go down the avenue of this X-1 trial that John was alluding to. So basically murepavadin would be combined with ertapenem in the study arm, and compared to meropenem as an active. It would be a non-inferiority trial in the mITT populations and only in the patients that really have confirmed Pseudomonas. There needs to be a diagnostic test in the trial to identify these patients up front.

And the issue that John raised about the need for empiric dual coverage would be managed by allowing amikacin in both arms up to 72 hours with the caveat that the decision needs to be made before randomization.

So taking all of this together, I think it is challenging, but we concluded with this joint effort of the different parties -- clinicians, regulators, and us -- that it is challenging, but it can be done.

One open question was about the utility of ertapenem to be the joint partner for murepavadin. Obviously, ertapenem is not active against Pseudomonas.

That's why it makes it the right setup in this study design. It doesn't work against Acinetobacter. It happens, of course, fortunately, only in very few patients. The issue was, do we have enough coverage against Enterobacteriaceae?

And in collaboration with Paul Ambrose's groups, we modeled planned dosing regimen and came to the conclusion that it would be okay and suitable and medical justifiable to combine our drug with ertapenem. So that design would lead to conceptually to compare the efficacy of our medicine, murepavadin, as a monotherapy against Pseudomonas protected by ertapenem against other possible pathogens.

The study would run primarily at what we call a usual drug resistance center. These are centers which do not have high incidence of MDR for the simple reasons because actually if you end up with too many patients in double coverage, the interpretation would be difficult. So we would go primarily to those sites.

And obviously from that perspective, we will have only very few patients that are truly MDR or XDR. They must be studied in another way, which we are

discussing with the European colleagues. And we think that we are close to cracking the problem. We don't underestimate, we really don't underestimate, the logistic challenges to run such a study, but we feel that this is one of the most encouraging parts of the story, that the joint efforts of industry, clinicians, and regulators, that we can address this problem and develop this novel antibiotic that may be important for patients and physicians.

So thanks for your attention. And I think we'll discuss later about it. And sorry for my voice.

DR. NAMBIAR: Great. Thank you, Dr. Wallnofer, for sharing your experience with trying to develop this compound.

So our third speaker in this session is Dr. Robin Isaacs, from Entasis Therapeutics. Dr. Isaacs will be discussing their experience with developing a drug that targets Acinetobacter, Sulbactam ETX2514.

Dr. Isaacs has extensive experience as a pharmaceutical executive in the development and launch of vaccine and infectious disease products. During the 18 years he spent at Merck, Dr. Isaacs was involved

with the worldwide development, regulatory submissions, and approval of many anti-infective products, including vaccine products.

Thank you, Dr. Isaacs.

DR. ISAACS: Thank you. And thank you very much to the organizers of the meeting for inviting us to present. Entasis is a relatively new biotech company that was formed in 2015, and our lead product is Sulbactam ETX2514 for the treatment of Acinetobacter.

Before I proceed any further, I just wanted to put in one disclaimer, that the thoughts that are presented in the following slides represent the thoughts of Entasis, and represent the evolution of our thoughts and how we may develop Sulbactam ETX2514 for the treatment of Acinetobacter.

As Dr. Boucher has already pointed out, Acinetobacter baumannii is a significant unmet medical need, and that's reflected in the new WHO update to the list of pathogens. It's one of the six ESKAPE pathogens. There are approximately 60- to 100,000 infections in the U.S., and approximately 130,000

infections in the EU5 per year based on Decision Resources data. And commonly, Acinetobacter infects the bloodstream, lung, urinary tract, and skin.

There is a significant difference between Acinetobacter and Pseudomonas on two parts. One is the extraordinarily high level of mortality associated with Acinetobacter in critically ill patients. In treated patients, currently the mortality rate is approximately 40 percent, and in untreated patients, it's approximately 80 percent.

The second is that approximately 60 percent of the Acinetobacter isolates in the U.S. -- and in some countries in the world, greater than 90 percent of the isolates -- are multidrug-resistant. So the issue with Acinetobacter is it's a relatively limited pathogen in terms of number of cases, but the majority of the cases represent multidrug-resistant cases.

And the data that I'm showing here on the bottom is just to give you some notion on a worldwide basis of imipenem as an example of a broad-spectrum highly effective beta-lactam and resistance amongst Acinetobacter where you can see that on a worldwide

basis, approximately 64 percent of strains are resistant.

Sulbactam ETX2514 is in clinical development as a pathogen-specific drug to treat Acinetobacter baumannii. As Dr. Rex pointed out, it's difficult to get broad-spectrum agents which cover everything you want them to cover, and Acinetobacter and Pseudomonas are agents of infection which have proved particularly difficult to cover with broad-spectrum drugs.

Sulbactam will be familiar to many in the audience. It's a beta-lactam that's widely used as a beta-lactamase inhibitor in the combination product Unasyn. But as a beta-lactam, it also has intrinsic activity, and one of its most remarkable features of its intrinsic activity is activity against Acinetobacter baumannii.

ETX2514 is a novel non-beta-lactam, beta-lactamase inhibitor, and its unique feature relative to other available beta-lactamase inhibitors is its broad coverage of Class D beta-lactamases in conjunction with very broad coverage against Class A and C. And data from looking at panels of contemporary Acinetobacter

strains indicate that if you're going to resurrect a beta-lactam against Acinetobacter, by covering beta-lactamases, you need to be able to cover Class D as well as Class A and C.

So, for example, in a large panel that we published last year, almost all of the Acinetobacter had Class D expression, but only 12 percent of those expressed only Class D beta-lactamases. The other 88 percent expressed Class D along with either A and/or C, and in most cases, more than one example of each of the beta-lactamases in each class at the same time.

ETX2514 is very potent in vitro and in vivo and restores the activity of Sulbactam against contemporary multidrug-resistant Acinetobacter. Sulbactam alone in our panels had an MIC90 of 64. When you add ETX2514 at 4 mcg/ml in the wells, the MIC90 came to 4 mg/L, and that is the breakpoint for Acinetobacter in the Unasyn combination. And greater than 99 percent of over 1,000 isolates from 2014 had an MIC less than or equal to 4 mg/L.

So the preclinical profile is very strongly suggestive of efficacy. The question is, how are we

going to get it to market and prove that in a way that satisfies the requirements of the regulatory environment?

So what are the challenges? The challenges are that you have to be able to identify patients with Acinetobacter baumannii infection. They represent approximately 2 percent of hospitalized Gram-negative infections on a worldwide basis in the U.S. and the European countries. The patients are sick. They're usually hospitalized. They generally have compromised health. They're often in intensive care units. They generally receive broad-spectrum coverage. And patients may have renal impairment, leading to the need to be able to have renal dosage estimate early in clinical development.

About half the patients will have pulmonary infections either with or without bloodstream infection. And when we look at this, what you're basically looking at is a very sick group of patients in intensive care settings where, as has been pointed out this morning, it's difficult to run clinical studies.

So how do we translate this into a development program? Clearly, identification of patients with Acinetobacter baumannii infections is important. How do we enrich for that? Well, in the case of Acinetobacter baumannii, I'll remind you what I pointed out earlier, multidrug resistance is actually the norm rather than the rarity.

The target of a new therapy is to meet the unmet medical need, in this case, multidrug-resistant pathogens. And although Acinetobacter baumannii infections are relatively uncommon, multidrug resistance is very common. And so if we can use routine microbiology to identify Acinetobacter baumannii within 48 hours, we can enrich for multidrug resistance by allowing up to 48 hours of prior therapy to enroll patients in the studies.

Enrollment can be done prior to knowledge of the resistance pattern of the Acinetobacter because approximately 60 percent on average of what you enroll will be multidrug resistant.

To reinforce points that may have been made previously also, rapid bedside diagnostic would help

enrichment, but it's not going to help increase the incidence of disease actually in the community. So it's really a way to try and enrich for populations while minimizing prior antimicrobial therapy. It would be helpful, but isn't essential. There are a number of companies, as you all know, that are working in the rapid diagnostic space, and we continue to monitor this as a potential way to assist in the clinical development program.

The other element of identification, a key element of identification, is where to find the patients. And there are some hot spots for Acinetobacter in the world. In the U.S. and the EU, it's in intensive care units, where they account for somewhere between 5 to 10 percent of ventilator- and hospital-acquired pneumonias.

But on a much more broader geography basis, and as this slide shows, which is taken from a review by Chung et al., you can see that in countries such as Thailand and Taiwan and other countries in Asia, that actually Acinetobacter is either the number one or the number two pathogen in hospital-acquired and

ventilator-acquired pneumonias.

So geographic siting of the clinical studies becomes a very important factor in identifying patients with Acinetobacter.

The last point I wanted to make before I sort of get to the general plan that we think is achievable and workable is to discuss the fact and to reinforce the fact that we're enrolling patients who are sick with significant co-morbidities. It's important to understand pulmonary penetration, because pneumonia is such a common feature, and renal dosage estimate early in the development strategy so that when you start Phase 3, you can enroll all of these sick patients without having to restrict enrollment because you don't have a renal dosage estimate, for example.

There needs to be substantial and substantive preclinical efficacy data prior to the clinical studies to establish PK targets likely predictive of efficacy and to establish clinical dose using robust modeling of Phase 1 pharmacokinetic and preclinical pharmacodynamic targets. This is to reinforce points that have already been made by each of the speakers this morning.

While establishing Phase 3 readiness, it's important, I believe, to generate a limited amount of safety data in relatively healthy patients so that you have a baseline to review safety data in the much sicker population that you will see in the Phase 3 program.

So then how do we establish efficacy? And I'm just going to walk through this slide because it really is a sequenced discussion of what the Phase 3 program would look like. In taking advantage of preexisting guidance and the unmet medical need, an event-driven study based on multidrug-resistant pathogens, enrolling patients with proven Acinetobacter baumannii infections, and focusing on common infections, that is, lung and/or bloodstream, and a non-inferiority comparison against a standard of care regimen, and utilizing a hard endpoint such as 28-day mortality, would require, if you make reasonable assumptions as to how many patients you can enroll and are prepared to take a certain level of statistical risk, about 200 patients in a 1:1 randomization, so 100 patients in each arm, to provide approximately 118 to 120 patients

with multidrug-resistant infections.

This study would be set up as an event-driven study. And what do I mean by that? You would enroll all-comers with Acinetobacter, but the primary efficacy endpoint would be analyzed based on multidrug-resistant isolates, so essentially you would be counting the number of patients with multidrug-resistant isolates, and you would trigger the final endpoint analysis when sufficient had been enrolled.

The study would have 80 percent power. It would assume a mild degree of superiority of 40 percent mortality in the comparator group, similar to what Achaogen showed, and that data was shown earlier by Dr. Rex, and a small advantage of 35 percent mortality in the experimental group.

The other key thing that I feel is -- and this goes to a point that Dr. Boucher made -- you need to get data which really helps physicians understand how the drug works, and although the primary efficacy endpoint would focus on lung and/or bloodstream infections, patients who aren't eligible for that comparative arm, so patients with other infection

sites, could be enrolled into an open-label arm where they all get therapy with Sulbactam ETX2514, and that data would then become supportive and would provide efficacy and safety data in a population beyond the primary efficacy population.

So then what might an NDA package look like built around that single Phase 3 study of approximately 200 patients in a comparative arm? And what other supportive data could be generated from the subjects who aren't enrollable in that comparative arm? Clearly, a strong microbiology package; strong evidence of in vivo efficacy in relevant animal models; and robust demonstration of PK/PD parameters based on in vitro hollow fiber and in vivo animal models.

We don't have the ability really to dose range in a conventional manner with this kind of study population, so the dose for Phase 2 and Phase 3 would need to be based on high probability of target attainment using robust modeling of preclinical and clinical data, similar to the approach that was mentioned a few minutes ago.

The safety database, assuming that the drug is

generally well tolerated, of approximately 300 to 400 patients consistent with published FDA guidance documents and demonstration of efficacy compared to standard of care in a Phase 3 non-inferiority study with a step-down to superiority once non-inferiority has been proven.

Finally, you clearly need to be able to justify the non-inferiority margin based on comprehensive review of the available data. And as has been pointed out by Dr. Rex and others, that's challenging, but there is actually quite a lot of data out there that one can work through to establish a non-inferiority margin.

The last point that I want to leave you with is that this is not easy, it's complicated, but we do believe that as you focus down and drill down to the kind of 200-patient study size, this is something which is achievable over an 18- to 24-month time period. It's important to focus on a relatively short time period because if the study needs to run too long, then you start to end up in the situation of changing resistance patterns, changing background therapy, and

an increased reluctance to enroll subjects in the study. So it's important to get a study size which is enrollable in a feasible amount of time, and we believe that that's possible with the kind of approach that we have suggested here.

Thank you so much for your time.

DR. NAMBIAR: Thank you, Dr. Isaacs.

What we would do is take 5 minutes to see if there are any clarifying questions that people have to ask of the presenters before we move to the next session. I think we're running just on time, so if there aren't any major concerns or questions that need to be addressed right away, we can do that in the afternoon session.

(No response.)

Lessons Learned and Considerations for Animal Model Development

DR. NAMBIAR: All right. So seeing none, we can go into our next topic, which is on "Lessons Learned and Considerations for Animal Model Development." We have four speakers in this session.

Our first speaker is Dr. Judith Hewitt, who is

the Chief of the Research Resources Section in the Office of Biodefense Research Resources and Translation Research in the Division of Microbiology and Infectious Diseases at NIAID.

Dr. Hewitt's group is responsible for several division-wide resources that provide research agents, animal models, and screening services to facilitate the development of vaccines and therapeutics or diagnostics for infectious disease other than AIDS.

Dr. Hewitt.

Lessons Learned from the Development of Animal Models of Inhalational Anthrax, Pneumonic Plague, and Tularemia

DR. HEWITT: Thank you. I am going to tell you today about an animal model of pneumonic plague. I want to tell you first of all that this is one of the lowest hanging fruits in terms of things that have been accomplished under the Animal Rule and hopefully will convince you that even though it was low hanging fruit, it was still not simple or fast.

Here is my disclaimer. Very boring Federal employee.

So I'm going to give you the bottom line up front about what we were able to accomplish this. I will later get into some individual animal data. Then I'll do some study-to-study comparisons to convince you of reproducibility of the model and how we compared this animal model to human disease, and with some recommendations, lessons learned.

This African green monkey model of pneumonic plague is rapidly fatal. So I have two fluoroquinolones plotted here on this same graph. And so the control groups die quickly, in a matter of 3 to 5 days, where the treated animals -- and the treatment is indicated by the bar across the top -- is very effective. There was one animal in each of these studies that died, one due to a catheter failure, so it was not adequately dosed, the other one was sacrificed because it had non-plague-related complications.

This timeline slide gives you a sense of both the time that it took to conduct the studies as well as get through the regulatory process with the Animal Rule.

So early on -- and I want to draw your

attention to the scale of this timeline starting in 2002 and ending in 2016. So right after the anthrax letters, an NIH/FDA working group got together, decided on a number of already approved antibiotics that could be tested in a model. The blue boxes are about the funding stream. NIAID then opened up pre-IND file with FDA in 2003 and began the work.

So the first thing we did was all the yellow boxes along the bottom of this timeline show the actual animal study work. The first thing we did was a natural history study where we looked at the disease in these African green monkeys. We did a number of PK studies for the various drugs that we tested. And the ciprofloxacin efficacy study was conducted around 2005.

We then did a number of additional natural history studies in the 2007 to '08 timeframe because we wanted to be able to test additional drugs at different sites. The levofloxacin efficacy study was conducted in 2009.

Our initial guidance from FDA was that it was easiest to work with a drug sponsor. We had been working with Johnson & Johnson on levofloxacin all

along. That drug was still under patent at that time, and so we went with them to their pre-sNDA meeting where FDA communicated their desires for what should be included in the sNDA package. One of those things was an additional PK study. I'm going to come back to emphasis on PK later. So that's the last of the levofloxacin PK studies there. Really, it was a bridging study that addressed some concerns lacking in the earlier studies.

So during these discussions then FDA came up with a pathway for NIAID to submit generic drugs. So we quickly put together the ciprofloxacin package in a pre-IND, and both of these packages were submitted to FDA around the same time, went to advisory committee thereafter, and the levofloxacin sNDA was approved within a month then. The cipro sNDA was approved somewhat later due to the actual NDA holder, which was not NIAID, putting together their package and updating their label.

The other thing I want to point out here is I also indicated moxifloxacin here as well. That efficacy study was done in 2013, and that approval came

in 2015. So clearly, with all of the discussions that we had on this animal model, we laid the pathway for subsequent drugs to have a quicker route to approval.

So what did the sNDA submission look like? 400 megabytes and 250 files may not be very large in typical drug development, but this is an Animal Rule submission, and there is really no clinical trial data in here. As Sumathi mentioned earlier, these are approved drugs with a lot of clinical use. We didn't have to do any new safety data. So 79 percent of the submission went into Module 4. We have 18 reports that are listed here along with references, datasets, case report forms.

Then the important thing that we also submitted was a white paper into the clinical module, and this is where we described clinical disease and compared clinical disease to animal disease, and I'll come back to that.

So this is a summary of the four natural history studies that we did in the African green monkey model. The other thing that I need to point out is that we struck gold with this very first model. We

didn't need to pursue a number of different animal models. So what you can see here are the challenge doses that we gave all of these animals and the mean time to death and mortality across these studies. In this slide, I want to point that in the very first study we had two animals that survived the challenge, but as you can see in the last column, those two animals were neither bacteremic nor febrile. So what we were able to establish here is a very good correlation between bacteremia and fever as signs of disease and correlating very well with mortality.

So here are some individual animal data from that first natural history study. We monitored bacteremia daily. We were limited in terms of the number of blood draws that we can take from these animals. So all of the animals that got sick were bacteremic by 72 hours, and you can see the two surviving animals that survived got lower challenge doses and were never bacteremic during the course of this study.

These animals also had telemetry implants, so we were able to monitor a number of physiological

parameters in real time. So in this slide, I'm showing you body temperature for two animals. The red upper line is one of the animals that succumbed to disease, and the blue lower line was one of the survivors that got the lower challenge dose. And we also have baseline data in addition to the data I'm showing you here, which is just post-challenge.

And you can see a nice diurnal pattern in temperature early after challenge, and that's disrupted around 72 hours, which is the typical time that we see fever in animals that get sick. There is some noise in temperature here with a survivor, but that animal returns to a typical diurnal pattern and survives.

This next slide shows you heart rate in those same two animals. And what you can see here is there is not quite the distinction between the survivor and the non-survivor as there was in the temperature data.

We also measured respiratory rate in this study visually. And what you can see here is that the two animals that survived had a normal respiratory rate throughout the study. But beginning sometime after 72 hours, really more in the 96-hour timeframe, the

animals that died of pneumonic plague had rapid and dramatic increases in respiratory rate. Each of the animals was anesthetized when they were showing signs of clinical disease, around 80 or 83 hours. And so we were able to take a chest radiograph at that time.

So this is the series from one of the animals. So zero hour was at the time of challenge. An independent blinded radiologist reviewed these radiographs later, rated this one as normal. Then at the 83-hour time point, the rating here was mild disease. And then the last radiograph was taken just prior to euthanasia of this particular animal, and the independent radiologist rated this one as severe disease.

So this slide puts together all of the endpoints that we measured along with except for the chest radiographs, this is the same animal. And so you can get a sort of comprehensive picture of what's going on. Around 72 hours or 76 hours, animals develop a fever, and they're also bacteremic at this time point. It takes more than 24 hours, typically 48 hours, to culture the bacteria on a plate, so we really use the

telemetry indication of fever to give us a trigger to treat in subsequent drug studies.

So now I'm going to show you comparisons across the four natural history studies. So here is the Kaplan-Meier survival across those studies showing consistent time of death, typically in this 3- to 5-day time range, where the highest challenge dose studies resulted in death somewhat earlier than the later challenge dose studies. And you can see the two survivors here.

When we look at bacteremia across all of the studies, we start to see some bacteremia on day 2. The study -- the light blue bar at the far right there is the highest challenge dose study, and in that case, all of the animals were bacteremic on day 2, but the vast majority of animals are bacteremic on day 3.

This graphic shows you the impact of challenge dose on time to death. So you see something of a relationship in that higher challenge doses lead to a shorter time to death in this model. And that's depicted again here in this slide, where we're showing mean times by studies, so the left edge of each of

these bars is the mean time to fever in a study. The right-hand edge of the bar is the mean time to death. And so what we're showing here is the therapeutic window in this model, which ranges from 30 to 40 hours.

We also had a pathologist review all of the slides from all of these natural history studies. I'm just showing you the lung pathology here. What you can see is that in the 34 animals that were diseased, the vast majority of them showed bacteria, edema, hemorrhage, and inflammatory infiltrates in the lung.

So, again, all of this data taken together summarized the clinical course of disease in the animal models. I summarized this while a clinical colleague went to the literature to find descriptions of human disease because there really aren't that many cases of pneumonic plague in these times.

But there was some information on outbreaks in the modern era. There were significant outbreaks in China in the 1800s, and a lot of those outbreaks were transmitted through shipping routes, and we had some outbreaks here in this country in the early '20s, and there were excellent clinical descriptions at that

time. And I'm showing you here one of the references that we used in our clinical description. This publication from 1926 had very good descriptions of human pneumonic disease. I need to point out that most cases of infection with plague result in bubonic disease, which is a less severe form.

So my clinical colleague used these descriptions to summarize human disease. We treated our drafts and were both astounded at what we were reading and how it basically mirrored what we had just written for the other species.

So here is a disease comparison of human disease and African green monkeys. The time course is the same, temperature is elevated in all cases. Yersinia pestis is present. In the human cases, it was mostly examined microscopically from sputum, whereas in our animal studies, we were culturing from blood or lung and nasal fluid. Heart and respiratory rate were elevated in all of the cases. Pulmonary infiltrates are seen in both models. And the lung pathology is very similar.

So now to get into bridging from animals to

human in order to test these drugs. What's imperative is that you can use human PK information to determine a dose to give animals that gives you a similar PK profile so then when you test animal efficacy, you can be assured that you are not exceeding the drug profile that you would expect to see in humans. And then, of course, you are trying to convince the FDA that this translates into human efficacy, the question marks here reflecting that we may never really know how effective these drugs are in humans.

So what did we learn throughout this decade-long program? We need to talk to FDA early about the selection of an animal model and the correlation to human disease. We were in constant communication with FDA about this. The correlation to human disease in this particular case we wrote up closer to the end of the program. FDA would like to see that earlier.

You need to standardize methods and reagents as early as possible. We did this to a large extent. Quality in how the animal studies are conducted is extremely important. And the pharmacokinetics are also extremely important. If there was any weaknesses in

any of our studies, it really came in this pharmacokinetic bridge. And there are some suggestions here for how you should pay attention to that.

And my last point here is that pivotal Animal Rule studies replace Phase 3 clinical trials. And so it's really important to understand that the expectation around these animal studies on FDA's part are really the same as how they would treat Phase 3 clinical trials. Blinded, randomized, case report forms on individual subjects, validated assays: all of that is really important as you're thinking about these Animal Rule studies.

So this slide really recapitulates many of the same points, but it's a little more forward looking. If you were beginning a program, how would you go about this?

So first you have to understand the human disease that you're trying to model, including the route of infection. As you develop that animal model, it's important, as a community, to identify the relevant strain that you want to use, characterize that strain well, and use master and working banks of those

strains.

You need to select one or more species that are going to be most relevant to humans. In our case, we were very fortunate that we hit that with the very first species we worked on.

You need to collect data to identify relevant endpoints. I showed you some of our data on fever and bacteremia.

You need to establish the reproducibility of your model. A model isn't a model if it only works in one laboratory.

Ideally, you would have proof of concept for intervention and be able to establish a trigger for treatment.

I can't emphasize the selection of an effective dose. Again, this is where PK becomes extremely important, and the quality systems to support efficacy testing of these drugs under GLP is also extremely important.

My colleagues wanted to emphasize some of the timelines around conducting these kinds of studies, so we came up with a generic timeline for GLP challenge

efficacy study. There are ranges here for different models. And you can see that it takes anywhere from 6 to 24 months to conduct one of these studies.

Clearly, the complexity increases as you are going up in species. You can have savings of time if you can overlap some of these different phases of the studies, or obviously if a study is more complicated, that's going to lead to increased times to get your final study report.

And with that, I will stop and thank you for your attention.

DR. NAMBIAR: Thank you, Dr. Hewitt.

Our next presenter is Dr. Meister, from the Battelle Biomedical Research Center. Dr. Meister has more than 15 years of research experience in viral and bacterial pathogenesis and in supporting medical countermeasure development. Dr. Meister currently serves as a principal investigator on multiple U.S. Government-funded programs that are focused on developing in vivo models of infectious diseases and in assessing efficacy of candidate treatments against biodefense pathogens.

Dr. Meister.

DR. MEISTER: Thank you very much. Thanks to the organizers for allowing me to be here today. It is an honor to talk about some of the work that I was fortunate enough to participate in over the past decade or so.

I'll start with my disclosure statement. I do work for Battelle. We are a nonprofit organization, and we do provide contract research services to numerous government and commercial entities. I don't have any personal financial conflicts of interest. I have no financial relationships with any of the companies we do work for or investments in any of products that we test.

And that kind of leads me into kind of another caveat. I'm going to be talking about Bacillus anthrasis in inhalational anthrax. And a disease, from a bioterrorism perspective, was pretty well studied, leading up to the development efforts of a therapeutic animal model. And so I will say we -- and when I say "we," the folks, to include U.S. Government, sponsors, the regulatory agency, the CROs, like Battelle,

performing the work in the BSL-3 laboratories, and the product sponsors developing the products -- had a pretty good foundation of information regarding the pathology of the disease, some of the animal models that were available. And so really I'm going to talk today about how we kind of refined and tried to optimize what we had at the time to suit the needs of the product sponsors, the regulatory community, and U.S. Government in building stockpile needs.

That being said, we're all well aware of what happened in 2001, and this initiated obviously an onslaught and a lot of research interest in understanding what we had available to combat Bacillus anthrasis and inhalational anthrax considering, despite the best medical care, about 50 percent mortality rate following Amerithrax attacks.

There was obviously an unmet medical need. There were some programs evaluating vaccines, so some antimicrobials. And at the time, antibody passive protection of monoclonal antibodies or immune globulin therapies -- and I make a distinction here when I talk about treatment indication.

I believe in 2002, 2003, 2004 timeframe, there was I think an emphasis put upon vaccination in the sense that if we were to immunize, we could probably protect the general population with an effective vaccine, but I think that also became very clear that that was going to be very costly, very time-consuming, and there was a lot of pressure I think to prepare us for the, quote, next attack if there were to be one.

And so there was a renewed emphasis put on optimized treatments, therapeutic treatments specifically. And so when moving into that area, we were fortunate in understanding kind of the mechanisms of pathology, for those of you not familiar with Bacillus anthrasis and what the toxin does. And I will caveat my talk today, I am going to be focusing on the work that was done for the monoclonal antibody that was developed, the monoclonal antibodies that were developed, for the treatment of inhalational anthrax. It was predominantly based on the toxin.

And so we heard earlier about the Animal Rule and having to understand the pathogenicity of the disease, having to understand clearly how your product

ameliorates that disease process. You've got to be able to demonstrate the effectiveness of your product in multiple animal models that would reliably predict an outcome in humans. You have to have a very well-defined endpoint. And then you have to link kind of your PK -- you have to have a firm understanding of what your pharmacokinetic profile is so that you can bridge what you're observing in animals from an efficacy perspective to what you're seeing in humans from a PK perspective.

And so we knew that inhalational anthrax was a toxin-mediated disease to some degree, and there was some initial work suggesting that utilizing an antitoxin shortly after infection or exposure to Bacillus anthrasis via the inhalational route of exposure could be efficacious, it could actually improve survival rates quite substantially.

I think some of that early work was questioned because it was early intervention. We saw a case in 2001 where I think the data strongly suggested the longer you wait, the less effective the current standard of care was. And so I think there was an

emphasis put on developing an animal model in which we could begin to understand treatment after diagnosis, what the effect of these products would be. And so this mechanism of pathology and knowing this helped us when working with the monoclonal antibodies.

But as we began to think about modeling therapeutic treatment, there were I'll call a few requirements that were asked of us. We have to define the disease at this point in time, early 2000s. I think there were a few publications out in the animal models clearly articulating the pathology, the terminal pathology, observed, and I think there were very strong links between the bacteria, the toxin, and those pathological observations, and many of those were very consistent with the limited clinical cases that we had.

But there was a gap. There was I think a lack of understanding of what occurs at the time of exposure to the time of, quote, presentation, if you will. And so some of the work that I'll talk about today is what we did to define the disease progression, at least in New Zealand white rabbit.

And in addition to that, at the time of this

model development initiation, there were two ways in which we thought about potentially diagnosing the disease, a chest x-ray in a rabbit and/or a positive blood culture. In a truncated disease such as 3 to 4 days until animals succumb to disease, waiting 16 to 24 hours for a positive blood culture could severely hamper the perceived effectiveness of a product. And so there was a lot of work put into developing a rapid diagnostic that could be used somewhat as a surrogate for a blood culture, and I'll talk a little bit about that later on.

