TRANSCRIPT OF PROCEEDINGS

IN THE MATTER OF:)
)
BACTERIOPHAGE THERAPY:)
SCIENTIFIC AND REGULATORY ISSUES)
PUBLIC WORKSHOP)

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BEFORE THE DEPARTMENT OF HEALTH AND HUMAN SERVICES
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IN THE MATTER OF:)
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BACTERIOPHAGE THERAPY:)
SCIENTIFIC AND REGULATORY ISSUES)
PUBLIC WORKSHOP)

Room 1D-13
NIAID Conference Center
5601 Fishers Lane
Rockville Maryland

Monday,
July 10, 2017

The parties met, pursuant to the notice, at 8:37 a.m.

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C O N T E N T S

P R O C E E D I N G S

(8:37 a.m.)

DR. KINCAID: Good morning, everyone, and thank you for coming. As the agency host for this meeting, I want to welcome you all. Dr. Peter Marks, the Center Director of Biologics at the FDA, will be giving some more in-depth opening remarks, but I just as a representative of NIAID, one of the co-sponsors, I just wanted to welcome you all and thank you for attending.

At NIAID in particular, our programmatic efforts are driven by a combination of recognition of unmet medical needs and scientific opportunity. I think we all can recognize that antimicrobial resistance is a present and growing unmet medical need that needs attention, and this meeting and you coming here today, you are bringing us scientific opportunities that we wish to capitalize on and programmatically develop projects to move forward, and so I'm very hopeful for this meeting to give us some ideas and concepts that we, in conjunction with our sister agency at the FDA, can move forward to better prepare the ground for regulatory science that will enable the development in this whole category of countermeasures.

So I wish you a very productive and pleasant and entertaining and educational meeting, and I'll turn it over to Peter.

DR. MARKS: Okay. So welcome, everyone. Thank you all for coming to this workshop on bacteriophage therapy that's covering both scientific and regulatory issues.

The workshop's co-sponsored by the Center for Biologics Evaluation and Research at the Food and Drug Administration, in collaboration with the National Institute of Allergy and Infectious Diseases at the National Institutes of Health, and before I go much further I'd like to take a moment to thank those at both institutions for all of their work and their efforts putting together what should be a very engaging and informative program over the next two days.

I also want to take the opportunity to thank all of those who will be presenting and serving on panel discussions for making the time to travel here and to do so, all of those who have braved Metro to make it here, thank you, even those coming from locally.

Antibiotic development got underway seriously in the 1940s and reached its heyday in the

1950s and 1960s. Although initially it was the source of miraculous cures for a variety of infections that were previously difficult or impossible to treat, it was not long before the problem of resistance to antibiotics that had been developed occurred.

Over the past two decades, such antibiotic resistance has really escalated to crisis-level proportions, and we now have the development of Methicillin-resistant *Staphylococcus* aureus that's present in many communities at high levels, vancomycin-resistant enterococci, and a variety of gram-negative organisms, such as *Klebsiella* and *Pseudomonas* species, that are resistant to multiple different antibiotics. In fact, some are remarkably resistant.

Ironically, though it was discovered a bit over a century ago before the modern antibiotic era, bacteriophage may turn out to be important therapeutics in combatting antibiotic-resistant infections.

First discovered by the English microbiologist Twort in 1915 and then characterized further by the French-Canadian scientist Félix d'Herelle, it was not until decades later the details of the lytic mode of action of phage were understood.

Such limitations in mechanistic understanding, combined with the ready availability of antibiotics following the Second World War, led to the development of phage therapy for the treatment of human infections in the United States and Western Europe to really slow down and to be shelved for a number of decades. The work on phage therapy continued in some Eastern European countries, including Poland and Russia.

Over the past decade, however, the investigation of potential phage therapy has seen a renaissance globally as certain infections have proven to be quite resistant to our existing complement of antibiotics and the discovery of novel antibiotics to combat such resistance have become increasingly challenging from a practical perspective.

On the other hand, phage therapy appears to be non-toxic in humans and in animals, and phage have the benefit that their bacterial specificity allows sparing of the remainder of the beneficial microbiota. In addition, there's the potential to either select or engineer phage to target bacteria that develop resistance to these agents.

Over the next two days there will be talks and discussions covering the scientific, manufacturing, clinical, and regulatory issues

surrounding the use of phage therapy. Though challenges clearly remain in the development of phage therapy for the prevention or treatment of infections in humans, their potential clinical utility seems quite promising in a time when other options seem much less so, and that's particularly in certain circumstances, and thus -- let's see -- the adage "What's old is new again" seems quite appropriate when describing the situation with phage, or said more modernly, "Old is the new new," and we look forward to a very informative and productive workshop over the next days.

Thank you very much, and we really appreciate your coming today.

(Applause.)

DR. KINCAID: Good morning, everyone. My name is Randall Kincaid, and I'll be serving as moderator in the first session today. Just so that you're aware of it, we have an additional room, a companion room which is connected by VTC, and it is possible that if questions arise from those in that room, we will be entertaining sort of alternate questions if that comes up.

Before we begin, I was asked to make a couple brief statements. First of all, that each

speaker outside of the government was asked to submit documentation outlining their financial interests. This is an important matter, and these records are all in place. And secondly, that the proceedings of the workshop are being recorded for purposes of transcription and these will be made available at the end of our sessions. Well, it'll actually be made available after we've compiled them and all of that.

So, without further ado, I'd like to introduce our first speaker, Dr. Ry Young from the Center for Phage Technology at Texas A&M, who will provide us an overview of bacteriophage then and now.

DR. YOUNG: So thanks for putting me in a position of leading off this, I think, historic conference. Since I work on phage lysis, I usually am the last talk in a phage conference, and that's because most people don't care about lysis and a lot of people have to leave early to catch flights. I couldn't figure out why I was chosen as the first one in this case. There's lots of other people who could have been more appropriate choices for the first speaker, but given the Metro problems and the security problems, I think Randy thought a lot of people might be late, and so it's better to have a buffer in the front.

So we're going to -- this is going to be largely historical. I'm going to -- I'm not sure exactly why I chose to do it that way. I guess I saw the title "Phage Then and Now," and I felt pretty intimidated by that because I actually did not know much about how phage therapy had been conducted in the Phage Therapy 1.0 in the first part of the 20th Century, so I spent a lot of the last two weeks or more boning up on it, and I felt like I needed to pass what I learned or at least what I think I learned on.

But then I also got this stern email from Randy saying I had to not only identify my conflicts of interest but also make it clear at the beginning of the talk. I think that makes sense. So I started thinking about my conflicts of interest, and I think really you're asking for bias, and I wanted to show this slide because I am a direct descendant of the Delbrück-Luria-Stent school. I was a Ph.D. student with -- where's the pointer? Is that the pointer right here?

Okay, I was Ph.D. student with this man, Hans Bremer, who was the first American post-doc brought to the United States by Gunther Stent, who was, of course, kind of the Luca Brasi if you view the phage group as a gang, Gunther was the Luca Brasi of

that gang, and he went around recruiting talent in Germany and devastated Western Europe and brought them and seated them all over the United States, including in Texas, where I ended up after my Navy service. This is me in 1972 as a Ph.D. student. I had a little more hair. As you can see, the receding hairline was already going back, but I was imbued with the philosophy of the phage group. The place I did my training was a hotbed of phage biology; essentially, all 20 scientists worked on phages of different types.

And this is a quote from, a famous quote at the end of the introduction to a textbook that essentially everybody in my generation used as the textbook for phage genetics, and that was, "The strange bacteriophage therapy chapter of the history of medicine may now be fairly considered as closed." So, if there was ever a -- and this was in the 1960s, right. So, if there was ever an attempt to put something to sleep, this was it. Phage therapy sleeps with the fishes.

So, before I go on, I want you to notice, by the way, this was 1972 that this picture was taken. I want to acknowledge that there are -- I know two of these people, Betty Kutter and Carl Merril are here. I thought Shankar was going to be here. Is he not?

Menaj is not here. Well, it's too bad. So I want to point out that all three of these people have a much deeper background in the applications of phage therapy. They were all committed to convincing the scientific public that there was a future in this 20 years before or 10 years at least before I even thought of it.

In fact, since I was up until quite recently biased by my training in the phage group, I was actually very anti-phage therapy, and if you want to notice here, these are publications in very respected journals published in 1968 and 1969 when I was still in the Navy. And, in fact, that's three years before I was back in graduate school, so these people have a much better background and perspective and should be giving this talk.

And, in fact, this is the way they all looked in 1972. The fact that they're so well-preserved.

(Laughter.)

Dr. YOUNG: All right. So the real conflict of interest in terms of, I guess, finances came when I was recruited to GangaGen. So, up to 2002, at the ASM convention where I was giving a lecture, I met Janakiraman or J. Ramachandran, who is the founder and

CEO of the company GangaGen, a company at that time based in Bangalore, and those of you who know him, he has a charismatic personality and was very eloquent in convincing me that there was a future for phage therapy.

He first recruited me to the Scientific Advisory Board, and I've been active on that even to about 2009. I actually served as a research director for this company in my one year of biotech, and the most important thing to note is the company actually survived that spectacularly incompetent year of mine. And, in fact, they are still in business. They have products in clinical trials, and they have said hello to me, but they haven't asked me for any further input from me for about eight years, which shows that his acumen was even better.

But it did lead very importantly to me being able to convince the Texas A&M Board of Regents to establish a Center for Phage Technology, and I think we're the only state institution, more than $5 million was in setup money, and four faculty positions and an annual budget have been committed to the notion that phage biology can be put to important translational uses.

So, overall, I have three biases, three

conflicts. I have the original. By training, I was very anti-phage therapy. I still am a stockholder and member of the SAB of GangaGen, so there is a second bias. And then the third bias is I'm actually running a center that is meant to promulgate the use of phage translation. So that's the truth.

So the main sources for this talk are these here. I won't go through reading them. I'm primarily going to be focusing on the Eaton and Bayne-Jones *Journal of American* -- this is a review commissioned by the American Medical Association back in 1934, and it's the one that's regularly cited as being the death knell of phage therapy, of Phage Therapy 1.0, and some others. I'm going to leave these for people in the PDF file, those that can look these up.

I am not going to be talking about, I think, a much larger group of data about phage therapy in Eastern Europe and in Europe and in France. I've only recently become aware of how deep and scientifically complex all of that record is. It was explicitly ignored in the 1934 report, and since I'm trying to bring you up to speed on what happened to phage therapy in the United States, I really couldn't, and I don't have the expertise really, and Dr. Gôrski is going to be following up and I'm sure he'll be talking

about that.

This is the outline of the talk and I'm going to -- first, I'm going to do basically a review of phage therapy in the United States, I call it Phage Therapy 1.0, a brief word about the methods and formulations that were used. I'll spend the bulk of the talk reviewing the Eaton and Bayne-Jones report. These are the conclusions. They concluded the scientific basis of phage biology was in dispute, the commercialization was premature, and I think, I hope, I should be able to convince you that there was actual bias against both phage theory and d'Herelle in this report, and then I have a one slide 20/20 hindsight on what went wrong, and then a brief segment at the end about Phage Therapy 2.0 and how it's different and proposed standards.

This is the first paper. This is the beginning of it all. There's d'Herelle himself. This paper was read in September 1917. It was in the French National Academy of Sciences. I believe it's the first time where bacteriophage is actually written, and even here d'Herelle is already citing some of the properties of phages that we know are critical in our attempt to use them. That is, they're very often highly specific, and he also noted they

could be acclimatized. In a lot of ways, that means you could, by passaging them through target strains, you could adapt them to grow more efficiently on those strains.

I should point out that Gunther Stent even did -- so d'Herelle passed away in the mid-1940s, and phage therapy was really not active in the United States, at least in major publications, for the entire period of the '50s, '60s and '70s. Even then, Gunther Stent found it necessary in a review of a biography of d'Herelle to vilify him if you read these statements. This was written for a review in *Science Magazine*, and he couldn't say things like widely reviled, he had nothing to do with the conceptual ideological origins of molecular biology, which was ridiculous because he invented the plaque assay, without which we would know nothing about what goes on. He dearly enjoyed accepting undeserved credit. Well, we all do that.

(Laughter.)

DR. YOUNG: So you can see that Luca Brasi still had his knives out for this guy. But at the time -- this was long after d'Herelle, d'Herelle didn't know that Gunther Stent was going to try to trash his science and his reputation. d'Herelle, he had contemporary enemies. It's always bad when you

have an enemy whose face is on a stamp.

(Laughter.)

DR. YOUNG: And so it turns out Jules Bordet, who was high up in the Pasteur organization and a Nobel Prize winner, and I'm pretty sure that Bordetella is named for him. So he's a Nobel Prize winner who really despised d'Herelle and, in fact, was the major mover in pointing out that Twort had discovered phages two years earlier than d'Herelle. And John Northrop, who was a later Nobel Prize winner, was very much an opponent of the so-called d'Herelle-Twort Theory that phages were viruses, and then Albert Krueger, who was a prominent bacteriologist at Berkeley, was a protege of Northrop and basically his hit man, and wrote many, many anti-phage and anti-d'Herelle tracks. And it didn't help that both Northrop and Krueger were editors of major scientific journals at the time.

So this is their theory. They had a completely -- a theory they view was an Occam's Razor Theory, that is, a much simpler idea, and that is that basically that what phage is was a self-replicating endolytic enzyme, so that there's an enzyme that would degrade a bacteria and then in the process of degrading that bacteria to create more enzymes from

degrading the large molecules in the cell wall. So, effectively, it's analogous to the autocatalytic formation of trypsin from trypsinogen.

So, basically, their idea was lysozyme is a prion. This is the first prion theory from many, many years ago, and this was very -- because these people were so prominent and because their academic credentials and circles of contacts and editorships had a lot of sway, whereas d'Herelle didn't even have a college degree, it was difficult for d'Herelle to compete with this.

So d'Herelle went on to aggressively promulgate his ideas for using phage as a tool against bacterial infections. He was widely successful. In 1928, he sold his company that he'd set up to make phages for a million francs, and that was the same year that d'Herelle also took his job, full professorship at Yale, and apparently he did not tell his dean about this conflict of interest in terms of -- so I actually think -- somebody may correct me here, but the company was run very badly for a while. Then he eventually took it over again. But in any case, the laboratory bacteriophage, which was the company that was making up to 10,000 doses per day in the late 1930s, including a phage Phi X174, which

turned out to be a really key experimental phage.

So they sold what they called polyvalent phage capsules against dysentery, carbuncles, sinusitus, et cetera, and they supplied other companies, including Eli Lilly in the United States.

There were a lot of problems with these things, as you'll -- that basically we couldn't -- you expect for a premature commercialization. All of their so-called phages were simply filter sterilized lysates. There was no purification at all. There was no quality control, no standards. Assays were usually yes or no in terms of whether they worked or not.

The so-called polyvalent phage mixtures, which by their definition had multiple different phages, each targeted against a different bacterial potential pathogen. Sometimes these were grown together in mixed culture, and that led to simplification of the mixtures, and then the companies that distributed often put in disinfectants to preserve the phage lysates, but, of course, it does preserve them dead.

And this is a famous advertisement that I always showed in my phage class every year. This is the actual -- this is the advertisement for different polyvalent phage cocktails, including intesti-phage

and pyo-phage, and I think these are at least ancestrally related to the -- Betty, isn't that right? They're ancestrally related to the ones --

DR. KUTTER: In the 1930s.

DR. YOUNG: -- to Billeci. Yeah.

DR. KUTTER: We still have some of the vials.

DR. YOUNG: Right. And the intesti-phage and pyo-phage mixtures have been developed and matured and also sequenced, I think, now, so we now know what was in them, at least in some of them.

Anyway. So this is a phage for whatever ails you. I notice here that this is 1936. It says it's under the control of Dr. d'Herelle. So this might be after he re-took control of the company.

But one thing I noticed in reviewing this literature was that there was a corruption of the word "polyvalent." So polyvalent was originally meant to mean multiple different phages, each targeted against a different strain, a different bacterial genus, so the pyo-phage would have phages against all possible or as many possible important bacterial enteric pathogens.

But polyvalent come to mean as in general usage as any phage preparation that would attack

multiple different "races" or strains of the same species. So you have to be careful when you're reading the literature. They say polyvalent phage X, and you realize it's not really a polyvalent phage, it's a phage that plays against several host strains.

Okay. So there was, because of this lack of standardization and the other practices, for about a decade, there were many, many clinical studies done or treatments done and reported using these commercial preparations. There were at least four companies involved, and it led Margaret Straub and Martha Applebaum to publish in 1933 in *Journal of American Medical Association* their standardization. They went out and bought three samples. I think they had three of the four companies represented: Eli Lilly, Squibb, and Swan-Myers. Squibb called their preparation polyvalent phage for staph. Eli Lilly called it staphyl gel.

And they basically tested these off the shelf. They didn't ask the -- they bought them and tested their activity, and their findings were that the Lilly products contained an antiseptic that simply killed the phage and all the bacteria they mixed it with. The Squibb phage were highly variable. The one batch they bought had virtually no phage activity in

it. The next had high phage activity. And Swan-Myers had a potent staph phage in it, but it had, even though it was reputed to be effective against B. coli, it had no activity against any kind of colon bacteria.

So this was kind of a warning shot that the stuff that was being used at least from commercial sources wasn't reliable. It is interesting that they showed their bias a little bit here because they wanted to say that the reason why they're doing this is is they wanted to protect the reputation of genuine bacteriophage. So I thought that was -- it made me feel good that they actually cared.

So I actually ran -- I encountered a 1930 sales manual for Eli Lilly which I thought was interesting. There are industry representatives here, and Lilly arguably is one of the most certainly prominent bio and pharmaceutical company. So this is a manual that each new salesman was given in I think it was a week-long training course. So it's organized by lessons, and the next to last lesson, Lesson 38, is bacteriophage because Eli Lilly was selling bacteriophage, and so I highlighted some of the things here.

They were very cagey about what they said these things were. They wanted their salespeople to

be able to talk to doctors and be able to answer questions, and they carefully note here that the Twort-d'Herelle hypothesis that these are basically viral living particles is not accepted yet by other investigators, and then very explicitly in italics, this is their italics, "It is too soon to evaluate phagotherapy," even though they're selling these lysates.

So these are the lysates that were sold and I believe at least two of these were directly from the bacteriophage company in Paris that d'Herelle had founded. So here it shows how they're prepared and, in fact, merthiolate is added to preserve, and I'm sure, although merthiolate's not hugely bad for phage, if you have it around for a month, I think it probably will kill it. It's very important to note that under what name are the bacteriophage filtrates licensed by NIH, and they're licensed as bacterial antigens, never as bacteriophages. So that was something that each salesperson had to say. These are not phages, these are antigenic lysates.

But they did come to the same conclusion that when you had failures with phage it was because there was a mismatch between the phage and the targeted bacteria. So they were of obviously a very

mixed mind about the whole process.

Okay. So this is the report. It was commissioned by the AMA, the so-called Council on Pharmacy and Chemistry. It was published over three issues of the *Journal of American Medical Association* with the explicit endorsement of the council, and I'm just going to highlight the things that are important.

They set out at the top to evaluate two things: the bacteriophage phenomenon, which I was a little surprised by, I didn't realize that they were going to evaluate the basic science; and then the therapeutic usefulness of bacteriophage.

And I did a little work looking into the background of the people who wrote this, and it turns out that Stanhope Bayne-Jones may have had a conflict of interest or at least a little bit of a question mark about him being assigned to do this. He had just taken the bacteriology position at Yale that had been vacated under very hostile circumstances by d'Herelle in 1933. The dean at Yale had basically invited d'Herelle to leave. He had first become afoul with him when he found out about his commercial connections, but also because he essentially never stayed there for more than a month at a time, and he also had a superb talent for pissing people off.

So Stanhope Bayne-Jones was already -- at least you could argue that he might have been a little bit -- he may have tended more to find fault with d'Herelle than if he wanted to make his new dean happy.

He actually is a very stand-up guy, ended up a brigadier general. He was a multi-decorated war hero from World War I. He was the first person to study phage lysates with millisecond imaging, so he's a hero of mine, and he was actually a major mover in the 1964 Surgeon General's report that started the anti-smoking campaign. So, I mean, I don't want to cast any bad -- he certainly was a very consequential figure.

And so they really point out that they are summarizing about 100 studies, and so the first thing that hit me was, my gosh, that's already selected. There were more than -- it turns out there were more than 100 studies out there, and they only wanted to review the ones that they felt were most significant. But I now worry that this meant that they didn't review the Eastern and French literature for this very reason. And it also might be a language problem.

Okay. So this is the organization. I'm not going to go through it all, but basically the first

part of it is evaluating the theory and the rest of it is evaluating the actual on a disease-by-disease basis, and, finally, there are summary and conclusions. Up to now I had never read anything but No. 6.

So I'll just quickly summarize what -- the first part where they are looking at the mode of action, the theories, the origin, it was all kind of a hodgepodge. Its literature is of 1933, and when I read this, I was quite pleased because they were very, very measured. They came to conclusions that were perfectly reasonable in terms of specificity, the fact that you could adapt them by passage and phages. They even had the size of particles approximately right, and the fact that they're antigenic themselves, and that they were in most part robust in storage but were sensitive to antiseptics.

And then they pointed out that you could get phage-resistant variants after treatment, and those variants could have either increased or decreased pathogenicity. So up to that -- when I finished reading that, I thought, wow, these guys were really on top of it. And so then you read this.

The last sentence, "It's obvious there is great significance and importance. However, it's been

exploited detrimentally by manufacturers." So I thought, uh-oh, that doesn't sound good. And then I moved on, and then they end this section by saying in the composition that the phage preparations are just lysates and that part, it was accurate, at least at that time.

So the dagger came in the start of the section of a review of phage therapy in terms of practice. The first dagger comes in the first section about *in vitro* experiments and they cite, there were 12 studies cited. Other than d'Herelle, there were 12 extant studies, and they find uniformly that serum or blood and also bile either eliminated phage activity or greatly inhibited it, and by name they explicitly refute d'Herelle's experiments that had been published 10 years before as being unreproducible. And, first of all, I had never heard this. I never heard that serum inactivates phage.

And then the second section was *in vitro* bacteriophage therapy in experimental animals. There were 21 cases of animal experimentations cited. All of them were negative. In each case, it was done the right way. They were active on the bacterial strain and infection model using a variety of animals and a variety of disease-causing bacteria, and their

conclusion was uniformly negative that in no case where animal model experiments had been successful, and explicitly again named d'Herelle's S. pullorum experience in chickens as being unreproducible.

So, bottom line, phages are inhibited by blood and have not been shown to have efficacy in animal models, and at that point, the game was over because for the rest of the report they're simply going through and trying to explain away any positive results because, as far as they're concerned, if it didn't work in experimental animals where you could do controlled experiments, the rest of it was just confirmation bias.

So here's the next section, there's the eight different disease things, and we obviously can't go through that in a very short time, but the whole experimental evaluation in human infection starts with this: "The many good reports make it difficult to assert that lytic filtrates are without effect." So they're complaining about the fact that they can't just say there's no effect.

But then they go through and find for each disease system here why the positive results that were reported were not significant, and so basically they were so biased by their finding that animal tests had

been uniform failures, they concluded phage therapy had undemonstrated validity.

They do point out that in the cases where negative they did the right thing. They said in most of the negative cases it's very likely due to the way the experimenter or the physician was using them, that the phages simply didn't work *in vitro* on the bacterium, and they, in fact, concluded in this very negative diatribe that for this reason *in vitro* lysis should always be demonstrated. So flash forward to Phage Therapy 2.0 now, that's sort of the way we're working, that we make sure that the disease bug is sensitive to the phages that are used.

So I'll skip down here. There were more than 80 citations, including 70 studies involving thousands of patients. I've said already that they're essentially either inconclusive or negative value. They make a big point to refute d'Herelle's success, the most highly publicized success with both cholera and plague, and in all these cases which appear to be well controlled, including double-blind experiments, they said they were unable to reproduce d'Herelle's results, and mentioned him by name. The one exception is local infections, boils, furunculosis, staphylococcus.

So they had so many and apparently well done studies with staph infections and boils and skin eruptions that they were unable to come up with any saying that they were possibly efficacious. Nearly uniformly positive. And in this case, they used both commercial phages and home brewed phages where the physician just went and isolated phages from sewage and used them directly.

Okay. So there were 11 summaries and conclusions after this long thing. I'll just show a few of them here.