And then I think one of the things that we heard clearly from a CRO perspective from both the product sponsors, the government sponsors, as well as the regulatory world, was we want to mimic a clinical scenario.

I think the Animal Rule strongly suggested that you need a model that mimicked what was observed in human cases, and that included clinical presentation. Were you able to diagnose these subjects as well as appropriate timing of medical countermeasure intervention? Was that going to mimic what was likely

to occur upon presentation? And I will caveat all of this by saying that the understanding of the clinical scenario was really based on a very limited clinical database.

So I'll move to some of the work that we did. The details of this particular graph and the lines I don't think are critically important. What I want to impress upon you is that we collected as much data from the New Zealand white rabbits as we possibly could. The top left graph represents body temperatures that were collected on an hourly basis.

We looked at CBCs, to include total white blood cell counts and the neutrophil-lymphocyte ratio in the top middle and top right graphs.

CRP as a nonspecific indicator of inflammation was assessed, bottom left panel.

We did cage-side observations every hour beginning roughly 12 hours post-exposure.

And then bottom right, we collaborated at the time very initial stages of the animal model development program with the CDC to evaluate circulating LF levels.

And we looked at this data and we said, wow, that's very intriguing, we see these changes, we see this increase in body temperature, we see this phenomenon which we observed was a decrease in total white blood cell count, increase in CRP and NL ratio, fairly consistent albeit individualized onset of clinical observations. And this hallmark increased plateau, and then secondary increase in circulating LF levels.

And in looking at each parameter independently, it was kind of a neat finding to observe, but when we pulled all of them together, we defined the disease progression.

And so to orient you with this particular figure, I've taken all of those parameters and I've added to that a positive bacteremia culture on the top axis to illustrate in black the body temperature profile.

So the body temperatures increase at roughly 24 hours post-exposure. They seem to correlate very, very well with a positive bacteremia culture, which I will admit is actually confirmed retrospectively

because we do have to wait roughly 24 hours for a positive blood culture.

And both of those correspond with an increase in LF, which is illustrated here in pink. And many of those fall in line with changes that are -- other clinical changes to include CBC serum chemistry changes that were also alluded to in 2001, or at least observed in 2001, alluded to as maybe predictors or at least correlates of inhalational anthrax infection.

And so what we did is we took the data from the exposed animals and we compared them to unchallenged New Zealand white rabbits, and there was a distinct clinical profile. And now we wanted to be able to use that clinical profile, diagnose subjects on an individual basis, and then intervene with a medical countermeasure.

And so those were our next steps, demonstrate that that anthrax antitoxin treatment following confirmation of disease was effective, and then evaluate the PK of an antitoxin in the context of the disease because to that date, the PK of the product was observed in healthy human individuals as well as naive

New Zealand white rabbits.

And then some of the constraints, traditional diagnosis of inhalational anthrax was impractical, and so we did spend a lot of time with NIAID and some of the product sponsors developing a surrogate diagnostic assay that actually detected circulating PA that correlated extremely well with positive blood cultures.

And then I will say that in any animal model, there are limitations in data collection, and so we had to work very closely, depending upon the objective of the studies, to prioritize the data and the data type as necessary.

And so this is just a Kaplan-Meier curve representing the results that we observed when we tested raxibacumab in New Zealand white rabbits as part of the pivotal monotherapy study. The green line and the red line represent survival in the two dose groups, active treatment arms. The black line represents survival observed in the placebo-treated controls.

And so we did not do this data, but I put this up here to try to illustrate how the product sponsor looked at the pharmacokinetic profile of the monoclonal

antibody to orient you with this. The red lines represent the pharmacokinetics observed. And then the orange and the green line represent the actual terminal levels of PA or toxin in circulation that were observed in the studies that were conducted leading up to the efficacy study, trying to demonstrate that there was coverage of the monoclonal antibody over the toxin throughout the course of the disease.

This data was taken to the FDA during an Anti-Infective Advisory Committee meeting. And when discussed and kind of looked at in detail, there were actually two additional observations made, the first of which was when you looked at inflammation or the inflammatory response in the meninges, in treated animals that succumb to disease, we actually saw an increased observation of inflammation compared to the placebo-treated animals that succumb to disease, potentially suggestive that there is something going on that needed to be further investigated.

And in addition to that, many of I think the Advisory Committee panelists wanted to see more information regarding the clinical scenario, and the

clinical scenario being that a monoclonal antibody was not likely to ever be administered by itself, but actually in combination with antibiotics.

Studies had been run to evaluate that, but those intervention times were actually optimized at the time of diagnosis, and you can see from the bottom right graph that levofloxacin alone compared to survival with raxibacumab plus levofloxacin were actually the same.

And so there was no really data available at the time to suggest or support the fact that a monoclonal antibody when given in combination with antibiotics would be potentially additive or superior or provide an improvement over antibiotics alone. And so that started a whole new quest for information and refinements to the model as they were currently used. And the goal was to demonstrate that the antitoxin added benefit to antimicrobial treatment alone.

There were some constraints or some requests. A marginal outcome following treatment with antibiotic alone was a request based on the 2001 experience. We were asked to use a humanized dosing of antibiotic.

And then the third pillar of that stool being statistical significance observed or a statistically significant improvement in survival observed in the combination treatment versus antibiotic treatment alone. And I will say that moving into this or going into this, we felt strongly these were going to be high hurdles to overcome.

And so when we thought about the designs of the study, we took into account three things: antibiotic dose, antibiotic duration, and treatment intervention time.

And so looking at this scale, if we're looking at the New Zealand white rabbits, the top line represents when we might administer antibiotics. The second layer is the dose that you're going to provide. And then the third layer is how long you're going to provide it. And I think in the first few studies that were conducted looking at this potential added benefit of antitoxins, PEP, or post-exposure prophylactic, dosing was administered. So antibiotics were given actually early, 7 to 12 to 18 hours post-exposure; an optimal dose, and when I say "optimal dose," it was a

dose that was I think close to a pharmacokinetic profile that you would see in humans; and then a short course of antibiotics to mimic kind of patient compliance.

When we did this, we realized that we weren't given the antibiotics in a therapeutic setting. The dose was close to humanized. Outcome, survival outcome, observed after cessation suggested that animals that succumb to disease were likely succumbing to disease because of a lack of antibiotic coverage in a later portion of the disease progression. But we did observe added benefit. Unfortunately, this did not meet the three requests of the model.

And so we looked at therapeutic dosing, and that would be dosing upon diagnosis. We kept the dose and the duration. The dose was close to humanized. Antibiotic efficacy was near 100 percent, and, therefore there was no margin of error or margin of difference that we could observe, therefore, no added benefit could be concluded.

And there was, I guess, a potential for assessing antagonism, which I think was a discussion

held at some level.

And then, lastly, we tested delayed treatment. And so we gave a dose that was close to humanized. We looked at the efficacy of the antibiotic, and if we delayed dosing long enough, we could get near 50 percent survival in the antibiotic treatment alone.

And then we actually did observe an increase or an improvement in survival empirically. I will note the P factor, or you will note the P value, is not near 0.5, as we had hoped to achieve, but we did observe an increase in survival in combination treatment versus antimicrobial treatment alone.

So with that, just to summarize, we were -- and when I say "we," all of us in the MCM community that were working on this particular problem -- did have historical data that was the foundation of optimizing the models that we eventually used to assess efficacy.

The indication of the product dictated the development pathway. We knew how the products were going to -- or we thought we knew how the products were going to be utilized, and we tried to develop animal

models that would mimic that. Many iterations were required before we got to the final model.

I can't overemphasize enough that a quality management system was critical to the successful regulatory review of these studies. We put a lot of time and effort in documentation practices, verification, accuracy, data integrity, and I can tell you sitting across the room from FDA inspectors, having to run GLP efficacy studies, not just safety tox studies, is a very stressful situation, but in the end I think the data stood for itself.

And then, lastly, this was a collaborative effort. This wasn't anything that was done by a single entity or organization. It was done by many, many people, not only at Battelle, but across the MCMI. So to that end, I just want to acknowledge the Battelle team, the U.S. Government agencies, all of which that participated, and obviously the product sponsors.

Thank you.

DR. NAMBIAR: Thank you, Dr. Meister.

So our next speaker is Dr. Julie Hutt, who is a veterinary pathologist with over 15 years of

experience developing small and large animal models for infectious diseases. Dr. Hutt is currently at the Lovelace Respiratory Research Institute, and she has been there since 2002.

Approaches and Important Considerations in Animal Model Development for Bacterial Infections

DR. HUTT: Thank you. And, again, thank you to the organizers for inviting me. Today I'm going talk about the development of a non-human primate model of inhalational tularemia. We developed this animal model, non-human primate model, of inhalational tularemia. The work was performed over approximately a 4- to 5-year period, and it was funded by NIAID.

I have a quick disclosure statement. Like Gabe, I work at an entity that performs regulated research for commercial and government sponsors, but I have no financial conflicts of interest myself.

So an overview of tularemia in humans at least. It's caused by the species Francisella tularensis, subspecies tularensis, the type A strains. The clinical presentation in humans generally takes one of six forms, with the glandular and ulceroglandular

form being the most common, and the pneumonic form being generally the most deadly.

I should also mention here that there are currently no new drugs or vaccines under regulatory review, so my focus today will be on the animal model development process and qualification process. I wanted to mention that once the model has been qualified, it will be available for use by the general scientific community for developing and submitting new vaccines and therapeutics under the Animal Rule.

So for our studies, similar to anthrax, we were fortunate to have a pretty substantial historical database from work that was done in the '40s through the '60s as part of the bioweapons program. We selected our strain as the highly virulent SCHU S4 strain. And we selected the cynomolgus macaque as our primate species.

As I said, there was abundant historical data on primarily using rhesus and cynos back from the '50s and '60s. At the time that we started these, the cynos were much more readily available for the studies than rhesus macaques were. And they're also a very robust

species to use in the laboratory.

We sourced our animals from mainland Southeast Asia. We specifically aborted the animal source from the island of Mauritius because these animals have been geographically isolated for many years, and they have developed some unique and distinct immunologic characteristics that aren't necessarily representative of the species. All monkeys were prescreened for pre-existing humoral and cell-mediated immunity prior to initiation of the studies.

Our approach, as I said, based upon the abundant historical literature, was first to establish an LD50 in cynos after head-only inhalation exposure to aerosolized SCHU S4 and then to follow that up with a natural history study in which we had one arm of the study in which the animals had surgical telemeters surgically implanted to measure heart rate, respiratory rate, and core body temperature. And then we had a second series of studies that we did where we euthanized animals at predetermined time points for blood collections for hematology, clinical chemistry, and quantitative microbiology.

Page 114

So for our LD50 study, we used 28 male and female cynos. They were approximately 2 years of age, 2 to 3 years of age. Our presented doses ranged from 1

6

to up to 10 CFUs with particle size of 1 to 3 microns. Death occurred on these studies between day 3 and day 46 post-challenge. And our calculated LD50 was less than 10 colony-forming units.

What we discovered from the LD50 studies was that the clinical presentation, the disease course, time to fever and death were highly dependent upon the challenge dose, not surprisingly. Animals that were

5 6

challenged with the very highest dose ranges, 10, 10 CFUs, generally died within 3 days. Their clinical signs were dominated by the clinical signs related to the respiratory tract, and they had a severe bronchopneumonia at death.

The animals exposed to the lowest clinical doses, on the other hand, less than 100 CFUs generally, they animals could survive up to 6 weeks. Death was primarily attributed to the disseminated disease in the liver and spleen. And the clinical signs were primarily related to anorexia, weight loss, and just a

generalized malaise.

All animals had pleuritis. All animals had pyogranulomatous to necrotizing lesions in the lungs and other organs after dissemination. The lesions varied in chronicity depending upon how quickly the animal died after exposure. Overall, the lesions were similar to what has been reported in the literature for the human disease.

So this example shows some of the great lung lesions that we got, and shows some of the extremes of the disease. On the left, the animal shown here has a very multifocal pneumonia. This animal was exposed to 30 colony-forming units and survived for 10 days. As I said, the lesions were fairly well distributed. Most of the lung is relatively normal here, and the lesions were very chronic. This animal had very extensive lesions in the liver and spleen.

The animal on the right was exposed to over 300,000 colony-forming units and survived for less than 3 days. This animal had very few lesions in the liver and spleen, but as you can see here, a very extensive, very acute bronchopneumonia at death.

However, both animals died from tularemia.

So from the LD50 studies, we selected a target challenge dose of 1,000 CFUs. Part of the rationale for this was to be able to -- 1,000 CFUs was a reproducible dosing regimen. It produced a reproducible disease and reproducible time to death. It allowed the animals to survive long enough for us to actually be able to intervene and evaluate and compare the efficacy of vaccines or therapeutics.

And the other reason we chose this again goes back to the historical database. There were studies done in the '50s in both humans and in macaques where they were testing the efficacy of the live vaccine strain, and they used a challenge dose of 1,000 CFUs in both the humans and the monkeys.

So for the first part of the study, we had the animals implanted with telemeters to measure core body temperature, respiratory rate, and heart rate. What we saw again was a fairly consistent fever onset between 2 to 3 days post-exposure, with that lost of diurnal variation in core body temperature. This correlated with an increase in heart rate and respiratory rate.

As the disease progressed and the animals got sicker and sicker, their core body temperature eventually started to decrease as they approached death. This later became useful as a euthanasia criteria for subsequent studies.

And, again, one of the things that we identified, as was done for the plague studies, was that the onset of fever became a very useful and consistent feature for later antibiotic efficacy studies as a trigger to treat.

So this shows some of the data from the telemetry studies. On the top is a summary of the results for the 12 animals. As you see, the average time to fever onset was 47 hours plus and minus 4, and the average time to death after that was 113 hours after the onset of fever. And this was pretty consistent and resulted in the death of the monkeys generally between 6 and 7 days.

On the bottom left, you see the variation in core body temperature. The time of exposure to tularensis is marked at the bottom as the challenge time. You can see the normal diurnal pattern that we

monitored for 6 days prior to challenge, and then at about 48 hours, the loss of that normal diurnal variation with the onset of the febrile response. And then out at about 160 hours, the core body temperature began to decrease as the animal was essentially cooling down to room temperature.

The two slides to the right of that show the corresponding for the same individual animal, changes in C-reactive protein. It's a measure of the acute phase response. And increase in white blood cells, particularly neutrophils, that increased again in that same 2- to 3-day timeframe.

So the next part of the natural history studies were done as, as I said, essentially a serial pathology study. We had 16 monkeys, and we euthanized four each on days 2, 4, 5, and 6 post-challenge. We did quantitative blood and organ culture. We did gross and microscopic pathology on all of the organs.

What this showed us, again, it was very useful in that it showed that there were multiple portals of entry after head-only exposure, not just the lungs, which isn't surprising in retrospect.

Many of these animals had secondary bacterial infections in the nasal cavity associated with presumably commensal staphylococci. We saw local inflammation initially at the initial site of deposition. This was followed in time by progressive inflammation of the draining lymphatic vessels, and followed in time again by progressive inflammation of the draining lymph nodes, and ultimately resulting in hematogenous dissemination to macrophage-rich tissues, such as the liver, spleen, bone marrow, and other lymph nodes in the body.

And, again, in this study, it correlated with increased white blood cell counts, activation of the acute phase response between 48 and 72 hours. And terminally, fairly consistently an increase in liver enzymes, as these animals had fairly substantial necrotizing inflammation in the liver.

So the challenges and lessons learned, don't laugh, some of them seem very obvious now, but they weren't necessarily at the beginning of these studies.

Having a well-characterized starting material, in vitro growth conditions, and aerosol generation

conditions is really, really critical for reproducibility of your disease model.

Related to the aerosol challenge, again, the presented dose is a calculated dose. It's based on plethysmography. The amount deposited and the location of deposition vary significantly with breathing rate and death, which, in turn, varies with the depth of anesthesia of the monkey.

The particle size dramatically influences the site of deposition and ultimately the LD50. The particle sizes we used in our studies were -- we were targeting between 1 and 3 microns. If you get up to larger size particles, 5, 10 microns, and above, you're going to be depositing higher in the respiratory tract as opposed to the deep lung, which means your mucociliary clearance is going to be greater and your LD50 is going to go up.

And then, as I said, it's obvious to us now, but head-only challenge allows the organism a portal of entry, not just from the tracheobronchial tree in the deep lung, but also from all other mucosal surfaces that were exposed to the agent.

From the standpoint of telemetry, some of the things we learned were with respect to where the chips or where the probes were implanted. Initially we planted the probes on the diaphragm. Well, if you get an animal with extensive pleuritis, as the disease progresses, they're going to start to breath much more shallowly, which means that the diaphragmatic excursions are going to be less, and you're going to lose your signal.

We did some studies where we compared subcutaneous temperature chips to telemetry to detect the onset of fever, and, again, it's obvious now that when you're implanting a temperature chip in the subcutaneous region, at the onset of fever, you're increasing the core body temperature, so the sub-Q chips really aren't going to work very well because it's shunting that blood away from the skin.

And then also realize that human activity in the room is going to impact some of the physiologic parameters that you're measuring. So in our case, heart and respiratory rate pretty consistently went up when we had people in the room cleaning cages.

Prioritize your sampling. In these monkeys, we took blood samples over a few days up to daily for the first week. We calculated that we could collect 2 mls of blood from each animal based upon their body weight, using a publication that describes what I refer to as the Diehl guidelines.

The Diehl guidelines for blood collection are written for healthy animals. Animals with a subacute to chronic inflammatory disease don't regenerate the way that a healthy animal would. So you just need to monitor your hematocrits in real time and prioritize the sampling because you don't want to continue to be drawing blood from an animal with a hematocrit of 15 percent, or you're going to be impacting the course of the disease.

I can't stress enough then also the establishment of endpoints for these types of studies. I cringe when I see the term "moribund euthanasia" because "moribund" has very dramatic differences in meaning to different people. Use a clinical veterinarian for the first studies to really get a good handle on what is a good indicator of when it is time

to euthanize. And then once you establish those euthanasia criteria, be very consistent in following them.

And, finally, this is from the perspective of the pathologist, collect tissues widely for histopathology. If you aren't looking at tissues, you're not going to see lesions in them. Keep in mind that the studies will eventually be used to detect -- to examine the safety of your test article, of your antibiotic, as well as the efficacy. So you're going to want to have a very broad list of tissues that you're examining.

In our case, it helped us to identify that there were multiple portals of entry, it helped us to explain some of the differences that we saw in clinical presentation in the disease course with differences in initial dosing. And it also helped us to identify some potential background infections.

Monkeys, in particular, are not pristine inbred animals. Background lesions and infections can impact the disease outcomes. There's a very good example in the literature describing a nosocomial

infection of an indwelling catheter with Serratia for anthrax studies in an African green anthrax study, in which case animals that were not being treated and should have died did not die, and it was primarily because of nonspecific immune stimulation from the indwelling catheter contamination.

We had a similar experience, at least we think we had the similar experience, with the "tuli" studies in that we had one set of studies where the housing facility where we purchased the animals had had an outbreak of Chagas disease, and the animals, in fact, when we went back and looked at some of the tissues around the heart, the animals did show evidence that they had previously been infected with Chagas.

And, finally, as Gabe mentioned, these are very large studies, and it's really a team effort. And thank you.

DR. NAMBIAR: Thank you, Dr. Hutt.

So our last speaker for this session needs no introduction. Dr. Edward Cox is the Director of the Office of Antimicrobial Products at CDER, FDA.

DR. COX: Thanks, Sumathi. And I'm just going

to make a few comments here from the table. And for folks that are watching on the webcast, I don't have slides. I'm just going to speak a little bit here.

And I want to just first start out by thanking Judy, Gabe, and Julie for really the excellent work and the description of all that was done in the animal model development work that you've done, and to thank all the folks that have come here to join us today.

And when we started out on this, folks may notice we've got colleagues from NIAID and BARDA at the table, and we brought this idea to them, and the good news it was is that it was enthusiastically embraced, in fact, the response we got from the NIAID and BARDA folks is, "Yeah, we're already thinking about this, we're glad you called."

So it's good to have everybody trying to work through this, and also the industry folks that have been pushing along and trying to help us in thinking about potential solutions for species-specific drugs for Pseudomonas aeruginosa and Acinetobacter baumannii to try and solve these problems.

I'll ramble a little bit, but I think I'll try

and make some points that I hope are of interest to folks. And I thought we would first talk about some of what we just heard in the presentations. And as you can see, there was really a lot of work that went into developing the animal models described.

And one thing that is I think noteworthy in these particular animal models is that the particular pathogen that folks are working with is intrinsically virulent in the animal models, and that helps a lot. It helps a lot when you're trying to get to a lethal animal model that in fact you have an organism that is highly pathogenic in the animals.

And if you look at the animal models for anthrax, they were able to develop animal models that if you line up the sequence of events, the tissue pathology, and what's going on, many features of the disease, there's a lot that seems really quite similar to what we see in humans. And we've been able to use these models really to evaluate the efficacy of both antibacterial drugs and antitoxins, so they've been particularly useful.

And another thing that comes into play or

another factor to think about is that at least for antibacterial drugs, oftentimes we know about their utility in the treatment of other human infections. It's also another very important piece of information to be thinking about the antibacterial drugs that are approved for post-exposure prophylaxis of anthrax or plague, they're approved for a number of other human infections that occur every day, and that occur in many of the same body sites.

Similar to what we learned about the anthrax animal model, the plague animal models, again shows many similarities to human disease, and that again helps. And we've similarly been able to use that model for the approval of some drugs in the evaluation of their efficacy.

So these are some of the cases that are probably most amenable to the development of animal models of infection because of the intrinsic virulence of the organisms that we're dealing with here. In essence, you don't need to fight mother nature in order to develop an animal model of infection.

And one last comment before we leave plague

and anthrax and things of that nature, we often think about these organisms in the setting of bioterrorism, but if you think about at least plague, there are, in fact, opportunities and folks who have done some clinical trials out there in the real world. Clinical trials are usually small, difficult to do, take a long time, but there are some studies out there, including a prospective trial, mostly cases of bubonic plague, so it's not inhalational route of exposure.

So it's really not that no clinical assessment can be done, it's really more that doing a powered study, even if you accept a fair degree of uncertainty, is not really practically feasible for these conditions. So you can see that there are some diseases that have some degree or some component of disease that occurs naturally, but it's really still not practically feasible to conduct a clinical trial where the Animal Rule has been applied.

And even in situations where the animal model is similar in many ways to the disease in humans, there still really is a considerable degree of uncertainty as you're extrapolating to humans. And to some extent,

that can be minimized to a significant degree if you have a drug that is approved for a variety of other indications in humans, based upon evidence from human clinical trials.

Now, I want to transition just a little bit and talk about an area that may be less familiar to folks that have been particularly involved in the area of antibacterial drug development, and that's some of the issues that have been encountered as we've tried to evaluate the work that's gone on out there to develop animal models for viral diseases.

And for the purposes of illustration, it's probably most helpful to jump to one of the most challenging circumstances, and that is the development of animal models for smallpox, you know, a unique pathogen that's been eradicated and has really a tremendous specifies specificity for humans.

We had a workshop and an advisory committee meeting to talk about smallpox drug development, smallpox animal models, that would be utilized to evaluate drugs. And just in general, the process served us well because we had a particularly

challenging issue to try and solve, so starting with a workshop, discussing some of the science, moving towards an advisory committee.

It would provide an opportunity for transparency, for learning, and sharing with the world what some of the challenges are and what some of the solutions are and what some of the options are for solutions and why in this circumstance it's a reasonable way to proceed.

And you can see here, the pathogens we're dealing with today are not smallpox, but you can see we're trying to use a similar process to solve some of these problems.

So we've mentioned really the strong species specificity of smallpox for humans, and if you want to take variola virus, smallpox virus, and to cause pathogenesis, a lethal model, in non-human primates, you essentially have to give a huge dose of smallpox

virus. You have to give like 10 plaque-forming units in order to get lethality, somewhere in that neighborhood.

And really folks started to look at that and

think, what's -- I mean, that's a fairly extreme situation to have to get to. It also involves working with smallpox virus, which raises a bunch of other considerations.

So the discussion in the advisory committee also talked about the role of other -- using other pox viruses such as monkeypox in monkeys, mousepox in mice, rabbitpox in rabbits. And the idea here was, I mean, you can see we're sort of using a surrogate virus in a surrogate animal in order to predict efficacy. The idea here was to be able to get to a model that was able to be utilized in order to evaluate the efficacy of these products for this really exceptional circumstance of trying to evaluate a therapy for treatment of smallpox.

So that's sort of at the other end of the spectrum. I just wanted to throw that out there so that folks are aware. Obviously, we're hoping not to be at that other end of the spectrum, but there are a range of different development pathways that have been considered under the Animal Rule with the degree of exceptionalness, if you will, dependent somewhat on the

pathogen and how it behaves in the particular animal species, the animal models that are being utilized.

And if you think about just animal models in general, animal models are generally designed and developed in order to be able to show an effect. And you choose the inoculum size. The species that you choose, their degree of intrinsic resistance or susceptibility of the particular pathogen has obviously a huge impact upon the model, and at what point in time of disease you intervene. I mean, all of these things are chosen in a way in order to be able to show an effect.

And the other thing that we run into sometimes, too, that also adds to the challenges is that oftentimes animals may clear the drug differently. So if you're trying to match the human exposure, you may have to give a mini dose somewhere in the interval between when you're going to try and hit sort of a next peak level. So there are a lot of things here that do add to the challenges.

And from the Ebola experience, we gained some insights into animal models of infection, and without

going into too much detail, I can tell you it's very humbling to have a finding in an animal model of infection that you think correlates with what should be human effectiveness only to see when a compound goes out, even in a trial that's fairly messy, but it looks like the drug didn't have the effect that we had thought we had seen in the -- or that we did see in the animal models. So understanding what effect you need to see in animals, because sometimes you can see an effect, and what correlates with human efficacy can also be quite challenging.

So I think as we think about Acinetobacter and Pseudomonas aeruginosa, we do have some things that can help us. We'll get to hear more about the quality and characteristics of animal models as the day goes on.

But another thing that we have here that could be helpful is that we do have some drugs that have performed sort of marginally, if you will, and have that. So we've got drugs that we may be able to utilize in the animal models of infection in order to be able to help us understand, can the model discriminate between a drug that seems to have marginal

efficacy and one that's doing better than that?

And we all would love to see an informative clinical trial, even if that trial is associated with considerable uncertainty. And really consistent with the desire to think about solutions first, to be able to have a clinical trial conducted was the July 18 to 19 workshop that we had last year where we talked about trying to do everything possible to try and make a clinical trial feasible.

But really what we're here talking about today is sort of two components, if you will: the supportive information that we can gather from animal models, and then also trying to think about the circumstance of if in fact a clinical trial is not feasible and what role animal models might play in the overall assessment of efficacy for a drug targeting a species such as Pseudomonas aeruginosa or Acinetobacter baumannii. And if we in fact find that these clinical trials can't be done, we will need to be able to rely to a greater degree on the animal models of infection.

If we end up there, using an approach that relies on a greater degree of understanding of efficacy

from animal models of infection will lead to a greater degree of uncertainty that everybody grapples with: developers, regulators, health care providers, patients, payers, everybody. But that could potentially be the reality here that we face.

And I think where we are today -- and in Helen's slides, she showed the WHO priority pathogen list. Where we are today with multidrug-resistant Pseudomonas aeruginosa and Acinetobacter baumannii, I mean, in this really limited circumstance, it is reasonable to take on a greater degree of uncertainty so that the development of new options can move forward and provide safe and effective options for patients with serious infections who lack alternatives.

And we thought about this a fair bit, and as you start to think about, well, what does the data package look like for such a product? And it's interesting, almost sort of regardless of what route you end up taking or how things come out, the package really is probably fairly similar.

And you've seen this on some of the slides projected already today. I mean, you really do the

best preclinical work that you can do, you'll do the best PK/PD that you can do, you do the best animal models of infection that you can do, you know, looking for response in the animals, you do the best human PK that you can do, and also try and get some PK from sick patients, and then you'll try and do everything you can to do the best feasible, practical clinical trial, you know, within reason, to be able to look at human response.

And you can see that folks who have thought about this a lot are trying to do everything they can to attend to the details of trying to make the trial to be -- increase the likelihood that the trial is likely to be an informative clinical trial. And I think that's really what this is all about.

It may still be that there is so much concomitant therapy or prestudy therapy that the trial is difficult to interpret, but I think every step is being taken in order to be able to try and make the trial interpretable.

And you've heard from the folks at Polyphor and Entasis and the steps that they've taken to try and

design their trials and take steps to increase the likelihood that the clinical trial will be both feasible and provide interpretable data, even with a greater degree of uncertainty. So I think that's a really important part of the effort here.

And I think everybody recognizes it's really in everyone's interest to demonstrate efficacy from a clinical trial, but also recognizing that if in fact that's not achievable because of the small numbers of patients in the trial, prestudy therapy, the time it takes to make a diagnosis, concomitant therapy, or patients who have mixed infections who require concomitant therapy, that the reason that we're here today is to talk a little bit more about what the role of clinical trial data -- or how clinical trial data can be complemented by data from animal models of infection.