First of all, and I think the tell tale of the bias in this, their very first statement was, "d'Herelle's bacteriophage is probably an inanimate enzyme, not a virus parasite." So the Prion Theory. So there was not a shred of evidence evaluated in this entire long review, and they started off saying that it was undemonstrated. Now they're coming down on the side of the Northropites and the Prion stories. I just find that amazing.

It says it repeated their finding that lytic action was inhibited by blood, and they find positive results only for local staphylococcus infections and possibly cystitis. They just say they would be convincing. Importantly, there's no evidence that

phage lysis or phage killing or propagation occurs *in vivo* at all, and then favorable results may have been due to immunizing action of the bacterial proteins in the broth filtrates.

So this is my sort of 20/20 hindsight version of this. First of all, I think the fix was in. I think if you read this, you know, it's not an airplane read, but if you ever sit down and read it, I've read the whole thing and I've read about a third of the cited English language studies, they never miss an attempt to specifically denigrate d'Herelle or d'Herelle's hypothesis, and as I said, they ignored the Eastern European and French studies that were far more positive and had, I think, irrefutable anecdotal data in terms of statistics.

In almost every case that I've read so far, no matter what the physician said they used, actually, they would often claim to use polyvalent phage, but, in fact, in almost every case I think you can presume that they used a single phage, and in most cases, they did not test that single phage against the bacterium in every case of infection that they tested.

So very likely these were simply -- many of the failures were due to specific mismatches or you could certainly get -- with a single phage, you will

almost certainly get rapid rise of resistant bacteria. And the frequent successes with staph, I think, can be due to the fact of the omnipresence of phage K. So what's phage K?

So phage K is I call the mother of all polyvalent phages. Phage K is actually closely related or related to the very first phage that Twort identified back in 1915. It's a large 130 kb DNA myophage, which means there's a contractile tail. There are 30 whole genomes that are greater than 90 percent identical in RefSeek and Genvac. All right. So 99 percent of the -- the phage K is 99 percent identical to Team One, which is a staph phage in the Tbilisi cocktail, and it was actually first described in the Vurnet Laboratory in Australia in 1935. Based on just a very quick survey, this phage has been patented many times.

(Laughter.)

DR. YOUNG: And so why are phage K and its relatives polyvalent phages in itself? The main reason is that the receptor for phage K is an N-Acetyl glucosamine in the cell wall of teichoic acid, and the key thing to that is that that is essential for the viability of -- so this phage has found a receptor that can -- essentially very difficult to change it or

lose it because the bacteria will be inviable.

The other thing that's unique about it is that there's not a single instance of GATC in the 130 kb genome. If there's evidence for intelligent or at least sadistic design, this is one of them. So by random statistics you expect 300 of these in that sequence and there's not a single one, which gives us automatically immune to restriction by the major restriction enzyme of the staph aureus.

And finally there's what I call type dominance. If you go out and look for virulent phages in any sewage or environmental sample that plate on a large collection of staph, you'll always get phage K. There's another type, a small photophage that you can get, but they're usually a very narrow host range. Phage K is recognized in wall teichoic acid. It's what you get and it doesn't matter where you isolate it, in Japan or United States or anywhere else. So just somehow for some reason there's been a bottleneck in the history of staph aureus, and it completely went to phage K and its friends.

Okay. So I think in the interest of time I'm going to skip down to the next section. I do say I am still confused and I would love to have somebody help me think through this, why the serum results were

so uniformly negative and surely there are modern data that somebody's published that can refute this, and why were the animal experiments so uniformly successful.

The simplest Occam's Razor argument is that they were careful in what they cited and that there was anti-confirmation bias, but I don't want to conclude that until I know more about what was actually going on at that time.

So, in the last few years, obviously, there's been -- so that Phage Therapy 1.0 died and was put to death basically mainly by that 1934 report which I find was highly biased. In the last few years, there's been an acceleration because of the onset of multiple drug resistance, as you all well know. We had this wonderful meeting like this just two years ago, in July. It already feels like a decade has passed in terms of what's going on, and just this last year there have been successful emergency IND phage treatments, which you're going to be hearing about in detail.

And so I had no role in this except as somebody who had emails come in and sent emails out. That's basically what I did. So this is a picture of the -- and this is my total experience with phage

therapy, so I don't have to do a meta analysis. So I'm just going to state the facts, and most of you have read about this. Tom Patterson contracted a *Acinetobacter baumannii* infection, spent months in the ICU, and then the physician, Chip Schooley, who will be taking to you very soon this morning, obtained permission via the eIND mechanism for attempting phage therapy. The strain which we will call TP1, Tom Patterson 1 was isolated and it was shown to be multiple drug resistant and was shipped to -- at the end of February 2016, the Patterson team contacted two agencies, our agency, Center for Phage Technology, and the BDRD at the U.S. Naval Medical Research Center for phages having obtained, I guess, the eIND permission. So this was the last week in February.

So what we did was we solicited phages from everybody we could think of because we didn't have any, and we got a bunch of phages from AmpliPhi. I have to say that it was a uniformly yes answer, forget the paperwork, don't worry about IP. Instantly people ran to the mailboxes and sent their phages, and we tested, I think, 40 phages from around the world and one of them worked against this strain and that was the one from AmpliPhi, and we spent about a month isolating new phages.

The Navy already had a wonderful, large, complex collection of AB phages, and they have a very efficient plate reader-based semi-automated liquid growth testing system. So they were contacted later, and they were able to identify phages much faster.

And then the two sources prepared phage cocktails. It was a nightmare. It shut our laboratory and center and academic activities down for two full months. We eventually provided four phages each as cocktails and mixed the phages together and made phage cocktails. They were eventually shipped to UCSD Hospital mid-March 2016, and some of the cocktails were actually purified by organic solvent extraction to lower the endotoxin levels, and that was primarily done by people in Forest Rohwer's lab at San Diego State University.

By mid-March 2016, the CPT cocktail was administered through an abdominal drain, and the Navy cocktail was administered IV, and I think the data suggests it was the most important component. Within a week or so, there was a bacterium isolated from -- was that from the blood or from the drain? I can't remember. It was a drain bug, and the strain TP3 was resistant to all the phages that were used, all eight phages that were used in the original cocktail.

The Navy then used their rapid system to isolate another phage that grew against TP3, and was able to then use that to modify the IV cocktail. Phage therapy continued for eight weeks, and the patient recovered, and there he is. I think the Superman designation should be on her chest because she is a super woman, and she and Dr. Chip Schooley need to have -- in fact, I think there's going to be a movie about them. Isn't that right? Who's playing you, Chip? Is it Tom?

DR. SCHOOLEY: Jack Black.

DR. YOUNG: Oh, Jack.

(Laughter.)

DR. YOUNG: All right. So that's Phage Therapy 2.0 from my point of view. There are some others and we'll hear about them here.

So I have questions for you. I know that half, more than half of the people here are regulators or people involved in this.

The single patient eIND pathway, is there a limit to the application of this mechanism? We'd like to know what happens when there's a negative outcome. From our point of view being non-regulators, it looks like it's going to continue. I get daily, I get appeals for phage therapy. There are always

emergencies. There's more MDR bacterial infections that are occurring and phage therapy is likely to work, so it seems like we sort of fit the bill for eINDs.

However, the only propagation that we used because of the urgency of our task was whether or not the phages would grow in liquid culture. We did no characterization of the phages. Of all the nine phages at the viral or molecular level, all eight phages used in the first strain were all very similar. We think they are all large myophages and probably use the same and/or linked receptors, probably an outer membrane lipopolysaccharide. And so, in this case, Phage Therapy 2.0 was the same as Phage Therapy 1.0.

So I'll just come to the bottom line here. I think we ought to have -- since we have this now 100 years of advanced -- there's no longer any doubt about the molecular and biological nature of phages. We have detailed knowledge of the moving parts of phages and how they work, although I think a lot more needs to be done on phages not of E. coli and B. subtilis, and we have or can develop rapidly tools for determining receptors, affinities, DNA modifications, et cetera. These are my suggestions.

I think we should have available phages for

eIND applications that have complete annotated genome sequences beforehand. They should have EM images sufficiently accurate to determine tails, tail fibers, and other attachment appendages. We should know the nature of their DNA modifications. There should be established purification and titer requirements, and I think in the long run the cocktails, that if we had that as a starting material, when the balloon goes up and an eIND is in motion, the cocktails that are assembled will have a much better likelihood of efficacy and redundancy so to avoid resistance, and the long-term, if we had hundreds or thousands of these cases, the retrospective value of having the genomic information raises an enhancing possibility that we might be able to predict efficacy strictly from genomic information.

So thanks for that. I hope we have -- I hope I haven't bored you too much with this retrospective, and I appreciate your patience. Forty-eight seconds left.

(Applause.)

DR. KINCAID: Well, first, I'd just like to point out that the choice of you as the first speaker was not because we expected people to be hung up on Metro or anything like that. I think you were

selected primarily for the strength of your intellect and, as only a true Texan could, to cover a century of events and in a story-telling manner.

I don't know if there's anyone who would like to ask Dr. Young a question. I think we have time for one as it turns out.

All right. Well -- oops.

AUDIENCE MEMBER: One place in your slide you mention that the company who are making this lysate indicated that this is a bacterial antigen. Did the title mention that these are actually a vaccine type of agents?

DR. YOUNG: So Lilly was very carefully telling their salespeople to not claim that these were phages that would lyse bacteria. But instead, these were lysates produced by phage lysis that would immunize the host. That was the official.

Yet, on the other hand, all of their warnings and everything suggested that the phage had to be targeted properly and should be shown to grow *in vivo* -- *in vitro* beforehand, so they were clearly confused, and I don't think in these days a company would go to the market with that much confusion built into the products.

AUDIENCE MEMBER: Thank you.

DR. KINCAID: All right. So we're going to move on and we'll learn from a very practical point of view the experience that has taken place in Poland over many decades, and so we're privileged to have Dr. Andrzej Gôrski from the Institute on Immunology and Experimental Therapy and the Polish Academy of Sciences. Andrzej.

DR. GÓRSKI: I wish to thank my colleagues from FDA and NIH for inviting me here.

Well, in our work, we sometimes refer not only to Félix d'Herelle, and I don't need to waste your time by going into details which were already covered by Dr. Young, and you know it from your own knowledge, but also to another famous, eminent Canadian doctor and scientist whom you know, Sir William Osler, and his accomplishments are well known in terms of his medical achievements. He's been also recognized as a philosopher and ethicist, and some of his profound statements are listed here. And we believe that our purpose in treating patients with antibiotic-resistant infection was not merely to eradicate infection but to treat the patient who has antibiotic-resistant infections, so do not treat -- do not eradicate the infection at all costs.

This is the institute where our center is

located in Wroclaw. The center, the institute is more than 50 years old, and our therapy center has been opened 12 years ago, and we operate under the umbrella of the Declaration of Helsinki and respective regulations of European Medicines Agency, as well as Polish regulations which are contained in the legislation of medical profession and the Polish Constitution and so on.

And I wish to emphasize again that what we are doing is experimental therapy, which is also known in Europe as compassionate use, and in America, it's often referred to as expanded access.

This is a kind of summary of our thinking about why experimental therapy is so important for further progress in phage therapy even though it does not formally yield the data which, let's say, could be considered as fully scientifically relevant according to the standards of evidence-based medicine.

Why? First of all, we have already achieved the data which support the notion that phage therapy is safe. We know that side effects are not very frequent and we know the side effects. We know what we can expect when giving the patients phages orally, indirectly, or topically.

We also learned a very interesting lesson

about the relationship between phage administration and antibody production. As you well know, there has been a common belief that antibody production to phage should be a limiting factor in the success of phage therapy, which we know it's not that simple.

Of course, experimental therapy can provide the idea and planning for optimal clinical trials which we have not been able to accomplish yet simply because of lack of funding.

Another very interesting area, and we have been deeply engaged in this area for the past 10 years, is the relationship between phage and immune response, how immune system reacts to phages, but also how phages act in immune system. We have published an interesting paper recently which is available online, "Phage and Immunomodulation," and I suggest that perhaps you will find a minute to go over this paper.

So phage and immunomodulation is something we believe which may be also a future application in phage therapy which is not directly related to eradication of infection. And, of course, the experimental therapy is important for our promoting knowledge and fund raising.

I know from my own experience that the average understanding of what phages are at least in

Polish medical and lay communities around the globe, many doctors do not know what are phages. Medical students have very limited knowledge. So I believe by engaging in experimental therapy we also serve this very important purpose of raising awareness of what phages are, what is their current possible use, and perhaps what are the hopes for the future.

In my younger years, I've been facing the development of organ transplantation in my country, and sometimes I believe that the current development in phage therapy are quite similar. I remember how it was tough at the beginning to transplant a kidney. In Poland, you had to ask the prosecutor for personal approval of each transplant; otherwise it would be considered illegal. And so now we have a very active, very fruitful organ transplantation like everywhere else. Hopefully, the phage therapy should follow the same route.

For those of you who might be interested in this quite interesting field of ethics review of compassionate use, I would refer you to our paper which is now in press in *BMC Medicine*, which addresses specific aspects of the ethical review and dilemmas of experimental therapy.

Now there's nothing exceptional in our

approach in terms of the let's say production of phage lysates. The scheme is presented here. We also use purified phage preparations, but they are, of course, much more costly, so it's quite controversial whether or not you should charge a patient for a product which is much more expensive, yet today we don't have formal proof of efficacy. So, of course, for clinical trials, that's another issue. But for experimental therapy today, it's kind of difficult dilemma.

This is the current bacteriophage collection of our institute. As you may see, it's quite rich. We have more than 800 of total phages, and the specificity and range is shown on the slide.

And the spectra, yeah, we are very glad of our anti-staph phages, including MRSA. We cover almost 100 percent of the Polish strains, and quite high coverage for other bacteriophages, except perhaps *Pseudomonas aeruginosa*. We are not happy. As you see, we can cover slightly more than 50 percent of the spectrum.

Quite recently, we wanted to increase the efficacy and the range of our bacteriophage preparations by propagating our phages on the bacteria that were rendered plasmid- and prophage-free, and without going into the details for which I do not have

time, you may see that when you propagate the phage on such strains, the phage titer and positive lytic reaction may increase. So this is the initial phage titer on a host bacterium and then on a bacterium that was cleaned of PPE, and you see the increased titer and the increased positive reaction.

In fact, we have a short abstract describing this phenomenon, and the text of the abstract is presented here, and the main information is highlighted in this fragment of the text. Probably in summary we can increase the efficiency of our phage preparations in future by working on purified strains rather than standard initial strains.

The specification of our final phage preparation is depicted here. Activity, stability, degree of purification, and -- well, that's kind of our local pharmacy.

Now the philosophy regarding phage therapy indications, contra-indications, and termination. This has been presented many times already and, in fact, if I remember well, I presented this data in this room two years ago. They have been published, so I don't know if I should go into the details. For those of you who are interested in those details, already five years ago they were presented in our

paper published in *Advances in Bioresearch*.

But generally speaking, the philosophy and the practice is straightforward. There's nothing exceptional here, maybe except that so far most of our work has been done on monotherapy. We've been using monovalent phage preparations rather than cocktails, although we have some preliminary data on cocktails which I show you later on, but again I beg for your understanding because they are really preliminary.

Now, of course, what about phage therapy patient monitoring? What we do when we watch our patients? Of course, we perform clinical detail, clinical evaluation, and as you probably know, we don't have many patients because we have to spend at least one hour, and probably more, with each patient explaining to him. Sometimes there are patients from abroad, from Germany, also from U.S., so you need to explain everything starting from scratch. What are the phages? What are the pros and cons and so on and so on. So really, it's a kind of really hard work, like in our profession, of course, that's nothing exceptional, except that you really need to have to spend a substantial time to satisfy your patient and yourself.

And, of course, very frequently, because

most of them are very complicated cases, we must seek external consultant opinion. Then pathogen isolation and testing of use, and then we perform detailed lab monitoring, including CRP, blood analysis, organ function.

Well, regarding our patients with chronic bacterial prostatitis, we perform old 4-glass test, which enables urologists to localize where is the infection located in urinary tract.

Now it's a kind of historical test. I don't think it's performed in United States, but it still has its value, but we live in times when doctors have little time, so it will be probably unrealistic to recommend the testing, but it has its value and, of course, you can gain important scientific information by obtaining fluids from data and other part of the urinary tract.

Immune monitoring. There is, of course, an important question whether or not the effects that we are observing in response to phage therapy are not simply let's say the immunostimulatory effects of bacteria that remains on phages themselves, and according to our experience, and the experience has been published and we have quite a few papers addressing this issue, although there are, of course,

reactions of immune system to phages, we believe that we cannot ascribe the beneficial effects of phage therapy to mere upgrading, upregulation of immune system. We don't think it's that simple.

We categorize the results of our treatment into seven major categories. They are listed here, and again I will not go into details because I believe they are straightforward. Of course, we hope to obtain the Category A pathogen eradication and full recovery, but it happens rather rarely in about 10 percent of cases. Overall, we consider Categories A through C as good responses to phage therapy, and D-G are considered as not a great response to phage therapy.

What I already mentioned and there's nothing unusual in this statement. We in our material, and this is not only in material from our phage therapy center but also historical material of the past because phage therapy in Poland is almost as old as discovery of phages. What is notable is the remarkable safety of phage therapy. Here, you see that on our material of almost 160 patients we observed good tolerance in almost 80 percent of cases, and the lack tolerance in less than 4 percent of patients, which force us to terminate treatment.

And we also have in press another paper which includes more recent data from the past three years. This is the characteristic of these almost 150 patients, the indications for phage therapy, and the routes of administration, type of applied phages. Maybe we don't have time now to go into details but just to give you the general idea of the patient types and the way we administer our phages.

And those results, which have been published, so there's nothing new, in fact, in this data which I present except that we have also results from the most recent cohort of patients which have been treated in years 2011 through 2013, and you may see that it's amazingly close. The results are amazingly similar. Almost 40 percent of good responses in years 2008 to 2010, and almost the same result obtained with most recent patients.

So, in summary, in about 40 percent of cases we obtain something which we categorize as a good response according to the description I showed you in an earlier slide.

And now this good response translates to quite interesting and promising results in patients with genital and urinary tract infections. Most of those patients, although not all of them, are those

who had chronic bacterial prostatitis, and they have been treated using indirect administration. In this group of patients, our results seem to be quite promising, and other responses shown in other clinical settings.

Now I would like to stop for a moment on this slide because here again I ask for your understanding that the data are very limited and the number of patients is small. But I think it's something new and something potentially, not perhaps relevant today but potentially relevant, and it may give food for thought for future work.

Here, we compared the efficacy of the monovalent phage lysis versus phage cocktail. The description of the content of this preparation is provided here. Right, non-purified monovalent phage lysates, and non-purified phage cocktail, which contain a mixture of three staph phages.

So you see here that again using this very limited material there is very little difference between the monovalent and cocktail. However, when we used the purified cocktails, this difference was significantly higher. The results achieved with the purified phage cocktail were much better.

However, for that and other reasons, the

titer of the phage preparation was also higher, so it's difficult for me to say whether or not those better results is due to the purity or higher phage content or both. One way or another I decided to present to you this data simply because of the fact that you can achieve more than 50 percent of success using purified phage cocktail.

Of course, using purified phage cocktails versus monovalent cocktails, you can easily organize a conference to discuss this controversial issue, and, again, we don't have time probably to go into this philosophy today, I know. But one word of the caution, I can cite the most recent paper by Oechslin regarding this issue because there is a kind of over-enthusiasm in recommending cocktails. We'll see.

Now this slide shows you the changes in phage profile and acquisition of phage resistance. What is probably most important is how muted we are regarding the application of phages once the resistance develop. This resistance develops, of course, here, and these two panels show you the percentage, but still we are able to identify a phage in our phage collections to continue the treatment if it's necessary, and the percentage of this success is shown in this slide.

Now antiphage activity of sera from patients receiving phage therapy, we have published four or five papers already, so again I will not go into details, but what is probably most important is following oral administration the level of antibody is very low. You may also find quite low antibody production using inter-rectal administration, which is maybe unexpected, but that's the fact.

And most importantly, there appears to be no clear association between the level of antibody -- antiphage-producing antibody in serum and the outcome of therapy, which is shown here, right, with a patient with a high level of antibodies, yet the result of treatment was good, good response, clinical improvement. In another patient who had high responses -- by the way, you may see that this level of antibodies may drop subsequently, and again we had good response to therapy.

So phage therapy and antibody responses is a complex story. You cannot simply say that antibody response is limited because it probably depends on a variety of factors. Some of them are listed here. Certainly, patients in neurological status, we cannot forget that at least in our material 50 percent of patients which come to our center are immunodeficient.

Then there is a question of route of phage administration, which I've shown. We don't get high level of antibodies using oral therapy, phage immunogenicity, phage immunogenicity varies, purified preparations versus lysates, cocktails versus monovalent phages, and type of antibody involved in phage binding.

Now the question how long the phage antibody persists, we show that they can eventually drop, and a kind of provocative statement because who knows whether the good antibody response to phage therapy is a good prognostic sign. Maybe. Who knows? Maybe it simply signals that the immune system recovers. It's able to provide, to offer, to mount a good immune response which does not inactivate phage, at least not at the level of periphery. Right? Who knows? We need more data.

Another interesting aspect is phage interactions with phagocytes. We just published a review on this, so, again, I will not go into details, but I'll show you the results from one patient, how phage therapy can contribute to, and this is one sign of let's say improvement of immune system following phage therapy regarding ability to kill bacteria by polymorphonuclear cells and monocytes.

So, during the successful phage therapy, as you can see here, the patient could recover -- the patient's ability to kill bacteria could significantly increase, and another, I mention phage and immunomodulation, and we have quite interesting data regarding the potential effects on phages on the indices of inflammation. Here, you see the CRP levels initially and following nine the use of therapy the value drop from 35 to 14, and then after second round of therapy to almost normal, even though the eradication has not been achieved.

So there is a -- again, I repeat -- a very interesting area, how phage interact with immune system, and we have published data showing that they can inhibit a reactive flux against phages.

We published a book on phage therapy which received a good opinion in *Lancet Infectious Diseases*, in *Clinical Infectious Diseases*, and a number of papers. One of them presents the present and future of phage therapy, and maybe I'm kind of selfish because I was the first author, but to some extent this is a kind of visionary paper.

And, of course, we realize that observational studies are not the evidence-based medicine. Yet I would like to cite here the statement

made by Dr. Califf about the value, potential value of such work, and I will in the end, I will refer again to this paper published recently in *Future Microbiology* about phage translocation from the lumen of intestine through surrounding tissue and gut-associated lymphoid tissue, and this has in our philosophy, in our thinking, not only -- this is not the only mechanistic let's say movement of phages, but on the way they interact with cells in immune system, which is shown here, and they may beget powerful, powerful immune reactions. We were very glad to see that this theory of phage translocation is already cited.

Well, what is the future? It's difficult to tell the future, but I'm sure you know the report prepared by Wellcome Trust and the projections. What are the alternatives to antibiotics for using wild type phages according to this forum only 9 percent of success? If this is so, if this is true, what is the future? What we should do in parallel to developing clinical trials?

This is a letter in Polish, but also it has an English portion. This is a letter which was sent by Minister of Health of Belgium to the Minister of Health of Poland. Of course, it went through the

bureaucracy of our Ministry of Health and this is the result, so I apologize. You don't need to read the Polish text, but you can concentrate on this portion which tells you what is most important.

And what is most important, and this is probably my message, I find the message that we need to develop clinical trials, but I believe, and I'm glad to see the Minister of Health of Belgium agrees, that we also need to expand the existing programs of experimental therapy. Thank you.

(Applause.)

DR. KINCAID: Do we have any questions for Dr. Gôrski? Could you use the microphone, please?

AUDIENCE MEMBER: Thank you, Dr. Gôrski. I have a question about your source of your phages for your institute. Are you constantly going out to environmental sources like waste water treatment plants to isolate new phages?

DR. GÓRSKI: Yes.

AUDIENCE MEMBER: Or what's your procedure about that?

DR. GÓRSKI: Well, the procedure is standard. I don't think I will go into technical there in details. You know, it's standard procedure for phage procurement which has been described in

details in a series of papers published by *Frontiers in Microbiology* recently. There is a paper by Beata Weber-Dabrowska, et al. under the title "Phage Procurement for Therapeutic Purposes." I think it gives you the most updated information.

AUDIENCE MEMBER: That's the primary source for your phages is waste water untreated sewage?

DR. GÓRSKI: We have also historical phages which are very old, which we inherit even from the time of Ludwik Hirszfeld, who worked, who founded our institute and before we were here, he was already working also, and he's been already engaged in the collection of the strains of phages, so part of our phages are historically related.

AUDIENCE MEMBER: You mentioned that you have seen a specific -- a phage-specific antibody response in about 17 percent of patients treated with single phages and about 43 percent of patients treated with cocktails. Since phages can differ in their immunogenicity, were the same phages used in the cocktails that were used individually? Is that a direct comparison?

DR. GÓRSKI: Yes. Yes. Yes. So it's a kind of learning. If you believe that the peripheral, peripheral antibody are peripheral -- and in the

presence of antibodies peripherally may be a little bit impacted for the success of phage therapy.

AUDIENCE MEMBER: Dr. Gôrski, thank you so much for very useful data on clinical use. It's very impressive. And my question about the allergy testing, what allergy --

DR. GÓRSKI: Allergy?

AUDIENCE MEMBER: Allergy. You mentioned that you test your patients.

DR. GÓRSKI: Very good question.

AUDIENCE MEMBER: And my question actually has two parts. What allergen do you use? Is it like phage, something from phage, and what method do you use for this?

DR. GÓRSKI: As far as allergy, we did not prohibit IgE responses, but interestingly enough, when you monitor the leukocytosis in our patients, in none of these patients we have increased value of eosinophils, which was striking. In no patient, I repeat, we had increased value of eosinophils.

And I'm also aware of the work of my associate, Krystyna Dabrowska, a very bright molecular biologist, and I know that she just presented a very impressive poster which she will be presenting in Evergreen, that in experimental animals, when you

inject phages, when you administer phages, I think she had some data, if I remember well, that there is no specific allergy to phages in mice.

So, very unexpectedly, there appears to be no strong allergic reaction as measured by the data. In contrast, you have a decreased CRP value, and clinically relevant allergic reactions, they can appear, but they are relatively very rare. As you mentioned, less than 4 percent patients develop such reactions that cause us to terminate the treatment.

AUDIENCE MEMBER: So you did not use test allergen in --

DR. GÓRSKI: Sorry? I did not hear you.

AUDIENCE MEMBER: You did not use test allergen made of phages, right? You just used indirect methods to see the allergy.

DR. GÓRSKI: Yeah.

AUDIENCE MEMBER: Okay. Thank you.

DR. KINCAID: I think what we're going to do is we're going to go to a break right now, and for those of you who do have questions, I encourage you to speak with Dr. Gôrski or Dr. Young, and we will return at 10:15. Thanks a lot.

(Whereupon, a short recess was taken.)

DR. KINCAID: Thank you very much. I'm also

going to take this moment to mention to all the speakers and to those asking questions, all of those good thoughts will go to waste, certainly as relates to the ability to be transcribed, if you don't speak into the microphone, and I would suggest to the speakers that they listen for the resonance because that's what really allows you to know that you're being heard.

So, without further ado, I'd like to introduce Chip Schooley from the University of California, San Diego, the Department of Infectious Diseases, who has an absolutely stunning story, one that many of you are already familiar with. It's a story that was given its initial promotion by Ry Young earlier in the day. So, without further ado, Chip.

DR. SCHOOLEY: Thanks very much, Randy. I'd like to thank Randy and the rest of the organizers for the opportunity to talk to you a bit about Tom Patterson, who you heard a bit about this morning from Ry Young. This is going to be a very clinical talk. I was asked to kind of give people a sense of how these cases play out in the context of an emergency IND and to talk about some of the strengths and weaknesses of this approach.

I am going to show pictures of the patient

and of his wife. They both are not just giving consent, they're actually quite enthusiastic about this.

So the story began in Egypt in November of 2015 when they took a vacation during Thanksgiving down the Nile, and this is the inside of the boat that they were on.

(Laughter.)

DR. SCHOOLEY: Let's see if we can get this to go here.

Okay, we'll get this back in a second. We'll have to stop the timer here.

In any case, Steffanie Strathdee, the patient's wife, and Tom took a vacation over Thanksgiving 2015 to Egypt and decided to take a barge down the Nile. As they're pulling into Luxor, he developed abdominal pain and fever. Tom is a friend of mine, as is Steffanie, and they texted me and said they were concerned about Tom and wondered whether this could be gastroenteritis or something else.

As this played out, it became more and more clear it was something else, and it was suggested that they get to a hospital. He was a 68-year-old diabetic, a little bit overweight, and it sounded more and more like gallstone hepatitis, gallstone

cholecystitis and pancreatitis.

They went to the hospital in Luxor. They had been seen by a ship physician who saw him and gave him Gentamicin. This always works. In this case, it didn't. He showed up in the hospital and the same physician actually was running the ICU and this time gave him some fourth generation Cephalosporin, some fluids, stabilized him, and then he was evacuated to the university hospital in Frankfurt, where Stefan Zeuzem and his colleagues took care of him.

When he got to Frankfurt, it was found that he had a large pancreatic pseudocyst, shown here with the green tags behind his stomach, both in this plane and in this, a relatively large fluid collection. On the second hospital day in Frankfurt, they threw an endoscope into his stomach. They then used an endoscope to put two stents through the wall of his stomach into this fluid cavity to drain the abscess cavity into his stomach.

In this first acquisition of fluid, they grew *Candida glabrata* and *Acinetobacter baumannii* that was resistant to most antibiotics except for colistin, tigecycline and meropenem.

He continued to be febrile, was on pressors, was delirious. The following two days later they did

an ERCP, noted that his biliary tree was partially obstructed by a pre-papillary stone which was extracted, a stent was placed to establish drainage of his biliary tree. These cultures also grew *Acinetobacter baumannii*. Colistin was added. Imipenem was changed to meropenem based on some of the sensitivities that were emerging, and arrangements were made to transfer him to San Diego.

By the time he got to San Diego on December 12, the organism was meropenem-resistant. The GI consultants felt that he needed surgery. We felt he needed surgery. The only people who didn't were the surgeons. They were concerned about the fact that he was quite unstable, and not being able to do the surgery ourselves, we had to go along with their plan.

So the plan was to try to manage him medically and to drain the abscessed cavities as they evolved as you'll see percutaneously using interventional radiology throughout the course of this. He developed a pancreatic cyst. This too was drained. The *Acinetobacter* continued to develop increasing resistance as we treated him with rounds of antibiotics.

He then began by the middle of January to

grow *B. fragilis* from a number of these drains. We didn't have good control of the source of his necrotic pancreas. He had an episode of septic shock and was transferred to the ICU, and at this point in time was found to have *B. fragilis* bacteremia.

He shortly thereafter developed emphysematous cholecystitis. Another interventional radiology drainage tube was placed. One of our colleagues in the Department of Pediatrics did some synergy studies *in vitro* and showed that if you squinted, that if you used azithromycin, colistin and rifampin together, there was some evidence of synergy, so these were added to his antibiotic regimen. Developed some renal failure. The colistin was held and then restarted later on. He then developed increasing abdominal distension. Paracentesis revealed that *Acinetobacter* was now in his peritoneal fluid.

By middle of March, he had the multiple intra-abdominal collections being drained through five IR drains. We continued to try to convince the surgeons to go in and do a definitive drainage procedure, and the cycle we got into was that when he was sick they said he's too sick to operate on. Call us back when he's better. We called them back when he

was better. They would say he's getting better. You need to just leave him alone, let him continue to get better. So we got into this cycle through February and March in which we were not able to get him, in our view, adequately drained.

As time went on, though, he continued to deteriorate. Additional organ systems began to fail. He became stuporous. He ended up on two pressors. He ended up intubated on a ventilator and began to develop hepatic dysfunction in addition to his renal dysfunction.

This kind of gives you a sense of his course. You can see his fever curve with multiple fever spikes throughout. White count kind of bouncing around between 10- and 25,000, often in conjunction with isolations of organisms from his bloodstream and peritoneal fluid in other places.

His wife, Steffanie Strathdee, is really the hero of this story. She had kind of been watching this and continued to read. She is an infectious disease epidemiologist, trained as a Ph.D., not as an MD, but she is really quite versatile in microbiology and continued to read about *Acinetobacter*, and came across a paper that was published in *PubMed* about the use of *Acinetobacter baumannii* in the treatment

of -- I'm sorry -- of phages in the treatment of *Acinetobacter baumannii*, and she sent me this paper by email.

This was about mid-March, and my response to her at the time was, you know, it's not like we're really knocking the socks off this infection, we're willing to try anything at this point, and certainly there's been a lot of history of phage therapy in other places, as we've heard today. Very little evidence that it will do any harm, and certainly we're not getting where we need to go with our current approach to therapy.

My inward thought, however, was that the chance that we're going to find somebody to make us phages in time to be able to use them and to deal with all the bureaucracy both in terms of the local bureaucracy at UCSD and the regulatory bureaucracy outside UCSD was slim, but given the fact that we had really very little to offer and Steffanie needed some hope, we decided to go ahead and go full steam ahead.

She got in touch by email with the group in Georgia. They referred her to Jean-Paul Pirnay in Brussels because they had been collaborating. Dr. Pirnay said he'd love to help. In fact, he had some phages that were active against Middle East-derived

Acinetobacter and that they were in the hands of Ry Young's laboratory at Texas A&M, and she suggested that the organism be sent to Ry.

She telephoned him, caught him in his lab and talked to him for a little over an hour, and over the phone he decided to go ahead and try this out, and said he would commit his laboratory for the next couple of weeks to see if he could come up with something that might be used to treat her husband.

The organism was sent to Texas A&M, and the phage search began. Unfortunately, at the same time the phage was being -- that Dr. Pirnay had was being shipped from Belgium to UCSD, preparations were being made and it was found that that particular phage did not have activity against our patient's organism.

In the library at Texas A&M, one of the phage from AmpliPhi was found to have activity. Dr. Young got in touch with AmpliPhi and they very quickly said, "Of course, we'd be happy to let you use that phage." And then he looked for environmental sources of additional phage that could be used in a cocktail against this patient's organism.

At that point, I called the FDA to get in touch with them to tell them that we would likely be asking for an eIND to give a home brew cocktail of

bacteriophage to a patient with a multidrug-resistant *Acinetobacter*. The FDA reviewer, who will be running a panel tomorrow afternoon, Cara Fiore, was very supportive, in fact, and said she wanted to organize a conference call with CBER to talk about some of the issues that they had been thinking about in terms of phage therapy. That was on March 1.

By March 4, she had made some internal discussions and said they really only need to know about if endotoxin assays had been done, what we knew about that, and what was being done about sterility. Otherwise, they were ready to go and they didn't want these stipulations to get in the way of starting phage therapy, and we provided that information a couple of days later and had approval from the FDA to proceed relatively quickly.

Meanwhile, back at UCSD, there was a lot of back and forth about what this was all about. Luckily, the patient's wife was one of the deans at UCSD and she got in touch with the chancellor, who was anxious to keep her happy, and he instructed the lawyers to make sure this worked. And so we had the university attorneys on our side very early on, which was very helpful, and actually a very positive interaction with the attorneys at Texas A&M.

I then had to talk to the investigational drug pharmacy that would be administering the phage. They independently then contacted the Institutional Biosafety Committee, who told me that they would be meeting in three weeks to discuss the application to use -- to allow the phage into UCSD, and in the meantime, an MTA was being worked out between UCSD and Texas A&M.

And then, at that time, the FDA reviewer said that she also heard that there was some additional places that *Acinetobacter*-directed phage were being developed and suggested that if we wanted to, it might be useful to talk to the combined program that the Army and the Navy had been leading down the road, and she provided me with a couple of phone numbers and we decided to go ahead and do this. This was at a time when we're still full speed ahead down at Texas A&M, but we didn't yet have a phage cocktail that we could use.

So I got in touch with the two programs. The Navy was willing to have us ship the patient isolate to them, and we did. In the meantime -- this actually was actually sent by Ry's lab to the Navy, the first one, and they began to screen for phage as well.

Fairly soon, this is -- you'll see more of this tomorrow, but this is just using the Navy's Biolog approach on the Texas A&M phage. You can see that compared to the control conditions, the phages individually or in a cocktail were able to suppress the *Acinetobacter* that the patient was growing. The Navy also were developing phage, as I'll show you in just a minute.

The phage that -- the cocktail that was constructed were the AmpliPhi phage and three environmental phages that Ry's lab had come up with after they started the screening.

The plan was to go ahead and ship this cocktail to us and to do the endotoxin testing kind of as the phage were in transit. This is the Navy cocktail showing the four Navy phages and the cocktail together with the expression, "This work being done by Biswajit Biswas," who will be talking to you tomorrow about some of the issues related to preparation of the phages and selection for resistance.

We were back in touch with the FDA and one of the concerns we had was that each of the phages would have to be treated as an individual product, which would have required eight INDs. We were very pleased to hear that they were willing to consider the

therapeutic approach to this patient's organism as a single -- would be covered under a single IND and that we would only need to submit another one if we decided to switch and treat a different organism. Luckily, he didn't have a different organism that needed to be treated, so this entire process was carried out under a single eIND.

Then we began to run into trouble with the endotoxin. About the time the phage cocktail arrived from Texas A&M the initial endotoxin assay showed quite a bit of endotoxin in the phage preps. We weren't sure whether this was an issue related to the endotoxin itself or whether there was an artifact in the assay. The assay repeated at San Diego State again showed quite a bit of endotoxin in the preps.

Forest Rohwer's lab at San Diego State then was engaged to try to scrub these preps and did that with an octanol extraction that, as I'll show you in a minute, was quite successful.

The first batch of Navy phages arrived shortly thereafter. They too had an unacceptably high level of endotoxin in them and, when measured at San Diego State, in fact, even a log higher than was seen in the assay done at the Navy.

The Texas A&M phage were scrubbed at San

Diego State with an octanol extraction and tangential centrifugation. The Navy then made a second batch of phage and scrubbed their phage using a cesium chloride gradient, which is their approach to phage purification. And at this point, we actually had phage that were really quite clean, giving you a sense -- so this is the Navy cocktail, all of them together, showing you the endotoxin concentration per milliliter. These are the individual phage from Texas A&M. Again, much improved over the previous batches.

So, at this point, we had two sets of four-phage cocktails. The Navy cocktail is shown here. Each of these were environmentally obtained *Myoviridae*, as was suggested. These may well have been very similar. We really didn't have time to try to look for phage that were quite different in terms of their tropism and mechanisms of action. Same thing happened from Texas A&M for other *Myoviridae*. Again, environmental samples and shipped simultaneously.

The Texas A&M cocktail happened to arrive several days earlier than the Navy cocktail, and we decided that -- and then a second generation cocktail that Dr. Biswas will talk about tomorrow included a phage that was active against one of the organisms, the *Acinetobacter* that was selected for resistance to

all eight of the initially employed phage.

The phage arrive on March 15 and we have the data related to the endotoxin scrubbing, and this is the interventional radiologist, Andrew Picel, who was giving the phage into the three cavities that at this point were still being drained. The approach taken was to irrigate the cavities, then to introduce ten to the ninth plaque-forming units into each of the abscessed cavities, cross-clamped the cavity for about half an hour and then allowed the cavities to drain.

We saw a change in the characteristics of the drainage fluid, the pseudocyst, for example, before and after the phage were administered. Whether this is causal or not, we don't know, but certainly we did see a change in the characteristics of the drainage fluid.

About two days later the Navy phage cocktail were ready to go. The patient had in the meantime stabilized but hadn't gotten demonstrably better. We had been seeing over the previous four to five weeks kind of each day things were gradually worse. In the two days between the administration of the intracavitary phage and the time the Navy phage were ready, there were no changes in his clinical status. Whether or not it was causal or just happenstance,

hard to know, but we still felt that we were not where we needed to be in terms of getting him better, and we knew that we had isolated *Acinetobacter* from his peritoneal cavity, which would be outside the field of the phage being introduced into the abscessed cavities. Every sputum that we obtained was full of *Acinetobacter*. *Acinetobacter* had been isolated from his urine and from time to time later on from his bloodstream. So we felt that if we were going to make any headway that we needed to switch to a parenteral administration approach and decided to give the Navy phage cocktail intravenously.

This is the ID fellow, Melanie McCauley, giving the first dose intravenously. He tolerated both phage administration routes quite well. He gradually saw fewer and fewer pressors over the course of the next 24 hours. Got gradually better, and then on Sunday evening actually woke up and recognized his daughter, who was sitting by the bedside.

At that point, we found that the *Acinetobacter* was now sensitive to minocycline. That was added to have maximal benefit from antibiotics as well. But Sunday morning things got worse again, and he again began to require pressors. His mental status declined and by 8 in the morning he was on three

pressors and unarousable again.

I was very concerned we'd done something with the phage. We stopped the phage therapy, cultured the bags to make sure that there were no bacterial contaminations, but also aware that he was someone who could certainly have intervening complications of being on the assay use, so we broadened his antibiotics and lo and behold found that he was now -- the next day he was growing anaerobic gram negative rods from his blood, probably again from his necrotic pancreatic bed, turned out to be a *Bacteroides thetaiotaomicron*.

He got better by Monday night, but again Tuesday morning he was in shock, requiring three pressors. This time he was noted to be in atrial fibrillation. The pulmonary attendings and fellows were sure it was the phage therapy again, and when we looked more carefully and it was found that he was in atrial fibrillation, I said did he -- I asked them whether he had become hypotensive before or after the atrial fibrillation. They said it was after. I said why don't you correct his rhythm and I bet you'll find that his blood pressure improves, and it turned out he got much better and it was really because they had diuresed him and had potassium depleted him that he

had flipped into atrial fibrillation.

The only reason I'm getting into this is that patients like this have multiple complications that always get blamed on the new therapeutic, and that's what was going on throughout this first week of therapy.

Here he was by that evening, the next evening awake and interacting again with his family. He began to grow *Acinetobacter* less frequently, but we didn't have really good quantitative cultures, over the course of the next several days developed phage-resistant *Acinetobacter* that you'll hear about tomorrow from Dr. Biswas. We did some phage PK to give a sense of how this is just phage being given intravenously at time zero and then monitored in his bloodstream you can see cleared by 60 minutes.

His course after that was relatively chaotic. He was a sick guy. He had another bout of *Acinetobacter* sepsis associated with the drain that was in his biliary tree, migrating into his liver, but he gradually got better and was discharged in August.

Here he is leaving Las Vegas, and here he is in May just before returning to work with his wife, Steffanie Strathdee, both big fans of phage at this point in their homes. I'm sorry they can't be with us

today. It would be much more fun to have had them present this case than me.

So lessons learned. It's feasible to develop a strain-specific bacteriophage cocktail if you have two academic groups turn over everything they're doing for several months for a single patient. The therapy was well tolerated and I'm convinced really turned his course around given where he'd been going over the course of that period of time, and that people like this seem to be very complicated to both treat and assess in the context of eIND therapy.

So, to finish, the strengths of eIND therapy, you benefit patients individually. Certainly, Dr. Patterson was benefitted by this therapy as far as I could tell as his physician. The eIND is very flexible. You can treat many different kinds of patients if they require therapy based on eIND considerations. And from the regulatory perspective, it's relatively straightforward.

The weaknesses are, however, that every patient's different and it's very had to aggregate patients and make sense of the data you collect prospectively. Every time you do this at a new place it's very complicated. The nurses were sure we were going to kill him with the phage. The pharmacy was

sure that we were going to contaminate the pharmacy. The Institutional Biosafety Committee had my name on a list for a while, and I had been the previous chair of that committee. And the other issue is that none of the regimens are going to be standardized if you're treating one patient at a time.

You don't collect the data in a standard way. You don't have standardized end points, and it's not sustainable. You can't have academic laboratories doing this over and over again, and we had no resources to do this. We were shipping things back and forth using personal accounts. So there needs to be a more sustainable way to approach this.

So I'll stop there. We'll talk about more of this, I hope, on the panel and just say this was really a village that did this. Multiple people engaged in the laboratories that were making the phage. Scott Salka at AmpliPhi made the phage available. Quickly needed advice from Dr. Merril was extremely helpful in terms of the overall therapeutic approach. We were very fortunate to have the help of the FDA in approaching this. We had helpful lawyers, which is like an oxymoron sometimes, and very helpful people in our administration, which is also an oxymoron, and finally, a bunch of extremely

enthusiastic physicians that made all of the difference and allowed this to go forward. So thanks very much.

(Applause.)

DR. KINCAID: That's an impressive number of stars that were aligned at the right time for that gentleman. A very interesting story.

I think what we'll do now is we'll go on to Dr. Narayan from Yale University, who will provide another example of treatment under eIND, which is obviously an important tool available under such desperate conditions.

Did you have something, Marcus, for me? Okay.

All right. At any rate, I'd like to introduce Dr. Deepak Narayan from Yale University, School of Medicine, and he'll give us an overview of another case, quite a different case involving a *Pseudomonas* infection. Deepak.

DR. NARAYAN: Good morning, everyone. Before I get started, I'd like to thank a whole host of people who have enabled me to be here. Randy, Roger, and most importantly Peter Marks, whose connection with Yale enabled me to move things along, as you will see.

So just a brief description of my talk. This is not going to be laden with scientific data, as Dr. Gôrski's was. It is a mixture of an apology for surgeons, some clinical storytelling, and some history which I think you'll find interesting.

So, as someone pointed out, paraphrasing Halliday, God must have really loved viruses because they are the most numerous replicating entities on this earth. The phage structure that we all learned in high school pretty much holds true now, and this is my first contact with phages when I learned about Twort and d'Herelle when I was in the 11th grade.

Coming from India as I do, I have personal experience with phage generation from the Ganges where you see sights like these where people drink directly out of the Ganges and never seem to suffer any ill effects, but it is when we drink water from New Haven, we seem to work up a huge host of gastroenteritis regardless of what else we do.

So the funny thing was that Hankin reported that a substance in the Ganges River prevented cholera and this was remarked upon by a fellow in New England named Mark Twain in his *Tramps Abroad* book. But the real hero of all this, as has been pointed out multiple times this morning, is d'Herelle, who was

also the model for *Arrowsmith* by Sinclair Lewis, and interestingly was brought to New Haven by Dean Winternitz, which was pointed out by an earlier speaker, and owing to sharp practices that Gunther Stent wrote about in his review of Bill Summer's biography was asked to leave.

The most interesting coincidence, in fact, this whole episode has been a list of multiple coincidences, sort of like Swiss cheese holes lining up, but in a good sort of a way. His office was right next to mine when it was created about 100 years ago. He didn't really do too well at New Haven, probably drank the same tap water that I did, malaria -- phrenic nerve palsy and sort of moved on to France.

So the big issue is why am I as a plastic surgeon talking about all this stuff, and what might not be admittedly obvious is that we deal with a whole host of infectious problems, including necrotizing fasciitis, abscesses in the head and neck, infected craniotomy plates and so forth, as well as dealing with infected prostheses on a fairly regular basis. This is an example, and I apologize for the goriness of the pictures. You cannot have a surgeon talk without gory pictures.

For instance, this case of an infected

prosthesis which was referred from a local hospital. As you can see, a fairly large volume of foreign material, open wound ring, pus, and a standard treatment for this is to wash it out a few times, move what's called a muscle flap, in this case a gastrocnemius muscle, close it, and with a full expectation that it will heal, which it does most of the time.

We have other problems that we deal with, sort of more appropriate to the case that we're discussing. This is a veteran who presented with basically pus around the Dacron graft, an aortic valve replacement going all the way down, and this whole thing smelled like a sewer, and for a few days we were concerned that he had a colonic fistula.

Again, the treatment is to wash it out repeatedly, make sure you get good by-fill control, flip the pectoralis major muscle into the wound, close it using a wound vac, especially because it's infected, and then have him present with a well-healed wound approximately two months after the procedure.

And one final note. Another area where Dacron grafts are often used, vascular bypass grafts for lower extremity ischemia, and in this picture you see the graft right about there. That little line is

to show the radiograph is where the graft actually is, and the groin is probably the most commonly contaminated site of vascular graft infections.

So the treatment, once again, as with the chest, is to wash it out, get as much control locally as you can, and flip a nearby muscle in order to deliver antibiotics appropriately, with the antibiotic regimen continuing for about six weeks after the procedure.

We discussed these traditional approaches approximately 10 years ago now, and the reason for doing that is, again, because of the aging population we're beginning to see a greater number of these patients presenting with graft infections, and for the most part, and I want to emphasize this, is that we don't really need to resort to out-of-the-box thinking, such as phage therapy, in terms of treatment.