And one thing to keep in mind, too, that if the drug fails in a clinical trial as opposed to the trial not being feasible or not being interpretable, I mean, if the drug truly fails in the clinical trial, it wouldn't be appropriate to try and rescue the program

with an animal model of infection or data from animal models of infection.

And I think what we're trying to get to, and I think we're really quite close and perhaps even there in some ways, is a route to be able to move forward now. You know, the clinical trials, the design considerations and getting to a clinical trial that is feasible and possible, I think you've heard some really excellent ideas about how to make that achievable, recognizing that there will be a greater degree of uncertainty.

We'll hear about the science of animal models of infection here. Folks may have noticed, and you'll hear more about this in a later presentation, we have a Request for Information out there about animal models of infection and additional research that could be done.

And I think we're looking at this sort of with a two-pronged approach. One is, what are some of the short-term refinements that may be able to be done relatively quickly that can help improve the animal models of infection that can help folks that are

developing drugs now? And then also thinking towards the longer term of things that may go into the future with regards to development of animal models of infection for A. baumannii and Pseudomonas aeruginosa.

So there are paths forward here, and we're working to minimize the degree of uncertainty and increase the likelihood of a successful program and evaluating drugs that are species-specific for Pseudomonas aeruginosa.

And then if we think down the road, I mean, for drugs that do show safety and efficacy, it will be very important to have full transparency on the limitations of the data that were generated in order to evaluate a particular compound.

And I think that the tools that LPAD provides will be very important here, provide tools to mitigate the risk and uncertainties for drugs that have shown safety and efficacy using a more limited and streamlined pathway. And it will also be an important signal to the health care community because the health care community obviously will play a very important role in the prescribing choices that they make to use

such products.

So just to sort of briefly summarize, I think there are pathways. There are still challenges in development and uncertainties that will be encountered, but as you can see from some of the proposals with regards to clinical trials for species-specific drugs, we're getting to some reasonable options here given the circumstances. And we're also considering if the clinical trials turn out to be infeasible or uninterpretable, how animal models of infection might be able to be utilized and with a greater reliance upon the findings there.

So I want to thank you for your attention. And I'll hand it back to Sumathi.

DR. NAMBIAR: Thanks, Ed. I think we're running a few minutes late, so if it's okay, we'll take a break right now and if possible, reconvene at 11:15, if that's possible. Thank you.

(Break.)

Pathogenesis

DR. MILLER: Okay. We're going to have two talks on the pathogenesis of these opportunistic

infection organisms. And the first is Joanna Goldberg, from Emory University on the "Pathogenesis of Pseudomonas Aeruginosa."

Pathogenesis of Pseudomonas

DR. GOLDBERG: Thank you very much. And thank you for the organizers for organizing this what seems to be a very interesting and timely meeting. I am going to be telling you about the general background of the outline of pathogenesis of Pseudomonas. And I have nothing to disclose.

So the genus Pseudomonas is a predominant microbe in the environment. It has numerous niches and can interact with a variety of hosts. As shown here in this cartoon, Pseudomonas aeruginosa, which is the focus for today's discussion, can live on dead plants and insects. It can also promote plant growth and, in some cases, cause diseases in plants. It's also involved in bioremediation and can produce several novel secondary compounds. Most importantly for today is that this bacterium is the cause of human disease.

It is ubiquitous in the environment. It has minimal growth requirements. It can actually even grow

in water. It can grow at temperatures between 4 degrees and 42 degrees. It's a facultative anaerobe and can grow anaerobically in the presence of arginine or nitrate as a terminal electron acceptor.

Most importantly, it is highly antibiotic resistant and it's quite resistant to antimicrobial agents. This resistance is due to it intrinsic outer membrane of the outer membrane impermeability, and it also has numerous efflux pumps as well as its acquired resistance.

As previously mentioned, carbapenem-resistant P. aeruginosa has just been listed as a Priority 1 Critical Pathogen on the WHO priority pathogens list for research and development for new antibiotics.

So when we talk about Pseudomonas aeruginosa, as we're doing today, there's a large amount of infrastructure available. The first sequenced strain of Pseudomonas aeruginosa was PAO1, and that was sequenced in 2000. It has a large genome, large circular chromosome, of about 6.6 megabases, and it also has a large number of open reading frames of over 5,580. It also has a high G+C content.

This strain PAO1 was a spontaneous chloramphenicol-resistant mutant of the original PAO strain, which was isolated in 1954 from a woman in Melbourne, Australia.

Since that time, there has been a large international effort put forth to sequence thousands of Pseudomonas aeruginosa genomes. The International Pseudomonas Consortium is a repository of isolates from various infections and environmental sources, including from plants and from animals. The strength is that this resource actually has a large amount of metadata associated with it, including its source as well as -- its source, the bacterial phenotypes and genotypes, as well as the clinical characteristics of these strains, including their antibiotic-resistant profile.

As of this last week, there were 1,588 isolates in this consortium, and 979 genomes that were available.

In addition, this is not the only -- could you give me a hand with this?

(Technical interruption.)

DR. GOLDBERG: Okay. Thank you.

Okay. So not only is there a large amount of genomic data available, but there is actually a fantastic bioinformatic resource that's available to the Pseudomonas community. Pseudomonas.com is a website that was developed by researchers in the field that provide annotation, transcriptome, and pathway data analysis. The site is manually curated and continuously updated and is basically the gold standard in terms of genomic analysis for any microbe. And it currently includes 184 complete genomes.

In addition to the various genomes that are available, there are two different ordered transposon mutant libraries that have been developed. One is based on PAO1 and another one is based on strain PA14. PA14, another genomic sequence strain, was isolated from a burn patient, but is also shown to be virulent in a number of different animal, mammalian and non-mammalian models, including infections in the plant Arabidopsis, zebrafish, and wax moth larvae.

The Pseudomonas field also has state-of-the-art genetic tools, including a myriad of vectors for the construction of mutants and for the transcriptional

and translational fusions. There are both luminescent and fluorescent constructs available for monitoring the expression or looking at bacteria as single cells or for looking at the bacteria in animal models of infection.

In addition, there are transposons available for transposon sequencing experiments, and with these available genomes, there are a large amount of RNAseq projects that have been undertaken.

Now let's talk about the pathogenesis of P. aeruginosa. Most importantly, as it's been said, it's an opportunistic pathogen. So unlike the situation that we heard about plague, anthrax, and tularemia, this means that this bacteria generally doesn't cause disease in healthy individuals. Infection occurs pretty much only when there is a compromise to the innate immune system. This can be immunocompromised such as individuals undergoing chemotherapy or a breach of the skin or basically any mucosal surface.

With respect to animal models, like humans, most animals are generally resistant to infection. For example, intranasal infection of a wild type P.

aeruginosa strain in a typical healthy inbred mouse is lethal at about 10^5 to 10^7 colony-forming units, but if this mouse is made neutropenic, the lethal dose is about 10^2.

P. aeruginosa can cause infection at many sites. It is the most common cause of bacterial keratitis after injury either by trauma or in some cases by damage by contact lens.

It can cause swimmer's ear and infection of the outer ear canal that develops when water remains in the ear after swimming.

It can cause intra-abdominal infections and nosocomial urinary tract infections as well as bloodstream infections and other catheter-related infections.

It can cause skin and soft tissue infections varying from the non-lethal hot tub folliculitis to, most importantly, life-threatening infections following wounds and burns.

P. aeruginosa is one of the most common causes of hospital-acquired acute pneumonia, especially in mechanically ventilated patients, and these infections

can disseminate and be associated with a particularly high mortality rate.

It is also the cause of chronic respiratory infections in patients with cystic fibrosis or with COPD. In CF patients, these infections are the most common cause of morbidity and mortality.

Interestingly, eye infections and chronic lung infections in CF patients almost always stay localized to the site of infection, while infections in these other sites can often disseminate.

Numerous virulence factors have been recognized in P. aeruginosa. This slide shows some of the select ones that have been associated with acute infections. For the most part, these have been defined and characterized by the construction of mutants lacking individual factors and then tested in, in vitro or in vivo models of infection.

This cartoon shows a sampling of some of the secreted factors, like proteases and the secreted toxin molecule, like pyocyanin. It shows one of the siderophores, pyoverdin. It also shows some molecules that act as adhesions, including the flagella, the

pili, and the LPS.

It also shows the secretion systems. Of particular interest is the Type III secretion system that injects exoenzymes directly into the host cell. However, the situation with P. aeruginosa is even more complicated than is shown in this slide, as certain strains from certain types of infections express particular virulence factors, for example, strains from corneal infections generally express ExoU and do not express ExoS.

Also not shown in this slide are the extracellular polysaccharides, including alginate, Psl, and Pel, all of which have been shown to have a role in biofilm formation.

The situation is even more complex when you compare strains from acute infections or from the environment to those that emerge during chronic infection in cystic fibrosis. Those from early infections are generally similar to those found in the environment, and the environment is probably the source of the initial infections in CF patients. However, during chronic lung infection, the P. aeruginosa evolve

and adapt to the lung.

This cartoon represents some of the changes that have been noted during longitudinal studies of isolates from cystic fibrosis patients. Most predominantly, as you can see on the left, the strains make a low level of the exopolysaccharide alginate, and on the right, this green bar represents the polysaccharide being overproduced, giving the bacteria a mucoid phenotype.

The bacteria tend to become nonmotile and lose their flagella and pili. Strains tend to mutate, the LasR gene involved in quorum-sensing, and therefore their quorum-sensing is differentially regulated. The chronic isolates secrete less proteases and less toxins. They change from making a lipopolysaccharide that's complete to making one that's LPS "rough" and rendering these strains serum-sensitive, and they also change their lipid A to make it less inflammatory.

Interestingly, loss of these factors make these strains less virulent when tested in acute models of infection, but it's obvious that their loss has an important role in the pathogenesis of chronic infection

in cystic fibrosis.

So there are a number of issues I think we need to think about as we discuss the development of animal models of P. aeruginosa. We need to remember that healthy people are generally resistant to infections, that some acute infections can disseminate from the initial site of infection and cause sepsis, while other infections can stay localized; that P. aeruginosa adapts during chronic respiratory infection in CF patients, and that this situation is actually even more further complicated by the fact that cystic fibrosis itself is a disease that can be caused by one of over 1,700 different recognized mutations in the CFTR gene in the human population, so not all mutations in CF are actually equivalent with respect to their severity.

And, finally, strains from particular infections may express different virulence factors that may be important in different types or sites of infection or at different times during infection. And I think these are the most important things to consider as we develop a standardized animal model.

DR. MILLER: Okay. We'll move on to our next speaker, Dr. Robert Bonomo, from Case Western Reserve, who will talk about the pathogenesis of Acinetobacter.

Pathogenesis of Acinetobacter

DR. BONOMO: Thank you very much. Unlike to contrast to Pseudomonas aeruginosa, in Acinetobacter, having dealt with this pathogen for close to 20, 25 years in the clinic, I have a little different view about Acineto. This is an organism where resistance and virulence are converging in what we consider now a critically important pathogen.

These are my disclosures. I would like to thank the FDA, Mark Adams, Brad Spellberg, and Phil Rather, whom I've worked with, with Acinetobacter over the years. The funding sources. And I do preclinical work for some pharmaceutical companies to basically do biochemistry on their beta-lactamase inhibitors.

So Acinetobacter. You know, in my mind, when you think about this and you have to talk about an organism that you've studied for 20 years, I sort of got a little philosophic. I thought about this, and I said, my god, this is one of the most complex

pathogens.

And when you look at it, you can make a statement like that because this organism has a really sordid past. It went by the name "Mima," then it went by the name "Herella," and then it went by "Bacterium anitratum." It even picked up a letter and a number. It's almost like somebody trying to avoid the police.

(Laughter.)

DR. BONOMO: And high diverse, it's a very diverse organism. There are more than 50 species that are known. Some species cause infections. And it's really difficult in this field because the microbiology labs often identify something as Acinetobacter X, Y, or Z, when in reality it's Acinetobacter A, B, C.

And there's a lot of mystery and misconception. The term "Acinetobacter" comes from the term "akineto," and everybody knows those are the actual Greek letters, I can't read Greek, but I'm told they're really Greek, and where this comes from, and this means that it's a nonmotile rod.

And probably one of the most dubious distinctions is that the organism loves an environment

that is warm and wet, so I don't know what you want to think about that, but it's probably not something that other organisms actually go into.

And thinking about Acinetobacter over a number of years, I hate to use the phrase, it's characterized by some quantum spookiness. And you have an organism that when you look for images of what it looks like on electron microscopy or scanning electron microscopy, the red image that you see looks nowhere like the green image that you see, and both of them are Acinetobacter.

This is almost one of those things where we really can't tell what we're studying sometimes because its genetics are so complicated, its classification is so complicated, and its morphology changes.

And it's fraught with misconceptions. It's supposed to be nonmotile, yet the images that you see on this slide, and if you shine blue light on it, Acinetobacter moves. Believe it or not, it moves to photo stimuli. It moves when you change the temperature in an environment. And it also moves when you change the consistency of the agar that it's being plated on. So there are a variety of complexities that

we have with this organism.

And, in addition, it causes a variety of human infections, with increased attributable mortality: hospital-acquired or health care-associated pneumonia, and it even causes community-acquired pneumonia. It has a mortality of up to 70 percent. And if you are in parts of the world where there is heavy alcohol use, even in the community setting, it can cause significant disease. It is characterized by bloodstream infections, and in some areas of the world, neonatal sepsis. It can cause burn infections, skin and soft tissue infections, meningitis, osteomyelitis, endocarditis.

And when the clinician is faced with these challenges, the most difficult thing is not only are you not certain which type of Acinetobacter you have because the lab is not 100 percent sure, MIC testing is challenging. You get things like skip-wells, that you will see the cutoff point is at 4, and then all of a sudden you pick up the pathogen again at 8, and at 16 you lose it, at 32, and you pick it up again at 64.

The organism, if you put an Etest on, you find

out that the organism that was susceptible demonstrates heteroresistance, and there are a lot of inconsistencies with the measurement of colistin.

So this singular successful pathogen brought this in my mind unique combination of resistance and virulence. And in Acinetobacter baumannii, you have a resistome I think that you have to face when you develop an animal model, and you have a virulosome.

And in this, you have to keep in mind that this organism has this remarkable capacity to acquire and rearrange genetic determinants that play a critical role. The Acinetobacter that you have at the beginning of the infection may not be the same Acinetobacter that you have at the end of the infection because you have these mobile genetic elements that are hopping around.

So how do we know that Acinetobacter is virulent? What are the clues? Well, good observation is important. Finding models and defining virulence traits are the key to this. So a long, long time ago in a kingdom far, far away when we first started working with the Walter Reed Army Medical Center, Dr. Colonel Kraft there sent us these isolates, and we were

very, very fortunate to get these isolates from him, and we did a genetic determination of the antibiotic resistance genes.

It occurred to us in a separate manuscript that, you know, it's actually funny that some of these resistance determinants were associated with particularly bad outcomes. So we didn't know what this meant at this time because we were very young, a lot younger than I am now, almost a decade or more, we didn't know that carbapenem resistance was associated with the need, not the success or failure, but with the need for mechanical ventilation.

We also discovered that certain particular carbapenemase genes were associated with ventilation, longer hospital stays, ICU stays, complexity, durations, and changes made to the antibiotic regimen. We had no clue. We had no clue what this meant. This was an observational study, but it sort of got the ball rolling.

So we thought that, well, maybe, just maybe, blaOXA-23, or the carbapenemase gene, is a marker, and we started thinking about the link between bacteria of

proteins in the periplasmic space and the cytosol, and could there be any virulence determination that comes from that? But regrettably at the time, we focused more on the resistance side of Acinetobacter than we actually looked at this sort of interesting virulence thing.

But the rest of the world went on luckily. And then Acinetobacter started being described as doing some really strange things, like developing outer membrane vesicles where part of its outer wall forms another little bubble, and that bubble gets popped off the Acinetobacter, and in that bubble is a resistance gene, and then people started looking further, and not only were resistance genes in these little lipid bubbles called outer membrane vesicles, but other proteins associated with pathogenesis and virulence and specialized secretion systems for the delivery of virulence factors.

So what's emerging here is an incredibly complex organism. Resistance is the center of what defines it in the lab. Its genetics change constantly due to mobile genetic elements that are hopping around.

And it's put together in probably a way that -- I don't understand Pseudomonas as well as I should -- but in a way that's probably very unique, and similar to something I've had to think about.

So how do you put together an understanding of models of infection? Well, we all recognize here that they are important to identify virulence. These help us identify the factors that are important between host and Acinetobacter. But there is a caveat, the in vitro assays, including adherence to human cells, cell invasion, biofilm formation, have often lacked correlation in the current models with in vivo virulence in Acinetobacter when studied head to head.

And I think that this observation or this statement actually made by Brad Spellberg, in a publication in Clinical Microbiology Reviews, speaks not because the models are bad, because the organism is very complex. And the models of infection that we have, this is a pictorial representation, a mouse, rat, a waxy worm, and C. elegans.

Some of the mice models, they're useful in some ways, but they're challenging. You have to give

sometimes a high inoculum infection at 10. That's not really relevant to what patients actually face. To make mice neutropenic to actually study an Acinetobacter infection, well, clinically, or at least in the real world -- in the clinical world -- the clinical world is the real world sometimes -- in the clinical world, neutropenic patients don't get Acinetobacter infection, they get other things. So making a mouse neutropenic to give them an Acinetobacter infection is limiting.

The diabetic mouse may be a little bit more relevant because diabetes is one of the risk factors and it can be used for antibiotics. To inject Acinetobacter into the intraperitoneal cavity using porcine mucin is a stretch, is a stretch, because, first of all, Acinetobacter doesn't really cause a lot of peritonitis in humans.

It's rarely recovered from a belly infection or spontaneous bacterial peritonitis. It's got other niches, it's more the lung. And so giving it an irritant and recovering it from a belly infection doesn't really help us understand virulence all that

well.

But there are strains of mice that are intrinsically susceptible due to diminished cytokine responses, and these are actually probably a good model. And there is also another strain of mice that is also a very good model, but we're not sure.

Rats actually have little bit better models of infection. The pneumonia model in the rat, you can give a rat pneumonia and non-immunocompromised. There have been rat models of skin and soft tissue infection to study burn. And there have also been wound models using to make the mice a little bit immunocompromised with cyclophosphamide and using particular strains.

And there are non-mammalian models. And it's really interesting that the waxy moth, even though it's non-mammalian, actually for some studies, like in assessing virulence and for some studies, assessing antibiotic efficacy, has done reasonably well. I can't speak to C. elegans or zebrafish larvae, but I understand they perform similarly in this way. We have had experience in our lab with the waxy moth, and once you get a handle on how to deal with it, it's actually

pretty good.

But these models lead us to define virulence. And when you think about virulence in Acinetobacter, I wish I could say it's one thing, but just like the complexity of naming the complexity of the genome, there are a lot of virulence factors. It can persist in dry environments. It expresses motility when it's not supposed to.

The virulence genes get turned on by ethanol. Now, your mind is not supposed to go to the spot, but just like humans, I guess.

Biofilm mechanisms are at play. Iron acquisition pathways. Polysaccharide membrane. Alteration in penicillin-binding proteins. And pilus formation. And these are all complex things that unfortunately we can't put together in a single model yet as to why all these different factors are working. It's more like there's a constellation of factors working at the same time. And I'll show you a paper at the end that shows this really very well.

And then there's the essential role of LPS. And I love this figure about LPS because when you go to

medical school and you study biochemistry, and you have to memorize all these little sugars and how they go around in circles and how they get chained, and it's so complicated. Just look at a molecule of LPS, and you say, "I'm done."

(Laughter.)

DR. BONOMO: There are too many chains, too many O's, too many circles. It's hard for us even to understand this on a chemical level and its modification, but be certain that LPS has a critical role in Acinetobacter in evading the host immune response and triggering the host inflammatory response.

And then there's the central role of OmpA, and this is one of the most important proteins that has been studied so far. On this slide, I have a summary of the things that it's been involved in: serum resistance, biofilm formation, adherence. And the Spellberg lab has shown that recombinant OmpA conserved across 90 percent of strains generates a humoral immune response that is protective. So this is an important protein and the genesis of it.

So when you start looking at all the papers

that are written and you start identifying virulence factors, the complexity begins. You have what I've mentioned, LPS and OmpA, and then you have superoxide dismutase. And then as you begin to go through those virulence factors, you see that some of these virulence factors are also involved in antibiotic resistance, OXA-24, pmrB, which is involved in colistin resistance. You have a variety of other genes involved in this, and even aminoglycoside-modifying enzymes, as well as efflux pumps.

So this is an organism that has its virulence, its virome sort of interacting with its resistome. But we do have immunologic factors that can go against it: cytokines, avoidance of polys, macrophages. Zinc and magnesium sequestration are important.

And to lastly put it together, the sort of integrated view is that there is a very important role for the capsule in Acinetobacter baumannii. The capsule is the primary defense against complement. It helps ward off white cells. You have the LPS. You have the fact that in many, many infections, it has been observed that there is a large infectious

inoculum, so the more Acinetobacter you have together, the more virulent it can become. And this is almost a predictable consequence of not being able to treat this organism with an effective antibiotic. You can't get rid of it in the wound, and the numbers grow, grow, grow, grow, then you get these quorum-sensing signals that are triggered, and you go into sepsis.

So effective therapy helps the host rapidly clear the bacteria, avoids damage, whereas ineffective therapy enables the bacteria to persist at higher levels, become more dense, and triggering host damage.

And what about targeting virulence and resistance? Inhibiting LpxC protects mice from resistant Acinetobacter by modulating inflammation and enhancing phagocytosis.

A very important paper that asks the question, Can LpxC inhibition block the ability of bacteria to activate the sepsis cascade and enhance opsono-phagocytic killing of bacteria and protect mice? And the answer is yes, and it was based on very important science that was published in Cell by Christian Raetz a number of years ago, who developed this approach to it.

This was unappreciated at the time, how important this pathway was. Now I think that people are beginning to think about LpxC inhibition in the -- my screen just went away. It went white.

(Technical interruption.)

DR. BONOMO: So basically what I was going to get to is that, yes, this actually works, and you can interfere with the inflammatory cascade by interfering with LpxC inhibition. And this is a way to sort of get at sort of a novel approach and to get to the punch line basically.

When you start to integrate all these various pathways together, you find that, well, when you look at the genomic plasticity that this organism has, and you look like at its responses to therapy, I think it's time to start developing a novel way of looking at Acinetobacter, and I think that's the role of this committee today, because there is significant urgency.

And the urgency is here, a very important outbreak that was described. I think Dan is going to talk about this later. This was a huge outbreak of Acinetobacter strains. They found that the strains

were genetically related to strains that had previously went around the world. In murine models, these strains were very, very virulent. They had a high virulence. They used iron metabolism -- they discovered iron metabolism genes, protein secretion, and they also developed a PCR assay in this paper that could help them identify this.

So we come back to this sort of synthesis in this organism where many, many genes have been found, about 300, that were required for survival and for growth of A. baumannii, and there are many transcription factors also required for this. And the subset of these transcription factors that are involved in virulence are also involved in resistance.

So I think this is a way to look at the virulosome of Acinetobacter and to begin to think of it in different ways.

So in conclusion, resistance and virulence I think actually are very linked in Acinetobacter. I think the way to approach this is to start to delve into systems biology approaches that can reveal these incredibly tight associations between what's happening

on the outside of the organism and what's happening on the inside.

I think we need to start thinking about different ways, antisense therapies, or siRNAs. They have genes that are involved in resistance have dual roles. We need to begin putting our heads around what is the role of outer membrane vesicles in transporting information across.

I think there are unexpected patterns of infections that we have to be aware about, and in one strain of Acineto, in one infection in a patient in Cleveland, we discovered that the patient was actually infected with two genetically different strains but was bacteremic with Acinetobacter by two genetically different strains circulating in the bloodstream. This is a way to think about this. And actually that was published by Mark Adams in an mBio paper. And I think that we have to link resistance to virulence in novel strategies.

So the take-home point, what I think is the future for Acinetobacter. Well, I think we have to take a three-pronged approach -- we have to think about

immunotherapy, we have to think about biologics, and we have to think about drugs -- because it's a very cunning pathogen.

And I made a very simple figure, you have your resistance genes on this side, you have your virulence genes, you have the link between, and I think it's a teeter-totter how to think about this. And I encourage this committee, this group, to think about Acinetobacter in a broader sense.

Thank you.

DR. FARLEY: For folks on the WebEx, we are having technical difficulties, and you'll be able to see the screen after lunch, hopefully. But you should be able to follow along with the slides that are available on the meeting website.

We'll now turn our attention to short formal public presentations. These were requested in advance per the meeting announcement.

I would like to invite Dr. William Weiss, from the University of North Texas Health Sciences Center to the podium.

Public Presentations

DR. WEISS: So 5 minutes to put my 2 cents in on this subject of animal models for Pseudomonas and for Acinetobacter. And Joanna and Robert just did some excellent presentations on pathogenesis of both these strains, so I won't belabor the point on this first slide other than to say that the organisms share common clinical infections and indications.

And the main areas of concern would be UTI, pneumonia, bacteremia, and skin and skin structure infections. And along those lines, there are resistance determinants in the strains we're encountering today that are of big concern.

In the case of the Acinetobacter, we're talking about a whole host of beta-lactamases, Class A, B, C, and D, particularly the Class D with the OXA-23 that Robert just mentioned.

In the case of Pseudomonas, it's a high percentage of infections seen in the hospital settings they cause with pneumonia, UTIs, and bloodstream infections, and, in particular, eye, ear, nose, and throat. And, again, wound infections and multidrug resistance is a bigger issue.

Now, the Preclinical Services Group at the University of North Texas runs a host of these animal models in evaluating new therapies for treating these types of infections, and I've been doing this for 35 years. I wanted to share some of the ways if you're developing a new animal model or the way we've developed the animal models and have run them that I feel are very important, particularly for these two organisms.

So in running these animal models, there's a need to have representative current clinical indications. You're not going to run a Pseudomonas against some type of infection where it doesn't occur. And this goes back to the UTI, the pneumonia, the bacteremia, and the wound. These are the models of concern, these are the models you want to have running.

You want to make use of relevant clinical strains. ATCC strains are great, but if you're going to test a new therapy, a novel therapy, you want to do isolates that are of clinical relevance, that they came from a site of infection.

If you want to develop a UTI model, you're not

going to take a Pseudomonas that came from a bloodstream infection. The best possible case of success in developing one of these models is to use an isolate that came from that indication and to acquire that and put that into the model, and that's what we do.

The other need is to use current strains of concerns. Again, you want resistant strains. You want something expressing an OXA or an NDM-1. You want something that maybe has an efflux mutation in it. This is what you're trying to treat. A susceptible strain, whereas it might develop an infection, is not that relevant to evaluating new therapies. So there is a need to acquire those strains. And this is where I would encourage people to share some of these strains if they have them and not hoard them. We need to put these into the models to develop the proper efficacy study.

We need to involve proper doses and dosing regimens. Now, for a lot of the evaluations we're doing early on, we don't know humanized doses. It's hard to do so. You want to have a dosing regimen that

you can show a cause and effect. We always try to have a dose range that gives a maximal effect and minimal if we can do it. That's a true indication that the model is working.

The humanized PK or adapting to PK needs to come later on after you've done your initial screening.

You need to define the measure of efficacy. What is your endpoint? And in a lot of these cases, it's bacterial titers. Efficacy is defined as a reduction in those bacterial titers at the site of infection following therapy.

Survival is good, but it's an all-or-nothing. You can't really tease out any little idiosyncrasies of the model if you're just doing survival.

So with that in mind, I would like to go over four of the models, the common models, but it's four of the models that we work with on a regular basis, and we work with on a regular basis with Pseudomonas and Acinetobacter, and how it can be done, and different ways to do it.

Robert talked about different strains of mice, and, yes, there are different strains of mice that work

better than others. He talked about neutropenic versus non-neutropenic. In the case of the lung model, you can do both. You can do normal mice in a lung model, Pseudomonas in particular works really well. Neutropenic is usually needed for Acinetobacter. They're a little bit tougher to establish that infection.

Intranasal inoculation. Other people can use tracheal. Intranasal inoculation, again, it's a common route of infection for people. So we try to do it by intranasal route.

Treatment can be delayed. We don't want to do a lung model where you treat immediately. That's really just knocking down the inoculum that you're putting in. So minimum of 4 hours out to 24 hours, depending on the etiology of your strain, is acceptable.

Single/multiple dosing ranges over time. What that will tell you with multiple ranges is if the compound you're testing with will accumulate in the lung to a high enough percentage to inhibit, it's more clinically relevant.

In terms of endpoints, again, lung titer. Survival can be used. In a mouse model, a lot of these strains will go bacteremic and cause death.

PK/PD in the lung model is very common. You can measure both plasma and ELF to get a measure of efficacy. And, again, we have this working with several strains that came from endotracheal aspirates or bronchial lavage from a clinical study. The relevant strain works much better.

The UTI, or what we run is an ascending pyelonephritis. It can be done in normal mice. It can also be done in diabetic mice. Particularly for Pseudomonas, the diabetic mouse model works very well. It's transurethral inoculation, so it really is a source of infection, and ascends to the kidney to cause infection.

Treatment, again, we don't start until 4 days later. We're talking about an established infection. You don't go to the physician just getting a UTI, you already have the UTI, it's already there, you already have an issue. So it's important to wait that amount of time.

Your endpoint isn't just kidney. We want to measure different aspects of it. Where does the compound go? Where is it most effective? So we're looking at kidney, we're looking at urine, and we're looking at the bladder tissue itself in case there was a biofilm type formation maybe with the Pseudomonas there. And, again, we have urine clinical isolates we have obtained and put into the diabetic model that give very good reproducible results for both new therapies and common molecules.

Skin and skin structure, there are a couple of different ways to do skin and skin structure. One is a superficial, the other is a subcutaneous abscess. You're looking at bacteria titers, you're looking at wounds, you can delay infection, you can delay treatment of infection, and then start. We have wound isolates that we're using. You could also do mixed infections, which are common, Pseudomonas and a Staph aureus to do wound infection models.

Then, lastly, septicemia. It can be done by IP infection, which delays the onset of it that produces a steady infiltration of the bug. Robert

alluded to it, gastric mucin in the GI tract is not quite the way to do it. You could also hematologically provide this infection.

Treatment has to be a little bit quicker because this goes pretty fast. You can do a survival endpoint. I know I said survival endpoints aren't necessarily great, but we also do time studies where you can track CFU in the blood and the spleen to look at efficacy of your agent.

And here again, we have this working with Acinetobacter and Pseudomonas of various resistance mechanisms, including the ones provided.

So I think that was my 5 minutes. Thank you.

DR. FARLEY: Thanks. Dr. Jennifer Hoover, from GlaxoSmithKline.

DR. HOOVER: Thank you. I want to thank the workshop organizers for giving me the opportunity to speak today. I lead the Bacterial Infection Model Group, which consists of seven scientists, including myself, in Antibacterial DPU at GSK.

What I wanted today was give a brief overview of the pneumonia model that we use to evaluate our

compounds against Pseudomonas and Acinetobacter.

We induce the pneumonia by placing a bacterial suspension in molten agar deep into the lung using a nonsurgical intubation technique. The technique can be a little tricky for someone to learn, but it is very doable, and once someone is skilled, they can perform it quickly and easily.

We did recently publish an article in the Journal of Visualized Experiments which details this model. It includes both a written manuscript as well as a video demonstration of how to perform the technique in both rats and mice.

One of the advantages of this model is that many different isolates will actually establish infection, and this gives us a lot of flexibility because we can choose and change the isolates that we put in the model based on an MIC for a particular compound or a particular resistance phenotype that we're most interested in.

In the graphs shown on the slide here, you can see the growth of representative strains of Pseudomonas in the top panel and Acinetobacter in the bottom panel.

The lighter colored bars represent the CFU from the lungs of the animals at baseline, which, in this case, was 1 or 2 hours post-infection, but that could be delayed to give a higher bacterial burden at baseline, as desired.

The dark colored bars represent the CFU from the lungs of the animals at the end of the experiment, which, in this case, was either 48 or 96 hours. We typically do not see bacterial clearance of these organisms within 48 or 96 hours using this model. And the model is highly reproducible and it's very consistent both between animals as well as from experiment to experiment. Typically, we use immunocompetent animals for this work as well.

In terms of validation, we have tested commercially available antibiotics and shown that they do not work against isolates which are resistant, and they work against isolates which are susceptible, and this is using doses in the animals that are relevant to the PK that you would get in humans using relevant clinical doses as well.

We've done a little bit of histology work in

rats, which shows that the pathology using this model does appear to be similar to that seen in human pneumonia. What we would really like to have would be some additional validation work based on achieving PK/PD targets. We have a little bit of that data, and it looks very good, but we would like to have more of that for additional compounds.

We would also like to see some additional pathology work to, for example, look at mice to look at the time course of infection as well as to better understand the impact that the agar has on the infection and the disease progression.

Because of the time limitation today, I'm not actually going to show you data from the model. I would refer you to our JoVE, where we do have representative studies that you can go and look at. And I'm happy to speak with anyone either today or sometime after this workshop to share any data that we have.

In summary, we feel this model offers another approach to evaluate compounds against Pseudomonas and Acinetobacter preclinically. And it does have

advantages over some of the existing models. Our goals from the presentation today are threefold. First, we would simply like to raise awareness of the model. Second, we would like to see collaboration with other scientists who might be interested in either this model or other animal models. And, third, we would like to offer our expertise and continue to be part of the ongoing dialogue regarding these animal models.

In my group at GSK, we have been doing these types of models for over 20 years, and we would really love to share our experience as well as our industry perspective.

We are very committed to high quality animal work and robust models, and we fully support using those models which will best help us translate from the lab to the clinic. Thank you.

DR. FARLEY: Thanks. Dr. Lynn Miesel, from Eurofins Panlabs.

DR. MIESEL: Hi. I'm Lynn Miesel, and I'm from Eurofins Pharma Discovery Services. And I'm going to tell you about our Acinetobacter and Pseudomonas infection models.

So Eurofins Pharma Discovery Services is a preclinical contract research labs. It's an international network of labs in the United States, in Europe, and in Taiwan, and we offer in vitro and in vivo testing services to evaluate the safety and efficacy of test articles. And we service multiple therapeutic areas and have over 40 years of experience in contract research. And our infection models are performed at the AALAS-certified or -accredited laboratories and Panlabs in Taipei, Taiwan.

Can you hear me? Okay.

So Eurofins Panlabs performs anti-infective drug discovery services in areas from target ID and validation through to candidate selections. It includes both in vitro and in vivo testing services as well as the safety, testing, in vitro, and in vivo non-GLP.

So we have over 40 models that are already validated and ready to go, and we readily develop custom models upon request. And we work with over 160 Pseudomonas and Acinetobacter strains for these models, including 130 strains that we just recently obtained

from the FDA-CDC AR Bank, and we're very grateful to the CDC and FDA for this great resource. The infection models that we work with include a variety of infection types, and we work with both susceptible and MDR organisms, Gram-negatives, including KPC, NDM-1, and MCR-1 strains, Enterococci, Gram-positives, and fungi.

So the lung and thigh infection models are the workhorse models that we offer to clients for evaluating initial efficacy as well as PK/PD analysis. And examples are on the left-hand side, a model with Pseudomonas, a cystic fibrosis carbapenem-resistant Pseudomonas strain, LES-431, that we use for both the lung and thigh infection model.

And with all of our models, we perform validation studies to demonstrate an increase in bacterial counts between the initial inoculum and the final data point at 24 hours after treatment. We like to see at least a 2 log increase in bacterial counts. And we strive for minimum data scatter as well as reproducibility from day to day, and dose-responsive efficacy of a standard of care agent over realistic concentration ranges.

And, again, we have these models with MDR strains, Pseudomonas and Acinetobacter.

We also perform the survival and infection models, such as the peritoneal model. And I agree with the comments raised before about the rapid loss of survival in untreated animals with these models, but these models offer the benefit that they're performed with immune competent animals, and in that way, it allows us to evaluate efficacy of vaccines and immune modulatory agents, that are not so effective in the thigh and lung infection models with neutropenic animals.

Again, these models are optimized to demonstrate dose-responsive efficacy. And we have 160 MDR strains available for testing in this model, although the optimization is done with classical strains.

So I am here to discuss any questions that you may have or any inquiries about our services. And I want to thank the organizers for the opportunity to present.

DR. FARLEY: Thanks. Our final presentation

is by Dr. Craig Rayner, from D3 Medicine.

DR. RAYNER: Thank you very much for the opportunity today. So just in terms of disclosures, I am the President of D3 Medicine, A Certara Company. We work with many, many biotechs and pharmaceutical companies as well as the life science investment community, and also with governments in providing strategy and stewardship on complex drug development programs. But today I'm here in the capacity of representing the Australian Department of Defense, the Defense Science and Technology Group, or DST.

And it's quite a wonderful opportunity to announce something proactive and progressive in this area, and that is, as part of the Australian Government Next Generation Technology Fund, with about $750 million appropriated, there has been approximately $40 million which has been allocated for the support of the development of medical countermeasure products, of which clearly multidrug-resistant bacterial pathogens, of which we're talking about today, is well and truly in scope.

So it's the intention that this initial fund

will be leveraged to more than $100 million, and also to create a self-sustaining public-private partnership so that this will be something which will continue into the future.

So firstly I would like to thank and acknowledge a number of people in this room and also others on the phone because we've been working on this for quite a long time. It's involved quite a lot of advocacy over a large number of years, and we've been able to get to this point.

And what I'm here to do today is to really just acknowledge the community and, second of all, encourage you, if you're interested in finding out more about it, if you're interested in collaborating, becoming involved, is to reach out directly me. My email address is up on the website. And I would just like to thank the organizers for the opportunity to present today.

DR. FARLEY: Thanks very much. We will turn the meeting now back to Dr. Miller and Dr. Bonomo for an opportunity for some panel discussion.

Moderated Panel Discussion (with Audience Q&A)

DR. MILLER: I thought I would start out by making a few comments about the morning since it's been so informative.

First of all, I think if we can contrast the animal model development and the FDA approval that we've done for plague and anthrax compared to what we hoped to do as a group or a community for these pathogens, this is obviously a much more complicated problem.

In terms of the bugs, both plague and anthrax are very highly evolved pathogens. They maintain their stability on plates, because they have evolved to cause pathogenic disease, and we could easily develop, relatively easily develop, new animal models.

Now, with these pathogens, they're unstable on plates. We know that they lose their genetic integrity and develop deletions as they're passed on rich medium, probably because they're environmental organisms that live in a nitrogen- and carbon-poor environment in the soil and water, and when they move either to a hospital to adapt or to human tissues, there is more food and a specialized environment that causes them to change and

adapt.

So not only are they diverse because all the environmental sources out there are extremely diverse, so we've got all this diverse genomic and characteristic features of these organisms because of their diversity in nature, but then they become more diverse.

And if we look at clinical trials and think about clinical trials that we might seek where these are causing greater diseases at a greater incidence, we know that these organisms have the ability to cause epidemic outbreaks where a single clonal organism evolves over 10 or 12 years, changing its genetic material in the hospital, perhaps adapting to the hospital environment to adhere to plastic, to patient lung tissue.

Then when we think about the clinical disease, we've got this huge diversity of disease as well where we sort of have two classes of patients. There are many, many more classifications we could do, but if we think about how we might classify them, there are the immunosuppressed patients with typical -- they're on

chemotherapy or they're neutropenic, who are highly susceptible to these organisms. And then there's the class of barrier integrity loss, which is where they may be extremely healthy people, the person who gets Pseudomonas pneumonia after intubation for a routine surgical procedure that's an elective surgery, the person who gets Acinetobacter due to getting a catheter.

And so any animal models that we want to develop or try to take into account have to take into account this organism diversity and at least probably have to fall into these two categories of barrier function, loss of integrity, and immunosuppression if we want to accumulate that data.

And I think that a point of discussion that we could hear more about from other people and begin to think about is how we could combine some clinical efficacy data in humans with the animal model data.

Of course, we've known for a long time that many organisms are susceptible in vitro to an antibiotic, the classical example being Salmonella being susceptible to first-generation cephalosporins on

a plate but totally resistant in vivo because we know that microorganisms change when they get into the environment of animal or human tissues.

So we can't exactly reproduce in these animal models human tissue, but it's closer, and if we use some diverse animal models, I think we can get a lot of good data about this that might be more consistent in many ways than some of the human trials because the human trials are going to have extremely diverse patients in them, and that is going to confound some of the data.

So this is a very thorny problem. And I'll turn it over to anyone else who wants to comment about these difficult issues that I've raised.

DR. COX: We're going to bring you a microphone so folks can hear you. There are some folks online, too.

DR. MAIRA-LITRAN: Thanks. This is Tomas Maira, from the Brigham and Women's Hospital in Harvard Medical School. So basically I was going to talk a little bit about our experience with Acinetobacter and the animal models primarily. We have been working with

lung infection models on bacteremias. So although Acinetobacter has been considered for a while I guess as a low virulent pathogen, we've seen in our experience that obviously depending on the strain you use for infection, the LD90s can vary dramatically. So we've seen the strains in the 10 range, but we've seen also many strains in the 10 and 10. So I guess you've seen one of these fairly virulent strains is going to help in these types of infections.

Also, in the mouse strain, we've seen also like it's very important, and we compare a panel of different strains, for example, in our studies, and we clearly see that this has a great impact in the outcome of infection, for example, strains like C57s are much more susceptible than C3H or (inaudible) in mice.

And, again, also I agree with the fact that using neutropenic model of infections are not really -- I mean, they are not clinically relevant since we know neutropenia per se is not a risk factor for infection.

And, finally, we've seen that working with mouse models of infection, that sticking to the same vendors and even sticking to the same rooms in the

vendors help us a lot to get consistent results. So the microbiome might play a role as well here.

PARTICIPANT: Can you identify yourself?

DR. MAIRA-LITRAN: Yes. Tomas Maira, from the Brigham and Women's Hospital, Harvard Medical School.

PARTICIPANT: Thank you.

DR. REX: So as a mental model, I guess, it feels like we've got a choice between two poles. One pole is that you say to yourself, I'm not going to get happy with Pseudomonas or Acinetobacter until I can do something with the clarity of plague, you know, you say that's what you want. Your other pole is to say that no matter how many variations I do, every one of them is going to have something wrong with it. It's going to be not enough strains or the strain varied or it's only in the lung or it's not in the -- there are going to be lots -- there are going to be quirks around each one of them.

And it feels to me like if you sort of lay those two things out, you could spend a long time trying to get -- and hearing the presentations, I think you could spend a long time trying to get to the

clarity of the plague with Pseudomonas or Acinetobacter. It's not obvious to me that you would get there. It would be nice if you did, but everything we're hearing says you won't.

And so rather than drive ourselves berserk trying to achieve that level of clarity, it does feel -- you know, the great value of this conversation is that it says that in fact what you need to do is do 10 different animal models with Pseudomonas, with your drug, just do it a bunch of different ways. And if each way, if it's all consistent across all of those, then great.

And also, I don't get too hung up -- you commented a second ago about neutropenia. Neutropenia for me is not meant in an animal model to mimic neutropenia in a human being; it's simply a tool to enable the organism to become aggressive. And I just view that as yet another way to set it up so that I can do something more sophisticated than work just in a test tube. And I think use a variety of models and look for parameters, PD parameters, that seem to cut through them, and you include in those models reference

drugs, sort of standard benchmarks, standard candlesticks, and make it all line up. Because I feel like otherwise we're just going to spend forever trying to get these models to work beautifully.

DR. MILLER: I think that's right. I think you could today design -- How many? is the question, and how many bacteria? Because you're going to have to use some diverse different bacteria that you know bacteria that have caused major outbreaks, bacteria from the environment, bacteria that are current clinical isolates. You're going to have to use -- and they will need to be well characterized to understand what's different about them so that that data can be applied in the future if things change.

So you start getting this matrix of, how many bugs and how many animal models and much is that going to cost? Now, it's way cheaper than clinical trials, and maybe things would have to make it through some sort of hierarchy like that before people would spend the money on a more limited clinical trial which would show enough efficacy that people would use it or used in a different way.

I mean, getting -- I don't know how the FDA has dealt with, say, stool transplant in C. diff, even where there's a clinical trial showing its efficacy, whether we would start having to give some of these drugs an ID after they were effective in an animal model unless there's a better pathway to get them approved.

DR. COX: Yeah, so the goal here really would be to study the drug and gather the data that would support an application that would lead to approval to get out there. What we talked some about, too, is some of the tools that something like LPAD offers, which would help to identify the drug as being a product of a more limited database.

But recognizing that for patients with Pseudomonas aeruginosa or Acinetobacter who don't have other options, it's a reasonable risk-benefit scenario to consider using a drug when alternatives are not available for patients who have those serious infections.

DR. ZURAWSKI: So I would like to second everything that John just said. I think it's really

important. Our experience with the Army with Acinetobacter, the goal of our program was basically to take a strain that we felt comfortable with and we showed it established an infection across seven different animal models. And so the real key is seeing this consistency across all the models and doing all the models, like John suggested. You can't just do one, you've got to do multiple ones so you can feel good about the drug or whatever therapy that you're testing.

The second thing I would like to say is about the neutropenia. I again second what he's saying. It's a temporary neutropenia. Anybody who has done these models can tell you that the white blood cell count comes back on day three, both max and neutrophils comes back on day three. And all it is, is a tool to allow you to start with a very low inoculum and then build up to what is a classical infection.

So what we see in the animals, what we call a

classic infection, is anywhere from 10, 10 program of tissue. So to get to that point, you want to start out with a lower inoculum, and what we do for our wound

models 10 we do for our lung models 10.

We're allowing the bug to go through its normal stages of pathogenesis. How does it attack a cell? attach to a cell? disrupt maybe the barrier, epithelial barrier? things like this that are involved in the pathogenesis that then allows it to establish that infection and get to that amount.

So by having models that start with a lower inoculum and then build, I think that really has to be incorporated in.

And, finally, I would like to make a comment about strains. One thing, as a microbiologist, as a Ph.D. researcher, you're always kind of limited to the strains you get from people. Right?

So when we get strains that we want to research, oftentimes that comes from patients that may have passed or had a serious infection, and somebody is pulling that isolate maybe a week, 10 days, after the infection is established. It's usually from the bloodstream where you've got massive sepsis and that patient is going down. That's not really the isolate we want to study.

Oftentimes, that isolate, especially with these two organisms, where their genomes are very plastic, they change rapidly, and certainly in the stress of the host they're actively changing, you want earlier isolates that are involved in again that early pathogenesis and how they establish an infection.

So I think you can get lucky and sometimes get those isolates when you're looking at a wide range of strains that you're getting from numerous places, but I do want to caution that just taking a bloodstream isolate from a patient that's 14 days old, it may not be the same bug when you started, from the beginning.

DR. GUINA: I would like to make a comment. I agree that it's really important to look at many different animal models at this stage if you, as a committee, are deciding on what animal models may be most appropriate, but I think it will be more clear after the afternoon's presentations how well characterized are these models. I think that eventually hopefully the field will come to several models reflecting back to the monkeypox and smallpox models that were used to approve drugs and vaccines.

I think that disease progression could better characterize some of these models. Pathology has almost been done to almost an extent in some of these models. The committee may want to agree what are the important disease biomarkers that are going to be quality checkpoints. So there is still lots of work to be done.

So while I agree that at this point if you're a researcher, it's good to test your strains in different models, but I believe that if you approach this systematically, we may end up with two or three models that may represent different stages of disease or different maybe even to some degree patient populations.

And, of course, strains are absolutely important and where they're coming from. So that's why we have basic researchers here at the panel, because they have to impress that upon us, that it's really important. We can have totally different disease if you use different strains.

DR. MILLER: Just to underscore Tina's point, even when you take a classical pathogen, like

Salmonella typhimurium, and you take an animal model such as the (inaudible) mouse where one organism will kill that animal, if you get 10 clinical isolates from people, they all have different pathogenicity in systemic infection model in terms of how many it will kill.

So you're looking at -- and this is something where we understand the pathogenesis pretty well, and we don't know why that's the case for these organisms and what the basis for any of this is. And so we are going to have to pick some models and standardize them to some degree, and this is going to be particularly challenging with these organisms because of their plasticity and because they're not used to living on plates, on growth media, and stuff, and they will adapt very quickly to that.

DR. MARRA: I'm Andrea Marra, from Melinta Therapeutics. And just building on Dan's point -- and I think the discussion is coming around to this -- but I've been doing this a long time, developing animal models of infection for antibacterials, and I think the key thing is the strains, and this is what we've been

talking about. And I think the most helpful thing, especially with these two organisms, is a repository that we all have access to.

So those of us in biotech, we don't have access to the strains that people in other areas do. And I can't get the clinical isolates that, say, Dan or other people in government can get, and we don't have the resources and budget and time to put lots and lots of strains through these models. So I think the best way to move forward would be to have access to the strains that we can actually use to test our compounds.

DR. BONOMO: I know they are developing right now biorepositories of strains that have been supported by the NIH through the Antimicrobial Resistance Leadership Group. There are at least 100 strains of Acinetobacter and at least --

DR. MARRA: (Off microphone.)

DR. BONOMO: Well, they were the same ones that came from the wounded soldiers at Walter Reed Army Medical Center. So you could check what happened to them, but that's all published data, whether they were bloodstream infection or wound infection or pneumonia,

it's all listed as part of the metadata that's part of that collection.

DR. MARRA: No, I appreciate that point, but I know that if I get 50 strains in, I might find one that is actually mouse virulent. And like I said, we don't have the time or the money or the resources to do that. And I know from Dan's experience that that's the case. So a repository of mouse-virulent strains would be the most helpful thing.

DR. BONOMO: Well, mouse virulence and human virulence are different. Mouse virulence and human virulence, there could be a different nuance there, and how some of these drugs act in a mouse are very different than how they act in a -- they could be different than how they act in a human.

DR. MARRA: Yeah, they need to add to the mouse.

DR. COX: Yeah. It seems that both are agreeing on that, that there are differences in the way the bacteria may behave as you move from the human to the mouse. And I understand. So the idea was to have a repository of strains that could be available to

folks developing drugs.

Is it directly relevant to the last comment?

PARTICIPANT: Yes.

DR. COX: Okay. Is yours?

PARTICIPANT: Yes.

DR. COX: Okay. Then we'll go in order. We'll be right back with you in a sec.

DR. CAMPBELL: I guess what I was wondering, and we talked a lot about animal models, but I know there are more and more people are developing organs on chips. I'm Joe Campbell. I'm a program officer in Judy Hewitt's section. Developing organs on chips that do start with human tissues. And I was just wondering if relevant to some of this -- and especially Dr. Rex's comments got me thinking about this -- that we're going to have multiple models which all maybe have some weaknesses, if human organs on chip models are something that should be discussed.

DR. MILLER: I mean, I think there are advantages, particularly for Pseudomonas and Acinetobacter, particularly for even just polarized epithelial cell models or tissue wound models that are

-- because it's human tissue derived. So, I mean, those would be early stage, but it probably isn't going to substitute for animal models or lead to actual approval of a drug, but I think they could be useful models for early testing of compounds that wouldn't involve animals, which is advantageous.

DR. ZURAWSKI: I think you just have to do both. I mean, I think it's your compound or whatever you're bringing forward, you have to do as many things as you can to feel good about it. We are personally -- like our lab now is moving towards doing some human cell work, tissue culture cell work, and the goal would be to correlate what we're doing in animals with that. So I think if you can have a correlation again across the board and feel good about it, yeah, do it and feel better about your drug. I don't think it's a bad thing to do.

PARTICIPANT: I just wanted to point out the antibiotic resistant bank offered by the CDC and FDA. It's a collection of 130 Pseudomonas and Acinetobacter organisms that are serious, a lot of different antibiotic resistance mechanisms. The genome sequence

is known. I think it's a fantastic resource that the FDA and CDC group are offering. Oh, and available for free in the United States.

DR. COX: Yeah, we're aware of that. I don't know the full extent of it, but we can certainly get you a contact, too. We'll try and do that before the day is out.

And then another comment? No. Okay.

DR. MILLER: We're going to have a lot of discussion later, and we were running a little bit late. So maybe we should break for lunch and we'll continue the discussion later.

(Lunch.)

Approaches to Animal Model Development, Future Direction/Next Steps

DR. KNISELY: Welcome back from lunch, everyone. It's 1:35, so I think we better go ahead and get started, and hopefully others will trickle in. So this afternoon we're going to have a kind of whirlwind tour of what's out there, at least a glimpse of what's out there in terms of existing animal models for Pseudomonas and Acinetobacter. And then we'll have a

panel discussion about the features of those models and where we might need to go.

So our first speaker this afternoon is Dr. David Andes. He is a professor in the Department of Medicine and Medical Microbiology and Immunology and Head of the Division of Infectious Diseases at the University of Wisconsin, and Director of the Wisconsin Antimicrobial Drug Discovery and Development NIH Center of Excellence. His research programs are multidisciplinary and strive to identify strategies to combat antimicrobial drug resistance.

PK/PD Consideration for Animal Model Development

DR. ANDES: Great. Well, thanks very much for the invitation to come today. This has been great so far.

Can you hear me back there? All right. Now can you hear me? Great. All right.

So I've been asked to talk about use of mouse infection models to answer PK/PD questions. Here are my disclosures. I primarily work with a variety of drug companies doing PK/PD. And here's what I would

like to discuss. So first, what PK/PD questions can these infection models help address? What study variables can impact the PK/PD answers we get? And can these models and PK/PD analysis be used to predict clinical efficacy in patients?

So first, why do we conduct PK/PD infection model studies? And simply put, it's to improve the probability of a positive therapeutic outcome in our patients.

So what do we do? Simply put, we tie drug potency, in this case, MIC, to antimicrobial exposure looking for a relationship between exposure and effect.

You've already seen the two workhorse models introduced during the public comments earlier today. These include the murine lung and thigh infection models. They roughly mimic soft tissue infection, sepsis, and pneumonia respectively.

They are neutropenic. This allows the growth of organisms that otherwise are not pathogens in these models. With neutropenia, they support the growth of most bacteria. We utilize organism burden at the site of infection as the primary endpoint. And I can tell

you that this correlates very closely with survival in these animals using a much lower number of animals. You would have to use anywhere from three to five times the number of animals for statistical significance using survival as an endpoint.

A large number of comparator antimicrobials have been examined in these models. And, importantly, as I hope I'll convince you in a bit, that outcomes from PK/PD analysis in these models have been useful for forecasting efficacy in patients.

So this is roughly our study design. Here on the left, we have an image of a mouse getting a thigh infection. And we initiate therapy in these animals 2 hours after infection. This allows the organisms to grow into the log phase of growth.

These are both acute models. And the endpoint after therapy is at 24 hours, where bacterial burden is assessed, followed by an analysis of the relationship between the pharmacokinetics at the infection site to outcome.

So how do we determine how much and how often to administer an antibiotic? the first PK/PD question

we ask.

So to do this, we, in these infection models, vary not only the dose level, but the frequency of administration, varying in what's called a dose fractionation design.

Here's an example of data output from one of these studies. This is with a cephalosporin antibiotic in the thigh infection model, and you can see here on the right, on the X axis, we see increasing doses. And in these dose fractionation studies, we've administered five total doses of drug fractionated into four different dosing intervals. And what you can see is as the dosing interval is shortened from once daily to every 3 hours, you see a shift in the dose-response curve to the left, indicating enhanced efficacy. So administering smaller doses frequently would be the most effective dosing strategy.

We then examined these exposures pharmacodynamically. So each of these dosing regimens is expressed as one of the three traditional pharmacodynamic parameters, percent time above MIC, Cmax/MIC, and AUC/MIC, using a Sigmoid Emax Model. And

you don't need to be a mathematician to see that in this case, the tightest data fit is with time above MIC, as you would expect with a cephalosporin.

And these types of analyses have, I would argue, been completed for every antibacterial that has come to market. And this study design has been, without an exception that I'm aware of, been useful for discerning the pharmacodynamic driver. Is it time above MIC, AUC/MIC, or Cmax/MIC?

So the first question, again, is, Which is the driver? And I think it's clear from the published literature that dose fractionation in these models reliably defines the PK/PD driver. This works with Pseudomonas and Acinetobacter as well.

The second critical question is, How do we define the pharmacodynamic target, or how much drug do I need for efficacy? So to do this, we used the same infection models and the same type of analysis, in this case, introducing additional bacterial isolates, preferably isolates with MIC variation. And then we calculate the amount of drug required for a variety of endpoints, varying from stasis, which is the burden of

organisms in these tissues at the start of therapy, to killing endpoints, 1 and 2 log kill relative to the amount of drug required relative to the burden of organisms at the start of therapy.

As I mentioned, I would also like to discuss some of the pharmacodynamic variables that can impact the pharmacodynamic target, and I'll discuss a few of these. I'll discuss those related to the organism and some of those related to the host and how the host handles these antibiotics.

Let's first start with the organism. And so here is data again with the cephalosporin with a Pseudomonas infection, in this case, the thigh model. And we have a treatment study with two organisms.

So the Pseudomonas strain on the left you can see with over this time above MIC exposure, the response curve has shifted to the left, compared to the Pseudomonas isolate number 2. What this results in is a stasis target for the strain 1 of 16 percent, and almost 40 percent for strain 2. So which of these, if either, is the pharmacodynamic target? And how do we address this?

To address this, we add additional strains, in this case, 14 strains, again in the same model. And you can see here in doing so, we reduce the variability and identify the pharmacodynamic target for stasis, in this case, is a time above MIC of 30 percent, moving to 40 percent for a 1 log kill.

So how many strains do you need to incorporate into these models to robustly define the pharmaco-dynamic target? I would say that's a difficult question to answer definitively, but you would certainly like to have enough isolates where your median and mean approach each other and have the lowest coefficient of variation as possible. I probably wouldn't put less than 8 to 10 of these strains in these models because of strain-to-strain variability to define the pharmacodynamic target.