In fact, over the years, over the last 15 years we've dealt with approximately 150 graft infections, only one has been lost because of an infection with *Pseudomonas* as a matter of fact of the anastomotic site.

As you all know, there's a dramatic decrease in antibiotic drug approvals, an increase in

antibiotic resistance, and I will not belabor the issue, and despite encouraging slogans such as "Bad bugs need new drugs," really not much has been forthcoming.

Pseudomonas aeruginosa is of particular interest to surgeons because it's responsible for a significant number of nosocomial pneumonias, burn, wound infections, which is of particular relevance to plastic surgeons, as well as other immunocompromised populations, as you know. And this is a really clever bug which has evolved multiple mechanisms by which to thwart the efforts of surgeons and ID specialists and most importantly has been associated with the formation of biofilm, especially with prosthetic material.

Now there are a whole host of mechanisms by which *Pseudomonas* survives, which I will, again, not belabor this crowd with, and a partial list of the drugs effluxed by the -- multiple drug efflux mechanisms are listed here for you to see.

So the story again begins as another coincidence whereby I was contacted by Dr. Paul Turner and his post-doc, Benjamin Chan, to set up a treatment for diabetic wound infections, and part of this discussion centered around the isolation of a new

phage, the OMK01, which has been written about, which is the outer membrane polar knockout one, and this was isolated funnily enough from Dodge Pond in Connecticut, a site of Navy testing and apparently so toxic that none of the residents eat the fish from this pond anymore, and this is from a direct quote from a patient.

So the phage therapy approach, as you all know, can mimic the use of antibiotics. You treat it with a phage. You kill as many as you can. Resistance to phage is developed, and essentially what ends up happening is that you basically cannot use the phage to treat them anymore.

So these resistance-targeting antibiotics, which are basically a combination of these two, might actually help to deal with this problem. So the amazing thing about this OMK01 was that it actually latched on to the drug efflux pumps. And so the thought was that maybe we can use evolution to help us, and I want to emphasize that all this was done in Paul Turner's and Ben Chan's lab, and they were kind enough to lend me these slides.

So the thought was that if the resistant organism was forced to make a choice between resistance to antibiotics and resistance to phage, it

would lose because it was so closely intertwined that it could only be resistant to one, and, in fact, an *in vitro* test did demonstrate that this actually happened.

So I'm just going to briefly talk to you about the patient in question. This was a 75-year-old male, had a coronary artery bypass graft, was done in a neighboring hospital, along with an aortic arch replacement, similar to the picture that I showed you in the infected thoracic cavity.

Following surgery, he developed empyema and became extremely sick, requiring four pressors, at which point he was transferred over to Yale-New Haven Hospital. My boss happened to be on call, and he was leaving town, and so being the most junior on the totem pole, I was given the enviable task of taking care of this gentleman who -- which is now a matter of public record -- who was a faculty member and thereby obviously raised the strain involved in treating him.

So the patient was placed on antibiotics appropriately since he grew *Pseudomonas*, as depicted in these green shadows that you see on the cartoon here. So, after -- and I'm going to try to see if this actually works -- washing him a few times, you end up with this sort of a scenario where you have a

pus pocket, and I'm sorry if you can't see this back here, sort of the heart of the matter, if you will, which was the cause of the problems, as we'll talk about it. You can actually see the greenish block.

And so, again, in keeping with the previous treatment, keep washing him out as many times as we could. In this case, we did it about three times, and then used a muscle flap to close it. So, as a part of increasing the immune delivery in this area, we harvested the omentum through a laparoscope, which is the yellow plat-like substance that you see here, and during the course of this harvest we found that the field was filling up with blood, and, to my absolute horror, found out that he had actually ruptured his ventricle on the table, which, of course, prompted a repair with the help of the cardiothoracic surgeons, which you see out here. We used a piece of lung to patch that defect, and fortunately for all of us he survived.

So, despite actually having been discharged from the hospital, he was admitted at least four times for episodes of sepsis requiring IV antibiotics. He was placed on ciprofloxacin as a suppressive measure. He did present with one episode of bleeding which I thought was from the outer -- it turned out it was

just a rib poking through an intercostal artery, which we cleaned up.

He was then asked to follow up and multiple requests from his son, who had a Ph.D. from Yale in immunology, urged me to find other places to treat this gentleman. Again, as Dr. Schooley pointed out, cardiothoracic surgeons refused to operate on him, saying that he was doing well and should be left alone. We contacted surgeons in Tokyo, Zurich, as well as in Texas, asking if this aortic arch could be replaced, and all of them basically declined to operate.

His son, who was pushing for experimental treatment, you know, suggested new antibiotics, and during the course of this whole business, I met with Drs. Paul Turner and Chan, who, as I said, presented a project for treatment of chronic wounds, and a few days after the meeting I realized that this gentleman is a perfect treatment choice for phages.

We organized an eIND, and thanks to Peter Marks and Cara Fiore, who were extremely helpful in moving this along, we did get initial permission to proceed, but the patient was lost to follow up since he left the country and was not heard of until January of 2016. Apparently, by report, had been getting

intravenous ceftazidime as the patient could afford it, for over a year and a half intravenously.

So, when they presented again, this was due to bleeding from a fistula site that had never healed, and the *Pseudomonas* that was repeatedly cultured from this obviously was a potential source of a problem. And so the FDA obviously gave us permission to proceed. And one of the happiest emails that I ever received in my life was that I did not need to go through the HIC for approval.

So, when I sent this to the HIC and they promptly approved it, we decided to go ahead and treat this gentleman, and a whole host of bacteriophages were tested by Drs. Turner and Chan, and we created a three-phage cocktail. The endotoxin business was also of concern, but we had it independently verified by a laboratory in Cape Cod to meet EU standards. So the thought was that potentially we could use three of these phages, one to weaken the biofilm, one to potentially remove the colonists, and then use ceftazidime to finish the job off.

So, as it turns out, we ended up using just the OMK01, and with the help of our interventional radiologist, Dr. Mojibian, we accessed -- tried to access the abscessed cavity, which you'll see the

needle trying to go into that space that I showed you on the video.

Now, in a further twist to the whole thing, the day we were doing this when we organized the OR, the emergency and anesthesia teams, the interventional radiologist, who is from Iran, received a call from his wife urging him not to do the procedure. As it turns out, the patient in question was a legend in Persian medicine, Iranian medicine, if you will, and the thought -- the wife was worried that they would not be able to go back to Iran if something happened to the gentleman on the table.

So there was a hasty discussion about all this prior to the injection, but thanks to the fortitude of Dr. Mojibian, we decided to proceed. Despite that, trying to access this for over an hour, we were able to inject just a few milliliters of solution, so we decided instead to actually push the phages in through the fistula site, seal it off, and let the patient be.

So the patient was sealed off with this thing in place for over 48 hours, and he immediately left town to go back to his home country. It turns out that six weeks later he suffered a perforation of the aortic arch from a bony spicule which resulted

from a re-growth of the bone from the debridement that we'd done earlier. So the graft was partially replaced by surgeons in Iran, and the cultures just revealed *Candida*, not *Pseudomonas*. He was treated for the *Candida* and has been free of all antibiotics now for about 15 months.

So, in conclusion, obviously, this case with an N of 1 is hardly the basis of treatment of all vascular graft infection, but the important thing I want to point out is that there are conventional methods of treating these infections, as I mentioned earlier, over 150 infections treated fairly successfully, with the exception of one who burst his graft due to *Pseudomonas* infection, and the scope of phage treatment for these highly resistant infections remains to be explored. Thank you.

(Applause.)

DR. KINCAID: I think so that we can move more quickly to the panel discussion we will have an opportunity for Dr. Narayan, Dr. Schooley, and others to field a few questions at the beginning of the panel discussion.

At this point, I'd like to invite Dr. Gabard to the podium. This project that he is responsible for directing is a noteworthy project in the history

of phage therapy because it is a randomized multi-center clinical trial and, as we'll probably learn, not without its challenges. So I think this is a very important step going forward as it lays the foundation for a more rational data-based approach towards using phage for medical interventions. Dr. Gabard.

DR. GABARD: Good morning, everybody. Thank you for the organizers for inviting me to talk about what we do at Pherecydes Pharma. Of course, this is the usual statement.

So, first, I'd like to introduce my talk by explaining what we have been doing in the company for several years, the different types of approach we have been using for phage therapy. So the first thing you probably heard quite a lot about is the Phagoburn project. Actually, we entered phage therapy by starting through the standard regulatory routes with a fixed product like an antibiotic, and actually we were testing two products, each of them with more than 10 phages, so we call that complex product, and they were really specific to either *E. coli* or *Pseudomonas aeruginosa*.

Then the second category of products we have been developing are two other cocktails, and I'm just giving you the example of one of them, which are much

smaller. I guess the experience of handling the manufacturing of products with more than 10 phages has been a good experience, and we decided to go with smaller cocktails of four phages.

Very recently I show some data that probably we have not been showing yet. We have been also involved in two compassionate treatments with tailored products.

So, regarding the product of the Phagoburn study, from the point of view of the regulatory route, it was considered as a frozen cocktail with no possibilities of evolution, so of changing the phages within the product and, of course, it was quite unmanageable to be able to adapt the phage of that product with so many phages in the composition.

It's important to understand also what this definition is about of an active pharmaceutical ingredient and a drug product. If I take the example of an antibiotic, an antibiotic has usually a single API. Here, we are talking about drug products that were made of 12 and 13 API, which is a very, very big challenge on the manufacturing side and the regulatory side.

So that has been a challenge and we have had some issues regarding shelf life of the managing of

the APIs in the drug product. When you do GMP manufacturing for any types of products, you are supposed to provide the guarantee that your active ingredients are stable over time. We have not been capable of finding ways, technical ways to demonstrate that each active was being always at the same concentration within the product's shelf life.

We have been capable of doing that, of course, for each individual phage, but not for the phage inside the drug product, and if anybody in this room has a way to do that, has a technical solution to do that in the complex product of 10 phages, I would be happy to learn from that person.

Of course, when we started the first process, we heard about the endotoxin content. Our first manufacturing process was too high in endotoxins. We were not in the range of 45,000, but in the range of probably 30,000, and in order to be able to use that product in the patients, we had to go through a dilution, a dilution at the point of care so that the clinicians were doing a 1,000 full dilution before to use the treatment.

Now, if we move to the second category of cocktails we have been developing, I have been mentioning two of those here. The first one falls

against *Staphylococcus aureus*, and the second one is against *Pseudomonas aeruginosa*. Here, we have really severely reduced the number of phages, trying to isolate phages with broader spectrum of activity in order to have less APIs because, on the CMC point of view, on the manufacturing point of view, it makes your life much, much easier.

Of course, with such a low number of bacteriophages, evolution is possible, but under which registration frame, and I think this is very important that we address that issue during this workshop. If you want to make an evolution of a phage in a product, what is the status of that new phage? This is important.

And then, of course, we have been working a lot on improving the endotoxin content, the purification of the products, the GMP process overall, and now we can say that development of toxins we have is at about two in units in a range and that we have improved the yield through a new purification by a factor of 10 to 100 according to each phage. So we usually routinely yield phages at about 10 to the 11th, 10 to the 12th pfu per milliliter in the GMP process.

Now, if we move to the next very recent

change in the treatment approach, we have been doing very recently, early 2017, two treatments that I will detail a bit more after that, using product prepared, really tailored for the patient. For that product, the goal would be to use GMP products, but I will explain that we didn't have any GMP phages. We had GMP-like phages, and after discussion with the pharmacists from the hospitals, as well as the clinicians, we got the authorization to apply these products.

Of course, here, we are not talking about product evolution because, by essence, you are doing a diagnostic and you are just delivering the phages that are active against the infection. But then the regulatory status of these products is really something that we need to address during this workshop.

If you use no GMP phages like we have been doing but produced like GMP phages, we enter more or less in what we call in Europe or in France the magisterial formula, which is usually done in the pharmacy hospital. Then, of course, if you do GMP phage for single patients, it requires a regulatory frame, and what kind of regulatory frame can we use for that? I think this is the type of questions we

need to address.

So a few words on Phagoburn, you're going to be disappointed because I'm not going to deliver the data today. I'm going to provide some preliminary information. You know that the study was performed in 11 burn units across Europe. Actually, only six of them recruited patients, so it was a challenge, and I have been listing the most -- the major recruiters, of course, is the Percy hospital close to Paris, the military hospital, and the Queen Astrid Military Hospital in Belgium. We were talking about Jean-Paul Pirnay in a previous presentation, who is coming from that hospital. So they were the biggest recruiters in the study.

The time frame of the trial, of the project is explained above, and I think we were very much too optimistic, especially on the CMC manufacturing. We thought we would do GMP phages within 12 months, and we ended up doing GMP phages within 24 months, and you see that the results -- I cannot show -- I cannot talk about the data because, as you can see, the consortium met about 10 days ago and the preliminary clinical data have been shown for the first time to the consortium only 10 days ago. We have not time, we did not have time yet to review all the data and to

analyze all the -- especially the biological data. So we expect to publish all this probably before the end of the year. But anyway, you have some issues, as we have been getting through this clinical trial, and I can give some information about what we see.

When we started to do the study, we were doing two cocktails, one against *E. coli*, the other one against *Pseudomonas aeruginosa*, and the epidemiological infection data that we got from all the hospitals were in a way not realistic, and we know that only today.

Why was that not realistic? Because when we check in detail the epidemiological data for, let's say, checking how many *E. coli* cases the hospitals got, actually, they usually count an *E. coli* infection as a case when the *E. coli* infection is the major bug that the patient got. But in most of these cases actually the patients gets poly-infections.

So you're going to get data that says that is a patient that has an *E. coli* infection, but it doesn't tell you that on top of that the patient also had *Klebsiella* or maybe a *Staphylococcus aureus* at the level of the colonization, but when you have a product which is mono-specific, when it comes to the time to deliver treatment, you cannot include that patient

because it's not a mono-infection. So be very, very careful if you do start clinical studies with phage with monovalent product to really check the value of the epidemiological data, so which ended up for us adding only one patient included with the product against *E. coli*, which is called PP0121, and we decided last January to stop the arms of the study with that product. So all the data we are generating now and that we are analyzing today at this moment are really for the patients that have been treated with a cocktail against *Pseudomonas aeruginosa* only.

Of course, one other thing you need to understand is that it's the same case, by the way, for the compassionate use treatments with the type of patients we have been handling. We are talking about people that are severely burned. Some of them were burned up to 90 percent of their skin surface. In the red, it was probably in the range of 20 to 30 percent deep burn, infected deep burns. It's impossible to avoid using antibiotics. It's simply impossible. The ethic committees would not agree anything about that.

So you really have to think very early in your clinical process in your stratification that you're going to have to analyze against antibiotic, and not only against the antibiotic that might be

prescribed before you even include the patient because this is the case. The guy who was going to be treated may already be under antibiotics because of a respiratory tract infection.

You have also to stratify on the fact that these patients may get this respiratory tract infections when he is being treated by the local treatment, during the course of the treatment, and that makes your stratification even more complex.

Okay. Of course, the severity of the patients of the cases. When we started the trial, we didn't get too much in consideration and we had data safety monitoring board, an independent data safety monitoring board which was reviewing the ethical treatment of the patients during all the clinical trial. And after they met the first time, they said that we should really check the severity of the patient before deciding to recruit or not -- include this patient into the trial, and we decided to implement something which is called the SOFA test, which is some kind of a monitoring process that checks how bad the patient is, in which situation it is, and if he is really in a very bad situation, then the rule was not to include the patient because the chances that the patient die anyway are so high that you would

not be able to generate any data.

And then, on top of that, when we reviewed all the literature about the primary end points that have been tested for checking antibiotics, I'm not sure that primary end points that have been tailored for antibiotic checkings are exactly the same for phages because these primary end points have been set up for fixed molecules, and here we are talking about living organism, so that's also something we'll discuss when we show the data.

Okay. So here I'm going to switch now to the two patient cases we got early this year. First of all, maybe a few things about the regulatory status in France. With the Phagoburn studies, there was a special committee who met last year organized by the French regulatory agency. It's called a CSST, and this special committee agreed that the phages from our Phagoburn study can be provided to the patients for treating them.

So there is a possibility in France to now treat patients with GMP-produced phages from our company, and these treatments are only reserved to patients that are either critically ill, they may die from the infections, or that patients that have a functional risk of losing let's say one hand, one

foot, something like that. So really serious cases.

Unfortunately, we didn't have any more stocks of these products in the company and we ended up having the request from the hospital. I mean, we have requests all the time, but serious requests from the Center of Reference on *Staphylococcus aureus* infections in France, which is based in Lyon. And they asked us very recently, in February, if we could provide bacteriophages to treat one of their patients. And in that case, we didn't have any GMP product left.

So we talked with the agency and we said we can provide GMP-like products, and when I say "GMP-like," I mean they are really produced exactly the same way. There is not all the paperwork for the GMP, but all the quality control tests are exactly the same. And we said we can provide this. Is that acceptable to the French agency?

And the French agency said, I think this is not our responsibility because we are not anymore in the GMP stages. This is the responsibility of the pharmacist and the clinicians to agree or not, and especially the pharmacists to agree or not about the quality of the products you can provide. And the pharmacists providing the data we provided said that it was fine, that we could do the treatment, but I

must insist that these products were not GMP. They were GMP-like, and the process is really for us now that we have been experimenting that a couple of times the clinician makes a request that goes to the regulatory agency. It takes about a few hours.

Their agency asks us if we want to do the treatment. If we say yes, we just ask in a rush process to receive the strain, which is about half a day to get it in the lab. We do preliminary screening. We check what phages are available in our collection that are active against the strain, and then we send back those bacteriophages, which basically it take about 48 hours, and then we have to provide all the data, quality data that we have already for the phage in collections, and then we can provide the phages. Let's say in less than a week the treatment can start.

Here are the data. So this is the first patient which was the one probably to help us to set up the process with the hospital and the agency. In that phage, we got no more phage of the GMP produced through the Phagoburn project, so we used some phages that we have against respiratory tract infections, *Pseudomonas aeruginosa* respiratory tract infections in the normal phage project, and you see the efficacy of

the phages here, without treatment here, and the four phages here, or three phages, and this is the product, and we send the product not prepared in a cocktail but independently.

Then we check for the titer, which was in that old fixed -- they were higher in titer, but we put the titer exactly the same for each phage, so we dropped it from 10 to the 12th to 10 to the 10. Of course, for these phages, because they were in collection, they were fully sequenced, fully analyzed, the genome was fully characterized, and we know they were confirmed by sequence analysis without any lysogenic behavior. They were checked for sterility, pH, and contaminant, and as I said, contaminant endotoxin content was about two units of enzyme per whatever you need. The host cell DNA was undetectable and the host cell proteins were below 20 microgram per milliliter.

And this is the case that I'm talking about. This is a man who got cancer and had to have cement put into -- to replace -- how do you say that in English? Metastasize, is that correct? Yeah. Bone metastasize, it was removed, replaced by a cement, and the cement ended up bringing *Pseudomonas aeruginosa* infection, which was total resistant except a little

bit to colistin. It was still a little bit sensitive to colistin.

So we got the approval for sending the product, I think it was on a Tuesday. They did the treatment on a Wednesday, and we decided to go for four applications because, in that case, the wound was still accessible during several days and we decided to go for four treatments of four phages each time.

So they applied the treatment on the Friday. They did the first wound sampling because we have been doing monitoring in the wounds to see if we were getting any resistant. So they did the sampling after the first application. It was a Monday. We got the data on the Tuesday, and the Tuesday itself after the first application the wound was sterile.

So we still maintained the three other treatments and the patient was cured and saved, except that a few weeks later, a few months later he died from his cancer because he has a general cancer. So that was a success, but, I mean, you save the -- the guy die from not being infected anymore, which is a partial success I should say.

So this is the type of things that we have been doing. I will not explain too much. The debridement, the administration of the phage. I think

it was about 20 milliliters in the wound, and then you see what's happening after that.

And this one has not been described yet because it's more recent. In that case, it was interesting because it was contamination where we had to prepare two mix of phages against two bacterial species, so it was not a mono-specific product, it was a product against two species, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and you see this lady had a serious infection where you can see what's happening, and here in the infection we were detecting *Pseudomonas aeruginosa* and *Staphylococcus aureus* just a few days before the administration of the product.

So this is what the patient has been receiving. It was three phages against each bacterial strain and they were mixed just before the use at the hospital facility by the pharmacist under a laminar sterile hood.

Well, the conclusion of all that is that today she has been treated for about three months now, and she is fine. We still have recently got the information that she has a *Staphylococcus lugdunensis* available, so we are going to check if the phages we have is efficient against that bacteria, but she's in good shape.

Okay. Maybe to expand on the discussion this afternoon, here I have been choosing a process that we have been through the company from standard fixed cocktail to precise precision medicine to just a little bit challenge the regulatory environment.

If we talk about the complex cocktail or cocktail which is described as an antibiotic, the regulatory frame is available and is ready for you to go through a standard process of market authorization.

But if we go to tailored preparation, you have seen that this type of magisterial preparation does not go, at least in Europe, through a market authorization or a registration process. It's an individual treatment for an individual person.

If we end up going for these types of personalized treatments, there is no real framework for approving that type of treatment. So I have been putting in that arrow the personalized drug product with viable evolutive phages that should be GMP produced under which type of registration, and I have been showing some examples that we are going to face in the future.

If you take the, for instance, the target bacteria A, which could be *E. coli*, you have a bank of bacteria phages, and the patients, you do a diagnostic

because we believe too that the preliminary diagnostic is essential and it's going to be something that is going to be requested anyway by all the antibiotic-resistant plan that all requests, without any exception, to do a preliminary diagnostic before applying the antibiotic. So it's going to be the same for the phages.

So the patient might have one bacterial species and you do a treatment with three bacteriophages, and another patient, which is more or less the case, the second case I was describing, has two -- here, I'm talking about three bacterial infections and you're providing phages against two or three bacterial infections.

And the third case is that patient number three which has maybe one bacterial infection which is being treated by a first treatment, but that treatment is not efficient enough and you have to go back with a variant of the phage you have been using for preparing the first treatment, and what is the status of that variant?

So I think the questions we need to really address today are more or less described into this slide. There is no process today, if we have let's say develop a data package, for getting the

authorization to treat the patient with a certain number of phages, and you generate new phages that could be either variant phages or that could be new-found phages into a sewage system but belonging to the same category, what kind of data package are we going to provide against these phages?

So there is the authority to say that we could start from a homologous group saying that you have some kind of a group which is representative of the phage family and that that needs to be fully characterized, but the new phages that belong to that group can get a short data package without all the treatments, testing pre-clinical studies in animals and all these things, and be eligible to get into manufacturing, or the same for the -- now, if we talk about the bacteria, the manufacturing process, there are ideas to go for validation of a manufacturing process that would be eligible to any phage being produced, but would that be the case for a manufacturing process which is defined for one bacterial species, or would that be eligible to any type of bacterial species even if you talk about making a gram-negative or a gram-positive bacteria, because then the manufacturing process is not exactly the same.

In one case, you're going to check for endotoxin content if it's a gram-negative bacteria. In the other case, you're going to check about hemolysins, for instance, which is not something that you're going to check eventually for endotoxin -- for a gram-negative bacteria.

And then what type of quality control level do you want to get for the set of reference phages? Everybody agrees that it should be a full set of data that has been trained to summarize with identity, toxicity, pre-clinical data, PK, PD, efficacy, sterility, but the phage that gets into this category of homologous group, can we just -- is it sufficient to do identity and sterility?

And I want also to bring some ideas of that. I'm not the father of these ideas. There is somebody in Belgium, Dr. Fauconnier, who has really some good ideas about the -- from the regulatory agencies in Belgium, that has pretty good ideas on how we can take bits and pieces from different regulatory process to build up a process for the phage therapy.

For instance, we are talking about banking. When we prepare the banks of bacteria that are going to produce the phages or the banks of bacteriophages that are going to be administered to the patient,

there is a process which is called the -- for the allergen extract prepared for a single individual where the source of material can be very diverse: pollen, molds, animal epidermals, insect, food, environmental, et cetera, and the extraction materials vary a lot according to the material you want to use.

Well, there is a process here which is available today in our countries for approving such products. So maybe for doing the banking of the bacteria and the phages we could get inspired from that process.

On the production process, you have something in the U.S., I believe, which is called the drug master file, which is something that is some kind of, if I am correct, some kind of a design, pre-design process of manufacturing which is not going to change and that you enter on one side your material and at the end you get your products out, and this is a fixed product. This is a fixed process.