We would also like to examine the impact of MIC variation. And here this slide represents a very large number of studies, so this is 65 dose-ranging studies, 65 organisms, several cephalosporins, penicillins, and carbapenems with MIC range for each of these drug classes varying more than 1,000-fold.

And in this case, each of these data points represents the time above MIC exposure needed for a stasis target. And what you can see is within drug class, that the pharmacodynamic target is in each of these situations relatively similar across the different MIC phenotypes.

We can dive deeper and look at the impact of specific drug resistance mechanisms on the pharmacodynamic target. Here's an example with strains expressing -- producing extended spectrum beta-lactamases, a large variety of them.

This is data with 20 organisms and 4 cephalosporins. And you can see the relationships are very similar on the left with the ESBL-producing organisms, and on the right, and, in fact, if you superimpose these two graphs, you see that the pharmacodynamic target in this case is the same regardless of whether or not an ESBL produced or you have a wild type strain without ESBL production.

Let's move to the host and how the host handles the drug as a PK/PD variable, in this case, the impact of protein binding. So this is data from the

mouse thigh model with seven fluoroquinolones. So for the fluoroquinolones, the PK/PD driver is the 24-hour AUC/MIC. And what you can see when one looks at total drug levels, it would seem that the amount of drug needed for, in this case, stasis in the thigh model is quite a bit higher for gemifloxacin and garenoxacin, but what you also see is that the mouse protein binding for these two drugs is quite a bit higher than the other fluoroquinolones studied in this case. However, when you normalize for free drug levels, you get a level playing field, and that across the board, you would need an AUC/MIC in this case. And this is a study against pneumococcus, you need a 24-hour AUC/MIC of about 25.

So it's important to consider protein binding in these studies. It's also important to realize while most commonly the degree of protein binding in mice is similar and approximates that in humans, there can be differences. So this needs to be accounted for in translation of these animal model PK/PD studies to patients.

Infection site. So for Pseudomonas, and

Acinetobacter, as we've heard earlier, the lung is an important site of infection. We are able to look at the impact of infection site and infection site PK in these models. This is data in an MRSA infection model, both the thigh and the lung, with an oxazolidinone, and each of these vertical bars represent the oxazolidinone AUC/MIC needed for on the left, the stasis endpoint, and on the right a 1 log kill.

And what you can see is you needed quite a bit more drug for the same endpoint in the thigh compared to the lung. These are from two different labs. And we speculate that the reason for this is because of the higher ELF concentrations relative to blood concentrations of the oxazolidinones in these infection models and in patients.

We can look at that more specifically in each of these infection models. Here is data with an anti-infective that's under development where we would predict efficacy would be the same in the lung and the thigh. And I say that because the ELF pharmacokinetics approximate the free drug plasma pharmacokinetics of the same antibiotic in the same mice. And, indeed,

that's what you see in treatment studies with this antibiotic where the AUC/MIC in ELF and free drug plasma is the same for stasis and 1 log kill are very, very close.

Now, as with protein binding, it's important to recognize that, again, while ELF pharmacokinetics or infection site pharmacokinetics can be similar in the mice and in patients, there can be differences as well, so you can't rely solely upon the mouse ELF pharmacokinetics to predict what's going to happen.

Patients, I think the best example of where that was a problem is with ceftobiprole, where in the mice, the EFL to plasma penetration ratio was close to .7, and it was much lower in patients, and I think most of us know how that drug fared in those pneumonia trials.

So the value, an additional value, of these particular models is that there is a large amount of experience in defining the pharmacodynamic target. In fact, there is experience with more than 100 individual drugs, more than 2 dozen drug classes, with a standardized panel of organisms, including clinical

isolates, ATCC isolates, and those with defined resistance mechanisms.

One of the points that came up earlier was the importance of studying these clinical isolates and the debate between whether or not you should study ATCC strains or clinical isolates. I think studying both is important.

The value of the ATCC strains is we have a large amount of experience across a wide variety of drugs. So if you've got your new drug, we can tell you how that performed against these other 100 drugs and how those did in patients.

So what I hope I've convinced you now is that these mouse models can define the PK/PD target, but, again, there are important variables to consider, particularly protein binding and infection site pharmacokinetics. But does this mean anything for our patients? Can we use the answers from these mouse models to forecast efficacy in patients? Why might this work?

Despite the fact that the mice are smaller and the pathogenicity of these organisms in the mice is

somewhat different, as long as we account for infection site PK and recognize and remember that the drug target is not in the mouse or in the patient, but in the organism, so if we express the exposure pharmacodynamically relative to the MIC, we should get the same pharmacodynamic target in the mouse and in patients.

And there is actually data to suggest that this is indeed the case. This is data that some of you may have seen before, and Dr. Ambrose presented this in the summer. And this is a comparison of the answer we get from the animal models and the answer we get from treatment trials of these same antibiotics in our patient.

So on the left here we have data from the mouse thigh model with an antibiotic called tigecycline. And you can see here as we increase the AUC/MIC, we see stasis and then we see up to a 2 log kill with AUCs/MIC greater than 100.

When we can then do, or what ICPD folks did nicely, is to look at the exposures with tigecycline in patients. So they look at infection site

pharmacokinetics from these clinical trials, and in this case looked at the MICs of organisms from these clinical trials, and you can see here that the exposure of tigecycline in the HAP/VAP studies would have been enough for the stasis endpoint in this infection site.

It's a bit worse when you break this out for ventilator-associated pneumonia than it is for hospital-acquired bacterial pneumonia. And it's not surprising that with these lower exposures and large amount of PK/PD variability that you might expect treatment failure, as was observed in this clinical trial.

The models can also predict success. So this is data in the mouse lung model. This is from Arnold Louie and George Drusano's group where with meropenem against Pseudomonas aeruginosa in this model, you can see a nice relationship between the free drug, in this case, infection site time above MIC, so the ELF time above MIC, with maximal efficacy up near time above MIC values at greater than 75, somewhere between 75 and 100 percent.

What were the meropenem exposures in the

treatment trial? In this case, I believe this was 2 grams every 8 hours, and one sees that exposure, while there is a large amount of variability, the mean exposure was up near the plateau for efficacy in this treatment trial, and one would have predicted in this case treatment success, and treatment success was observed.

So just so that you don't think that they were cherry-picking with these examples, here is a group of data that they pulled together from treatment trials for community-acquired and hospital-acquired pneumonia. So 20 different drugs, all of them but one studied for hospital-acquired pneumonia. There are 14 of these antibiotics for which treatment was successful and regulatory approval obtained, and six that failed to gain approval.

What does this data look like when you look back at predictions from the infection model PK/PD? So if you're a visual person, look on the left, and this is the relationship between the probability of PK/PD target attainment in the animals and the probability of NDA approval, with the solid symbols representing those

treatment trials for which the drug was approved, and the hollow symbols representing those failures.

So as you move up and increase the likelihood of target attainment, you see more drug approvals. Now, there are some drugs that had high likelihood of target attainment for which the NDA was not approved, in this case, due to, on the far right, safety, this is with garenoxacin, on the left, below here, is the daptomycin exposure.

And if you're numerically inclined, you can see here the probability of target attainment. And so by target attainment in this case, I mean you achieved a 1 to 2 log kill in the lung model with these antibiotics. And you can see if you achieve that target, you had a very, very high likelihood of FDA approval.

What have we learned from this type of analysis? I think we've learned that these mouse PK/PD models can be used to forecast efficacy in patients. And we also get some sense of where we should be, what the hurdles should be, in these infection models for the infection site. So if one is going after hospital-

acquired pneumonia, one should be probably looking for a 2 log kill in the infection site model in the mice.

But as you've heard in the discussion earlier, a mouse is not a human. There are differences. These infection models for PK/PD purposes are not meant to study organism pathogenesis or virulence. These mice are not susceptible normally to these pathogens. There are occasional strains that can produce infection in non-neutropenic animals, but most of these organisms need the absence of neutrophils for recovery.

And there can be pharmacokinetic differences between the mice and patients. But as long as you take that into account, differences, potential differences, in protein binding, potential differences in ELF, I think it's clear that these models can be used to forecast effective regimens in our patients.

And with that, I'll thank you for your time.

DR. KNISELY: Thank you, Dr. Andes.

Our next speaker is Dr. Matthew Lawrenz. He is interested in defining interactions between bacterial pathogens in the host immune system. In his research, he routinely uses animal models to study both

bacterial pathogenesis and for vaccine and therapeutic testing. Many of his efforts have focused on plague, but since 2014, he has also been using the mouse model for preclinical screening of novel therapeutics targeting MDR Pseudomonas aeruginosa.

Mouse Model of Pseudomonas Infection

DR. LAWRENZ: All right. I would like to start by thanking the organizers for the invitation. It's great to be here in a Pseudomonas field instead of just a Yersinia field.

The model that I'm going tell you guys about today was developed at the University of Louisville, and this has been done in collaboration with a good partner of mine, Jon Warawa, who is also in the Microbiology Department. And we have a team at the Center for Predictive Medicine that's shown on the left there that is our animal core that runs all of our animal experiments for us and allows us to do these experiments, as they're very labor-intensive.

I also want to mention that this model was designed in collaboration with NIAID, specifically to start to go after and look at new therapeutics for

Pseudomonas.

The model that I'm going to tell you guys about today, I want to highlight a couple things as we go along, and I probably won't be able to hit all of the different items that go into this model development, but this has been published, just so you guys are aware, in a couple papers that are on the bottom.

What I'm going to highlight today is a little bit on the installation method that we used to establish a lethal infection. And this is a lung infection, so we're looking at a pulmonary infection.

We have talked a lot today about the differences between immunocompetent and immunocompromised. We tend to use the immunocompromised model for all of our therapeutic testing, but I'll show you a little bit of comparison between those two. More of this is covered in the publication.

We spent a long time developing nonsubjective bio endpoints, biometric endpoints, for this model, and we did that because, as you saw, we have a large team

of people that we work with. We also have multiple health check time points over the course of this infection model. And so we wanted to be able to rotate people in and out without losing or I guess gaining variability based on behavioral characteristics that can be used.

And then at the end, I'll highlight some of the multiple parameters that we can use to monitor therapeutic efficacy in this model.

When we first developed this model, again, this is a pulmonary or lung infection model, and so there are different ways that we can introduce the bacteria into the lungs. And, of course, one of the most common is intranasal. We do that a lot for our plague model, but we have to worry about for certain infections whether or not there is going to be some complications due to involvement of the upper respiratory tract.

There is also the potential for variability in the inoculum as the intranasal or the droplet of bacteria descends the upper respiratory tract into the lower respiratory tract. And so we didn't want to

introduce those variables into the model.

The other method that's used widely is a conventional intratracheal model that is usually a surgical model. It can be a little technically difficult. It can be slow. And we were worried about the potential for blood contamination during that surgical procedure.

And so the method that we came up with is an intubated mediated instillation method, we call it IMIT. Essentially we intubate the animals, the anesthetized animals, and then guide the catheter down the trachea using an otoscope. We then insert a blunt needle into the catheter, and we instill about 50 microliters of bacteria through that using a Hamilton syringe. We also include about 100 microliters of an air pocket behind that, and so we're essentially causing the animal to take a nice little deep breath after the bacteria are instilled.

We can do this with a team of about two people, and when they're well trained, we can do less than a mouse a minute by this method. And so that allows us to be able to do large groups of animals when

we're using this instillation method.

These two images here show some of the benefits of IMIT. So one of the things that we notice when use this is that we get a broad distribution of the inoculum. So in the center, there are the lungs of an animal that was instilled with Coomassie dye, and then we harvested the lungs immediately after the instillation process. And you can see the dye is distributed throughout the lungs in this case.

The other thing about the IMIT method is that it's highly efficient at its delivery. So the graph on the right-hand side there shows the CFU that was intended to be delivered to the lung on the X axis, and then what we recovered in the lungs 20 minutes post-infection or instillation on the Y. And you can see with those different doses that on average we were able to recover about 98 percent of inoculum. So we have a very good idea of how many bacteria we're instilling in the lungs each time we perform this.

Furthermore, it's very reproducible. At each one of those doses, there are multiple animals in there, and you can see that it's very tight in the

inoculum. So we feel very confident that at least as we start out on the inoculation that we're not introducing a lot of variability into this model.

So using the IMIT model, we were able to establish lethal infection in both immunocompetent and immunocompromised animals. All the data that I'm going to show you from here on out is using a clinical isolate of Pseudomonas called UNC-D, which is a lung clinical isolate. On the left-hand side is just our BALB/c model, which we consider our immunocompetent model, and on the left is our leukopenic or neutropenic model that is established using cyclophosphamide.

And what I what to highlight here is, of course, as we've been discussing today, introducing neutropenia allows us to establish an infection with a much lower dose of bacteria. And, again, we tend to use this for all of our therapeutic studies. There are problems with the immunocompetent mice if we put large numbers of bacteria in the lungs and then hit them with antibiotic. The immune system doesn't really like dead bacteria and LPS floating around, so there are some complications based on that.

As I said, the other thing that we wanted to do when we established this model was to establish those biometric endpoints. And so I'm showing you data here on the temperature. With these animals, they all have transponders, so we monitor their temperature. We also monitor the heart rate and oxygen levels using a mouse ox system. And then we've developed a checklist based on the kind of natural history of this disease.

So the temperature is on the right-hand side. The colors match to the infectious doses in the survival curve on the left-hand side. The symbols that are open are animals that succumbed to the infection. The ones that are closed are animals that survived. And the grey box is kind of our normal range for temperature. And we did this for both heart rate and oxygen also.

Based on these, we knew that our primary indicator of morbidity is temperature, and we know that once we reach about 26.6 degrees, that those animals won't make it till the next health checkpoint. And so based on these criteria -- these are also I think fairly stringent -- we're trying to get as close to

death as we can get, and these allow us to get there. And so individuals that go in use this checklist in order to monitor morbidity.

In addition to temperature, there are a lot of other things that we can measure in this model to look at therapeutic, potential therapeutic, efficacy. So, of course, we have survival and mean time to death that we can look at.

The temperature, that's all from a lethal infection, but we have seen with therapeutics that we get a nice rebound in the temperature if those therapeutics are going to work.

We can also look at bacterial load in the lungs. So at the site of infection, our primary place where we want to look.

But we can also follow whether or not the bacteria disseminated in the bloodstream from that site by looking at the spleens.

And then, finally, for our animals, we also harvest the lungs and we look at pathology, so we can look at if there are differences in the host response or the development of pneumonia in these animals.

For the last couple minutes here, I just want to show you a couple of examples of some antibiotics that we've run through this model. The two antibiotics that I chose for today are polymyxin B and meropenem. The data that's on the slide right now is the in vitro EC50s of these drugs, and the MICs are shown at the bottom.

You can see with polymyxin -- again, I'll remind you guys, this is a multidrug-resistant UNC-D strain of Pseudomonas. And the MIC for polymyxin was at 1 mcg/ml, making it -- it falls into the sensitive category. With meropenem, it's a little more resistant, it falls into that intermediate category at 8 mcg/ml.

When we look at lung, the lung burden, and so these animals were instilled with a lethal dose of the bacteria. Three hours post-infection we began antibiotic treatment by subcutaneous inoculation, and we continued that treatment every 8 hours for 5 days, and then continued to monitor the animals 2 days after we ended treatment. The lung data here is pulled at the time that the animals were euthanized, so either if

they succumbed to the infection or if they made it to 7 days post-infection.

You can see with the polymyxin, which is on the left-hand side, that as we increase the dose of polymyxin over the course, we begin to see significant decreases in the bacterial load in the higher doses. And we see the same trend in meropenem even though it is in that intermediate resistance category.

I also want to point out on the meropenem side that you can see that there are several animals, the dotted line indicates the limit of detection, and so there are animals in this case that we were unable to recover detectable bacterial loads in the lungs.

So even though we were able to see a decrease in the bacterial loads in some of the higher doses of the polymyxin treatment, when we look at the survival curve here now, we were unable to differentiate any differences between treatment with polymyxin and the vehicle-only controls. We also saw no change in dissemination. So there was no control of getting to the spleen, and there were no significant differences in the development of pneumonia in these animals.

On the other hand, when we looked at meropenem, we see a nice dose-dependent increase in the mean time to death, moving from left to right, there is from lowest dose to highest. We can calculate an ED50 in these studies. We also saw that the higher doses of meropenem where we're seeing protection are protecting the animals from developing septicemic infection by the organism.

And I'm not showing you it, but the ability of the bacteria to protect the animals also results in a decrease in pathology in the lungs.

So I just want to leave you guys with a little bit here. The mouse model is very amendable to many different things when we look at it. So we can use several different routes of administration for these therapeutics in our hands. I showed you data from using subcutaneous treatment, but we've also done IP injection. We've looked at molecules that can be directly instilled into the lungs. We can do that either by intranasal, or we can actually go back and reintubate the animals and treat them by IMIT also. And we also have used nebulizers to look at aerosol

delivery into the lungs.

Importantly, I think this model has the capability not only to screen monotherapies, which I showed you today, but also combination or adjunct therapies. So especially with meropenem, where we're at that intermediate range for treatment, if there are potential therapies that might be thought to improve the ability of existing antibiotics to work against these organisms, the meropenem, at least in our hands and with this strain, allows us to do adjunct therapies.

Using this model, so far we've been able to test 11 different novel therapeutics, and I just wanted to highlight that those have ranged anywhere from novel antibiotics to small compounds or even biologicals. And even though this is a leukopenic model at the beginning, we're also able to test therapeutics that may potentially target the host as opposed to just targeting the bacteria, so an immunomodulatory type of compound.

So I would like to thank you for your time, and I guess I'll leave it up for the next speaker.

DR. KNISELY: Thank you, Dr. Lawrenz.

Our next speaker is Dr. Daniel Zurawski. From 2009 to the present, he has been contracted by the U.S. Army to manage a laboratory in the Wound Infections Department at Walter Reed Army Institute of Research. And he is also an adjunct professor at the University of Maryland Dental School in the Department of Microbial Pathogenesis.

Mouse and Pig Models of Acinetobacter Infection

DR. ZURAWSKI: All right. So good afternoon, everyone. I'm going to try and keep you awake here in the afternoon. After lunch, everybody is getting a little sleepy.

So I'm in the Wound Infection Department literally I think it's 8 years today, to the very day. I started March 1, 2009. And the work I'm going to show today was really the first probably 3 or 4 years of work that we did there. And the typical disclaimers here for a government employee.

One of the things that we care about obviously with the Army are IED blasts and soldiers getting

wounded, and then the consequences of that oftentimes is infection afterwards. And so these are just some of the numbers on that. And the typical bad actors that we see there are the ESKAPEE pathogens. I'm adding an E there now because of E. coli, where we've seen a big uptick of E. coli, but certainly Acinetobacter was a big player in this with the Iraq War, and we needed countermeasures, and we needed to understand the bug better.

As Dr. Bonomo pointed out earlier, we still don't, even to this day, understand the pathogenesis very well and how Acinetobacter does its thing, but we're learning more and more as more people are working on it now. So that's what I decided to focus on when I started.

But at the present day, our department is doing quite a bit of things. We're still studying basic science, pathogenesis. You can see that, it's on the left there of the slide, and that funnels into all these therapeutics that we're using and attempting to use. A small molecule program we have that's run by ET, Experimental Therapeutics. We have a pretty robust

phage program now. I'm working on monoclonal antibodies. And then we also have the kitchen sink there. And we also partner with pharma and academia also testing their therapeutics as well, all funneling into our animal models.

And I did want to bring up one point in the little intro here about Acinetobacter because some people have suggested that it's not a good pathogen or it's a low virulence pathogen. I disagree. I actually think if the situation is correct, it will take off and cause quite a bit of problem, and clearly the amount of patients where the mortality levels are so high, it's definitely an issue.

And I wanted to bring up that the CDC numbers are 750 deaths a year in the United States. That means every day two patients die from Acinetobacter. Now, that may not seem like a lot, but to me, it's a lot. That means today, just doing this meeting, two people died because of this bug, and it shouldn't be the case. We should be able to get over this and really start making new drugs and get on it. Those are people's families that are being affected.

I'm showing this slide because I wanted -- this is a recent study from Brazil -- I wanted to show that all these bugs are playing a role in wound infections. This is actually a wound infection study. But if you actually combine Acinetobacter species together, so you've got baumannii there, and you've got the other Acinetobacter species, it actually makes 12 percent of all the wound infections that they're seeing in Brazil. And this actually holds true, if you look at a lot of studies in Central America, South America, Asia, this number actually holds very true most of the time. So it's actually one of the things that from the world's standpoint is very important. We're seeing a lot of it in Third World countries as well.

So I'm going to go through model development. I'm going to take this into three parts: so strain selection, then talk about our murine pulmonary model of infection, and then go through our wound models. We have two wound models of infection, we have a mouse wound model and a porcine model as well.

So strain selection. We were kind of getting at this earlier. Which strain are you using? What are

you going to use? And so when I first started in 2009, I had tried using the ATCC strains in a lung model of infection, and I could never get the animals that die, and I'm like, how are we going to do this? We don't have a survival model to test anything. And I was really interested in virulence. I wanted to find the genes that were required for virulence so we can make monoclonals against them, and we're still doing this to this day. But if you don't have a strain that kills the animal, it's really hard to do a survival study to look at virulence.

So that really prompted me to go in and dig into these other strains, certainly the clinical isolates that we have the access to with the Army, we were getting them from patients very routinely. And then I set up basically a criteria of what I wanted that strain to be.

So I wanted it to be clinically relevant, it's got to be a recent isolate, it's got to have actually shown to have an infection in a human. I want them to be MDR/XDR to fit the current problem of drug resistance. I want it to be genetically amenable so we

can actually look at virulence factors. So it had to have basically some susceptibility somewhere so we can actually use markers to knock out genes. So we put that on the list. Cause some more infections across all different animals of infection.

So I'm going to show you these models today. But we've also done a rat wound model at one time. We did a rat fracture model with some collaborators. And this same strain carries through all these models.

We wanted something that's more virulent. So, of course, you want a more virulent strain to kill the animal if you're doing a survival study, but it's also about enhancing the therapeutic window. So if you can actually show a large difference between something that's treated versus untreated, that's really important. And with our wound model, I'll show you why that's important.

Can we predict efficacy? So Matt just showed some stuff where he took FDA-approved antibiotics already through his models and shown that they actually work and kill bugs. We can do the same with our models. And can it be standardized across more than

one model? And as I said, we did that as well.

So early literature. ATCC strains. Those strains are 60-plus years old now. They're from 1955. They're just completely not relevant at all. They're not virulent at all. It doesn't take into account any recent evolutionary changes.

And as I mentioned earlier, the Acinetobacter genome is extremely plastic. It changes within a patient literally in hours. It can pick up DNA very readily, incorporate it into its genome, picks up plasmids from all over the place, it will pick up a Klebsiella plasmid, it will pick up a Salmonella plasmid, and utilize it. And it can go into its genome or keep it as a plasmid. I mean, it's very, very volatile as far as its evolution goes. So when you're using strains that are that old, you're missing a lot of these recent events, and those strains are not -- they're literally susceptible to every antibiotic.

So we went into the recent literature, and some French groups were looking at clinical isolates for virulence. And then Tom Russo, at SUNY Buffalo, was also looking at a rat pneumonia model as well, and

he was looking at some strains. So we kind of used those folks to kind of set up what we did.

And so here is a list of 33 strains that we looked at. These are all military isolates. They all came from patients that actually had an active Acinetobacter infection. And they're basically set up here as far as their clade, and the clade is based on pulsed field gel analysis.

And if you look at all the military data from 2003 to the present, you can basically put Acinetobacter into 34 different clades. And there are four major clades. And so that's the first ones at the top there. And then there are 30 minor ones. And so you can basically group them into that just based on PFGE.

The PFGE is not necessarily the best way to characterize strains, obviously we use MLST and all the other things now as well, but this did give us a way just to look at variability.

And then these isolates, I chose them for their variability based on PFGE, but I also chose them for where they were isolated from. So we have some

that are from bone infections, some that are from blood, some that were from wound infections, to really give a nice diverse set here that we can use and look at.

And so this is just PFGE data that shows how different they are. And clonal complex there on the right is how the European groups originally started classifying Acinetobacter into three clonal complexes. Now there's a fourth complex, and you can kind of see the four complexes there, how they're laid out. And so these minor clades are actually little subtle changes in the genome within the four major clonal complexes.

And if you evaluate these strains looking at different in vitro characteristics, you can look at biofilm diversity here. So you can see all these strains make varying biofilms. Some make really great biofilms, some don't, but this is all in vitro.

And Dr. Bonomo mentioned motility before. So this is a slide showing motility, and it's also being kind of superimposed on top of optical mapping. So optical mapping is a way to chew up DNA and put it on a glass slide and look at it, and so there's a company,

OpGen in Gaithersburg, that's doing that. And you can actually sort your diversity that way just based on what the DNA looks like via the optical mapping. And if you do that and also put motility there, you can see how diverse they are. They just all do different things. They swim on plates differently, and there are different patterns. So clearly this set is very diverse.

But what we cared about was virulence, and so here's virulence in the wax worms. This is the first model that we started with because there was a publication by Anton Peleg about it. And you can see here that the strains all have varying virulence in this model.

We also have a little bioluminescent Acinetobacter down there. We use bioluminescent Acinetobacter as well in some our models. I'm not going to go into that as much as I probably should have today, but if you want to ask questions about it, you can ask questions later.

And this model is very black and white. They're either dead or they're alive. And the worms

either turn black when they die or they stay white. That's how it works. And you can see that there is one strain, the square bar there, that's going across that pretty much by 24 hours over 90 percent are being killed or over 80 percent at least are being killed by this strain. And that's AB5075.

We do like this model because sometimes it does appear to translate into mice later on. And we love the model because we can do tons of worms. They're super cheap. You can buy 1,000 worms for like $50. So like our N group of 20, I mean, you can do tons of stats on this and really evaluate and get some good data.

Okay. So on to the pulmonary model. So once we got an idea that the strain 5075 was more virulent, in this model, we wanted to then follow it up and see, does this really hold true into a pulmonary model?

So we're using an intranasal inoculation.

6

We're pretty consistent with this. We do 10 most of the time. We lose about a log sometimes in the nose and the areas, stuff like that, but we're pretty

5

consistent. We always get at least 10 into the lungs

every time. And we've measured at time zero.

So it is cyclophosphamide. It's a temporary neutropenia. It allows us to start with a real low

6

inoculating dose, 5 times 10, and then we see disease progress. And basically, with 5075, we see death in just 48 hours.

We can do clinical scores, our endpoints, our clinical scores, and CFU enumeration. We always take CFU day two. This is right before the animals basically start to perish. So we are getting our control group, and then we're also getting our treated groups as well at that point.

And I just wanted to show, here are bacterial levels in lung tissue, just between the different strains I showed earlier. So you can see 5075, the white box, has a higher CFU burden in all these strains than the other strains, and then that, in turn, leads to what we see as dissemination, enhanced dissemination, with that strain, and basically more death, more death by day two.

The histopathology, if you look at the lung, the lung sections here are A and B, and in B, there's a

small arrow there on the right pointing to basically what we see are droplets, they're almost like droplets of bacterium, which I think are actually biofilm-like, probably in the middle, and then you have planktonics on the outside as they're growing in the lung.

Typically, the bacteria are on the sides of the lobes of the lung that are closest to the trachea. So Acinetobacter is strictly aerobic. It does not do so well in low-oxygen conditions. It does okay, but it doesn't do great. So as far as the actual pathology of the model, you're actually seeing the Acinetobacter focus more in those lobes closer to the trachea.

However, after about 24 hours, getting into that day 2, we start to see dissemination from the lung. It then goes to other organs in the body. On the left there, that is the adrenal glands. On the right, D, that is the kidney. So it goes to both of those places, colonizes both organs. We also see a colonized spleen, liver, heart as well. So we looked at all the organs of the animal. And that basically leads to organ failure, and then death.

I would like to think that that's what's going

on in patients, in human patients. I don't know if we really know for sure. I don't know if a lot of work has been done clinically on how we looked at organs after patients have died and what kind of colonization we see. It's something we can maybe talk about later.

We also put through rifampin as a proof of concept, so we've got varying doses of rifampin here. And you can see that 5075 with sub-MIC treatments or subclinically relevant treatments will still kill the animal at 48 hours, but if you increase the concentration up to 10 mg/kg, we start to see clearance, and the animals survive. And so we can use that to our advantage when looking at things.

And so one point that I want to show is show that advantage as an application. We partnered with Spero Therapeutics and looked at their compound, SPR741. This is a new compound that potentiates, it actually pokes holes in bacteria and allows other antibiotics to get into the bacteria much more readily.

And in this case, we actually took advantage of the rifampin data that we had where at 5 mg/kg we only see 50 percent survival, but if we take that

concentration and then mix it with Spero's compound, you can see we push that up to 95 percent survival in this situation.

So understanding how the antibiotics work with your model strain, you can then use that to your advantage and always have clinical comparators throughout.