If you use always the same process, you can refer to that drug master file number and not have to explain each time how you are going to manufacture your product. That also is maybe a good idea for manufacturing of the phages.

And now regarding the third point of phage

therapy is product evolution. Product evolution, as you know, is fully agreed when you make a vaccine. Well, there is a multi-strain dossier that we have in Europe where you can change the component of a vaccine very easily without years to wait, just to adapt the treatment to the evolution of the threat. Here, it could be the same thing.

I mean, if you have an homologous group of bacteriophages and you want to change, make an evolution of one of that phage in that homologous group, maybe you have a process that we can copy to just adapt our regulatory process to a quick evolution with only a limited number of tests for getting approval of that modified phage.

So this is it. Thank you.

(Applause.)

DR. KINCAID: I think at this point it would probably be a good idea to have all of our speakers come up, and I'd also like to invite Dr. Doran Fink from FDA and Dr. Betty Kutter so that we can first field some questions because I realize there hasn't been an opportunity for all of you who might have questions, but we also have some topics that might be stimulating in terms of their potential consequence to development of phage therapy in the future.

So, if I could have the speakers come up here, please.

(Pause.)

DR. KINCAID: So, before we begin, I'd just like to invite anyone who has questions, who may have questions in particular for the last three speakers, to use this as an opportunity to ask those, and then we will move on to some of the topics that have been selected for this. Dr. Stibitz.

DR. STIBITZ: Yes. I just wanted to ask about something in Ry's talk. You stated, I think, in one of your last slides that you think there is value in characterizing base modifications for phage. Could you elaborate a little bit on what you think the value of that is for phage that we want to vet prior to using for therapy?

DR. YOUNG: Hello, can you hear me? There we go. Is it working? I can't tell from that.

So DNA modification is a major way in which phages can become insensitive to or can overcome host defenses beyond the resistance, classical resistance. So many virulent phages that especially have unusual DNA, some of them have, for example, no thiamine, only uracil as their DNA base.

But the methods for, high-tech new methods

for looking at protein and nucleic acids don't really work very well, the ones we have for assessing the modifications in these phages, but I think there are approaches that can be developed that are more based on classical nucleic acid chemistry that could be very informative.

If we had a way of rapidly checking a new promising phage for its DNA content and how much of it is modified and how much of it is normal, I think you could then eventually index that against many species.

DR. STIBITZ: So I'm just wondering to what degree. I mean, if it's being used as a -- by the phage as a resistance mechanism to host defenses, wouldn't that be captured just in the normal screening for *in vitro* activity?

DR. YOUNG: Well, yeah, but you wouldn't know what was causing it, right?

DR. STIBITZ: Sure.

DR. YOUNG: And so you could have a phage, you could have one gene change and then you would have a gain or loss of the ability to survive in that organism. I mean, I think having the -- sort of the classic way of just checking the pattern of resistance is certainly the thing you want to do, but we have the ability and I think the incentive to go beyond that to

the molecular level. If we had more and more data, even if it wasn't absolutely required for --

DR. STIBITZ: Right.

DR. YOUNG: -- the emergency application, we would be able to look back and start cross-indexing these molecular features with efficacy and with redundancy.

DR. KUTTER: Well, maybe I'm the person also to say something about that since I've been working since 1963 on the question of the role of hydroxymethylcytosine in T4 phage, and one thing that came out this past year emphasizes something that may be relevant in terms of thinking particularly about phages to be used in the gastrointestinal tract, and that was something that Sankar Adhya and a student from Florida had done, finding something called super spreader phages.

They found them when they isolated them from nature, that there were a couple of phages that tended to under rather -- under conditions that really looked for them -- to be able to spread plasmids for antibiotic resistance to all sorts of different kinds of bacteria, not just ones where it could be through phages carrying those.

And the way they figured out an idea of what

was going on is they went back and used phage that we had made about 40 years ago that were T4 that are able to make phage that are purely cytosine in their DNA, and they are missing a variety of different genes, including the genes to make the hydroxymethylcytosine but also the genes to shut off transcription of host DNA and the genes to degrade the host DNA that are cytosine-specific. And they found when they used that strain that was missing all of those, suddenly they could generate something that was not a full super spreader thing but that the T4 by itself showed none of that property, and they had three orders of magnitude more spreading when they were using those.

Now what hasn't been looked at at very many phage at all is the degree to which they degrade host DNA, and often you don't even know whether they have the nucleases to do it. There are other things that need to be sorted out more, like the ability to infect stationary phase cells and things like that.

So what's really needed, I think, is for NIH and USDA and so forth to fund a lot more of these really basic kinds of things, and what we have now is a few undergrads are working in my lab to try to look at some of the other standard phages and to see whether they can get the super spreader phenotype and

doing something like phage hunters, and getting undergraduate schools all over the country to be looking at some of these properties.

I teach at a -- for those of you who don't -- I'm Betty Kutter, by the way, and for those of you who don't know, I've been teaching for very long at a school where almost all of my work is done by undergraduates since 1972, and I'd like all of you to invite you to our Evergreen international phage meeting, our 22nd one of which will be, biennial meeting, will be in August, but bringing people from a lot of different backgrounds and really getting more young people involved in asking a lot of these questions that will never be done, I think, if we only have the major labs to follow them up.

Thank you for the opportunity to make an ad.

(Laughter.)

DR. KINCAID: Please.

MR. McCLAIN: Yeah, Bruce McClain, United States Army. You know, most of the applications that we've heard today were irrigations of a wound or irrigations of an infected body surface. I mean, I think there was only the single intravenous administration. And I know that in your manufacturing you're concentrating on endotoxin levels and stuff,

and yet these wounds are swimming with endotoxin. It may be that the endotoxin concentrations from a manufacturing standpoint is a minor component and you may want to propose to your regulatory, you know, colleagues that the endotoxin concentration may be really irrelevant for that type of therapy.

DR. GABARD: Yeah, we would have loved to be able to do that. Unfortunately, you have something called the pharmacopeia, and the pharmacopeia has some standards regarding endotoxin content and there is not so many standards, but there is at least one of them which is giving figures about the amount of endotoxins you may have when you do an IV administration, and then there are a case -- although the treatment was topical, the agencies considered, because these were seriously burned patients, that they asked us simply could the bacteria become septic. I mean, could it get into the bloodstream? And we said yes.

So they asked, and what about the bacteriophages? Can they go in the blood as well? And we said yes, they are going to follow the bacteria. So they said then the standard for your product needs to be for IV administration, and the endotoxin content must be about that level.

DR. SCHOOLEY: This even came up in the

patient that I discussed. As I mentioned, we had this very nice improvement on Saturday night. By Sunday morning, he was looking as if he was headed in the wrong direction again.

I called one of my colleagues from University of Colorado, Charles Dinarello, who has done a bit of work in this area, because I was concerned that this was endotoxin-related and was trying to talk to him about some ways to try to block this if this was what we had done by escalating the dose of phage, which I didn't get into today. His comment was, you know, with endotoxin there is tachyphylaxis anyway. Why are you worried about this?

So, you know, again, I think it's something to consider, but I also think it's technically feasible to scrub it anyway, so why not. You know, in the context of most situations, now that there are several ways to purify the phage, I don't see any real reason not to unless you're trying to do it kind of on the end of a hood in the back of your car.

DR. FINK: So, from a regulatory perspective, I agree with Skip's point entirely. From a safety perspective, what we worry about is the product characteristics and the intended use, and if someone comes to us with a well thought out scientific

rationale for why worrying about a particular impurity is not important and why trying to get rid of that impurity would be overly burdensome, then we would certainly take that argument into consideration. But I haven't heard such an argument yet for endotoxin.

DR. KINCAID: Next question, please.

MR. TURNER: Hi. I'm Paul Turner from Yale University. I had a question for Jérôme about -- and maybe Ry or others want to chime in for this. You mentioned the challenge of the evolution of the phage, but what about the more proximate issue of the competition among phages in a cocktail, how much of that have you studied and, you know, there's a possibility that it could actually negate each other's success during the treatment because they'll compete?

DR. GABARD: Very good question. We have not been doing that with the phages of the Phagoburn study, but we have been doing some other studies with some other phages from the other projects, and we have seen -- it's very preliminary, but I think there is some good work done in California. We have seen that if you have -- how can I put it? If you want to use four phages to fight a bacteria infection, and only one is active, and you put the three others, you may lose some activity, clearly. So it's better to use

phages that are only active against your strain and to limit the number.

MR. TURNER: Yeah, we were fortunate. We could only go with -- that we could go with only one phage in the case that Deepak talked about. But, okay, that's good. I think it's an interesting problem that needs follow-up. Thanks.

DR. KUTTER: We've done some looking at various individual phages, like three different kinds of *Pseudomonas* phages or T4 with several other kinds of phages, and you certainly find some cases where you wind up with a complete blocking of production over the short term of at least one of them.

Now, with the T4, for example, even though it would block all of the T5 and some of the other kinds of phages when they were simultaneously there, if you had a low enough MOI that there were a few percent of the cells that were only infected with one of them, then 24 hours later the T5-like phage was doing better because what happens is that when you're affecting T4 at high multiplicity, it has lysis inhibition and instead of lysing at 30 minutes it lyses at six or seven hours, and that allows the other phages to catch up.

So we found that there really were

advantages, but there are a lot of reasons why, for example, in Georgia with the cocktails, and they say to infect with a relatively low multiplicity so that you're looking at them having to expand and having to grow, and we had the same kinds of results that we saw with some more work with using phage and treating sheep, that, again, you found out that the optimal multiplicity was significantly lower than throwing lots and lots of phage at all the bacteria.

DR. GÓRSKI: I mentioned in my talk that we made such a preliminary observation which may suggest that patients and cocktails may have higher phage antibody levels than those receiving single preparations. Regardless of the outcome of the story what is the role of peripheral-blocked anti-phage antibody in phage therapy outcome, this is kind of information which is interesting because, in our work, we have found also that phages differ in their immunogenicity. It may well be that some phages that are present in a phage cocktail may act as adjuvants. This is something we need to consider in the future.

AUDIENCE MEMBER: I have a question for Dr. Górski and the French company about propagation of phages once you -- larger propagation in terms of actually using it in the clinic.

Do you find it more useful to transfer the phage to a different bacterial host, or do you tend to keep it in the original strain that you fished it out with? And I'm just curious if there's any usefulness in transferring it somewhere else for either higher phage production or something like that.

DR. GABARD: Well, the selection of the bacteria for production is important, clearly. Usually we tend to try to find phages that are being produced into a single bacterial strain just for manufacturing cost reasons. We couldn't do that for the first *E. coli* product for the Phagoburn study where we had to use, if my memory is right, seven bacterial strains for manufacturing, which was very expensive because then you have seven working -- well, a master and working banks.

So, in the solution process, when you have the choice, it's always better to go for one strain, and sometime the surprises of this manufacturing strain cannot be used as the titration strain, so you have to go from the one manufacturing strain, and you may have to go for a different strain for titrating your phage during the manufacturing process.

DR. GÓRSKI: Well, I think we have the similar policy. I also mentioned preliminary data

which, again, are very, very preliminary but interesting that when you propagate phages on a strain that is freed of plasmid and prophage you may get increased titer and broader host range. This is something good. It's very promising but again requires further study.

DR. BISWAS: Hi. My name is Biswajit Biswas. I am from BRD Navy. So I have a question for Dr. Jérôme Gabard. This is a technical question.

I saw in one of your slides that you are monitoring phage bacterial interaction by lysis method and you are monitoring it through the spectrophotometer reading. My question is when phage lyse the bacteria it produce debris also. So how relevant is this one for your monitoring system for phage efficacy?

DR. GABARD: I cannot answer that. I'm sorry. It's not my expertise. It's too technical. Send me the question and I will ask to our team because it's beyond my knowledge.

DR. BISWAS: Thank you.

DR. KUTTER: Actually, when you do lyse phages, lyse bacteria with phages, we often monitor it by OD because the OD goes way down at least for *E. coli* and *Pseudomonas* and *Staph*. At the time when your

burst of phage is complete, the OD almost totally vanishes, so it has to do with the way the bacteria interact with the light and the concentrations in the bacteria rather than just the three.

DR. BISWAS: Yeah, I understood your point, but we see in many bacteria and many phage, we have lot of clinical isolate. We see those clinical isolate when we lyse, not all the time they go through the complete lysis, sometimes they lyse but produce the debris which is targeted, and that is the problem because when you compare one phage to other and the phage lyse differently in the same bacteria, that is a problem. So that is my point. Thanks.

DR. KUTTER: Yeah, it doesn't lyse it totally but like eight-fold or something like that usually with the standard ones, but that's a good point, yeah.

LT REGEIMBAL: Good morning. My name is Lt. Jimmy Regeimbal. I'm from -- actually, I'm from NAMRU-6 now in Peru, but I was previously at NMRC here. And my question actually is much more general to actually the entire panel. Is it possible to take a step back and to actually not think about the product being tested as an individual cocktail, but instead your product is a library of phages from which

you have differentially compounded cocktails that are personalized or individualized as long as that main library has been characterized and deemed safe, and whatever that means, and whatever you think you need to find a safe library?

And so you're really personalizing everything because I found it interesting, Dr. Gabard, that you started with fixed cocktails. My guess would be that if you have good coverage in those Phagoburn trials it'll probably work fairly well, and if it doesn't have any coverage, you're probably not going to see much efficacy.

And so what if the whole point is to take a step back and go this isn't our product? Our product is a library, and maybe 20 years from now, like Dr. Young was saying, that you might find that every time you compound a cocktail against *baumannii* you find the three -- the same three phages are in it.

And so it's like okay, then we'll just start with those three. But to say we understand that now might be very premature. And so is there a framework from which you can say our product in our clinical trials need to test a library, not a cocktail in any stretch of the word, and so what you're really doing is much more -- like we're starting a new way of

regulating phages, not like antibiotics or drugs or anything like it, but it's completely new. Is that even possible?

DR. KUTTER: That's exactly what Górski does in Poland.

LT REGEIMBAL: That's what I understand, but I guess the point is, is that -- what's the point? You have to understand that the problem with specificity, the problems of resistance like, for example, rather than going and make a new variant to phage the rule has provided 10 to the 31st. Like why don't you just go find another one?

And so instead, if you have an iterative library that's constantly being updated over time like a flu shot or something else, you won't need to constantly -- but it will also change your CMC, it will change the characterizations that are required if it's in the same field, you know what I mean, so, just generally speaking, is that possible to do in the West or in the U.S.?

DR. FINK: Yeah. So, you know, what you describe is certainly different than the way that antibiotics have been regulated and licensed by FDA to date, but it isn't necessarily new. It doesn't necessarily require a new regulatory framework. You

know, the key question, and I'm going to talk about this a little bit more in my presentation this afternoon, is that if you have a large library of phages, you know, what are the data that you need to ensure that any phage that you pick out of that library is going to be both safe and effective for the intended use? And there may be some, you know, data that you can derive from a subset of phages in that library that will allow you to make that type of determination, but, you know, we're not there yet, and that's, you know, that's where the field, you know, really needs to get together and do some thinking.

DR. GABARD: And then, in addition to that, what kind of data package do you provide? If I go back to Phagoburn, we were lucky enough to initiate our clinical trials with cocktails. I think we had 16 phages in the first *E. coli* cocktail, so all of them characterized, sequenced and data package, at least technical package already available for these collections.

So, at the time being when we knew that we didn't think about this bank issue, and now we have some -- you know, when we have banks, small banks of bacteriophages. But if tomorrow you want to do that, a recommendation of let's say 50 phages, you're going

to do 50 phages in a cocktail and to do that in two pre-clinical testing in animal models. So that's where the threshold is. Can we just define a group in which when you do all these necessary data for toxicity safety, pharmacokinetics and so on, and how do we define that group, and how do we expand that group with phages that belong to the same group with a limited number of data?

DR. YOUNG: So that ultimately, if we do genomics correctly in a large enough set, we should be able to do it essentially by genomic analysis period, which is becoming ridiculously cheap, but we have to collect the data now for over a very large number of applications so we can start making those correlations. That's my feeling. Everything eventually is determined by the genome.

MR. CHEN: Yeah, my name is Rong Chen from Phagelux. I have a question to Dr. Jérôme Gabard actually similar to the previous question, but it's a more practical, real. You select a cocktail which is fixed number of phages, and they only target a certain strain of the bacteria. Now, when you do the clinical trials, multi-site clinical trials, especially multi-country, you will actually face the problem, likely you can have a different strain of the bacterial

infection. So, therefore, when the trial -- when you have such a situation, I'm wondering in the Phagoburn study how did you manage such an issue?

DR. GABARD: At the beginning, we decided to collect strains from all around Europe and USA so that we had some kind of a pretty big collection that could represent the genetic diversity of the bacterial species, but I think it's important for the next studies that may be run and conducted that a preliminary diagnostic is done before planning the treatment, and in our case, it was impossible to do it, but I think a diagnostic before preliminary treatment is a good idea so that you make sure that you recruit a patient that is really sensitive to your treatment.

MR. CHEN: And that's what you did in the study?

DR. GABARD: We didn't do that in the study for Phagoburn. For Phagoburn, we tried to make a wide spectrum cocktail based on selecting phages against a wide collection of bacteria from the same species.

AUDIENCE MEMBER: Could I ask about that? Did you do retrospective when the Phago -- if something didn't go right? Did they check to see whether the cocktail worked against the isolated

bacteria?

DR. GABARD: I think for phage therapy, it's important to remember you have a low number of recruitment of patients. If your number is already low and you don't check at the beginning of the strain the sensitivity to the treatment, then you end up with potentially reducing the number of efficient patients.

AUDIENCE MEMBER: But did you check?

DR. GABARD: No, we didn't.

AUDIENCE MEMBER: I mean afterwards.

DR. GABARD: Afterward, of course, we did.

AUDIENCE MEMBER: And so you could correlate failures with absence of --

DR. GABARD: This is going to come in the paper.

AUDIENCE MEMBER: Yes, nice try.

DR. GABARD: Good try.

AUDIENCE MEMBER: Yeah.

(Laughter.)

DR. KINCAID: Please.

AUDIENCE MEMBER: So I'm going to be a little bit of a heretic, I guess. I've seen a lot of examples of compassionate use for phage. Makes sense. I've heard about the banks being formed. The question I want to ask and it may be both transnational. Is

this going to be more than an academic national effort to develop phage therapy? In other words, what are the economic incentives for industrial development? Part of the issue with antibiotics has been, of course, in the early days, a lot of big companies were involved in antibiotic development, but as that become less lucrative they all dropped out. Many of them have dropped out. Any new antibiotics are basically reserved for, you know, third-line use when it's absolutely necessary to use it.

So what's the economic incentives for developing phage therapy at an industrial scale for, you know, the vast population as opposed to compassionate use?

DR. KUTTER: I can answer. One piece of that, when we first got involved in this back in 1997, two people came from Tbilisi and brought their phages, and we got a bunch of bacteria from cystic fibrosis patients from Children's Hospital in Seattle, and theirs had been used in wound care, and what we found was that all but one of those was very effectively hit by the group of phages in both Pyophage and Intestiphage, and the one that wasn't hit later on when we did the genomic analysis of the 16S RNA turned out not to be *aeruginosa* even though it had been

diagnosed as such. That's not true with all kinds of bacteria. There certainly are some, but that's something that companies need to think about as they're developing it.

Similarly, against *E. coli*, a bunch of similar ones have been isolated against O157 from countries in every part of the world, from Iran to Korea to Australia to Evergreen, and some of those between Evergreen and Belgium were very similar. So it seems like most of the phages wind up going to a lot of different countries.

DR. SCHOOLEY: I was just going to say I think, you know, we have to be a little careful about trying to get so general that you can't get to specifics about could it ever be used. I think there are some clinical indications that you could think about that might be first pegs in the board.

For example, if you find that you can more reliably sterilize prosthetic joint infections by adding a phage to an antibiotic directed at an organism that doesn't require 16 phages to cover it, like *Staph*, you may be able to find a product there that has a much more traditional paradigm, development paradigm.

As you begin to do that, then you can start

filling the blanks around that as people develop a bit more comfort with the general therapeutic approach and as more of the molecular data that Ry is talking about evolves and you can start thinking about how to extrapolate from that situation.

So I think it would be a big mistake to shoot our feet off before we start trying to walk by saying it'll never be scalable and why would pharma ever do this. So I think it's great to raise it, but I hope nobody outside the room heard it.

(Laughter.)

DR. KINCAID: I'm going to take a small prerogative and just put a footnote on our first phage workshop that was held two years ago. We did receive interest from major providers of solution sets for surgical intervention. So it's one of those cases, as Chip just pointed out, where there are people always who are looking for an opportunity if they feel that it's going to make their products better or improve or, in a contrary sense, to reduce the liabilities associated with their products. They'll probably in a very measured way take whatever measures are necessary to consider phage as potential, you know, adjunctive elements to their products.

So I think I agree with Chip that we have to

wait and see how these things play out as more and more people become familiar with the nature of the potential for the product.

MS. EMRICK: Good morning, afternoon. I am Robin Emrick, and just a member of the interested public. And listening to you guys this morning got me thinking about something and, actually, Dr. Schooley, you sort of hinted at it. I hear about the problems of the specificity and nailing it down, and I thought I wonder if somebody isn't already looking at and solved the idea of having a less acute circumstance where like, okay, you're going to have this kind of surgery in three weeks and we're going to start you on some kind of a, I'll say generalized phage therapy that's going to knock down the prevalence of resistance plasmids that may or may not be present in your system. Just kind of prime your body to already be a little more responsive to the antibiotics they already have.

DR. KINCAID: So that turns out to be a rephrasing of one of the topics that we had talked about discussing here. We've had such a good response I didn't want to break that flow, but in a more general sense, it would be useful for the panel to weigh in on the sort of scope of phage use that could

be considered in a preemptive way as a prophylaxis. Is this something that one might consider, whether it be surgical intervention or decolonization of at-risk patient populations? I mean, quite apart from the business model. What's the feeling from surgeons and others?

DR. NARAYAN: So that's a thought that I brought up with Randy earlier on. The problem, though, is that if, as we pointed out earlier, you start administering these phages way ahead of surgery, then you can potentially build up other resistant organisms, as we've seen with antibiotics. It seems to make complete sense to sort of rid your body of all antibiotics before you proceed to interventional procedures which implant large volume of foreign substances, but it's never been shown to be effective since antibiotics are useful if you give them immediately before surgery and doses during surgery.

And so the question is could we do that with phages as well, as opposed to doing it *a priori* and then building up resistant organisms?

DR. SCHOOLEY: I'd like to invite Dr. Narayan to join our Department of Surgery because our surgeons often are not that concrete in their thinking because they put antibiotic beads in everything, and

the data supporting that is relatively modest.

If you think about it, there are situations, though, where phage are not going to be -- as far as we can tell aren't a big problem. If you have a prosthesis you're putting in, you do get periodically *Staph epi* infections, for example, of hips. But the difficulty in clinical development is if you have a good surgeon and you have good antibiotic and you have good antiseptic conditions, the instance of that is low enough that showing that by sprinkling some phage in that you've decreased the instance of that complication is complicated, is very difficult.

And so I think the clinical development paradigm is complicated even though theoretically it makes a lot of sense as long as you do it at the time and don't get way ahead of yourself and allow for second and third generation organisms to populate, which is what Darwin's all about.

DR. KUTTER: I think one other thing that we've been thinking about, and we've done some work and published one paper on working with treating diabetic toe ulcers with phage, and we started by using just pure *Staph* phage. We did that even though we knew there probably are other bacteria besides the ones that are coming out and what they're saying, but

the podiatrists see *Staph* as the head of the snake in these kinds of particularly very poorly aerated toe situations and so forth, and what we found is even though we had Pyophage available with others if we needed it, the staph alone has been enough to treat all 11 patients we've tried before whose only other possibility was amputation, which normally then within five years even if it's just a toe to start with leads to death.

And so I'd really like to see some of those kinds of situations. I mean, I've seen *Staph* and diabetic foot being a logical target since I saw my first experiment in 1996. It wasn't an experiment, it was a treatment by the leading surgeon in Tbilisi, and what amazed me was not only that it worked to treat a foot that had come in for amputation, but he was 95 percent sure it would work.

In other words, you're talking there with a situation where it may be that *Staph* is indeed the head of the snake and simply making a bed that allows other bacteria to grow as well, but the wound's all healed. That's the final thing that we've used so far even though half of them have very obvious osteomyelitis. There's clearly bone infection and the *Staph* is getting into the bone.

And I would really like to see, and I think several groups, both AmpliPhi and Pherecydes are talking about really going to that model, and what we're just starting to do now is to do metagenomics and look at the wounds before and after and see in that case what bacteria are really present and to what degree our model is true.

I think we need to choose some of those simple situations and make it possible without it costing a million dollars right away for trials to be run that are simply adding something to a standard treatment that's happening and not being an expensive clinical trial or even very expensive processes. He's just been doing it in his office, and it's simply something that we add without any extra expense. And I think we need a lot more of that kind of data and not just the data that's come from things that are what will be necessary for the companies wanting to make a lot of bucks about it.

I'd like to see a simple *Staph* phage thing be in effect like the -- like it's used with vaccines or even with aspirin where it's -- you know, what we use is about $5 worth of phage from Tbilisi to treat, and I'd like there to be situations where we can get a lot of this kind of data that's done very generally

and for relatively little money and be able to really build up an understanding better of what's going on.

DR. NARAYAN: I'm going to circle back to the commercial question as well as reply to the previous question. So a good scenario, for instance, is ventral hernias are a very common surgical problem. You have a big operation. A significant number, especially in this day and age with obesity being so high, develop ventral hernias. And so you treat ventral hernias by putting in prosthetic mesh, and then we see this sort of cycle of when it gets infected you have this core population that cannot get rid of an infection.

So, to Randy's point, that might be a situation where you can actually apply certain phages specific to the bug that you've sort of identified. In fact, there are matrices available now, rifampin-coated prosthetic meshes which sort of address the issue of MRSA, for instance, and that might actually be a commercially viable proposition given the increasing number of surgeries that you see, as well as addressing the issue of, you know, prophylactically giving phage even though in clean cases the incidence of infection is really low, say, maybe on the order of 1 to 2 percent. These cases represent a fairly large

number that can actually be both commercially viable as well as treatable by phages specific to the particular bacteria.

DR. FINK: One last point, and I see we have another question. So, just to get back to the issue of preventative use of phages or use for decolonization, there's no *a priori* reason why from a regulatory standpoint a phage therapy product couldn't be developed for preventative use, and, in fact, it's serendipitous that the regulatory review of bacteriophage products is housed in the Office of Vaccines at CBER, so we have a lot of experience regulating preventative products, and, of course, the devil is always in the details of clinical trial design and selection of end points.

DR. KINCAID: Okay. We have time for one question according to my timer. So, Carl.

DR. MERRIL: I'd like the panel, if they could, to amplify a little bit more about the economics. I want to just make a comment. I'm carrying this because, in 2003, I was invited to give the Harold Neu Infectious Disease Conference lecture on phage. This was when I had just done a study that we published in PNAS showing that phage could be highly efficacious and we could even make special

phage that were long-circulating, and so they invited me to give this lecture.

But, in fact, the people from the companies, this was Glaxo and some other companies, said exactly what the previous questioner had brought up, that there just wasn't money in infectious diseases and they were cutting back on their antibiotic production.

But the reason I'm bringing it up as a question now is that there are factors that are affecting the economics, and I wonder if you could comment on them. For instance, the fact that hospitals are now responsible for hospital-acquired infections, number one; and number two, the time spent in an ICU can be far greater than anything anybody spends on any of these therapies we're talking about. I'm sure with Dr. Patterson his ICU time was immense.

DR. SCHOOLEY: He was lucky he was sick during a period when there was no cap on lifetime costs from insurance companies.

There are all kinds of costs that can be calculated into how this all comes back to us as a society. The problem is that they're all in different buckets, and that is where, you know, we have to try to figure out how to rationalize it so that we as a society can realize that the investment's worth it,

but the individual funders themselves don't see it in it for them.

Companies want something that you give people for life, so they don't like antibiotics that work. They like antiretrovirals because you have to give them for life, but they don't like anti-HCV drugs, for example. Phage that work and sterilize, they're not going to like any more than they like these fourth generation antibiotics that they give to six people twice a year who have bacteria you can't treat with anybody else.

As an infectious disease physician, I'm always arguing with the hospital that the reason we're there is to reduce antibiotic use so you have fewer people in the ICU with multidrug-resistant antibiotics, and therefore I need to find a way to pay the faculty in my division, and the department in the hospital always says, well, just bill the patients. We're the ones who collect the money for the hospital. Don't you worry about that.

So you end up -- I think it's really a multiple bucket issue more than it is an issue of is it worth it as a society to do this. I think it is worth it as a society, but I think we need to be more creative in terms of how we cost account it. That's

the extent of my physician/country doctor statement.

DR. GABARD: I'm going to take on that physician/country doctor statement role for a second. So, if you look at it from a strictly commercial standpoint, all you have to do is look around. The Epi-Pen cost has gone up 400 percent. Martin Shkreli is on trial now for increasing costs for generics over 7,000 percent. So there may be something in the market forces itself drives the economics of potentially using this in a commercially viable fashion, and so that's never been addressed and I hope it never gets out of this room that, you know, phages can be sort of overpriced, if you will, to make economic sense for the companies.

DR. GÓRSKI: One thing that surprises me always when we have this type of discussion is that, let's say someone gets cancer, you're going to increase his life by 12 months, and you're going to spend 20,000 bucks while doing that in treatment. So maybe he's going to lose his leg and you're going to spend 5,000 euros or dollars to preventative treatment, and this is too expensive? There is a problem.

DR. KINCAID: Well, I wanted to take this moment, first of all, it's an interesting note to end

on, so philosophical but also so real, and this is a classical concern for infectious disease generally speaking, not just phage.

But anyway, I want to take a moment to thank all of our speakers and our panelists for a very stimulating morning session, and I'm not exactly sure when we return. Do you have the number? One-oh-five according to Roger. Okay. Thank you very much.

(Applause.)

(Whereupon, at 12:15 p.m., the workshop in the above-entitled matter recessed, to reconvene at 1:05 p.m. this same day, Monday, July 10, 2017.)

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A F T E R N O O N S E S S I O N

(1:10 p.m.)

DR. CARLSON: The rest of the people who are still at lunch will filter in briefly in the next few minutes. So we're going to get started back up with Session 2, and you can see on the agenda that we're really talking about regulatory considerations for phage products in this session. So we're going to start with some phage characterization and CMC and then get some talks from FDA folks who are actively involved in regulating these things.

So, with that, I will start off by introducing Jason Gill from the Center for Phage Technology. He's going to talk to us about phage characterization.

DR. GILL: So good afternoon. I'd like to thank the organizers for inviting me here to give this talk.

So I was asked to talk about phage characterization, which is a relatively large topic, so I'm obviously not going to be able to cover everything, all aspects of all things that you can characterize about phages. Some of the stuff I'm going to be talking about was touched on this morning when we were talking about the development of phage

therapeutic products. But really I want to kind of give an overview of where I think we are now and what I see as some obvious paths for the future, and I don't want -- we don't want to intend this to be like some kind of, you know, dictat that I'm giving to the audience about the things that everybody has to do, but these are things that we've been doing in our program and I think they've been helpful for us.

All right. So, when we talk about phage characterization, really, there's -- you know, we're doing this for a reason, right, so the reason is really you want to increase the efficacy, right, so you want to try to pick phages that hopefully have highest efficacy or at the very least get rid of phages that you think are not going to have any efficacy.

We want to increase safety, right. So we want to obviously eliminate phages that might have any deleterious features, at least ones that we can identify now with our understanding of phage biology, and also to increase efficiency, which is not something which is often talked about, but really this has to do with keeping yourself sane and trying to pick phages that actually are going to hopefully be better behaved in the lab and ones you can actually

deal with, and to try to reduce your workload.

So, whenever you want to try to characterize a phage, I think especially when we're talking about phage applications here for some kind of therapeutic, you really want to have a reason, right. So, for example, if you characterize a phage for thermal stability, you know, unless you're planning on putting the phage in a 70 degree environment, it doesn't really help you that much.

So, really, you should have an end point and you should have an actionable outcome for the characterization. Are you going to use this information to make a decision about using the phage or are you going to be able to rank the phages or are you going to be able to, you know, make some kind of rational combination of phages?

So host range obviously is probably the oldest character of a phage that people have looked at. It's still, I think, one of the most important characteristics. It really is kind of the minimum requirement for any kind of efficacy, right? I think the basic requirement for a phage you want to use for any kind of antibacterial is that it has to actually infect the strain that you're attempting to treat, right? That's kind of the basic.

But the needs of host range will vary depending on the application you're looking for. So, for the more kind of company approach where you're trying to have like a mass produced and distributed product like, for example, Phagoburn, or the company approach, you know, you're really going to have a bias towards finding phages that have broader host range as much as possible. But if you're going for kind of the experimental, you know, personalized medicine eIND approach, having broad host range phages in a collection is convenient, but it's not necessarily required because if you have a phage that only infects that strain that you're trying to treat, but if it works well, that's really all you need.

But even then host range doesn't nearly give you all the information that you need, right. So you want to have all the phages that infect one target strain, and so this is going to work. Yes.

So, if you can imagine, here this is a KPC1. This is actually the NIH clinical center outbreak strain here. So we have a lot of phages that infect this strain. These are six phages that are isolated in our group over the last few years, and they all infect this strain. So, if this is the strain you want to treat, you have a lot of phage options. And

so these tells you these are phages you could use, but you may not necessarily want to use all six. That might be redundant. And you probably want to use more than one. And even if it does infect the strain, you don't have any guarantee it'll actually have any efficacy *in vivo*.

So another assay that you can -- factor that you can characterize is, you know, virulence, and so this is basically the ability of the phage to inhibit bacterial growth in liquid culture. This is probably one of the -- this is probably the second oldest character that's used for phage therapy. This is done in test tube, so we have very, of course, sophisticated ways of measuring it now. This is done in an automated plate reader where you can take a time point every 10 minutes. But really the result you're looking at is the integration of the absorption rate, latent period, and burst size of the phage altogether in this liquid culture.

So you can have this high throughput, but the principle is really the same as it was in the 1930s, but if you optimize the method a little bit, you can get some distinguishing between different phages. So here on the left these are all phages that infect -- these are KPC positive *Klebsiella*

pneumoniae, a clinical strain. So, on the left, you have a high input MOI of phage to bacteria. All the phages look roughly the same, so you can see they're all what you would call virulent in liquid culture.

But if you, you know, tweak your inputs a little bit and you have less phage going in, you can see and you can start to separate these phages, and then this may give you some kind of indication of efficacy of these phages, and you can see this one phage down here, which is called "Pharr," suppresses growth better than the other phages, and so this might give you some indication that maybe this phage would be -- if you have to pick one of these phages that all infect the same strain, maybe Pharr would be one that you'd want to look at more closely as opposed to other phages.

So, when you're determining host range, I mean, the classic, the good-old spot assay, which I'm sure a lot of people here have done, it gives you a basic measure of phage sensitivity, but you can actually couple your virulence screening with your host strain, which is something we've started doing and Dr. Biswas has also started doing this as well in his nice -- the Omnilog System where you can kind of just do the virulence assays in a high throughput way and you can get an

idea of the host strains and the virulence of the phage in one go.

So here are two different *Salmonella* strains with the same phage. You can see this strain here, 3003 is insensitive to this phage, right. You don't see any difference in growth when you add the phage, whereas here you can see an inflection of growth and so this tells you that this strain is sensitive to the phage, and also, if you have a number of phages that are sensitive, that give you some growth inflection, you get some idea about how sensitive they are or how active they are against that strain.

So that's kind of where we are now and that's really how we're selecting phages. You have to remember these are really criteria that were developed, you know, in the '30s, and what we do now is much more sophisticated ways of measuring it, but the principle is about the same, and so we think this is kind of the minimum for being able to deploy a phage, but we really should be able to try to get more information about the phages now, like the genome sequence and phenotypes of phage insensitivity and receptor use.

So one thing I didn't mention before is detection of temperate phages. So this is something

that the early phage therapists that were talked about this morning in the '30s didn't really have to deal with because they didn't know it existed yet.

One of the kind of classic things you see sometimes in the literature is that you can just look at a plaque morphology and turbid plaques mean temperate phages, and that is really not true. There are a lot of virulent phages that will make turbid plaques, and we have temperate phages that will make very nice and clear plaques.

So, in our experience, the virulence assays, like I showed you earlier, one way to -- the temperate phages will tend to fall out in those kinds of assays because you'll have really rapid growth of lysogens that will come up very quickly and they will look just bad in the virulence assay.

Another method that we use is to just isolate phage insensitive, you know, mutants, what we call air quote "mutants" of a bacterial strain, and then we just look in the culture supernatant for the presence of that phage because, if it was actually insensitive to the phage because it formed a lysogen, most temperate phages will spontaneously induce from the lysogen stage at a low rate, and so just overnight culture supernatants will have phage that you can then

spot them back on the parent, and so if you have that phage there, that's a good indication that it actually formed a lysogen, and also you can use PCR to screen as well if you have the genome sequence.

So this is one way to look to see if you have a temperate phage or a couple ways you can use.

So I'll talk a little bit about receptor use. So, if you know what the phage’s receptor is, that really can help predict the interaction and also maybe help you plan ahead to overcome bacterial resistance in the future. So our experience with the Patterson case, of course, is that we didn't have any of this information, so it's not essential for use, but it is nice to have that information, I think. So, if you have the opportunity to get that kind of information, I think it would be beneficial to get, and there's a couple ways you can get at this. You can get it on a purely phenotypic level, which is usually looking at cross-resistance, and you can get at it on the more genetic level.

So, if we look at just doing this straight up kind of classic cross-resistance, so these are -- this is a panel of phages against *Salmonella anatum* from my lab, and here we just want to look at what's boxed out here.

So we have a bunch of phages here on the left, and we isolated phage-resistant mutants against all these various phages here, 6, 9, 12, 15, and 27A, and so you can see that, for example, the Mut15 phage is now insensitive to phage 15, as you'd expect. But we want to look here at phages 6 and 9, so if we have a mutant that's insensitive to phage 6, it's still sensitive to phage 9, and we have vice-versa. Mutant that's sensitive to phage 9 or that's become resistant to phage 9 is still sensitive to phage 6.

All right, because it's kind of reciprocal cross-resistance here, so when you look at these kinds of phenotypes, this can help you design a phage cocktail that'll help you maybe overcome phage resistance because we know that if the cell becomes resistant to one phage it will still be sensitive to the other one and vice-versa.

It's a pretty low-tech method, all right, so you're really just, you know, you're just plating stuff out on agar plates and picking surviving colonies. It's not super-complicated, and it doesn't really tell you what the receptor is, but it just tells you what the phenotypes are, and you can see this is borne out in these kinds of, you know, liquid virulence assays again. You can see phage 6 and phage

9. If you expose them to the bacteria, you get resistance, survivors come up after about eight to 10 hours, will start growing up, but if you mix the two of them together, you suppress the arrival of that resistance, at least for the 12 hours that we ran the experiment.

You can see that if you were worried about phage resistance you can get around that. You can kind of get ahead of the game by rationally designing the phage cocktail if you know what the resistant phenotypes are.

Sometimes it's not super-cooperative, so this is a whole bunch of phages we isolated against KPC *K. pneumoniae*, and we found that pretty much all of the phages here -- so here we have the bacterial strain, here it is on the left this time. These are all resistant to each of these phages and the phages here are on the top.

So you can see of this whole phage collection here every strain that became resistant to one phage became resistant to every other phage in this panel, which is a little disappointing, but it means that all these phages are probably using the same receptor and one thing it does help us out with is it means that there's probably not much point in

mixing a bunch of these phages together because, if the host becomes resistant to one phage, it can become resistant to all the other ones, and really that then guides us to actually, you know, doing a new phage hunt and taking one of these phage-resistant mutants, then finding new phages that will infect this strain from the environment or generating a mutant in the lab, and this is what we have done. We were able to go out into the environment using one of these hosts and we can find phages that infect these strains just fine to overcome that resistance.

So actually genetically determining the phage receptor is a little more arduous to do, but it can definitely be worthwhile. So phages can really -- they can recognize pretty much anything on the cell surface. That can be carbohydrates like a capsule or LPS. It can be an outer membrane protein or membrane protein or any kind of cell surface extension like a flagella or a pili.

So there's a few ways you can get at genetically what the actual receptor feature is. If you have existing knockout libraries, for example, of a convenient host that are already knocked out in all your known surface features, you can just spot the phages against those on a plate and you'll find its

resistant to some of them and you can get to the receptor that way if you happen to have that.

You can do Tn5 mutagenesis or isolate spontaneous knockouts or spontaneous phages with the mutants, then just re-sequence them to get to the nature of the phage receptor, right. It's a little more work to do, but if you actually know what actual surface feature of the phages you're going after, you can do some neat tricks, right.

So one is that if you know what the receptor is, you can maybe predict if the resistance is linked to other phenotypes, like reduced virulence or sensitivity to antibiotics. So this is a table from a paper from a few years ago, and these are phages against a *Klebsiella pneumoniae* strain, and you can see the phage-sensitive strain here. We're looking at the LD50, right, so this is how, basically how virulent that strain is.

There's a wild type strain here as 1.5 times 10 to the 8th, and when they made phage-resistant mutants against the phage here called phage NK5, you see that the LD50 went up by a lot, right. So, basically, this strain became 50 to 100 times less virulent once it became resistant to the phage, and they hypothesize that the receptor is probably the

capsule or the LPS, which are important for virulence in *Klebsiella*, but if you know what the receptor is, then you can actually maybe pick phages that are going to go after known virulence determinants on the cell surface.

And another, you know, great trick from -- this is much more recently, is looking at this phage which is actually specific for this outer membrane protein which is an efflux pump that caused antibiotic resistance. So, if you have a phage specific for that particular efflux pump, right, so when the -- and this is the strain here, and when it becomes resistant to the phage, you can see that it becomes sensitive to tetracycline, right. So, again, you have a huge fitness cost associated with the strain becoming resistant to the phage. And so, if you knew beforehand that that's what this phage does, then you can actually look at doing co-treatment with the phage and an antibiotic.

So we like to do phage genomics. That's one of our fun hobbies that we do at the center. There are about 2200 phages, phage genomes in INSDC right now, which is really actually a small fraction when you compare that to how many bacterial genomes there are in the database, so they're really pretty

underrepresented still.

So right now you can have some predictive ability using phage genomics to pick out a phage that you might want to use. So you can increase efficacy and safety. So one thing you can -- by looking at the genome, it can also help tell you if the phages can be virulent or temperate, right. So we know enough about phage biology now that if you find a phage which looks exactly like T7, that's going to be a virulent phage, right. So we have some understanding on phage biology to that level.

You can look for toxins, virulence factors, at least ones that you might know of, and you can exclude phages that you might expect to perform poorly. For example, if you had a phage and it turned out to be an F-specific phage, it's very easy for the host to become resistant to those, and you might want to then put those farther down the list in terms of when you want to use for therapeutics, and it can also really increase your efficiency, and this is -- it's kind of a selfish reason for doing it, but still it really saves you a lot of work because if you have the genome and you can see that you have a bunch of phages that are almost exactly the same as each other, you may not want to necessarily continue developing all of

them, right. You might just want to pick a few representatives and go with that to prevent, you know, duplicating work, and you can also make some predictions on how the phage is going to function based on the type.

So I want to show you a couple examples from our own work of really how helpful phage genomics is. This is a genome of a phage called BcepIL02. When we sequenced it, it really had no homologs in the database. It's kind of a novel type. It's 63 kb genome, circularly permuted. Here is the map. So what's important here to notice is that where this red arrow is pointing, it has tyrosine recombinase, and actually next to it it has cI and Cro-like transcription regulators, so it really looks like maybe a temperate phage.

But before we had done the genome we had actually used this in a mouse model of *Burkholderia cenocepacia* lung infection, and it was therapeutically effective, right. This phage gave us about a 100-fold, two log reduction in bacterial load in the mouse lung, and, you know, I thought it was a successful study. But then, once we sequenced the genome, we found it looked like a temperate phage.

In the past in the previous assay I told you

about where you take your phage-resistant mutants and you look to see if they're lysogens. So it turns out that this phage is what we call a cryptic-temperate phage, so it is a temperate phage, but it's unable to actually form a stable lysogen in the host that we were using in the mouse model, and the reasons for that we think is because the *att* sites that this phage recognizes was not -- it didn't happen to be present in that strain, so it wasn't able to stably integrate, but it was nonetheless a temperate phage.

So we broke like one of our rules right off the bat here and accidently used a temperate phage for therapeutic use and while it worked well in the mouse model we would really have to think long and hard about it if we actually wanted to try to deploy something like this in the world.

So another -- this is, of course, the clinical intervention that Chip talked about this morning. So we were involved in this in February and March and April of last year. So the CPT got the call in and we received the strain from UCSD, as Chip had told us about earlier, and we isolated *de novo* three new phages and we had a fourth called AC4 which was supplied by AmpliPhi, and the U.S. Navy isolated four phages, and this was turned around in about 15 days

from the time we received the strain, which was, I thought, pretty heroic. But the infecting strain became resistant to all the phages after eight days, and as Ry talked about earlier, this whole venture really kind of turned the whole lab upside down for two, three months.

I'm showing these two people here. This is Jacob and Adriana. These are the two people that actually did most of the work in the lab, and I want to show you this picture because they haven't slept in about three days when this picture was taken. This is actually the first shipment of phage that was ready to be shipped in the FedEx, like same-day delivery pick-up guy was about to come. And so it was a huge really heroic effort, right, to generate these phages.

And it turns out that after the dust had settled and then over the summer and the fall of last year we sequenced the genomes of all the phages that we had and it turns out they're all almost exactly the same, right. They're all T4-like large myophages that infect *Acinetobacter baumannii*. There are some differences. They are not exactly the same. There is some variability here, but I wouldn't say the diversity is very high, and this is one reason probably why the strain became resistant to all the

phages at more or less the same time, was because all the phages were pretty close to each other.

So, if we had had this knowledge beforehand, this here is a map actually showing two of our phages. This is C2P24 and C1P12. You can see of the three phages we isolated they were almost 100 percent identical. They were very, very close to each other. Only a few SNPs different between them.

You can see here AC4 is somewhat different. This is showing this protein-protein similarity here. But if we had known this beforehand, we really could have saved ourselves, you know, a lot of stress and headache and heartache preparing four phages when we probably could have just prepared two or maybe even one, right. So that would have actually really streamlined the efforts a lot better and we could have had a much more efficient deployment of the treatment. We could have maybe anticipated that we would have this cross-resistant would come up if we had foreknowledge of what the receptors are, and so we're currently working on determining what the phage receptors are. I think Dr. Biswas is going to talk more about that this afternoon, so I'm going to leave it there.

And also the *in vivo* screening, right. So,

this is something that we still do. I think screening phages in an animal model is still the standard way to de-risk new treatments, but really I'm thinking about trying to do this kind of *in vivo* work in a different -- maybe with a different strategy, right. Most of the stuff that has been published to date is about seeing -- to show that the phages can work, right.

So it's like proof-of-concept type of thing, and you want to show the phages are able to control infections *in vivo*, but you could maybe start looking at trying to -- if you have a bunch of phages that you're pretty sure are going to work, then try to figure out which ones are going to work best, right. And so while you're doing this you also will be able to get data on dynamics and administration routes and also the phenotypes of phage-resistant bacteria and also maybe the immunogenicity of the phages as well.

So this won't be able to be done for all phages, right, so anyone who's ever done animal model development, it's pretty arduous. Not all the past strains you're going to be working with are going to probably work in the mouse model. So you're going to have to have a few kind of go-to strains and use phages that infect those strains to get some of this

information. So you won't be able to screen necessarily every phage, but you'll probably be able to screen at least kind of a subset of the phages you have or at least a representative of all the different types of phages that you have.

This is an example of some data from a paper from Debarbieux's group at Pasteur from a few years ago where they tested the same kind of idea. They tested a bunch of phages here. These phages up here that are color in the top are phages they isolated against this particular *Pseudomonas aeruginosa* challenge strain, and then down here they have other phages, and you can see some of these work better than others, right. So the ones that are up here in the colors work better. You had better survival of the mice following a bacterial challenge, and the gray ones down here worked worse.

And, you know, this also does kind of go back to they did those *in vitro* virulence assays and you can see the ones that did worse in the *in vitro* assays also did worse in this *in vivo* screen. So this can give you also some information about maybe what phages you want to use and not.