And then if you look at CFU, we can also measure CFU, and the combination there are the pink animals, and we see about a 5 to 6 log reduction with the combination, whereas the rif alone, it's about 4 logs. And so the combination is definitely allowing for a better hit on the animals, which, in turn, is leading to this clearance.

Okay. So that's the lung model. We're going to move to the wound model now. The same concept here where we do a temporary neutropenia. We inject cyclophosphamide day -4, -1. Concentrations there are 150 mg/kg, and at 100 mg/kg, that's basically throughout the literature for most of these models. Again, temporary neutropenia the first 3 days.

I wish I had brought the slide where we see

the white cell count just come up at day 3. It drops to about 1,000 or less, by day 3, it's up to like maybe 5- or 6,000, like it's already starting to skyrocket. Normal white blood count in a mouse is anywhere from 3- to 5,000. So it actually goes above that normal count with the infection there at that day 3 point, so it really comes roaring back.

The other note I wanted to make about the neutropenia is that there are other insults to injury. With the soldiers, we see massive blood loss. They're likely getting morphine because they have crush injury or blast injury on top of the actual wound infection and things. And we know that that systemically plays a role in basically dampening the immune response.

Okay. So both Pseudomonas and Acinetobacter, we know the innate immune response is clearing these bugs out. That's the thing that is you're a normal healthy human, clears these bugs out no problem, that's what's doing it, it's neutrophils and white blood cells. If you're getting morphine, morphine dampens that immune response. If you lose a ton of blood and a lot of blood replacement, dampens the immune response.

So all that neutropenia is doing is trying to temporarily dampen that immune response to kind of get something clinically relevant.

In this situation, so you can see the endpoints up there, wound measurement, CFU enumeration. In this situation, we're only doing 5 times 10 Acinetobacter, very, very low dose, we think very clinically relevant. We can kick that dose up to 1 times 10 or go actually more than that, probably like a log higher, we'll actually see dissemination from the wound, they'll go septic, and kill the animals.

So we had to play around to find that dose. If we went too little, we didn't see a nice wound infection. If we went too high, we were killing the animals via sepsis. So finding that dose was key. And we can use this model, by the way, for all ESKAPEE pathogens, not just Acinetobacter. And we had to do the same thing for all of the strains that we're working with.

We have an amazing team. They're rare. Our animal team right now consists of four people, and so it literally becomes like a factory assembly line where

each person has a different station.

And so one person anesthetizes and shaves the animals, the next person wounds them, passes the mouse on, where we measure the wound with an Aranz instrument. This is the older instrument. We have a better instrument now that fires a laser, actually two lasers, to measure the wound, and then we measure the wound over time with the instrument down the road. And then the next team member is then inoculating.

We also inject with a small amount buprenorphine to keep the animals pain-free, and then we put a Tegaderm dressing overtop.

So basically there are all these stations, and each person is doing their job. And we can roll through 60 mice in about an hour and a half, 2 hours. And it's amazing. Every week we're doing 60 mice. It's 52 weeks a year. Our capacity now is up to 300 mice if we really wanted to, that our team is expanding. So it's quite an effort.

And this is just some of the data that you can get. So we can take punch biopsies out of that wound, we can do SEM, we can do 16S for microbiome, we take

blood from the animal, look at systemic, if there is anything systemic going on for the immune system. We can certainly plate and do CFUs at different time points. You can just do gross pathology, look at the wounds itself.

So this time we've got antibiotic comparators here. So on the left side you've got your untreated nasty wound infection, goes all the way out post-day 21. We see it close maybe around day 25. Again, the animal is not dying. The wound eventually closes and heals. But if you treat with rifampin or you treat with doxycycline, these are two antibiotics that this strain is susceptible to, you start to see the wound close around day 15. So you can see that there in that picture.

And what that leaves is this big 10-day what I call therapeutic window where if you have an antibiotic or a new compound or something you want to test -- and we test lots of things, we're testing predatory bacteria now, we're testing bacteria phage, we're testing lots of different antibacterial approaches -- but if you want to get an idea of how well it's

working, it may not be as good as rifampin in this thing, your wound may not close at 15 days, but it may close at 18. And so what that does is it provides guidance back to our partners, hey, this worked well, maybe not as well as rifampin, maybe you could tweak your molecule a little bit, go back to the chemistry drawing board, make it, I don't know, more soluble, make it more efficacious, just give them more ideas to work with. So that's how we kind of work with the folks we work with.

For wound area, you can see obviously a big difference in treated versus untreated, and, again, that carries through those time points. And this is all published, by the way, in AAC, and you can see the reference there.

Histopathology, we can do the same thing and track wound closure and the re-epithelialization. I've heard some comments that, well, mice don't heal the way humans do because they have contractile healing. That is true. You can add a splint so there isn't contractile healing with mice, and then they will heal by re-epithelialization. All that being said, it still

is -- again, we're seeing efficacy and we can still use this to look at efficacy.

I wanted to show this. It wasn't in the original slide deck, but this is using a PNA FISH probe. We're actually look at Acinetobacter throughout the wound bed and even all the way down. So what's kind of cool about this, all the way down here is muscle and then bone.

And what we saw in patients, what we saw in infected humans, was the Acinetobacter actually progressed its way -- I was getting stuff from doctors all the time over at Walter Reed telling me, look, we see Acinetobacter going from the wound all the way to muscle, it's progressing throughout the tissue. It's not just staying in that wound bed.

And so when we saw the same thing -- and that's not the greatest slide, but we were seeing Acinetobacter in E, which is the basal bottom of that muscle down there, and we were even seeing it sometimes associated with bone. So we were seeing what we thought was very close to what's going on in human patients, and this is what I was hearing from the docs

over at Walter Reed.

Okay. So CFU. Obviously, we can take punch biopsies out of the punch that we made and then count CFU, and, again, there's a nice difference there. And everybody, of course, talks about wound infection and biofilms, and so we certainly wanted to evaluate biofilms as one of our methods, and we just do this SEMs, so we take that punch out.

You can see the biofilm is really nasty in the placebo, but with treatment, it kind of clears out when we use different treatments. What's really left there is just it looks like polysaccharide and red blood cells that are left when we treat properly.

So I want to give one example. This is not published yet, but I hope to publish it soon. One example reviews the wound model to look at something. So in this situation, I'm looking at a monoclonal antibody to a virulence factor of Acinetobacter, the type six secretion system. We add the antibody prophylactically day -1, and then we just run our wound model the way we normally would, and you can see that it actually curbs the wound size, it actually allows

for the wound healing to take place a little faster. We're still working on this. We're actually making a cocktail against the number of targets on the surface. But the proof of the pudding is always actually looking at the animals themselves, I think.

And if you just look on the left, these are really nasty infected Acinetobacter wounds, and this is actually at day 15, real nasty, but if you look at the antibody treatment, you see much less swelling, you don't see the necrotic tissue kind of expanding out from the site, and we're kind of keeping that infection more localized to the initial wound bed where we had it. And this is just one antibody dose prophylactic.

And if you measure CFU at day 3, you can see there is an effect on CFU. So it's only about a log, maybe a log and a half tops, but that's enough that it keeps that infection a little more limited. And so I just wanted to show you an example of where we've used the wound model in the past.

Endpoints of this model: time to close is obviously the big one; CFU/g for tissue; biofilm; gross pathology; histopathology. We can also do immunology,

animal weights we found to be very predictive. So if we measure the animal weight in actually the lung or the wound model, any infection, it doesn't matter what bug it is, we see 20 to 25 percent decrement in animal weight. Treated animals, if they look good, even at day 1, we won't see that weight decrement, so it's something to look into. And we can certainly look at microbiome as well.

Further optimization of the model. We're now starting to look at adding urinal nitrate to basically humanize excretion. So if you add this, this actually prevents the kidneys to work as well in the mice, and then you get a little more PK/PD more like humans as far as excretion is concerned. We've looked at other strains of Acinetobacter because I thought some people would ask about that. I said earlier if we increase the dose, we can get dissemination and sepsis.

We're also looking now at other mouse strains. We're using diabetic mice. Dr. Bonomo mentioned the A/J mice and this other mouse strain that are more susceptible. And we're also looking into humanized mice now, and we've done some work with them just

recently, and that's something where I really want to keep pursuing, because these humanized mice actually have a human immune system, and we're going to be utilizing those more. And that work is all funded through a DARPA project on predatory bacteria. So that's where we're going with that.

Finally, we want to look more at the immune response. So that's the wound model. I'm happy to answer questions about that in the mouse. I'm going to move to the pig now.

The pig model, again, very similar to all these other models except here we are lowering the cyclophosphamide dose. So unfortunately, there is a lot of, again, on-the-fly learning here on what's going to work best for a pig. Now, when I say "pig," I'm talking a Yorkshire pig. This is a 25-kilo, 30-kilo pig. This is not a mini pig. This is a pig where it's on a farm and you get bacon from them. That's how big this pig is.

Walter Reed has an amazing facility. It's a 100,000-square-foot animal facility in our basement. It's huge. Okay? No one has something like this. And

so it allows us to do all sorts of animals. We have non-human primates, pigs, sheep. We have all sorts of things going on there. But the pigs are really -- what I like to say about them is if there is a drug that you're interested in getting approved, there is an 80 percent correlation to FDA approval using a pig model. So if you can get it to work in a pig, they've seen 80 percent FDA approval rate there. Now, I don't know if it's going to hold true for this, I'm just saying that that's what's out in the literature right now.

So we wanted to make this wound model obviously in their Wound Infection Department, and it's doing the same kind of idea where we do cyclophosphamide, but what we had to take into account, mice have very robust innate immune systems. They clear everything. They live in the wild. They're a dirty kind of animal. Pigs are actually a lot more like us --

(Laughter.)

DR. ZURAWSKI: And so the cyclophosphamide we've got to use is very, very small, it's only one dose at 25 mg/kg. I told you before, we're doing 150

and 100 mg/kg in mice. So it's 4X, 6X in mice, and pigs -- and it's only one dose in pigs, but still at day -4. We based this off of a Haemophilus paper where they're looking at Haemophilus infections in pigs, but we had to really do some playing around with that dose to get it to go.

Where we've seen infection, so we're dosing

5

with about 10 Acinetobacter, and, again, the numbers

9 10

rise to about 10, 10 in that wound site. If we go too high, again disseminates sepsis, the pig dies unfortunately. If we go too low, we don't get those same high CFUs that would be associated with infection.

The other beauty of the pig is that we can do multiple wounds on the back of the animals, so it allows us to facilitate lots of wounding and lots of trials. We basically take a number of wounds at any given day. And it's a 12-millimeter punch biopsy, but then we can do again smaller punches within there. Those are 6-millimeter punches within the 12-millimeter where we can then take it and then do other data aspects afterwards.

This is what a layout looks like on the pig.

So you've got the actual layout that we have like in our protocol. We randomize it. So some people were complaining, well, if I do stuff on one area of the back of the pig, how is that going to reflect to the other area of the pig? So now we're just like forget it, we're just going to randomize it, we just do whatever.

And then you can see what the actual pig back looks like there. Again, it looks a lot like human skin. And, again, we can pull, we can do a punch biopsy out. That's us pulling the punch out. We basically set up a DuoDERM kind of layer on top where the wounds are being made, and that allows us to keep our inoculum localized to that area.

Once we do that, we put a dressing overtop. So that's the bottom. Then we put a bandage kind of over those dressings. And then a jacket so the pig doesn't actually like try to scrape it off or shake in his cage or whatever where he is. And, in fact, this keeps the wound closed and basically monoinfected with Acinetobacter.

I did want to mention that, too, a lot of

people say, "Oh, microbiome, you must have 200 different bugs in there when you're looking at this stuff." While true, we do have other bacteria in there for sure, Acinetobacter completely dominates. It's the only one that's there present above 10. So it's there at a huge level. It basically takes over that wound site irrespective of the other things that are there.

So, again, with this model, this is what it looks like. We can do punches out of that. And then we can do -- in this situation, we're doing placebo versus polymyxin treatment, which is typical, found in Neosporin. And here we did like a polymyxin gel. You can see that we see a CFU decrement over time.

And so to summarize, we're basically looking at a pathogen that we actually think is much more aggressive than people said in the past. Dr. Bonomo mentioned earlier there is one clade that actually killed people that weren't compromised at all. That's Colonel Lesho's work and my postdoc's work, Crystal Jones. And basically they had little wounds on their arm, and in 24 hours those patients died. It went septic and killed them. Thankfully, this is only one

clade of Acinetobacter. It's called Clade B. It's only 10 percent of what's out there, but it's out there. So it's something to take into consideration.

5075, the strain I'm using for all the work I showed here and for the rat models, it's an ST2 strain. ST2 is the most isolated ST group of all the Acinetobacter and all the outbreaks in the world. So, again, we're trying to use a strain that is clinically relevant.

We developed two murine models with that strain that rely on neutropenia, and we feel that that reflects the pathogenesis that we see with the organism when I talk to doctors over at Walter Reed. We also developed a porcine model using similar techniques.

On the whole, we've tested 14 different antibacterials in the last 2 years using our models and compared them to the standard of care. And this is just a list of them there. Some of them are blacked out due to our people that we're working with, but the bottom line is we've tested a lot of things in these models, and we've been doing it now for quite some time and know how things behave.

So I will end there. Any questions -- or probably we just have to move on.

I do want to acknowledge our group, which is amazing, and the former members who helped with all this. This is not a one-person job; this is a multi-person job. These animals take a lot of effort to do, and as I said, it's quite an ordeal. So I'm very happy and honored to represent them today.

DR. KNISELY: Okay. Thank you. Yes, we will move on from pigs to bunnies.

Our next speaker is Dr. Binh Diep. He is an associate professor at the University of California, San Francisco. His research is focused primarily on the epidemiology and pathogenesis of Staph aureus, but he has recently adapted his rabbit pneumonia model for preclinical testing of novel monoclonal antibodies for Pseudomonas aeruginosa.

Rabbit Model of Pseudomonas Pneumonia

DR. DIEP: Hello. Thank you very much for giving me the opportunity to share with you two different rabbit models of Pseudomonas pneumonia. These are my disclosures. We receive funding from the

NIH as well as various industry partners to develop, validate, and use different rabbit infection models.

So I first would like to make some comments on, why rabbits? Every year annually in the United States, about 140,000 rabbits are used in research compared to 10 to 20 million rodents. And so rodents offer distinct advantages over rabbits, including the fact that they are inexpensive, easy to handle. You can really do cool genetic studies with mice that you can never do with outbred New Zealand white rabbits.

And it's also long thought that lagomorphs, which include rabbits, are more similar to Rodentia, which include mice and rats, than they are to primates, based on their sheer morphological characteristics. So why go through the trouble of using rabbits? However, it has been shown that primates and lagomorphs are more closely related to one another when you align 91 autologous protein sequences from these different species, that they're more closely relate to one another than to the rodents.

And it's also known for many, many years that rabbits are rather similar to humans and chimpanzees in

their susceptibility to LPS. So, for example, in rabbits, 2 to 4 ng/kg of LPS injected intravenously is sufficient to induce a threshold change, physiological changes, like an increase in temperature as well as TNF-alpha.

So compare that to 1 to 5 ng/kg for human and chimpanzee, and you see it's quite similar. For mice, one would need to go to 500 mcg/kg to see a threshold change. And when you look at the lethal dose in rabbits as well as in swine, it's 10 mcg/kg. In mice and rats, it's 8 to 15 mg/kg. So you need 800 to 1,500-fold more -- 1,500-fold more LPS to cause lethality.

So in my lab, we use about 1,000 to 1,100 rabbits annually, mostly for Staphylococcus aureus. But recently we started working with Pseudomonas aeruginosa as well as Klebsiella pneumoniae.

And we use many different types of invasive models in rabbits, and they can be roughly categorized into two different groups. The higher throughput rabbit models for which we can do about 32 rabbits per week, it doesn't seem much when you compare it to mice,

but 32 rabbits per week, and these sort of animal models are suitable for initial lead candidate selection and validation. You need the high throughput. And this includes the rabbit model of acute pneumonia, rabbit model of severe sepsis, rabbit model of acute bacterial skin and skin structure infection.

We also have lower throughput surgical models for which we can only do 6 to 10 rabbits per week. And this is more suitable for further efficacy testing or mechanism of action type study. And this includes the aortic valve endocarditis model, prosthetic joint infection model, and more recently we started working with a ventilator-associated pneumonia model.

So what I'm going to do today is share with you data on testing the protective efficacy of a novel monoclonal antibody developed by MedImmune in two different rabbit models, the acute pneumonia model as well as the ventilator-associated pneumonia model.

So this molecule, MEDI3902, from MedImmune, is a multifunctional by specific antibody targeting Pseudomonas aeruginosa. The Fab portion of the

antibody targets the virulence factor PcrV, and the scFv portion of the antibody targets the persistent factor Psl. It also has a typical hinge-Fc portion that's required for OPK.

And I should mention that this molecule has been tested extensively in mice already, and it has been shown to be protective across multiple different invasive mouse models. So here what we wanted to do was to test it in a different species, in rabbits, and we wanted to know if MEDI3902 can reduce mortality and/or prevent major morbidity in a different species.

So first the rabbit model of acute pneumonia. In this model, the rabbits, these are New Zealand white rabbits, outbred, about 2.5 kilograms. They were randomized for either prophylaxis at 24 hours before infection or treatment at 1 hour post-infection with different doses of MEDI3902 or a control antibody. The control antibody is an irrelevant isotype matched control antibody.

These rabbits were challenged endobronchially with 9e7 CFU of Pseudomonas aeruginosa Strain 6077. This is an ExoU-positive cytotoxic strain. The rabbits

were then monitored every 2 hours, at least every 2 hours, for the first 36 hours post-infection, and then three times daily thereafter. They are then euthanized at 96 hours post-infection. The humane criteria for euthanasia is a respiratory rate greater than 90 breathes per minute, the animal is cyanotic and has a cough. Death in these animals is imminent, so they should be euthanized.

Here are the Kaplan-Meier survival curves. You can see that the animals treated with a control antibody have five out of six of those animals die -- unfortunately -- oh, there it is.

So between 12 and 24 hour post-infection, most of the animals die, five out of six die, and then the last animal die at 52 hours post-infection. At the lowest dose of MEDI3902, at 0.3 mg/kg, what we see here is 50 percent overall survival. And in animals that were treated at 1, 5, and 15 mg/kg, they all survive.

And then when you look at the lungs of these animals and take the weight of the lung, animals that were treated with 15 mg/kg, they all die and they have very large lungs. The lung weight/body weight ratio

for an infected rabbit is 4. That's like 10 grams, a typical weight of 10 grams, for the lungs divided by the body weight of 2.5 grams. So that's a 4. These animals, the lung weight/body weight ratio is between 15 and 24. So the lung goes from 10 grams to 40 to 60 grams.

And so why is it so heavy? When there is damage to the alveolar endothelial barrier, there is this massive influx of fluid from the blood into the air space, and that resulted in just respiratory failure and death in these animals. So the lung weight/body weight ratio is a direct measure of acute lung injury.

Animals that were treated at .3 mg/kg, three of the animals die. They're shown here in the closed symbol. The three that survive are in the open symbols. And you can see that the ones that die have bigger lungs. Now, animals that were treated with 1, 5, and 15 mg/kg have very significantly lower lung weight/body weight ratio.

So this suggests that MEDI3902, its mechanism of action in this model, the reason it's protective is

it's protecting against acute lung injury and death.

That was data for prophylaxis. Here are the data for treatment. What we see here is the treatment was administered 1 hour post-infection, and all of the animals die in the control group. Four out of six animals survive at the lowest dose at 1 mg/kg. And at 5 and 15 mg/kg, all of the animals survive. And survival again in this model was correlated with a significantly lower lung weight/body weight ratio.

So these are the lung CFU count. And you can see that at the highest dose of MEDI3902, you get quite a significant reduction in CFU count. Remember, we put into the lungs about 9e7 CFUs, so it's quite a drop, and it's very significantly different from animals that were treated with a control antibody. And that's all consistent with data in the mouse showing that this antibody enhances OPK.

So we also recently only in the past 6 weeks, 7 weeks, started working with another model, ventilator-associated pneumonia. We worked with this model for a while in Staph aureus, but we have adapted it for use with Pseudomonas only very recently.

And so in this model, rabbits were randomized for prophylaxis at 24 hours before challenge with a control antibody or MEDI3902, and here we only have four rabbits per group. The rabbits were mechanically ventilated with a lung protective low-tidal volume of 6 to 7 ml/kg, for 2 hours, and then they were challenged through the endotracheal tube with 2.5e7 CFU. So this is three-, four-fold less bacteria than required for the acute pneumonia model presumably because of ventilator-induced lung injury that predisposes the lung to this lower inoculum infection.

These rabbits were monitored continuously for 36 hours post-infection. Blood samples were obtained every 2 hours. And you can do this with a big animal like a rabbit. And we did comprehensive blood analysis for each of the time points. We do a five-part WBC differential. We measure blood gas, acid-base, electrolyte, creatinine, lactate, glucose, and various enzymes, markers of renal and liver injury. So I'll show you those data. What I'll show you today, I'll show you the rabbit.

So these rabbits were intubated under view

control with a pediatric endotracheal tube, a cuff, endotracheal tube, and then they are connected through a one-way breathing circuit to an anesthesia ventilator. And we have six of these ventilators, so we can ventilate six rabbits concurrently. And so the ventilator is set at an FiO2 of 35 percent, isoflurane 1.5 percent. The peak inspiratory flow is 4 liters per minute. Respiratory rate of 35. PIP, 15 centimeters of water. PEEP, 6. I:E ratio of 1:2. So all of the settings is really to achieve a low-tidal volume while also achieving normal ventilation, normal carbon dioxide in arterial blood of 35 to 45 mmHg.

So the carotid artery is cannulated for invasive blood pressure, but it also has a port here to be able to take arterial blood from which we then measure all sorts of things.

The rabbit is also cannulated on the ear artery for infusion of, in this case, a fluid with 5 percent dextrose. This is the basic setup for our ventilator-associated pneumonia model. And we also have a more advanced setup where we do transpulmonary cardiac output measurement as well as mixed venous

oxygen saturation, but I'm not showing you those today because those are what we do with Staphylococcus aureus.

So here are the basic data. So the rabbits that were treated with a control antibody, they all die. Two of the rabbits die at 12 and at 13 hours post-infection. And then one rabbit died at 17 hours post-infection, and another one at 24 hours post-infection. And they get different colored symbols so you can track the chronology of events leading up to death.

So all the animals that were treated with MEDI3902, they all survive, they all survive to 36 hours post-infection, when we euthanize them.

So here are the lung weight/body weight ratios for these animals. The two animals that die late, they have huge lungs. This one has a 40 grams lung, and this is 51 grams lung, and you can see it here. And the lungs are huge, and they are necrotic. It's likely that these animals die from respiratory failure, and I'll show you the evidence for that.

But, curiously, there are two animals that,

the blue and the green animals, they have small lungs that are not so much bigger than normal uninfected lungs, and yet both of those animals die. When you look at the lungs, even though the lung is small, there's a lot of necrosis. When you look at the lungs from MEDI3902, the lungs look relatively normal compared to infected lungs, very few areas of necrosis, so suggesting that, again, MEDI3902 protects by preventing this acute lung injury.

So we took serial blood samples so we can do serial blood cultures, and you can see that animals that were treated with a control antibody all develop a bacteremia that then peak at the time of death. Some of these are in the 1,000 CFU/ml in the blood, so it's quite high bacteremia.

Three of the animals in the MEDI3902-treated group had no bacteremia, but one of the animals did have a very high-titer bacteremia, but it did survive, at least to 36 hours post-infection.

When you look in the lungs at the CFU count, those animals that were treated with a control antibody have significantly higher CFU count than those that

were treated with MEDI3902. In the spleen, kidneys, and liver, no difference.

So what causes death in the four control antibody-treated rabbits? Although MEDI3902, the fourth rabbit that survived, what are the effects of the persistent bacteremia on the host? And we want to be able to answer these two questions. And so what we wanted to do was to look at the blood for some clues as to why these animals die.

And here these are complete blood counts. This is the white blood cell count. And you can see that animals that were treated with a control antibody, the white blood cell count dropped rather dramatically, especially at the time of death. Platelets remained -- platelets a little bit decreased. Curiously, one of the animals that was treated with the MEDI3902, but that survived, had high bacteremia. It has slightly elevated platelet levels.

But look at neutrophils. Neutrophils significantly decreased. There's neutropenia. And neutropenia, again, there's neutropenia in the peripheral blood, so the neutrophil is getting traffic

somewhere. And so often the case with ventilator-associated pneumonia, they get traffic into the lungs where the neutrophils actually participate in the acute lung injury. And we'll be able to confirm that this profound neutropenia in peripheral blood is actually due to the trafficking of neutrophils into the organs, and the histology will confirm that, which we don't have right now.

The monocytes, also there is a dramatic decrease in monocyte count.

Now, the pO2/FiO2, this is a very reliable clinical marker of hypoxemia. And you can see that three of the animals that were treated with a control antibody have very, very severe pO2/FiO2.

And there is also a concomitant increase in carbon dioxide in the blood, suggesting that these three animals die of respiratory failure, but very curiously, one of the animals, the animal in the blue, the control-IgG animal number 2, it has normal pO2/FiO2, and yet it has -- the carbon dioxide tension in the arterial blood is less than 10.

This is very severe hypocapnia. And

hypocapnia is, actually when you look at the old literature, hypocapnia, very severe hypocapnia, is a consistent feature of Gram-negative septic shock. So we wonder if it is the case that this animal died of multiple organ dysfunction, septic shock-induced multiple organ dysfunction. And do we have any clues to the multiple organ dysfunction in this animal?

So let's keep tracking of the animal in the blue. It has very severe base deficit. There's a massive increase in lactate level. So lactate, when it gets to this range of 19, 20, it really indicates very, very severe tissue hypoperfusion consistent with septic shock.

And when we look at the potassium level, potassium is filtered and excreted by the kidney. So this is a marker of kidney injury, of acute kidney injury, and potassium just increased very, very sharply. Creatinine also increased sharply in that particular animal, suggesting the involvement of acute kidney injury in this animal.

We also looked at liver enzyme alanine aminotransferase, and we see that it's also increased

for this animal, as well as the amylase. Amylase is produced in the pancreas. So this is suggesting that maybe there is also acute pancreas injury in this particular animal.

So taken together, so this is the blue animal. It has many of the features that are consistent with septic shock. And so the cause of death in that one particular animal seems to be septic shock. But for the other three animals, control IgG-treated animals, 1, 3, and 4, they likely die of just profound respiratory failure. It's pulmonary failure.

The animals that were treated with MEDI3902 look rather good when you look at these different blood markers, except one that has this persistent high bacteremia, but it didn't die, and it looks like lactate at the very end looks pretty good. So there's no evidence really of severe sepsis and septic shock in this one particular animal.

This work was done by postdocs and graduate students and undergraduate students in my lab, who are very dedicated. You can see that this is a 36-hour experiment continuous. And it's also done in

collaboration with Tony, Bret, and Ken at MedImmune.

Thank you.

DR. KNISELY: Thank you.

Okay. So from bunnies back to pigs, our last speaker in this section is presenting remotely. He is Dr. Gianluigi Li Bassi. Is he on the line?

DR. LI BASSI: Yes, I am.

DR. KNISELY: Wonderful. Great. So Dr. Li Bassi is board-certified in anesthesiology and critical care medicine. In 2013, he obtained his Ph.D. on the management and prevention of infectious interstitial and cancerous pulmonary disease. And he is the head of the Division of Animal Experimentation and Attending Physician at the Department of Pulmonary Critical Care Medicine at the Hospital Clinic, Barcelona.

Okay. Your slides are up and we're ready to go.

Ventilated Pig Models of Pseudomonas Pneumonia

DR. LI BASSI: Very good. Thank you, Sumathi, for the nice introduction. Can you hear me well?

DR. KNISELY: We can.

DR. LI BASSI: Okay. Perfect. So in the next

15 minutes, my main objective is going to be to try to convince you that we still need large animal models to study these Pseudomonas aeruginosa models of infection.

These are my potential conflicts of interest related to this presentation.

Next. So, of course, we learned from many of previous talks the importance of animal experimentation. And in my daily routine, of course, the Declaration of Helsinki, we have this declaration in our lab that clearly states the importance of animal experimentation before translating any result into the clinical setting.

Next. So in particular, the rationale for the use of pneumonia models, these models, they clearly elucidate the pathogenesis of the disease. They even characterize the interaction between the pathogen and the host, and, of course, even that we are in this workshop organized by the FDA, we can test new antimicrobials, new drugs, against these pathogens.

Next. So far, you guys have focused particularly in small animal model of Pseudomonas aeruginosa pneumonia, and we do have many models, say,

rats and mice and rabbits.

Next. I'm a critical care physician, and we primarily focus on pneumonia that develops in patients that are admitted in the ICU. And, of course, one of the main challenges to develop a model of these patients is that animals, and particularly small animals, that there are many challenges in ventilating this animal, even giving hemodynamic ventilatory support is important to these animals for long period of time.

Next. Many of the previous speakers presented many models of pneumonia and even other infections. I'm not going to go, of course, into any detail on this model.

Next. But I would like to particularly draw your attention on the number of publications throughout the years in small animal models and large animal models. As you can see in the top part of this slide, there has been an exponential increase throughout the decades of the number of publications in my mice, rats, and rabbits. Instead, if you look at publication in pigs, sheep, in general, in large animal models, there

was a steady decline in the number of publications.

And I was very happy to hear Daniel, from Walter Reed, that we have a list, 85 percent of FDA acceptance of new drugs, and when they are tested in large animal models. And as I said, one of my purpose here is try to convince you that irrespective of this decline in the number of publications, we still need these kinds of labs and these kind of animal models.

Next. Now, many of the previous speakers, they show you the benefit of mice models of Pseudomonas aeruginosa pneumonia. And we do know that these small animals, they have limited cost, and this is very important. They have potential for extensive genomic manipulation. This is very important because you can even create a specific condition that then you can find in the clinical setting and to look at their response of a challenge with this pathogen. However, there are also limitations in these models.

Next. And in particular, very careful, there are dissimilarities in the anatomy of these mice in comparison with humans. Even the physiology of the respiratory tract have some differences. And even the

immunatory system and their response to a bacteria challenge sometimes is different than the real response in humans.

Next. And some other limitations, in particular, limitations in my specific field of investigation, which is intensive care medicine, is that long-term mechanical ventilation in these models sometimes is absolutely unfeasible.

Also, you cannot model the same level of severity of patients in the ICU because when you reach a very high level of severity, these animals, they just die, and this is not the case in humans in the ICU. For specific diseases, like ventilator-associated pneumonia, you cannot even replicate the exact pathogenic mechanisms. And there are many scientists, that they are just skeptical on the possibility of translating these results in the clinical setting.

And one of the reasons in particular in the ICU is -- next slide, please -- one of the reasons is just that ICU patients are very complicated. There are many co-existing diseases. They vary for age. There are many supportive therapies. The severity of the

illness sometimes is so high they cannot even be reproduced in mice. So, of course, we need models that they closely resemble this condition.

Let me give you an example. Next slide. This is an interesting study in which they measure the growth of Pseudomonas aeruginosa with vasoactive drugs, and without these drugs. And vasoactive drugs, like norepinephrine or dopamine, are drugs that we daily use in any ICU. And as you can see here, when you use these drugs, for some reason, the bacteria growth was even higher.

Next slide. And even the biofilm formation was much higher when you use these drugs. And, of course, if you cannot even provide all this hemodynamic support on the small animals, then you don't have a reliable model.

Next slide. One other limitation particularly in the ICU is that you cannot use the same devices that we are using in the ICU, endotracheal tube, the same ventilator, and these, of course, are a limitation.

But particularly, my major concern -- next slide -- is the length of the mechanical ventilation.

We do not have ICU patients highly severe that they are ventilated just for a few hours.

And the only way to increase the length of intubation and keep these animals alive -- next slide -- is to prolong -- to change the model.

Next slide. And to change the model and use large animals: dog, baboons, sheep, pigs. So when you have doubts in science, it's always better to ask the experts.

Next slide. Let me give you some examples from highly brilliant mind. For instance, Traber, Daniel, he was a brilliant physiologist from the University of Texas, and he was very clear in his mind in what kind of animals we should use to model the ICU patient. Would you, as a critical care physician, accept data from a drug study on intensive care patients who was not only not resuscitated with fluid, but who did not even have blood pressure and heart rate monitored. And sometimes this is what happened. And you cannot resuscitate or give vasoactive drugs to these animals.

Next. And one of my mentors from the National

Institutes of Health, Theodor Kolobow, who was another brilliant physiologist, one day he told me that it's rather unreasonable to believe the results from small animal studies, started right after breakfast in the cafeteria and completed in time for dinner, could be safely translated into valuable therapies for ICU patients.

Next, please. So what are the ventilated animal models of Pseudomonas aeruginosa pneumonia that we use in our lab?

Next. Now, before starting with the models, you need to carefully consider some features. And many of the previous speakers, they highlighted the importance of the burden of the bacterial inoculum, the bacterial strain.

Mind you, unless you talk with an ID physician or a microbiologist, many physicians in many hospitals worldwide, they think that Pseudomonas is just Pseudomonas, and all the strains are the same. This is not the case. The bacteria strain, we know from many previous presentations, they can completely change the disease and the kind of burden to this animal.

And even you have to decide the right type of animals, for instance, sheep, they produce a lot of saliva, pigs, they do not. And even decide what the proper length of mechanical ventilation.

Next. Let me give you an example based on our data. For instance, we use many Pseudomonas aeruginosa strains, and some of these strains, they have a mortality of 20 percent, and some others are multidrug susceptible, they do not have a high mortality rate.

Next, please. Even for the need of a vasoactive drug like norepinephrine, some strains, they produce huge septic shock, and they need norepinephrine, and some others, they do not have these requirements.

Next. I believe that first the model and the gold standard for Pseudomonas aeruginosa models in large animals was developed from the (inaudible) set by Johanson.

Next. They actually -- this was (inaudible). They were not supposed to develop a model of ventilator-associated pneumonia. They tried to create a model of acute respiratory distress syndrome

injecting oleic acid.

Next. But then after a few days of mechanical ventilation, what they found was Pseudomonas aeruginosa -- well, they found ventilator-associated pneumonia.

And when they looked -- next, please -- at the positive pathogens, they found that the majority of these pathogens of this pneumonia were caused by Pseudomonas aeruginosa. So this is not a pure model of Pseudomonas aeruginosa. And there are many advantages and limitations of this model.

Next. Of course, I said that this is the gold standard because they have the same anatomy, they are very close in their response to the bacterial burden, but they just aspirate whatever pathogen they have in their oropharynx after intubation. So many times, there is not even the right -- there are heterogeneous amount of pulmonary challenge.

And, of course, using primates has high cost, there is scarce animal availability, and there are strict legislation. For instance, in our university, we cannot even use these models.

Next. A model that is matched easier and has

had huge development in the last decade was the Luna's Model. This was a model developed in pigs. These are animals mechanically ventilated for 72 hours, and they are managed exactly like in an ICU.

Next. So these are animals that they are challenged with Pseudomonas aeruginosa through a fiber-

6

optic bronchoscope with 15 ml of 10 colony-forming units per milliliter of a suspension of Pseudomonas aeruginosa, and this 15 milliliter into each lobe. They have five lobes, so a total amount of 75 milliliters.

Next. And after approximately 12 to 24 hours, they already have the first sign of infection. The upper part on the left, you can see the sharp increase in temperature, and on the upper part of the right, a decrease in pO2:FiO2 ratio, which is a marker of pulmonary failure.

Next. And if you can at the end of this study, when they dissected the lungs, they found extensive severe pneumonia in all the lobes.

Next. So the main advantages of this model, there is a rapid development of severe pneumonia. This

is a highly severe pneumonia. It's ideal to study efficacy and safety of new drugs, but there is an extensive pulmonary bacterial challenge, and many times this is not the real pathogenesis of pneumonia because patients, they do not have this kind of bacterial challenge.

Next. So in 2014, 2013/14, we decided to develop another animal model, in particular, of Pseudomonas aeruginosa VAP.

And our goal -- next -- was specifically to reproduce the pathogenic mechanisms of VAP. We choose it through aspiration of pathogens across the endotracheal tube cuff.

Next. So we use the same kind of pigs, which were oro-tracheal intubated and mechanically ventilated for 3 days. Standard sedation, analgesia.

Next. And this is very important.

DR. KNISELY: Sorry to interrupt. Can I ask everyone who is not our speaker to please mute themselves online? We're hearing someone else's conversation. Thank you.

Okay. Please continue.

DR. LI BASSI: Yeah. So this is very important, the position. When you keep the animal in this position, they have the trachea in an oblique orientation, so they start aspirating across the endotracheal tube cuff, and this is exactly what happens in humans who are mechanically ventilated and intubated.

Next. So we challenged not the lungs, but we challenged the oropharynx just 5 milliliters after 4 hours and 5 milliliters after 8 hours of a high concentration of Pseudomonas aeruginosa multi-susceptible. And then, as I said, they started aspirating this pathogen.

Then after 72 hours -- next -- we performed the autopsy. You can go ahead faster here because they are just a picture of the autopsy.

These are standard method, straight (inaudible). And then we obtained the lungs. In the (inaudible) samples, we biopsied each lobe of these lungs, and export the results.

Next. We found an exponential increase in tracheal secretion colonization, and this is the proof

that these animals, they were aspirating throughout the time on mechanical ventilation.

Next. But the most important data are regarding the colonization. This is not a broad high colonization by Pseudomonas aeruginosa like in the previous model, just the most dependent region, right medial lobe, right lower lobe, which are gravity dependent in this model, gets the highest colonization, and this is exactly like humans, that usually ventilator-associated pneumonia develops in one or two lobes.

Next. Histology confirm pneumonia in one or two lobes.

Next. And there are many advantages, as I said, in particular in this model reproduce exactly the pathogenic mechanism, but there are also some limitations: high cost, labor-intensive, and time-consuming, and you need 3 or 4 days and a huge team of investigators to perform this study. But it's ideal to study particularly preventive measures for this disease.

Now, briefly, just for the application --

please go ahead, and again -- we received consistent funding by health care industry, government agencies, and medical societies, but I would like to draw your attention particularly on the health care industry. As I said before, there has been a strong decrease in this kind of lab, particularly in the U.S., throughout the years.

And if you go to the next slide, among all the industry that we receive or we are receiving funding, the majority is from the United States because they are looking for labs that could perform this study because, as I said before, there are some limitations in small animal models.

Therefore, in conclusion, many of the previous speakers -- I'm sorry, go ahead -- many of the previous speakers clearly explained the importance of animal models of pneumonia. And several animal models, like the Johanson model, is one that represents a milestone in this field.

Next. But particularly for the pig model, ventilated pig model, we still think it's the most appropriate if you want to be 85 percent sure that

you're going to receive FDA approval or even you can translate your findings closely into humans I think because there are close anatomical similarities with the humans, good survival, irrespective of the severity, and this is the important possibility of prolonged mechanical ventilation. And in the second model, we presented reproduction of the main pathogenic mechanisms.

Next slide. Thank you very much for your attention.

DR. KNISELY: Thank you. That was fascinating.

DR. LI BASSI: Thank you.

DR. KNISELY: Thank you very much.

We're running a little bit behind schedule. Let's take a 10-minute break, come back at 3:40. Thank you.

(Break.)

Research Support and Resources

DR. NAMBIAR: Our first speaker in this last session is Dr. David Boucher, who is from BARDA, and he serves on the NIH Adjudication Committee, Data Review

Committees for Actelion and Medtronic, and is the Treasurer of the IDSC.

Dr. Boucher.

DR. BOUCHER: All right. Thanks a lot, everyone, for the organizers. Thanks for giving me a little time to talk up here. I'll try to keep it brief. I'm going to go off my slides. I'm only going to go into about the third one, so if it looks like it paused if you're online, don't worry, you're fine.

I'm with BARDA. That's the Biomedical Advanced Research and Development Authority. For those of you not familiar with our organization, we are a member of the PHEMCE.

Are typical mission space is to pick up promising programs from our partner agencies -- the NIH, NIAID, CDC sometimes -- at that IND phase, Phase 1, area; bring those through what's commonly called the "Valley of Death," this area of clinical development characterized by high costs, high attrition.

Our ultimate goal is licensure of products. For products without a commercial indication, we like

to be able to extend that to procurement, and hand it off to CDC for inclusion in the Strategic National Stockpile.

I have a slide here labeled "CBRN Profile," that's chemistry, biological, radiological, and nuclear countermeasures. I am personally within the biological portion of that. And even within biologicals, I do therapeutics, so this is a very biotherapeutics-centric slide. If it was somebody from another area of my division, you would see different countermeasures here.

But we are historically a biodefense organization. If you look at our portfolio, our more mature programs -- our anthrax, smallpox, botulism -- we have several countermeasures approved and in the stockpile. Recently, particularly with the response to Ebola and Zika, we've become active in the emerging infectious disease area. And also in public health, we have several antibacterials in late-stage clinical development, and we're one of the drivers of the CARB accelerator.

I think John Rex was one of the first ones to mention that Tier C/Tier D area of the Animal Rule. If

you kind of focus on the upper two-thirds of this slide, that's kind of where we spend a lot of our time. We rely heavily on the Animal Rule. We do a lot of animal studies.

The way we get that done is through a non-clinical network. This is a network of private and academic partners that we recompete every 5 years. We are looking at capacity, we're looking at capabilities, we're looking heavily at quality.

Membership into this network allows you to bid on proposals or Request for Proposals that we would issue in the form of task orders. Proposals from the network come back to us. That's a competitive process individually.

And for those individual task orders, whether it's an NHP efficacy study, if it's model development, if it's strain panel production, we're going to take a look at the offerors' capabilities, their relative experience in that area, we're going to pick the one that we think might be able to help us the best, and then we sign a contract with them. We work very closely through these contracts on the execution of the

task order.

That's kind of where I'm going to go away from my slides. I'm going to put this up. I'm just going to talk very loosely. When we do animal development, again because of the phase of development we're looking at, we're not so concerned with downselection of candidates. We're not looking at optimization of leads. We are looking exclusively at studies that will support licensure. We are going to be bringing these studies to the FDA, so we might have a little bit of a different slant on how we see the animal model development.

Typically, what we're going to do is we're going to start with the challenge material. We want recent clinical isolates. If mortality is a suitable endpoint for the pathogen that we're looking to develop countermeasures against or a model for, we want strains that were involved in recent clinical cases that ended in fatalities. Again, in most of the pathogens we're looking at, mortality is a suitable endpoint.

We're looking to push that model such that when we're using it, an untreated control animal, we're

looking for as close as we can get to 100 percent mortality. This raises the bar for therapeutics that we're developing and also reduces the animal numbers that we need to meet statistical objectives.

We will take isolates. If we're looking at a pool of potential isolates, the first cut we make is only those isolates that will be available and free to use. We don't want to have restrictions on those. We want to be able to share them with who we want to share them with, and that's typically anybody who asks.

And we want, again, clinically relevant isolates. We want recent isolates. For a specific pathogen, we downselect further. I was involved with C. difficile, and what we looked at was things like ribotype toxin production, drug resistance, that type of thing, to get down to a panel that we think is clinically relevant at that time.

We produce master and working cell banks. We do extensive characterization on this, including deep sequencing, other things. And we put those on stability. When we go back into liquid nitrogen a year from now, we like to know what we're taking out.

When we go to animal model development, again, we're looking at pushing those animal models so that we will be able to do efficacy studies that stand up to FDA scrutiny. And we are looking heavily at quality. When we look at who can complete this work for us, we want technical expertise, of course, but we are heavy on quality, and we are kind of hammering documentation constantly.

So in the interest of time, I think I'll probably stop there. I think there are probably a lot more people you want to hear about Acinetobacter and Pseudomonas particularly.

If you're interested in the C. difficile program, the Burkholderia slide that I had up just a moment, just grab me. There are a couple other people here from BARDA. Any of us will be happy to talk to you more.

DR. NAMBIAR: Thank you so much, Dr. Boucher.

Our next speaker to talk about research support and services is Dr. Tina Guina, who is a program officer in Drug Development Section in the Division of Microbiology and Infectious Diseases at

NIAID/NIH.

DR. GUINA: Thank you very much for inviting me. And I think there have been a lot of good discussions today. So, again, I won't also take much of your time.

I just wanted to share some information about important research and development resources, including development and application of medical animal models for testing therapeutics.

So I'm in the Division of Microbiology and Infectious Diseases, and as you know, NIH and NIAID, we cover basic research, early translational research, and drug development in preclinical phase and through Phase 1. And our division works on all infectious diseases except for AIDS, so you can imagine there are lots of programs and there are lots of models that we are working on.

NIAID Antibacterial Resistance Program is aligned with National Strategy for Combating Antimicrobial-Resistant Bacterial Infections. And we actually support development of new diagnostics, therapeutics, and vaccines.

We also have published recently new Antibacterial Resistance Program document that talks about "Current Status and Our Future Directions," and this document describe our intention to support new types of therapeutics, such as non-traditionals, including those that actually act on human immune system, for example, bacteriophages, and also narrow-spectrum antibacterials that are sort of topic of this workshop.

Since we actually provide support and cover development, both basic and translational and early clinical research, we have a number of different mechanisms to fund investigators and developers to lower the risk of product development. So there are a number of different types of grants that are available for more early research and development.

And then we often enter into contracts with sponsors or investigators that actually have some kind of therapeutic candidates or clinical candidates that are in more advanced preclinical development. We typically establish those contracts and fund them through early clinical and sometimes Phase 2

development, but most often after successful Phase 1, we transition it over to our colleagues at BARDA.

In addition to developing individual therapeutics and products, we also have a whole host of preclinical and clinical development services. And I just want to -- I'll take a moment a little bit later to talk about them because apparently lots of investigators are not aware of them, and I think they should be, because we can test your therapeutics in various different animal models and to help you with many other stages of development at no cost to you under confidentiality agreements.

So I'm not going to go much in detail on this slide. I just provided this slide so that you see what type of funding mechanisms we have, and also a couple of useful links. For sponsors, they're kind of looking ahead, and they already have some kind of, I would say, therapeutic leads or clinical candidates, and they have to put together very large proposals, and they're looking for source of funding a year ahead or 2 years ahead or 6 months ahead.

I encourage you to actually look at the NIAID

website on which we'll list our NIAID Council-cleared concepts because often at NIAID Council, together with external advisory board, we discuss potential funding opportunities that are up and coming. So this actually can help you to look ahead and plan your proposals.

In terms of product development services, actually we have an ever-expanding host of services that include various types of in vitro and in vivo models for testing therapeutics. We also have some additional chemistry support and development.

Then toxicology, we can do toxicology studies for investigators, GMP manufacture for biopharmaceutical products and vaccines.

We also can help you with regulatory advice and help you put together documentation for IND.

And we provide these services through actually establishing contracts similar to BARDA and some other organizations. We establish a network of providers that are either at different CROs or at different academic labs that actually have the capacity, and we heard about some of them today. And in the past, we actually have worked with them very closely also to

develop -- in collaboration, to develop models for testing of therapeutics for biodefense indications, as you heard this morning. So we work with them very closely. But we also use these contracts to either develop new animal models, or we establish contracts with providers that have these models in place to test your therapeutics and vaccines and potential diagnostics. So actually this is again at no cost to you, and it's done under confidentiality agreements, and you keep your IP, and all the data is yours.

And I should mention also that we also have some Phase 1 units and vaccine testing and evaluation units that actually we can provide support for Phase 1 testing of new therapeutics, but also we have abilities to perform clinical trials in some special populations.

And Antibacterial Resistance Leadership Group is actually on the network of investigators and clinical trial sites that is looking into different aspects of working in antimicrobial resistance space such as potentially evaluating new diagnostics or maybe new regimens or dosing for already existing antibiotics. Those are just some of the examples.

So these resources are available to everyone. They are actually gap filling. So, for example, if you were going to do your entire development through these services, it would take very long because there's a process at every step. You have to provide some proof-of-concept data and prove that you're eligible to go to the next step of development and actually use the resources. So they're not really intended as a sole source of development funds, but they can fill really important gaps that can help you get to the next stage of funding either with NIAID or BARDA or some other organization externally.

Everybody is eligible. It's open to academic, investigators, and from any other organizations. And also it's open to everybody around the world, so we are not limited only to the U.S. investigators.

And you do not have to have current NIH funding to be eligible. You just need to have some proof-of-concept data and good ideas, and we will be talking to you and helping you all along. And we also actually have lots of experts in-house who are very familiar with different stages of especially early

preclinical drug development, and they can provide lots of advice as you go along. And, as I said, this is all done under confidentiality agreements.

So NIAID has a website on which actually, believe it or not, all of these resources are listed, and I'm providing the website link here. When you go through this website, you can go through different resources and actually at every resource there is an email, a name of an actual person who is a contact and a program manager for that space. And they have been very successful lately helping the ever-increasing number of investigators in doing both development of therapeutics and vaccines.

And that's it. Thank you very much.

DR. NAMBIAR: Thank you, Dr. Guina.

Our last speaker for the day before we move into the panel discussion is Dr. Thushi Amini, who is the Associate Director for Research in the Office of Antimicrobial Products at the FDA.

DR. AMINI: So in 2011, FDA developed a strategic plan for regulatory science to facilitate the translation of breakthrough discoveries into

innovative, safe, and effective products for tomorrow.

On December 19 of last year, we issued a Request for Information to develop animal models of infection. The purpose was to solicit information input from our external stakeholders to evaluate the current state of animal models for different types of pneumonia due to Acinetobacter baumannii and Pseudomonas aeruginosa; and, secondly, to conduct studies of an animal model or models of serious infection caused by these two pathogens. We've extended the deadline until March 22.

In addition, FDA has an ongoing broad agency announcement. The purpose is to solicit research and development to support regulatory science and innovation. The deadline to submit white papers is by March 31, and full proposals are due by June 26. And this is an open announcement.

The Office of Antimicrobial Products research area within this BAA is Research Area 2.4. And we state that FDA's role in combating antibacterial drug resistance are to facilitate the development of new antibacterial drugs to treat patients and to advance

the science of clinical trial design.

Specifically in FY17, our research will focus on Section 2.4.2, which is to advance the science of in vitro animal model and/or PK studies to facilitate antibacterial drug development. Our research funding to develop animal models this fiscal year is approximately $5 million.

So what are the next steps? We will take all the information that we receive from today's workshop, as well as the responses from the Request for Information and review them. We encourage potential applicants to submit white papers through the BAA process. The deadline for white paper submissions is March 31 for funding.

We will then review the white papers on technical merit and contribution to FDA's mission. Those that meet the eligibility, we will then notify the Office of Acquisition Grant Services, or OAGS, to send a letter to submit full proposals. And the deadline that OAGS will send these letters is on June 5, and full proposals are due to the FDA by June 26.

We will then evaluate the proposals. We will rate and rank them. And then we will award the study through a BAA contract. And we will try to do this all during the summer so that we can initiate the research study by September.

If you have any questions, please feel free to contact me. My information is right here on this slide. All the information I've discussed today can be found on the Office of Antimicrobial Products research web page, which can also be accessed through the OAP main webpage.

That's it. Thank you.

Moderated Panel Discussion (with Audience Q&A)

DR. NAMBIAR: Thanks.

All right. So I think we have about 45 minutes for our panel discussion, and we're running a few minutes late. So we do have four questions that we were hoping to get input from the panel members.

So I think in the interest of time, what we could do is maybe have a discussion around Question 1 and 2, and then we can move into Questions 3 and 4, which sort of are more focused on what more work needs

to be done.

With Question 1 and 2, I think the main focus really is that we've heard about different models during the course of the day. We've heard the pros and cons, the merits and demerits, of using the mouse or using the pig model or using the rabbit model. So I think we just want to get a feel for, are there any other models that need to be considered when one is developing or trying to understand infection due to Pseudomonas and Acinetobacter other than the models we've already discussed?

And I think another important point to discuss would be whether or not we actually need to do studies in large animals. Dr. Li Bassi's presentation, it did appear that he is very much in favor of larger models, studies in larger models, and not necessarily in the smaller models.

So I think it would be very helpful to hear from the different panel members on your thoughts of what might be the role of smaller animal models versus larger animal models in the context of developing drugs that treat these specific organisms. So I will open

that up to discussion and see if somebody wants to start.

So, Dr. Li Bassi, it sounds like you do have a question for Dr. Diep about the rabbit model? Is that correct?

DR. LI BASSI: Yes, I do have a question about the rabbit model --

PARTICIPANT: He's on the phone. I'm not sure if we're -- are we muted?

DR. LI BASSI: -- large animal model. So the question is about you mentioned in your presentation that you intubated these animals for the bacterial challenge. After the challenge -- I didn't get from your presentation, after the challenge, you extubate the animals? You keep them alive up to 36 hours? Or these animals are continuously ventilated?

DR. DIEP: The rabbits are continuously ventilated for 36 hours post-infection. We also in our Staphylococcus aureus study, the animals are continuously ventilated for up to 60 hours post-infection. And we can ventilate them as long as you can do with pigs, so we can ventilate them out to 96

hours post-infection.

It just depends on the study. We don't have so much experience working with Pseudomonas aeruginosa. We only started working with Pseudomonas in the last 6, 7 weeks. But with Staphylococcus aureus, you can go out longer because it's needed to look at a variety of different parameters. But, no, so to answer the question, the endotracheal tube is in place for the mechanical ventilation for the duration of the study. They're not taken out.

DR. LI BASSI: Okay. And what about the septic shock of these animals? Do they develop such septic shock that you need to provide vasoactive drug? Or as soon as they reach this level of severity, they are at the end and they die?

DR. DIEP: So fluid resuscitation is going to be needed if we're going to prolong the animal, so keep them alive longer so we can test the protective efficacy of whatever it is, antibiotics or antibodies, and so we do, do that. Right now we are taking a very conservative approach to fluid management because it's a double-edged sword, as you know. Too much fluid can

induce acute lung injury.

So in this model, we don't have so much experience again with Pseudomonas, so we are very conservative. We put in 3 ml/kg/hr. But in the context of septic shock, perhaps it is necessary to administer more fluid.

Shock is defined clinically as a 20 percent decrease in cardiac output. Formerly, in the model of Pseudomonas aeruginosa, we have not measured cardiac output, but we do measure cardiac output with a thermal dilution catheter in our Staph aureus model, and there, there is an decrease in cardiac output as well as mean arterial blood pressure, all of these parameters we can measure. We just don't have enough experience with Pseudomonas, so we don't know.

DR. LI BASSI: And do you use vasoactive drugs?

DR. DIEP: No. We have not done that for either Staph aureus or for Pseudomonas. But that could be added to any treatment modality, of course.

DR. LI BASSI: Of course. Okay. And the comments about -- one of the problems, particularly in

critical care medicine, now we are talking about in general Pseudomonas aeruginosa infection, but specifically in the infection that they are ultimately treated in the ICU, in general, we had a lot of failure in many medications, not just infection, acute respiratory distress syndrome, septic shock, of many drugs that were developed and tested specifically in mice, and then they were translated in ICU patient.

So the ICU physician is particularly important to highlight in this FDA-sponsored workshop, that we still have a huge gap in this field of investigation. Many of the drugs that they are very efficacious in the mice model, they do not reproduce the same results in ICU patients. We do not know the exact reason why. Of course, we are a lot of (inaudible) because the complexity of these patients, but this is something that absolutely should be taken into consideration in this field.

DR. MILLER: I'd make a couple of comments as sort of a more neutral observer perhaps. First, I think in terms of antibiotic drug development for the FDA, the mouse models are not -- the anatomy is such

that I think that's going to be difficult to accept mouse model data just because of the anatomical differences. And that will be difficult to accept that as part of the Animal Rule.

So then I think we're moving toward the rabbits and pig models as reasonable models that have a reasonable LPS type response similar to humans but also have some anatomical characteristics similar to humans.

I think when you start to get to very high levels of bacteria that you have to instill as part of the model, then the model starts to get iffy, too, because in some of these models, you're instilling the equivalent of a dose of LPS that will kill the animal. And so then you start to get into a very difficult model as well.

What probably hasn't been explored enough, in humans, there is probably some lung injury often associated with the actual disease, and probably what hasn't been explored is the ventilating animals associated with some sort of lung injury as a way to establish the infection more acutely.

And I don't think there has been a lot done

with that type of thing, but it's probably more likely what's associated with the human disease at the time of surgery or in a particular situation, there's a lesion that occurs, a thromboembolus or actual damage from surgery or something that has contributed to the actual inciting part of the infection.

So I think there might be room for development of lowering the infectious dose with a combination of injury and ventilatory combination.

DR. LI BASSI: If I can just add, this is a very interesting comment. We do have two pig models for particularly acute respiratory distress syndrome that we create, for instance, bacterial challenge or saline lavage of the lung, and then we created the second hit with ventilator-induced lung injury.

But this is absolutely a great comment. Usually, animals, they many times in many diseases, in reproduction of many diseases, they need a two-hit model. That could be with the injury of the ventilation or sometimes even they need not just one single inoculum, but two or three inoculations in order to have that kind of disease similar, even a smaller,

lower concentration of the bacterial challenge. Very interesting comment.

DR. COX: Yeah, so just hearing the comments have been very helpful information. Just my crew and I were talking about this, and that's sort of the difference in animal models that may look at sort of antimicrobial activity/infection versus disease models. So if you're actually trying to sort of model the disease, and the greater role that an animal model might take, to get into the space, to have sort of similar physiologic conditions may help to get something that may have a greater likelihood of being predictive of a human response.

DR. MILLER: I like the way that you've distilled that. I think these larger models seem to be really impressive as far as pathophysiology and I think provide really nice complementary information in drug development. I think it would be hard to utilize them alone for dose finding. So I still there is a lot of value in the smaller models for the PK/PD studies and then use these larger models for validation studies, looking for a signal of, gosh, maybe this isn't going

to work.

DR. MILLER: And even these models tell you something, particularly if you get bacteria in the bloodstream, you're at least putting them in an environment different than a culture plate and different than the environment, and so you're getting some information even in the mouse models, no question about that. But for antibiotic development, you get -- but it's incremental.