So PhiKZ is actually an old, well-known, very virulent *in vitro* phage of *Pseudomonas*

aeruginosa. It's one of these giant phages, giant myophages. But if you had this kind of data, you might think, well, I'm not sure if I want to bother giving this to a person or to try to use this for treatment because it doesn't do very well in the mouse model, right. So it's again another way to try to increase your efficacy.

So the last thing I want to talk about is phage immunogenicity, right. So this is a classic Merril & Biswas, 1996 paper, right. So this showed that if you had phages, that you could select for phages that had longer circulating half-life, as shown up here as opposed to the wild type, and they had improved efficiency, as you see here. These are the long-circulating phages down here, and these are the wild type, and you can see the long-circulating phages perform better in a mouse model.

So maybe you could screen for phages that have longer half-life. You know, short duration half-life might be kind of a generic feature of phages, but maybe it would be worthwhile to actually select for phages that, you know, you could select -- these are all mutants, right, of the long-circulating phages. So you could just select for these perhaps and have these in your arsenal, long-circulating versions of

other phages you know that have already worked.

And another issue that was brought up was the antibody response to phages. So this might be an issue that if you do repeated treatments that you're going to maybe lose efficacy or perhaps you'll need to switch phages, so if one person becomes immune to one phage and you want to treat them again, you have to use a different phage. So perhaps you want to have some idea about what the cross-reactivity, the cross-antigenicity of the phages is before you do the treatment, right, so you'll know you have serogroups A, B, C, D phages, and if you treated somebody with A and B the first time, that if you're going to treat them again, you want to use C and D types the next time.

So this is just a proposed work flow that we use in our lab. So we start with a bunch of phages and we use restriction digestion actually to de-duplicate. So anyone that has identical restriction patterns, we just eliminate those or just keep one. We then do these assays, and then from here you'll pick some and then do -- say such as phage cross-receptor use and then perhaps other types of characterization as well.

So the current outlook. So recent

individual cases have shown that emergency use of phages is a viable treatment option, and really the near future -- almost all phage treatment that we can see until some of the company products come online, which will probably take some time, it's all going to be these kinds of emergency basis, and so the rapid turnaround time is really going to limit what characterization you can do. But if you have a standing collection you can characterize in the background, you can be prepared, right, and you can have the phages that you're already able to turn around relatively quickly, and then, as we gather more data, we'll be able to interpret that data better, I think, as well.

So, with that, I'd like to thank you for your attention and take any questions.

(Applause.)

DR. CARLSON: We have a couple minutes for questions if anyone has any for Dr. Gill.

AUDIENCE MEMBER: Hi. Nice presentation. I have one simple question. So sometimes we see when we do selective pressure using phage the bacteria start, you know, under selective pressure, there are receptors which reduce number expressed on the surface of the bacteria and that can, you know, it's a phage

bacteria is also growth competition, so in that case, bacteria will start over-dominating the culture or media. So how you predict those type of situations in the actual clinical scenario?

DR. GILL: This is like a physiological response, right, you're talking about? Yeah, so those are really -- I mean, those are the hardest ones to get at because they're not stable, right. So we have had phages where, you know, either their resistance is physiological or they just revert really quickly as soon as you remove the phage, and it's tricky. What we've had to do -- I can tell you at least the experience of *Klebsiella*, that we've had to kind of abuse them a lot, so we'll really kind of co-culture them with the phage for a long time so that hopefully that phenotype becomes permanent, or if it's something that reverts really quickly, they can accumulate some kind of compensatory mutations that allows them to maintain it without wanting to revert instantly.

But, yeah, phenotypic changes, they're tough. They're tough to deal with, and I think there's still a lot about the phage host interaction that we don't know. This is kind of the Rumsfeld style like unknown unknowns, right? There are still a lot of those out there.

AUDIENCE MEMBER: So sometimes it's just a simple phase variation --

DR. GILL: Yeah.

AUDIENCE MEMBER: -- sometimes.

DR. GILL: Yeah.

AUDIENCE MEMBER: Okay, thanks.

AUDIENCE MEMBER: I have a question about if you have a preexisting library or a set of phages that have been characterized and you get a new strain that comes in from the clinic no one's ever seen before, and you figure out that, you know, five different phages from all over your library work, do you envision a need to characterize how that specific cocktail works in the context of that specific strain in order to see efficacy? Like do you -- because if you do that every single time, you could imagine that that would be prohibitive if you're --

DR. GILL: Yeah.

AUDIENCE MEMBER: Do you understand what I mean?

DR. GILL: Yeah. Well, I think, you know, it's hard to say when the rubber hits the road, right, how this will actually play out. I think the hope is that if you have the phage -- the phage collection is relatively well characterized, then you could do some

kind of simple experiments just to see if that new strain behaves like it does against the other strains you've already tested, like, for example, if it's the simple kind of virulence assays, it gives you the same kind of phenotype as it does, and maybe you could do cross-resistance assays because you can generate those mutants, it doesn't take too long, right. It's really an overnight, and you can get those and you can test them within a few days, but, again, it depends on how much of an emergency it is, right.

So, if you have the time, then you could do a few more confirmatory or follow-up experiments, but if you don't, I think you just -- the idea is that having that library characterized already will give you at least some assurance that it's likely to work, more likely to work than if you just picked a random phage off the street.

DR. KINCAID: Yeah, I was just curious whether or not you'd developed any assays that assess biofilm disruption and/or whether or not that's worth any effort?

DR. GILL: Yeah, that's something -- it looks like we can talk about everything. So that is another issue, biofilm disruption. So there are these kinds of standard, you know, *in vitro* biofilm assays

you can use and we've used those. I mean, it's just in a 96-well plate, you know, for like crystal violet staining, and so we have some phages, for example, that have the capsular depolymerase enzymes on them that will degrade the capsule, and those are a little more effective at removing biofilm, but, yeah, that could be another aspect, though, of the characterization in addition to say a standard virulence assay is to look at biofilm reduction.

DR. CARLSON: Okay, great. All right. Thank you.

DR. GILL: Thank you.

DR. CARLSON: So, obviously, we can have more questions at the panel later. So up next we're going to have Susan Lehman from AmpliPhi Biosciences, who's going to talk to us about CMC and other considerations for phage products.

DR. LEHMAN: Good afternoon. I'm going to talk about this topic today in the context of what I think is a bit of a gap between everything we know about phage biology and what we need to get us to the point of having phage therapeutic products that are accessible for a large number of people.

One of the biggest challenges for phage therapy apart from maybe money, I think, is

integrating all of the R&D expertise that exists and all of the CMC expertise that exists. Jason talked a lot about phage characterization among the data that we can collect on the research side. What's the key data coming from that R&D environment that we need to collect in order to build a robust manufacturing program and put together a CMC package that can be taken to the relevant regulatory agency to get permission to proceed into clinical trials in humans?

There is a ton of fantastic phage biology knowledge throughout the world, and there's also a really well-developed infrastructure for commercial antibacterial development. I think bridging that gap between those two fields of expertise is a particular challenge for phage therapy. I think the gap exists partly because there's such a long history of human phage use, and that happened largely outside of the drug development sphere, and that's put phage therapy in a bit of an unusual position relative to other novel antimicrobial classes that come to the FDA or the EMA or any of the other regulatory bodies in the early stages of development because we just simply have so much other experience and there's an underlying belief that it works, and so I think that's led to some historical tension between the knowledge

that we have about phages and phage therapy and the traditional drug development pathway that an antibiotic would go through.

But I also hope that I can finish convincing a number of people who may not be convinced already that phage therapy absolutely can fit into a traditionally regulated drug development pathway and that also the established antimicrobial development community can adapt to all the strange little ways that phages don't quite fit in. They don't quite act the same way as a small molecule drug does, and they don't even really act the same way as a non-replicating biologic. There's always some extra complications when you've got something that's self-replicating.

When I make that statement that phages can fit into a traditional drug development pathway, I'm basing that on AmpliPhi's experiences. We are engaged in traditional drug development programs. We have two lead products, a cocktail for *Staph aureus* and a cocktail for *Pseudomonas aeruginosa*. Both of them have very high coverage across the various isolates of both species that we've collected, and that's been true as we've continued to collect new isolates over time.

Last year, we completed two Phase I safety trials with the *Staph aureus* product, one in chronic sinusitis patients in Australia and one in healthy volunteers in the U.S. toward the skin and soft tissue infection. In both cases, the product was safe and well-tolerated in the subjects, and following those trials and some additional conversations that we've had, we are well-positioned to move that product forward into Phase II clinical trials.

For our *Pseudomonas* product, we are hoping to enter Phase I trials next year. We've had a successful consultation with the MHRA because our partner is there in the U.K. and are well positioned to move forward in that, with that product as well.

There's also been some talk about compassionate use cases. AmpliPhi has responded to physician requests to use our products and our phages under expanded use schemes, and in those scenarios, we've had interest in a number of other indications besides the infections that are listed here and things like IV administration, and we are investigating those as well.

So you've done all this great work with the phage characterization that Jason was talking about. You've designed a great product and what do you need

to get from that data into a clinical trial to a product that hopefully works and you can get regulatory approval to take to market?

As you move out of that R&D environment into a more regulated manufacturing, testing, and clinical environment, everything becomes quite a bit more structured. Broadly speaking, you need to scale up fermentation and purification. You need assays that you can use to monitor your process and also to monitor the final product that you get out of it. You need to figure out how you're going to formulate and deliver your drug. You need to make sure that it's going to be shelf-stable for long enough that you have a useful product, and there are industry standards that govern all of these things: things like how your biological stocks are maintained and used; how your process validation is done; the quality systems that govern all of these; what kind of claims that you can make about drug delivery and drug dosing; how you store and test stability for your products; and how you test safety before you can get permission to move forward into humans; and then, of course, once you enter clinical trials, there are a number of regulations that govern how those trials are conducted and how the data is analyzed.

I'm going to start by talking a little bit about the first two stones on my little stepping stone pathway. The overall message for GMP manufacturing, the underlying principle is process consistency. You need to manufacture the same thing every time, doing the same thing every time.

I've put up an example schematic for a hypothetical four-phage product where there are two bacteriophages that are grown in one manufacturing host and two phages grown in a second manufacturing host. There was a comment made this morning about whether you pick a manufacturing host early in development or later in development. I definitely agree with the statement that it's better to do it early.

You don't -- as was said this morning, bacteriophages just can behave a little bit differently based on what you've grown them in, and you certainly wouldn't want to get to the stage of manufacturing and find out, you know, switch a host and find out that all that characterization data you've so carefully collected is no longer entirely relevant to what you're about to manufacture.

So, having said that, you've done all that work, you've transferred your phages and manufacturing

hosts to two of the GMP manufacturing environment, and set up a two-tier manufacturing system. You've got master stocks that are really well-characterized, working stocks that you're going to use to make each batch, and when you've used up all your working stocks, you can go back to your master stock and make a new set of working stocks.

Every time you make a new batch of phage you take one of those working manufacturing host vials, one of those working viral seed vials, put them together, do your fermentation and purification processes, get your drug substance out, and mix your purified phages in known ratios to generate your drug product.

There are a couple of things that this does. Two of the most important ones are that it limits serial passage, so you can maintain vertical consistency between those master stocks and every batch of drug substance that you make. It's particularly important for phages because there's a replicating genome.

The other really important thing that isn't entirely captured by three little boxes on a screen is how important your process consistency is through this step. Every time you make a new batch of phage you

follow the exact same process through the fermentation, through the purification. The process might differ for different phages in your product, but for a given phage, same thing every time, and it's extensively documented.

Having gone through the process of setting up that manufacturing system so that you are making the same thing every time, you need analytical methods so that you can demonstrate that consistency both to yourself and to others. You can't test everything. You have to rely on the processes you've set up and you have to have designed good processes, but you can test a number of key characteristics against established criteria to give yourself the confidence that everything has gone the way you've set it up, and a lot of those assays are going to be heavily dependent on the phage characterization that you developed early and the process development data that you gathered early on.

So it's important to keep that in mind during the early R&D phase. I think it's really easy in a research environment to get very caught up in screening phages, picking the best phages. If you're in the genetic engineering side of things, making the best phages. But everything that you pick is

eventually have go through the, you know, process of controlled manufacturing and analysis, and so you do yourself a lot of favors if you think ahead to these analytical processes while you're earlier in development.

Part of that thinking ahead is thinking about the difference between good research analytical methods and good analytical methods for GMP manufacturing. There are a lot of good research assays that have controls, standards, they work the same way when you run them again, they measure what you think they're measuring.

But when you move into a GMP manufacturing environment, you have to know a lot more. You have to have much more in-depth knowledge about the assay performance both in terms of trends over time and the way that the assay functions every individual time that you run it. You don't get to decide that you're going to run a gel, an agarose gel at a slightly higher voltage for a shorter period of time because you've got somewhere to go. It's got to be the same every time.

I don't think I've ever worked in an academic research lab that made everyone in the lab who did plaque assays take the same tube and test it

multiple different days and make sure that everybody in the lab got the same number within a pretty tight margin of variability and then said, oh, the lab next door that we collaborate with or the lab two universities over that we collaborate with, we're going to give you the same samples and make sure that you get exactly the same results.

We make an effort in those environments to make sure that our results aren't wildly different, but we don't really put hard numbers on those kinds of things, and in a GMP manufacturing environment, we have to.

There are also a number of scenarios in a research and development environment where what we're doing is somewhat exploratory. You're not just interested in things that have a yes or no output. In a GMP manufacturing environment, in a quality control testing environment, you don't want that fuzzy middle. You need something that has a clear readout and it's clearly interpretable as a yes or no answer.

And, finally, you -- well, not finally, there are a lot of examples I haven't given, but finally for this slide, is the assay actually helpful? Can it be run in a reasonable amount of time for what you need? Are the tolerances that you put on that

assay tight enough to detect problems when they exist, or are they so tight that you start flagging things that you shouldn't?

All of that assay development and process development occurs in stages. It matures over time, and the validation level of those assays also matures over time. Some of your assays are going to be industry standards. Some of them are going to be product-specific, and the product-specific ones are likely to be the biggest challenges.

I'm not going to talk about all of these. I want to highlight a few. Phage concentration, obviously, a key parameter for a phage product.

What host are you going to test it in? There was some conversation this morning about manufacturing hosts and assay hosts that people have found were not -- they didn't work if you use the same one, so it might work if you use the same one, it might not.

Are you testing a single phage? Testing the concentration of a single phage is fairly straightforward. What about if you're trying to test the concentration of three or four or five or 12 individual phages within a cocktail? That's a much bigger challenge. Identity and purity of your phages

are also obviously key. You want to make sure you've grown the phage you think you have. You haven't cross-contaminated any of your stocks.

How informative are the assays that you have? There are a lot of different methods available to assess phage identity and phage purity. They each have different strengths and weaknesses. Obviously, you need something that's going to differentiate among different phages. You also need something that's got the right capacity to detect a problem. PCR is great, it's fast, it has a nice, clear readout, but it's only based on a tiny section of the genome, so it's going to be useful for some kinds of assays, but it may not be useful if you're looking for changes that may occur outside of that section of the genome.

When it comes to removing impurities, are you at a phase of development where you have a really good idea of a couple of specific host cell proteins that your process -- that you can test for to tell if your process is working well and you've gotten good purification, or are you at a stage of development where you need to look more generally at bigger picture of what's going on in your purification?

If you're talking about endotoxin, how you plan to administer the phage product is going to

determine what your acceptable endotoxin limits are.

With microbial contamination, there are established test methods for bacterial burden and for sterility. That's good when you need to test that, but you also should think about where in the process you might have contamination occurring. Maybe there are points in your manufacturing process where it would be beneficial to do a risk-based assessment of what the likely problems are to arise, and so you can test as you go along for some of those most likely events and reduce the risk that you're going to get to the end of your process and have something that fails, fails a quality assessment, and you could have found out before you spent the time and money going through the whole thing. You could have found out early on that there was a problem with that batch.

The next three stepping stones on my little graphic are here. There's a ton of things to think about just in terms of the practicalities of developing phage products. There are guidelines that govern a lot of these, things like stability testing and device qualification if you're going to use a delivery device. I want to use this to give you a specific example of the ways in which a lot of these things can get more complicated than you initially

predict.

Our Phase I trial on rhinosinusitis patients administered the phage as part of a sinus wash. It was a sinus wash that's used as part of the standard of care for CRS patients, and they have about a cup of water and they dissolve a little saline pouch into it and run it through their sinuses in both directions, and we thought we'll put the phage product in the sinus wash. It seems really simple, and you think, okay, let's make sure that the saline, you know, components don't interfere with phage viability.

What about the water? Most patients use boiled tap water, municipal tap water to prepare that saline wash. The municipal tap water where we were going to run the trial, boy, even after you boil it, it's not so good for the viability of that phage product.

So, in that trial, we ended up providing every subject with a case of water that we knew would have no problems and they took that home with them. We did all the testing in advance, so we knew that we needed to do that, but it's a level of -- it's that extra level of testing for compatibility that you need to think of throughout these processes, and it takes time.

Clinical trials and clinical development in general are hard. They get harder when you add phages. Assuming you've picked a good infection target that matches phage biology really well, you've got a plan for your clinical trial progression that's going to get you to the indication that you're targeting, and you have a sufficient patient population to enroll those trials in a reasonable amount of time, what are you going to measure? Is your primary end point clinical? Is it microbiological? If it's microbiological, do you have a threshold for success versus failure?

Do you need new assays? Sometimes the infrastructure that's available in clinical microbiology labs aren't going to be equipped to handle phage-specific assays particularly well because they simply don't work with it. They're not used to it. So, if you have to qualify a new assay and potentially qualify a new lab, if it's a multi-center trial, are you going to try and qualify multiple labs at all of the sites, or are you going to look into centralized testing?

You made it through all of this. Before you can get permission that it's safe to proceed into humans, you need to submit all of that collected data

to the relevant regulatory authority. In the U.S., this is done under an Investigational New Drug application, or an IND. Different countries have different requirements, but the general structure is similar. You present your overall plan. An investigator's brochure communicates your core product and quality characteristics to the physicians and also to the institutional committees that are responsible for approving the trial, and all of the non-clinical data and all of the quality information from your manufacturing come together in that as well.

The structure of these elements will change over time. As you move through clinical development, you obviously acquire more clinical data, but the general structure is there, and the FDA, the elements to this aren't a secret. The FDA and the ICH have lots of really detailed information online about exactly what goes into all of them.

So, to end, I think my message here is that you can -- I mean, our experience so far certainly has been that you can fit phage development into industry standard processes, and a lot of times through this I was making reference to the fact that there are industry standards for X. I think a lot of times in the phage therapy community we tend to view those

industry standards and requirements as a hurdle, and I don't believe we need to see them that way.

Certainly, requirements that need to be met, and that takes time and effort and money. They're also the things that are going to let us treat a number of people to make phage therapy available to a large number of people.

There was a comment earlier this morning that the kind of really intensive, focused effort that's required for a compassionate use case isn't necessarily sustainable. The way we can make phage treatment sustainable is through this kind of approval pathway, to fit them into the drug development pathways that exist, and the good news in a lot of ways is that all of this, all of the infrastructure for this, does exist from the antibiotic world. There are ways that we need to fit phages into that and there are some specific challenges associated with that, but there's also a lot of CMC expertise out there that we can use and we can use to our benefit.

I promised I'd talk a little bit about some of the expanded access use for products as well. Our tendency so far has been to use GMP products when possible, and because the products that we have have such high coverage across different strains of the

species that they target, we think that it's quite feasible to use GMP products in a number of cases or at least a GMP-like version of those products.

In situations where GMP material isn't possible or isn't available, we still believe that non-GMP material can and should meet very high standards. When it comes to key attributes, such as understanding that, you know, phages are lytic, having really well-documented evidence of your product quality, using purification methods that are appropriate to develop material for human use, doing microbiological testing, handling things in a controlled way, a lot of the way that these attributes get handled in a non-GMP product may be a little bit different from the way they're handled in a GMP product, but we can still meet high standards of quality for those.

My message for today has been that the phage biology expertise that exists in the phage community is absolutely compatible with a drug development pathway. There are a lot of exciting conversations that I think are being had about additional ways to look at this. The concept of phage libraries is a really good one to talk about, and I think that having some development through some of the traditional

pathways is going to help move those conversations forward, which was mentioned this morning.

We have a lot to learn as we all move forward, and I'm very grateful to the FDA and the NIH for continuing to organize this workshop because I think we've gotten a lot of useful discussion and will continue to over the rest of today and tomorrow.

I think I have time for one question.

(Applause.)

DR. CARLSON: So we can take a few questions. We started a couple minutes late.

AUDIENCE MEMBER: So a nice presentation. So my question, specific question is that whatever you presented here is good for a fixed cocktail model. But if you are a dynamic, you know, phage library and you want to make a product out of it because we know from my experience a fixed cocktail model is not going to work all the time because the resistance for the phage is pretty often because the phage-bacterial interaction happen more randomly than the antibiotic, mainly the fungal products, and the bacteria.

So, in that environment, today's fixed cocktail will not work tomorrow's bacteria. So how you address this question, how you?

DR. LEHMAN: I think there are a couple of

pieces to that answer. One of the things that we've found with the cocktails that are predesigned that we have is that they have been very broadly active, and in terms of, you know, when you go and collect clinical isolates every couple of years, new clinical isolates every couple of years, they've maintained pretty broad activity.

The inherent frequency of resistance is pretty low, and you also have -- so you have a lot of cases where those are going to be useful. In cases when they're not, we are open to -- we want to have the discussions about whether having very well-characterized libraries of phages has a -- basically whether there's a way to fit that into an FDA-regulated system, and certainly, as I've indicated, we've been involved in some compassionate use cases where there's -- where permission to use something that's less well-characterized is easy to get.

I think there's also some difference to keep in mind between species. *Staph aureus* is probably a lot easier to hit with a defined cocktail in more cases because the organism itself is more homogenous. *Pseudomonas aeruginosa*, you start to get into an organism that's a little more genetically diverse, and you get to something like *Acinetobacter baumannii* and

that's even harder.

So I wouldn't want to get scared off by the challenges of *Acinetobacter* when we're looking at something like *Staph*. I think we need to talk in a very context-dependent way about the need for additional -- the need for, I guess, the relative utility of those pre-defined cocktails because there are a lot of cases where they think they are going to be very useful. At least that's been our experience with the data that we have.

AUDIENCE MEMBER: I basically wanted to ask the same question as him, but the data you have where you continue to see efficacy even when there's new clinical isolates emerge, are you doing that in animal models or are you doing that *in vitro*?

DR. LEHMAN: The bulk of it is *in vitro* because you can simply test so much more.

AUDIENCE MEMBER: But one would have to -- not to be belligerent, but one would have to consider that *in vivo* -- I mean, even manufacturing strains matter. So, if you shift the center of your population away from optimization to the target, it could be that *in vivo* that's -- or, sorry -- *in vitro* it's undetectable because diffusion is controlled in a liquid broth, for example.

But when you put that into an organism with an active immune system and CRPs and three dimensions, you might not see the same efficacy downstream and you might get different kinds of resistance emerging in an animal that's also adding selection pressures of its own vice a context where you're just in the broth. Does that make sense to you?

DR. LEHMAN: Yeah, yeah, and I think everyone agrees you can't do quite as much testing in an *in vivo* system as you can do in an *in vitro* system, and I think the testing that gets done in an *in vitro* system in that capacity needs to be chosen pretty carefully. For example, a mouse model is only going to support a certain size bacterial population, so there's only so much of that that may be informative in a mouse. But as you get to bigger and bigger animals, you can do even less.

So that's one of the areas that compassionate use cases may help us to understand a little better. It may help us to define the questions a little better because a human has -- it's -- ultimately our question is what is it going to do in a human being, and as we collect some data from a lot of these cases, we're going to have a better sense of what those questions are, which may let us go back and

do some more focused *in vivo* testing in animal systems as well.

AUDIENCE MEMBER: Coming to the *in vivo* question, one of the biggest challenges that we have in antimicrobial development, and certainly it'll apply to bacteriophage is in our patients how much to give and how long to give it. So, while in antimicrobial development we have very recently well-defined strategies and pathways to arrive at the PK/PD, what pre-clinical animal model systems PK/PD analysis do you do to be able to know how much to give and how long to give?

DR. LEHMAN: That's a real challenge. A lot of traditional PK/PD is done in uninfected animals, and that's not going to tell you the same things in phages that it will tell you with a small molecule drug because the dosing changes and the way that the phage can hide from biological clearance mechanisms changes when a susceptible bacterial population is present.