DR. COX: Just to agree with your comment, David, I mean, the idea of using small animal models of infection, you know, try and work things out, learn as much as you possibly can, and utilize the smaller animal models, the activity models, as sort of the earlier models to choose a compound, work on dosing, all those sorts of things, and then as you've learned enough from that, I mean, the paradigm of then moving forward to the bigger sort of physiologic models that are more difficult logistically and they use larger animals, it seems completely reasonable and a very rational way to approach this.

And the small animal stuff will add something,

but the bigger animals will -- or the disease models I should say, it doesn't necessarily have to be a large animal, but a disease model I think will help to get to that physiology you're trying to replicate.

DR. LI BASSI: Yeah. If I can just add a small comment. Usually, for instance, to give you some kind of cost of these large animal models, just for one animal, we usually -- the animal's sedation, ventilation, staff, this experiment, just one experiment, can cost between 3,000 and 6,000 euros, which is very close to $3,000, $6,000 U.S. dollars.

So absolutely you need to first know -- when the health care industry approach us for potential collaboration, we cannot even start if they do not have some kind of good results in mice, an idea, at least a broad idea, of dosage, because, otherwise, it's just unfeasible to test hundreds of these animals in a normal setting, it's just impossible. So you can do in one week 50 mice, 60 mice, but you can do just two or three pigs per week in these models.

DR. GUINA: I would like to make a comment. So I definitely agree, and I think we all agree, that

mouse models are very useful, and they're really critical for evaluating PK/PD and studying clinical doses and targets. I think maybe if we may could start considering -- could we start maybe discussing what sort of biomarkers we would look for in a larger model or animal model that would be more close to human physiology? I think there were a couple of great presentations, and actually almost no presentations people discussed that.

So if people are doing animal model studies, would you like to comment on what you think would be the critical biomarkers that would reflect more human disease? And I would like to hear from clinicians also, what is your opinion on that?

DR. ZURAWSKI: I think with the wound model and with pigs, Stephen Davis, at the University of Miami, has done quite a bit of work, I mean, over 25-years-plus work, looking at the host biomarkers as far as wound closure and healing. So if you're going for an SSTI indication for your FDA, I think you want to look at those kinds of wound-healing markers. And so there is matrix metalloproteinases and things like

that that are involved in wound healing that are conserved even in humans from pigs. They're not the exact same, but certainly you can use some of those as markers for healing with wounds.

As far as pneumonia and HAP/VAP, I don't know. I think I would be most concerned about sepsis because I think -- I mean, certainly, lung function is important, but we're clearly seeing this dissemination out of the lungs going into the bloodstream, and I think if you have markers for sepsis, which I know are out there, I think that would be important to look at.

DR. DIEP: So I would like to comment on the blood markers that can be used for ventilator-associated pneumonia or septic shock or severe sepsis in that case, and these are the standard blood tests, biochemistry tests that's done in the clinic. You look for, you know, if you think there is an acute kidney injury, you look for creatinine, you look for potassium, you look for BUN.

Now, these are the standard markers that's used in the clinic. If you think there is liver involvement, it's ALT/AST. And all of that can be

measured in any animal models. The biochemistry tests are the same. The only problem is if you're using a tiny animal like a mouse, there is only so much blood that you can get through like submandibular blood. That's only like 2-, 300 microliters that you can get.

And so the advantage of using something bigger like a rabbit or a pig or a dog or any of these animals is that you can take serial samples and look at the chronology of events leading up to the septic shock, leading up to death. And they tend to correlate very well with what you would observe in the clinic.

And I'm not a physician, but from reading the different papers on what are the predictors of survivors versus non-survivors, and you see those same correlates happening in the severe animal models, I'm not sure whether that is observed in the ventilated pig models.

It didn't seem like, from the data that was presented here, that the infection got to the point where it's very severe ARDS, because if I remember correctly, the FiO2, the pO2/FiO2 in those animals, it's still in the range of 200, and in that range,

there is not very severe -- it's classified, ARDS is classified, using three different tiers. And so you have very severe ARDS when the pO2/FiO2 is less than 100, and it's moderate ARDS when it's about 200, and you have very mild ARDS when the pO2/FiO2 is about 300.

So it depends on how severe you want to get. But these blood markers that are used in the clinic can be recapitulated in the animal model, you just have to do it.

The problem is again of course it's very costly. Every time you do a blood sample and you look at blood gas analysis and you do all of this biochemistry, it's about $100 at each time point. And for the study that I showed you there, it's 16 time points per rabbit. And so suddenly it's like $1,600 for just the blood work. So it can get expensive very quickly in these models.

DR. LI BASSI: Yeah. I'm sorry, I'm sorry. Go ahead.

DR. WALLNOFER: I wanted to add to the discussion. My impression from the presentations is primarily I think that the large models will allow to

investigate a limited number of questions. It needs to be focused with a clear plan in mind. And this can be useful actually to validate what we have observed in the smaller models, validate, for example, your PK/PD modeling.

And then the other aspect where it could be helpful is it could complement the clinical trials that you do. Now, what is more difficult to understand now from my perspective, how valuable that would be seen. I think to the clinical real data that you generate, those could be further substantiated, those specific questions addressed. We stated that they have been generating in such a large model. So that would be my reading from this discussion.

DR. LI BASSI: Okay. So for our model, usually we focus on the lungs. So these are models of pneumonia, and we specifically look at inflammation and (inaudible) and injury of the lungs. And one of the main advantage of this model is that you can get sequential bronchoalveolar lavage. And this give you some data on the inflammation, local inflammation, that you could even compare with systemic inflammation with

inflammatory markers in the blood and bronchoalveolar lavage. Then you can measure the Pseudomonas aeruginosa growth in the lungs through these bronchoalveolar lavage. And at the end of the study, you can even culture the tissue and do histopathology of the tissue, and you can look at the severity of the injury and severity of the bacterial burden in these animals.

In terms of clinically valuable data, now, many of the clinical trials on new drugs, they look at the improvement at 48 hours, 72 hours. And when we look at improvement, patient improvement, after 2 days or 3 days of treatment, we look at lung function, and usually pO2/FiO2 ratio is a good marker of lung function. And bacterial culture. Many times we either get tracheal aspirates or bronchoalveolar lavage of these patients to corroborate the good response. So these are the things that usually we can obtain from these models.

DR. NAMBIAR: To answer your question, Andreas, I think our main focus here, as we discussed today, is try to get the best clinical data we can

because that would really be ideal. But then we all realize from a practical standpoint that that might be doable or might not be doable, and there might be practical difficulties in getting to the numbers you had set out to do.

So I think this discussion is around, what is the best we can do with the animal models that we have? And what more needs to be done? What are the areas that we need to focus on so that at the end of the day, if someone tries to do a clinical trial and only once you attempt it will you know how successful you're going to be, how difficult it's going to be.

And if the trial is just not feasible, so we're not talking about a trial where the drug has shown it doesn't work, I mean, then the drug is dead, but if you have a drug that has the potential, but you just cannot do the human trials, then we want to have the best body of information we can get for how the drug performs in animals. So I think all kinds of animal models have their different rules. The smaller animal models play a different role.

So I think that's the purpose of this

discussion. It's not that these are to replace human data, but it's to sort of complement whatever human data you can get, which I think we all have to be practical and think that there might be a situation where you're really not going to get human data that's going to be interpretable.

And then you cannot start planning what next, you start planning now. And that's the purpose of having this discussion.

DR. ANDES: I think that before you can use these larger animal models to try and predict what's going to happen in patients therapeutically, it would be nice to see -- I saw some examples where people took a drug that was approved, treated the animals, and they did well. It would be nice to see, try and like has been done in the mice, take a drug that failed, take a drug that doesn't work, mimic the PK in patients and show that it failed, show that it's predictive, and look at then, what of those endpoints tracked along with that failure?

DR. NAMBIAR: That's a very valid point, something we've certainly discussed, having both two

kinds of controls, ones that you know. Unfortunately, we have a few examples of drugs that haven't worked in humans, so we can use those examples. And we also have examples of drugs that really do well in humans. So having both, I think, and testing them in animals is a good idea.

DR. MILLER: Since we were asked, one of the questions was other models, and so I think that we should also be working on bacteremic models because bacteremic models are very reproducible usually if you're injecting bacteria directly into the bloodstream in animals, and they have a lot of -- they're short term, there are good endpoints. Particularly with rabbits, the LPS aspect is very predictable for humans.

So I think bacteremic models have a lot of appeal. It's a short-term model compared to the ventilatory models, which are really complicated, which we need, but I think there are a lot of advantages to bacteremic models.

DR. NAMBIAR: Any other comments from the panel members for Questions 1 and 2? Dr. Rex, you have a comment?

DR. REX: I'm sort of thinking about the intersection of what David and Andreas were talking about, and trying to think also about the way that -- you know, the standard we have for drugs, about substantial evidence of efficacy based on adequate and well-controlled evidence.

And the words are not defined numerically. And we've talked a lot about justification for you could say larger NI margins or a different alpha. I mean, all those are sort of functionally equivalent manipulations of the statistical boundaries.

And maybe this is saying that one way to look at this is that if you've clearly developed your PK/PD argument in mice, and maybe do a couple rabbits, and then you do a tiny number, which is all you're going to be able to do, of pigs, and prove to yourself that in fact when I treat a 40- to 50-kilo pig, which is a reasonable fraction of the size of the human being and a lot of the same anatomy, it worked again, then that actually would permit you, if you could not do adequate clinical data, to say that a different dimension statistically -- it's sort of like saying it's Bayesian

prior, it's another way to say it, it's actually creating that sort of an argument.

And I actually do think it's important that we get down to discussing that because another part of sort of the whole scenario, game-playing this all the way out to the end, is that even being approved doesn't mean that other people will understand the basis of approval or why they should pay for it or use it.

So the fact that this very sophisticated group sits down with an agency and says, "Yes, you've done these studies, and, yes, you've got this little bit of data," it actually needs to be something that others can see reasonably well in terms of justifying whether or not they would reimburse for it.

So, again, it feels like that's the kind of logic that other people could get behind. It steadily adds up. So I'm very cognizant of the other stakeholders that have to ultimately decide that, yes, not only was it approved, but that it makes sense to me, too. The agencies do not have the last word in that regard; there are many others whose opinions really matter.

So I think I came in today not as certain that I knew why you would do the big pigs. And I had looked at Dr. Li Bassi's slides, and I thought, wow, that is a huge amount of work, but maybe there is a reason for doing a small set of studies there, particularly if you've really run that model well, if you've really characterized it and you know that a humanized exposure of X doesn't work when it shouldn't work and it does work when it should work, you've put in all your standard candlesticks. You've actually -- you've really measured it --

DR. MILLER: You have the controls --

DR. REX: You have the controls, you really know.

DR. MILLER: -- of antibiotics that work and antibiotics that do not work, and you have a few surrogate markers --

DR. REX: Yeah.

DR. MILLER: -- that are fairly equivalent, then I think you could approve it for an indication when there was not something else available that was approved, you could include that in the approval.

DR. REX: Well, but now, you've put in something that I'm sure I would be careful about that, something -- nothing else approved. I --

DR. MILLER: Well, not -- when there is no other agent available.

DR. REX: Well, for use when there is no one there to let it run.

DR. MILLER: Yes.

DR. REX: But I would be careful about saying that we can't approve something else unless there is nothing else anywhere in the universe that --

DR. MILLER: No, that's not what I'm saying.

DR. REX: Yeah, that's -- be careful about that.

DR. MILLER: Because there are clinical uses where approved drugs that would work cannot be clinically used for various reasons.

DR. REX: Right. Yeah. Dr. Boucher showed us one of those today.

DR. MILLER: Human allergy or many sorts of situations. So it would just be when another approved antibiotic is not available.

DR. LI BASSI: Yeah, if I can add to something. I think we should put all this discussion in the context of 2017. And as a clinician, we are very worried that many agencies, like the Food and Drug Administration, the European Agency for Drug Approval, and even health care industry, they are -- considering that we do have an increasing number of pathogens that they are becoming drug resistant, there is not a parallel improvement in the research, development, even approval by these agencies to facilitate the development of new drugs.

As a clinician, every day I go to an ICU where there is a patient with a Pseudomonas aeruginosa resistance to everything except colistin. So I think that one of the goals that Food and Drug Administration, and Europe as well, should have in mind, is to try to not make the entire approval process more difficult than it has been throughout the years, but much more easier, because if there is the development of drug, then we wait for mice, then we wait for drugs, and the multi-institutional randomized clinical trial, this just make the development of new

drugs so difficult that the health care agency and health care industry, they just shift to other kind of business that they are more proficient in terms of money. And so this is a very important conversation particularly in the era of multidrug resistance.

DR. KNISELY: Okay. Thank you. Oh, Ed, go ahead.

DR. COX: I was just going to say, I mean, a lot of what today is about is trying to balance benefits and risks, and recognizing the unmet needs that are out there for patients with serious infections who need new options.

So I think really what you're describing, Dr. Li Bassi, is consistent with what we're talking about here, which is trying to find feasible pathways here so that there are options out there for patients, recognizing the tremendous need.

DR. KNISELY: Right. So now let's move on to discuss Questions 3 and 4, which are kind of more forward-looking questions on what we need to do moving forward.

These have already come up, but what are kind

of the key highlights of research questions that need to be addressed in order to move this idea forward, including other sites of infection that should be prioritized? Dr. Miller already brought up bacteremia.

DR. DIEP: I'll make a comment. So in the Staph field, Staphylococcus aureus, the gold standard model for testing antibiotics is the rabbit model of aortic valve endocarditis. This model would produce a persistent bacteremia.

And the reason that this model is the gold standard model for testing new drugs against MRSA is because it's a biofilm-based model. It's very difficult to treat. And the rabbit model of aortic valve endocarditis also exists for Pseudomonas, for Klebsiella, for A. baumannii. And it's for the same reason, it's the biofilm. It's very difficult to treat.

And it's very easily adaptable for any bugs, and that's because of the way that the model is done, it's a low inoculum model. And the reason that it's a low inoculum model is the carotid artery is cannulated with a catheter, and the catheter is placed across the

aortic value, and that cause valvular damage, providing this deposition of platelets and fibrin, and it sets up a niche for bacteria to bind to. So you can use often

4 5

10 or 10-fold less bacteria to cause the endocarditis.

And this is also a relevant infection for opportunistic pathogen like a Pseudomonas. It occurs in injection drug users. Right.

DR. COX: Can I ask, in the last question, we were talking about the things that you might do early on, and as you move from sort of activity models, you would then move to disease models. Where would you put the model that you're describing, biofilms, aortic valve? Is that something you would do early or is that something you're thinking about doing later on just in the overall development program?

DR. DIEP: I can actually comment on that. So the sort of model that like a rabbit bacteremia model or a severe sepsis model, those are really easy to do, just like they are in mouse. It's just an intravenous injection. The surgical models are complicated, and they cost a lot more to do. For example, it was mentioned that for the pigs, it's 3- to 6,000 euros to

do a ventilated pig model. To do a rabbit ventilated model, it's $6- to $8,000 U.S. dollars. It's extremely expensive, more expensive than the pigs it seems, maybe because labor in the United States is higher than it is elsewhere.

(Laughter.)

DR. LI BASSI: That's a good point.

DR. DIEP: But I think cost is the key issue. And so if you can do -- you know, it depends on how much money is available to fund the study. An endocarditis model is, I would consider, a later stage type mechanism of action study. And I would use, for example, a bacteremia model first because it's a lot cheaper.

DR. ZURAWSKI: I would like to comment about the biofilm aspect. So when we talk about drugs that fail, oftentimes it could be because of resistance that we see. And one of the things that I used to often banter with Jared Silverman about was there is really no good model for resistance when it comes to animal models and when we're looking at antibiotics. We can do as much hollow fiber as we want, we can do a lot of

PK/PD in these models, but a lot of that is short term.

And I think one of the things that isn't -- that we should just invest more in are some longer term models, something like this, where we've got a biofilm that's going to change your -- actually, it's going to change your PK/PD because some of those biofilm bacteria are just not going to be able to get hit by the drug that you're looking at. And so we need something that's like a week long where a mutant can arise, all those kinds of things, and we just don't have good -- we're not addressing that very well right now.

There's a rabbit ear punch model that my colleague Kylie (ph) Young was doing at USAISR for Klebsiella, but you could probably adapt that to any of these ESKAPE pathogens if you used the right strain again. There you actually have a biofilm growing on the ear of the rat making a wound, similar to our wound models where we see biofilms.

We ourselves have not addressed that very well even in our models where we've looked at mice and pigs over a prolonged period of time where it would be nice

to do a study where we use rifampin, something where we know resistance pops up routinely at a certain number, see if we can recapitulate that. And then maybe where we've seen failures in terms of resistance, also see if we can recapitulate in the animals as well, that we've seen in humans.

I don't want to throw stones, but, I mean, like the Anacor compound, for example, that failed, it failed because of resistance reasons. I think if that company had done more work up front in animals and saw resistance crop up, it may have never gone to clinical trial, or it shouldn't have. So just something to bring up there. But the biofilm component is a huge component of pathogenesis that we're not really discussing, and it's a big deal. It's literally a 1,000-fold change as far as how some of the antibiotics work.

DR. COX: Can I ask another one? So we've been talking about activity in disease models, and it sounds like we've got disease models for Pseudomonas aeruginosa in the lung. And what I'm not so clear on, there are some nice public presentations, but sometimes

it's hard for me to tell sort of when we're talking about activity and when we actually move to disease. And I welcome comments from any of the speakers that are available to talk about this that presented earlier.

How are we doing for Acinetobacter for a disease model in the lung? Dan, you presented some nice models, but they were skin models, if I'm remembering.

DR. ZURAWSKI: Well, we have a mouse lung model that's similar to all the other ones that people are running for Pseudomonas that works very similarly.

There is a rat pneumonia model that Tom Russo developed at SUNY Buffalo that works with fairly low

inoculum. I think the inoculum is 10 I want to say. So the inoculum is still pretty high, but you don't have to do neutropenia, so you're taking that element out because the rats have less of an innate immune response than mice.

So I did want to bring that up because Question 1, we're talking about other animals that should be discussed. I think rat is left out quite a

bit. I like the rabbit quite a bit, but rats I think maybe should also be included. We've done a rat wound model that I didn't talk about today. We've kind of done that low frequency, but it recapitulates everything that we're seeing in mice and pigs, and certainly in humans, where we see this penetrative infection of SSTI. But as far as long goes, really it's just mouse and rat at this time.

I'm sure the models we saw for Pseudomonas lung today could be adapted to Acinetobacter if the right strain were used and you get the damage that Dr. Miller was talking about. I'm sure you could get Acinetobacter working in that setting.

DR. COX: Do you think that the rat model, lung infection, Acinetobacter, is it a disease model or are we looking more at activity there?

DR. ZURAWSKI: I think it's a disease model. I mean, what Dr. Russo showed, you have the Acinetobacter disseminating from the lungs into the bloodstream and killing the animal. I mean, I really think that's what's happening in ICU patients as well. So it's not perfect, but I think it's a very good first

step, even looking at disease.

I think where things go wrong as far as efficacy go is when we just do like a mouse thigh or a rat thigh, and, "Oh, I see a 2 log drop. My compound is great. We're moving on." That is not -- you need a pathogenesis piece because these bugs do something differently in every different part of the body. There are different stresses in every part of the body. I feel like you have to have a biofilm component. Some of that gets addressed in the rat pneumonia model.

I believe even when we do a mouse lung model, there are components of biofilm that are going on there with those bugs. I wouldn't call it a true biofilm, but you get these droplets, like I mentioned, that you probably have biofilm-like or stationary phase-like bugs and then planktonic around them. And we see that even within 2 days, 1 to 2 days pathology-wise. So they are disease models. They are not necessarily just efficacy.

DR. MILLER: But I think even for Pseudomonas, where there has been a lot more work done, the reproducibility of these models and their

standardization with specific bacteria, it just has not been there over the years.

And so there needs to be more work done with both of them if the models are going to reach the standard that you're probably looking for in more than one site and very specifically designed experiments I think to develop these models that could be accepted with the right bugs and developed so that there could be a specific model where you could point to and say, "This is what we think needs to be accomplished," because they haven't been using drugs that work or don't work, or they're a specific strain or a specific mouse strain, or just --

And any animal model, anything involving host-pathogen interactions can be difficult to reproduce biologically, and so you're going to have to be convinced that you can biologically reproduce it in more than one site. Just like in clinical trials, right?

DR. DREIER: I'm Tom Dreier, from BARDA. Just a question. It's not per se some of these questions that you have here, but who's going to decide what

bacteria strain you use for the models? I look at this and I thought -- and we've done a lot of work with BARDA and the FDA, but the first step is always you've got to define the strain so you can define your problem. So who's going to define the strain? Who's going to grow the strain? And who's going to put it on distribution?

DR. COX: Since I don't develop animal models, I guess I'm free to answer that question.

(Laughter.)

DR. COX: No, I'm just kidding around. It's getting late in the day. And I'll welcome the comments from folks who actually do develop animal models, but I would think that it seems like the host that you're studying and the strain go together. And we heard some discussions earlier about picking a strain that has certain properties based on its clinical behavior. And then it sounds like that strain needs to be well pedigreed in the animal model of infection and such.

But maybe some of the animal model developers, who actually do this, will tell me how embarrassed I should be for what I just said there.

DR. ZURAWSKI: I'll speak for Acinetobacter. I mean, even though I laid out those criteria, we just don't know enough how this thing is causing disease. Right? So, I mean, I would like to say that -- you know, I haven't -- we have data that we haven't published yet that we're working on where we've identified virulence factors that we know are pretty important. And there are some that I think may be important that we identified that could be important, let's say, and one of those might be barrier function. So breaking the tight junctions in the epithelial barrier, it may possess a toxin that does that.

So you don't want to use a strain that doesn't have that toxin. Right? So if that's a key part of its virulence and how it's -- there are going to be a percentage of strains that don't have that toxin. We know by looking at the 1,000 sequenced Acinetobacter that not all of them have it or they have different variations of it.

So how are you going to -- it's hard, I think, to get at this, to crack the nut, because we're still so early -- I mean, Pseudomonas, there's much more work

on that that's been developed on the pathogenesis side where we know a lot of the toxins, we know a lot of the things that it does pathogenesis-wise that I think we can exploit, but with Acinetobacter, it's more of an open question.

DR. GUINA: Well, I would like to just add to that. I think it's really important to pick strains that are obviously very low passage, they've been very well characterized, and also those that reproduce as close as possible to human disease in any of the animal models, that we pick based on some agreed upon biomarkers and endpoints that actually are the quality checkpoints for the model. Because there are so many strains, we'll never agree to agree.

DR. GOLDBERG: And I think you should not just rely on one strain, but you should always look at a panel of them to see how they're holding up to one another.

DR. MILLER: And that's part of the challenge of developing these models, is whoever is developing these models will consult with experts and decide on a set of strains, and that's what will get developed, and

once -- but they will need to be continually validated that they haven't changed, just like stocks, just like you would with any other biologic, there will have to be endpoints that it still has that biological characteristic when the animal --

DR. COX: It sounds like in part that speaks to the importance of having control animals and monitoring your strain over time, too, it seems like. Thank you all for helping me.

DR. LI BASSI: I have a question. Should the decision of this strain be also regulated by the agencies based on the prevalence of the strain in the human population?

DR. REX: No.

(Laughter.)

DR. REX: So John Rex says no. And you don't want to write something down like that and create such a hard rule because things may change over time. And I think that as we go along, what you want for Drug B will be different than what you wanted for Drug A because of the existence of Drug A. So you've got to be careful not to overspecify here.

DR. LI BASSI: Okay.

DR. KNISELY: Any other questions from the audience? Okay.

DR. GELHAUS: Carl Gelhaus, MRI Global. Previously, all the antibacterial products that have been approved under the Animal Rule use non-human primates. So we saw a non-human primate model briefly. Is there any thought about the need for a non-human primate model?

DR. COX: All right. Am I going to have to go first again?

(Laughter.)

DR. COX: So really what we're trying to get at are animal models that are predictive of human efficacy. So I think the question sort of, you can distill it down to, what are those potential animal models? And can you do that in animals other than non-human primates and do it in a way that's sufficiently predictive for establishing the likelihood of efficacy in humans?

So in some cases, it seems that the non-human primate is in fact the model that gets you to that

point of having something that really you've got good reason to think that it's likely to be predictive.

So I think it may depend upon the particular bacterial species, how it interacts with the host, and whether you can get information from -- or from which animal species you'll actually be able to get information that you think is based -- that's likely to predict efficacy for humans. And it may not always be a non-human primate, it may be something other than that. In some instances, it may be a non-human primate, but I think that's really sort of a scientific question, and folks here have described a number of different species and the animal models that they've looked at.

DR. LAWRENZ: Keep in mind, too, when you look at plague and tularemia and anthrax, there aren't a lot of human cases that you can move then and do those small clinical studies, where with these bugs, there are availability to move to that next step. And so I don't know that the requirement for a non-human primate is going to be as high when you look at these guys in the clinic.

DR. MILLER: And there is no evidence that these bacteria would be more pathogenic or more like humans in non-human primates than in pigs, whereas for other human-adapted pathogens that are really human specific, say like Francisella or something like that, then that makes more sense.

DR. DIEP: I would like to make a comment on that. Sometimes the non-human primate does not make the best animal model either. It depends on what is the target. In the field of Staphylococcus aureus, where I come from, there is a particular kind of toxin called Panton-Valentine leukocyte, and it's associated with this epidemic of MRSA that we saw in the past decade.

And this particular toxin targets a C5a receptor on human neutrophils, and also targets at the same level sensitivity to rabbit neutrophils. The C5a receptor for rabbits and for humans are rather similar to one another. And when you look at monkeys, monkeys are very resistant to this particular toxin.

And so to test an antitoxin against the Panton-Valentine leukocyte in the rabbit model is like

testing it in the mouse and the rat, which are also not susceptible. So sometimes monkeys are not -- non-human primates are not the best model. And it depends on the pathogen.

Like Staphylococcus aureus and Pseudomonas, those are natural pathogens of rabbits. There are outbreaks, natural disease outbreaks, that occur in rabbit farms. That's why it makes for a good model for that, also for that reason. But it all depends on what is the target and whether the host is susceptible to the target or not.

DR. NAMBIAR: No other comments before we -- oh, there's one more comment.

PARTICIPANT: Thank you. So I wanted to make two comments actually. First I want to address the O52 question because I was heavily involved with those studies, and we did an enormous amount of animal work. We did neutropenic mouse, lung, thigh. We did competent mouse studies. We did rat thigh studies. We did rat pneumonia studies. We did recreated human PK profiles in rat pneumonia studies. We did almost everything you can do. And we never saw the resistance

in those studies.

We even tried after the fact to recapitulate the resistance, and we couldn't get it to happen unless we spiked a certain number of mutants into the inoculum. So anyone who has worked with trying to resistance studies in animals, it's incredibly difficult to develop those models. So I felt like we really deserve a little bit of credit for doing a whole lot of work.

DR. ZURAWSKI: I understand. And, again, I wasn't throwing stones, it's just -- I understand. That's what -- it's a high bar. I mean, that's -- if we can get that, that would be wonderful, if we have a resistance model.

PARTICIPANT: Yes, I agree. I think one of the difficulties there is you need a high enough inoculum. We've done a lot of work with rifampicin, and even that notorious compound, you need to get at

8

least 10 bugs in there to really get any mutants to come back out. So it's difficult, and the animals tend to die when you get those high inocula, so you just can't follow through.

So the other comment I wanted to make was around this idea of picking a model and one strain. I think that's incredible dangerous, and I really would encourage us to not go that direction. Thank you.

Closing Remarks

DR. NAMBIAR: All right. So thank you, everybody. It's 5:00, and it's been a long day.

I just wanted to thank all the panel members for their participation and presentations today. Very helpful.

I want to thank the audience for their participation as well. Thank you for coming.

And I also want to thank people who are joining us remotely. I think this was an extremely helpful workshop, and we will take your feedback and discuss it and hope to continue the dialogue because we really need to find a way forward to get these products to patients who need them. So thank you very much and safe travels.

(Applause.)

(Whereupon, at 5:02 p.m., the meeting was adjourned.)

CERTIFICATE OF NOTARY PUBLIC

I, MICHAEL FARKAS, the officer before whom the foregoing proceeding was taken, do hereby certify that the proceedings were recorded by me and thereafter reduced to typewriting under my direction; that said proceedings are a true and accurate record to the best of my knowledge, skills, and ability; that I am neither counsel for, related to, nor employed by any of the parties to the action in which this was taken; and, further, that I am not a relative or employee of any counsel or attorney employed by the parties hereto, nor financially or otherwise interested in the outcome of this action.

MICHAEL FARKAS

Notary Public in and for the

State of Maryland

CERTIFICATE OF TRANSCRIBER

I, DEBORAH ARBOGAST, do hereby certify that this transcript was prepared from audio to the best of my ability.

I am neither counsel for, related to, nor employed by any of the parties to this action, nor financially or otherwise interested in the outcome of this action.

MARCH 13, 2017 DEBORAH ARBOGAST

j