AUDIENCE MEMBER: For example, in the wound models that you have and in the implant, we do have animal model systems for those. Were they developed in order to -- and explored in order to know again how much to give, how long to give?

DR. LEHMAN: In most cases not. Figuring out how to do proper PK/PD for phage therapy is a huge unknown and I think it's something that's understudied in the field in general, and we've got a lot to learn when it comes to figuring that out, and some of the work that Dr. Górski has been doing and his colleagues in Poland looking at what kind of antibody responses exist and how those antibody responses correlate to clinical outcomes has been valuable, and I think, you know, as companies go through controlled clinical trials, we have a lot to build on in that specific area. Or, I'm sorry, we have a lot to build up in that specific area.

DR. CARLSON: So we can continue with questions along these lines in the discussion session later and I guarantee we'll talk about phage, personalized phage therapy there. It keeps coming up in, I think, just about every talk, so I promise we'll come back to it at the panel discussion.

So up next we have Scott Stibitz from FDA CBER, who's going to talk to us about regulatory pathways and CMC considerations for bacteriophage products.

DR. STIBITZ: Great. Thank you. This is the point where I would normally thank the organizers

for inviting me, but instead I will thank all the other organizers for their hard work and for the CBER staff and NIAID staff, who have just done a fantastic job getting this all together.

So this talk constitutes what we kind of sometimes refer to as regulatory outreach, and when I was younger and more foolish, I said I would never ever give a regulatory talk, but I've now given quite a few and I actually really like them because it gives us a chance to sort of set the record straight in a way to really give accurate information. We often hear people talking about the FDA and what we think and what we'll allow and what we won't allow, and sometimes it takes us by surprise.

In terms of phage therapy, the most pervasive is -- which is stated over and over again -- FDA will never allow phage therapy. So I also have to just throw this disclaimer up here to let you know that my comments will not bind or obligate the FDA.

So, just to kind of address off the starting block some of these misconceptions, I put together a few points that address some of these issues we've heard.

So one is, you know, does CBER FDA have a history of regulating novel products and treatment

modalities, in other words, weird stuff? And the fact is, yes, we do. I think the most recent example of that would be fecal transplants.

Are clinical trials on phage therapy proceeding under FDA oversight? Well, I can give the positive answer yes, but only because those have been publicized by the companies involved. Otherwise, I would not be able to make that statement.

Similarly, we do allow compassionate use of phage therapy. Our preferred term is "expanded access," but I can kind of tell it's a losing battle.

(Laughter.)

DR. STIBITZ: So are there new and challenging aspects to clinical trial design? Absolutely. Are there novel CMC challenges or considerations? Yes. Does the FDA actually in-house have research projects on phage therapy? That is also true. A story for another day.

And so what I think all this adds up to is I hope that you are convinced and I think everything I've heard so far today and what you'll hear tomorrow is that the FDA does not have a preexisting negative position towards phage therapy.

So a brief outline. I want to talk about who at FDA is responsible for regulating phage

therapy, if we could put a face on the monolith, a brief overview of regulatory procedures, and then talk about CMC issues that may be special relative to phage.

So to the first. FDA contains many centers. I've thrown up some here. This is not a comprehensive list, but we are in the Center for Biologics Evaluation and Research. It occurred to me as I was looking at my slides today actually each of these other centers probably will have some interaction with us or something to say about phage therapy as well.

So we're the Center for Biologics. It's natural to ask the question what's a biologic. In almost all cases, a biologic is also a drug, but here's a definition from the Public Health Service Act, but the most critical statement in here is, and this echoes the definition of a drug, is that it's applicable to the prevention, treatment, or cure of a disease or condition of human beings.

So, within CBER, we are within the Office of Vaccines Research and Review, as has already been pointed out, and there are three divisions. The division in the middle here is Vaccine and Related Product Applications. They are the people who really manage the files, communicate with the sponsors, and

coordinate the reviews. The reviews include in almost all cases -- I mean, sorry -- in all cases I would have to say product review, and that's done in the divisions that actually do laboratory research. So those of us in those divisions, we have labs, we do experiments, and then we also do product review. And within DVRPA we have clinical review and, as needed, toxicology reviews and also, as needed, statistical reviews or quite often consults with other divisions that might have, for example, clinical expertise.

Okay. So when in product development does the FDA get involved? As many of you are aware, this is first in-human use. When that happens, it's supposed to be done under IND. That has at least two effects. One is that it's done under our supervision with our advice, but it has a legal ramification, is that it allows use of an otherwise illegal product in interstate commerce or in clinical trials. So the IND is basically an exemption from having to use a licensed product. But it's also important to remember that not all INDs are for product development. We get some that would be called research-only studies, I think, by most people.

So this is kind of a summary of the whole

picture. I want to spend a little time talking about it. We have the phases of IND research here, and the boundaries between these can sometimes be somewhat mobile. Certainly, many studies would span Phase I, Phase II or Phase II, Phase III, but the important aspect here is that through the IND process it's basically progressive implementation.

I have broken it down into three aspects here. One is effectiveness. So the trials that you're doing, Phase I, for example, could be simply safety. Phase II could be preliminary evidence of efficacy, and then Phase III, of course, is your pivotal clinical trial, collecting the data to support a license application.

Similarly, manufacturing consistency. In the beginning, it could be quite simple. During this process, there may be changes made to the product, dosing might be altered, dosing or formulation, but by the time you get to Phase III and are ready for your clinical trial this should be basically set.

Similarly for assay developments. The, you know, assays in Phase I should be basically scientifically sound. By Phase II, you start to think about qualifying, and by Phase III, assays to be used in the pivotal trial should be fully validated.

And then, if all goes well, license application leads to an FDA license, and, of course, that's not set in stone. BLA supplements after the license can be used to change things as long as it's not too terribly drastic, but there can be some changes to the product. There can be changes in manufacturing. There could be clinical studies to support a different dose or a different indication, changes in the manufacturing equipment, et cetera, et cetera.

And then I'll talk again about this more, but leading into all this, especially for people that don't have a lot of experience, we have opportunities to meet in what's called a pre-IND meeting.

So one of the things that I like to tell people because the term "CGMP" strikes fear in the heart of many would-be IND sponsors is that GMP is not GMP, is not GMP. In other words, what people think of as a GMP lot fully validated, you know, all the things that you think of when people say, oh, well, we need to get a GMP lot to begin studies. In fact, GMP in Phase I is not as rigorous, and so I've quoted this guidance which is out there. The approach described in this guidance reflects the fact that some manufacturing controls and the extent of manufacturing

controls needed to achieve appropriate product quality differ not only between investigational and commercial manufacture but also among the various phases of clinical trials, and boiled down, this means that for Phase I, CGMP is not required to be as extensive as for later phases or for an approved product.

Who sponsors biologics INDs? Big companies, small companies, individual bench researchers, individual clinical investigators, and other agency. And so the point that I'm trying to make here is that the regulatory expertise and regulatory support that's available to sponsors varies greatly, and this is why at critical points the opportunity exists and it's highly recommended to meet with us, for example, prior to submission as in a pre-IND or prior to pivotal studies, license application, et cetera, and this is where we really work out a lot of the details.

And just to reprise this slide to make this point that because biologics are so different from one another we can't have, you know, kind of clearly prospective milestones that will apply to all products and that, you know, the sliding scale or progressive implementation, the milestones on that are arrived at through conversations between the sponsor and the FDA.

Again, pre-IND meetings. The stated purpose

of these as per this guidance that's shown here is to discuss CMC issues as they relate to primarily safety of an investigational new drug proposed for use in clinical studies, and the way this works, some of you will have done this and are familiar, but the sponsor poses questions, provides a description of the CMC.

This is a case where you get out of it what you put into it. So the more detail that you can give us about where you are in product development, how much information you have, and what your real questions are to us, the more you will get out of it.

And so what happens is that CBER assembles a full review team, so that would be product, clinical, toxicology if indicated, statistically possibly at an early stage, and we do a full review of what you have submitted. This is good for everybody. It's good for the sponsor because they get a much better and clearer idea of what we're asking for. It's good for us because, when the IND actually comes in, the review is much more straightforward, and it's good for both of us because fewer studies go on clinical hold.

And so, if all goes well, the ultimate goal is an FDA license. For a product to be licensed, that requires three things: that it be shown to be safe and effective and able to be manufactured

consistently. And, again, the details of what a safety database would look like or what the demonstration of effectiveness is in any particular case will be based on the nature of the product and our discussions.

So, finally, just to get to some more phage-specific stuff, I tried to organize this around unique, as I see them, aspects of bacteriophage, all of which have been touched on already and will be. But phage are incredibly diverse. They're highly specific. They mediate genetic transfer. They're antigenic. They're generally assumed not to interact with human cells, and as part of that, there's basically a high expectation of safety.

So just to go into some of these one by one. Now some of these have positive aspects and some have not so positive aspects. In terms of diversity for many bacterial hosts, and again I think the dogma that there are billions of phage out there for any bug may be not so true for all bugs. I mean, certainly, Ry talked this morning about how *Staph* phage seem to have been dominated by the K-like phages, and I think Andy Camilli's work has shown that there is really a very small number of cholera phage out there, but this is a concept that we work with, that there are lots of

phage out there and it should be possible to find new phage. So I put inexhaustible in quotes because maybe it's not inexhaustible, but it's a big supply of natural products.

But the downside of diversity is that every bacteriophage-bacterial host pair is unique, and so it is, in fact, problematic to draw a priority or general conclusions, either good or bad, about phage in general from specific examples.

Specificity of phage, these will generally be pathogen-specific treatments, which raises a whole new issue when it comes to clinical trial design, which our next speaker will be speaking of. Now the good thing is that we expect less disruption to the microbiota, as has been stated. But it also, as has been stated, will usually require identification or diagnosis of the agent prior to treatment.

We generally talk only in terms of receptor actions, interactions, as dictating specificity, and in that regard, as was presented very nicely by Jason, there's a future for using receptor identification to inform cocktail composition, to avoid the issue of resistance to all phage in a cocktail simultaneously. But I just want to plant a seed that receptor is not the only source of specificity, and in work in our

lab, it looks like both gene expression and replication may be playing a role in that as well.

Bacteriophage are immunogenic. An adaptive response is likely. This may limit the length of use or re-use, but not that much is really known about this in clearly defined studies. I was extremely interested to hear all that Dr. Górski had to say on this, and I think that they're really getting at a lot of these issues, but I took away from that it's not as dire as it seems. I mean, in some cases, it does not appear to limit their therapeutic use. But it's also unclear at this point, I think, what safety concerns arise from immunogenicity. I'll leave it at that.

Okay. And so one of the most unique aspects of bacteriophage is the fact that they mediate genetic transfer. So those genes can be transferred. Transfer may be part of the phage genome itself. This is what's called lysogenic conversion, and the phage itself contains genes for antibiotic resistance, virulent factors, what have you. There are many, many examples of this out there.

So that by integrating its genome into the genome of the host, those genes now become part of the genome of the host, so special abilities often in terms of resistance or pathogenicity, should I say

virulence are bestowed.

The other way is by what's called transduction, and this is where phage particles move genes between hosts. There are generally recognized to be two kinds: generalized, in which all chromosomal markers of the host organism are transduced with equal frequency, and the other is specialized, and this refers only to lysogenic phage where once having integrated, imprecise excision will lead to phage particles that now carry genes that were close to the insertion site. And so, by restricting our use to non-lysogenic phage, we really get rid of two of these concerns and are left with that of generalized transduction.

So, just to make sure that we're all on the same page, generalized transduction refers to the fact that some bacteriophage when they infect a cell create new copies of themselves and then start to package that into phage heads, will sometimes pick up a copy or a hunk of host DNA. Those particles, so this lysate coming out of this infection would have infectious particles, wild type phage, but also these transducing particles, which will contain a hunk of the host genome. Those particles are not infectious, but they can adsorb and inject their DNA and have it

incorporated into the genome recipient bacteria.

So what are some ways that you can look at this? A microbiology approach would be to do a transduction assay. Simply take your phage, you propagate it on a bacterial strain that contains a selectable marker, such as antibiotic-resistance. You just take that transducing lysate or potentially transducing lysate, apply it to an antibiotic-sensitive strain and plate on media containing antibiotics. If the phage is capable of transducing that marker, you should be able to detect it.

From a molecular biology standpoint, one can just take your phage lysate and examine it using more sensitive techniques perhaps for the presence of host genes. So PCR can be used for that or you could even deep sequence.

For lysogeny, and Jason made basically the same points, look at your plaques. Dogma has it if they're turbid it's lysogenic, but if they're clear they're virulent. He mentioned exceptions that are turbid yet virulent, and we have examples of ones that are clear yet lysogenic. But what you do is you pick bacteria from the center. You see if any bacteria in that battleground are still alive. One of the ways they could be alive is if they're lysogens and

therefore phage-resistant, and then you test those for release of phage, either spontaneous or after chemical induction.

Most people that I ask how do you decide your phage is lysogenic or not, they say just look at the sequence. So they've obviously done the genomic sequence and analyzed for the presence of repressors, homology to other known lysogenic phage, and any indicators of lysogenic lifestyle, and you could have a hybrid approach where you take some of these survivors and then you determine that DNA sequence of a putative lysogen and see the phage as an insertion. That's an approach we've taken recently.

So the current consensus, I think I'm safe in saying that, I hear these echoed over and over, of what type of characterization do we want for phage for therapy. In terms of the phage phenotypes itself, non-lysogenic, non-transducing. For the phage genotypes which could be assessed by DNA sequence analysis, free of undesirable genes, such as antibiotic-resistance and virulence factors, and the phage preparations should be pure and sterile and, we believe, low endotoxin, although we're having an interesting discussion about that.

Phage cocktails have generally been proposed

to increase the spectrum of treatment, in other words, against more strains of a given organism, but also to avoid resistance, and regulatory implications are that each phage should have relevant activity. In other words, you don't just throw the stuff in there for good measure. Potency tests should address each phage in the cocktail, and stability testing, likewise, should assess each phage in a cocktail. Future inclusion of additional or replacement phage should be supported by CMC information.

And then, finally, other things that would be nice to have but I don't think are going to be requirements, and all of these have been referred to, so I'll just mention them. The idea of stealth, this is from the Merril and Adhya work showing that mutants could exist in circulation longer; from Andy Camilli's work, a nice example of using virulence factors as receptors so that resistant mutants are less virulent; and then, of course, the nice story about the MDR *Pseudomonas aeruginosa* treated under compassionate use, which actually used antibiotic-resistant proteins as a receptor. And, again, this is something I think we have an ongoing discussion about, but possibly being able to propagate on non-lysogenic, non-pathogenic, non-antibiotic-resistant hosts.

So, finally, last side, almost on time, this is a feel good slide. FDA is committed to facilitating the testing of phage therapy in clinical trials. We do not feel that, and hope that you do not, the FDA regulatory review presents an obstacle to the assessment of safety and efficacy of phage therapy. We believe that regulatory officials, scientists, and product development developers have shared goals and need to work together and to do that communication is vital, and as investigations begin, meeting with the FDA early is highly recommended.

Some resources which I will distribute because you can't write them down. Just wanted to thank my group and two people there in particular, Sheila Dreher-Lesnick and Roger Plaut, who helped me the most with the slides, and Roger with many, many other aspects of this workshop, and then all the other folks who were involved in practice sessions. So thank you very much.

(Applause.)

DR. CARLSON: We can take a couple questions for Scott before we have a break. You're on.

AUDIENCE MEMBER: Thanks, that was really nice. I agree we're all in it together and we should try the best we can.

You know, we haven't heard anything about RNA phages, and I'm wondering from your standpoint are they just off the table because you're worried about high mutation rate or just what you're thinking on that?

DR. STIBITZ: I don't think anything's off the table. They haven't come up that I'm aware of as people isolate phage that they think have the characteristics for phage therapy.

AUDIENCE MEMBER: Yeah, I think it has a lot of potential and people just don't bother to look.

DR. STIBITZ: Right. I mean, we will if those come on the scene and people are proposing to use them, we'll look at them.

AUDIENCE MEMBER: Yeah, your statement about the transduction is what reminded me because you don't really have to worry about the transduction if you're dealing with an RNA phage. But, okay. Thanks.

DR. STIBITZ: Fair point. Do you know of any that are in the running? Really? Okay. Can't wait to hear about that. Yes?

AUDIENCE MEMBER: All right. Simple question.

DR. STIBITZ: Sure.

AUDIENCE MEMBER: Does OVRR regulate

genetically modified bacteriophages, or will that go over to the gene therapy group?

DR. STIBITZ: Sure. I mean, in the extent that there will be among the phage that we will be regulating I'm certain genetically engineered phage.

AUDIENCE MEMBER: But will you share it with the gene therapy reviewers in the other office? They changed their name.

DR. STIBITZ: Well, I think we have ongoing discussions about who best to perform those reviews.

AUDIENCE MEMBER: Okay. So --

DR. STIBITZ: I thought you were going to ask the question, which is does a flag go up because they're genetically modified.

AUDIENCE MEMBER: Well, that is the indirect question, yes. I mean, this is the primary lead.

DR. STIBITZ: So we don't review them from the aspect of being GMOs or genetically engineered. I think we review based on the unique characteristics that that modification provides, not because as a class they're genetically modified --

AUDIENCE MEMBER: Okay.

DR. STIBITZ: -- if that makes sense. I'm not going to say they're the same as the wild type because clearly they've been modified, but we would be

reviewing what the effect of those modifications was.

AUDIENCE MEMBER: Okay. But who would review them from a clinical perspective then?

DR. STIBITZ: Beg your pardon?

AUDIENCE MEMBER: From a clinical, the clinical study perspective.

DR. STIBITZ: Are you asking if they have been used?

AUDIENCE MEMBER: No. I'm asking would OVRR take the lead or would the --

DR. STIBITZ: Well, like I said, those are discussions that are ongoing as to --

AUDIENCE MEMBER: Okay.

DR. STIBITZ: Currently, OVRR is doing the phage therapy.

AUDIENCE MEMBER: All right.

DR. STIBITZ: If there are indications that we would expand that, that will come.

AUDIENCE MEMBER: Okay. Thank you.

AUDIENCE MEMBER: Hi, Scott, another clear, positive speech. I think all the talk is -- I think it's referring to let's say pharmaceutical products. Now, if phages used together with a device, how that going to be reviewed, and if phages is going to be used in the hospital for hard service disinfectant,

how that going to be reviewed?

DR. STIBITZ: So I believe what you're talking about is a combination product, for example, where phage might be embedded in a matrix of some sort.

AUDIENCE MEMBER: Uh-huh.

DR. STIBITZ: Right. So those aspects -- the matrix itself, which would probably be considered a medical device, I believe there are biocompatibility studies that has to be done as part of that, and then we would collaborate with CDRH, Center for Devices, on review of that product.

AUDIENCE MEMBER: The last bit. If it's used as a disinfectant, how's that going to be reviewed?

DR. STIBITZ: Disinfectant like on surfaces in a hospital?

AUDIENCE MEMBER: Yeah.

DR. STIBITZ: That's a good one. I'll have to think about that. We are almost exclusively concerned with human studies, so it's not clear -- I mean, clearly, that would not require human study. Exactly what part of FDA would deal with that, it's not clear to me. But you're aware, of course, that CFSAN, the Center for Food Safety and Applied

Nutrition, has approved phage for use on meats and fish to decontaminate, but that's a little bit different.

DR. CARLSON: All right. So that's all the time we have for this session. We're going to take a quick break. I'm going to say we'll come back at 3 because we're running a little behind. If you have questions for Scott, you can obviously come ask him now, but we'll get back to all these topics during the discussion panel later.

(Whereupon, a short recess was taken.)

DR. CARLSON: Everybody, we're going to try and get started again, if you can take your seats.

(Pause.)

DR. CARLSON: Okay. We're going to continue with the FDA presenters. Next up is Doran Fink, also from CBER, a clinical reviewer, is going to talk about regulatory considerations for clinical evaluation of phage products.

DR. FINK: All right. Who's ready for some more regulatory talks?

(Chorus of no's and applause.)

DR. FINK: Yeah. Is everyone recaffeinated? Good to go? Okay.

So the usual FDA disclaimer. My comments

are an informal communication and represent my own best judgment, not Scott's judgment, that was his talk. This is my own best judgment, and, of course, what I say does not bind or obligate the FDA.

And, actually, as I've been listening to the talks throughout the day today, I've realized that pretty much everything I'm going to talk about has already been covered, so I might as well just skip to the summary slides. No.

(Laughter.)

DR. FINK: I'll throw in a few bits of wisdom or maybe not so much wisdom from the clinical regulatory perspective. So I'm going to start out just with a few introductory slides about key variables and overlying regulatory principles for phage therapy. The bulk of the talk will be about considerations for clinical development under IND and licensure of phage therapy products. I'll talk a little bit about personalized phage therapy in this section as well. And then I'll end with some discussion and some additional information about compassionate use of phage therapy products under our expanded access IND mechanism.

So these are not an exhaustive list of variables that are relevant to phage therapy products

that have been discussed, some in great detail during previous talks, so you can think about phage therapy in terms of spectrum of activity, whether you're talking about defined products, either a single phage or a cocktail active against one or more bacterial strains or species versus a personalized phage therapy product that is selected for activity against a single clinical isolate.

You can think about route of administration, whether it's topical, interlesional, inhaled, intravenous, oral, or others, and then, if you throw in that the product is administered with a device or as part of a matrix, that adds a layer of complexity.

You can also think about whether the phage therapy product is intended for use as a stand-alone product, which we really haven't talked about at all, or whether it's to be used as an adjunct to antibiotics or as salvage therapy.

No matter which of these variables you need to consider, there are a number of regulatory principles that will apply to all phage therapy products. First and foremost, that phage therapy products are by definition, and Scott showed you the regulatory definition in his talk, phage therapy products are biological drugs. They are biologics and

they're drugs. And so, consequently, any clinical use of an unlicensed phage therapy product in the U.S. must be conducted under Investigational New Drug or IND regulations. So, for all intents and purposes at the current time, this applies to all phage therapy products. We don't have any that are licensed for use in the U.S.

And in order to get a phage therapy product licensed for use in the U.S., we will require a demonstration of safety, purity, potency, which is interpreted to mean effectiveness, and consistency of manufacture. And I have safety and potency underlined because those are the attributes that are the chief concerns of clinical development.

So Scott had a slide that was very much like this one in his talk. I'm not going to dwell on it too much, only to show you that, as Scott said, while effectiveness in manufacturing consistency are attributes that are demonstrated in greater detail and with greater certainty, as the various stages of clinical development progress, safety considerations predominate throughout the development process, beginning with pre-clinical development and extending all the way through licensure and beyond.

So what are the safety considerations that

are relevant for phage therapy products? Well, we've heard a lot today that phage are directly active only against specific target bacteria and are presumed for all intents and purposes to be inert with respect to human cells and tissues, and certainly we have a lot of accumulated clinical experience, mostly anecdotal, that would appear to corroborate this presumption. However, for any new investigational product, I think it's still important to consider a couple of safety items.

Number one, whether certain human tissues might be sensitive to components of phage material. So, as an example, you might imagine a patient who has a fulminant lower respiratory tract infection with compromised airway function and whether introduction of a large amount of phage antigen might somehow inflame that tissue and at least initially exacerbate the patient's condition. This is a theoretical concern. It's not something that's been described, but something to think about.

Similarly, one might worry about the potential toxic effects of product excipients or impurities. We've had some discussion today about residual endotoxin in phage preparations. One might also worry about the potential toxic effects of a

device or a matrix that's used to administer the phage therapy product.

And then, in addition to these potentially direct effect-related safety concerns, you might also want to consider indirect effects, such as effects of bacterial lysis at the site of infection, whether the phage might be able to transfer antibiotic resistance genes, and, finally, whether the phage might result in some changes to the microbiome. This would obviously be a greater concern for a cocktail that has a much wider spectrum of activity than it would for a single phage therapy product, but again just something to consider.

So, when one is thinking about initiating clinical development of a phage therapy product under IND, the antibiotic development model will naturally come to mind, and in that model, first-in-human Phase I studies of investigational drugs are typically conducted in healthy volunteers and they focus on safety and, if applicable, which is usually the case for antibiotics, pharmacokinetics.

However, for a phage therapy product, it's unclear how relevant safety and PK data generated in healthy volunteers would be to patients with active infections where the phage may interact with and

multiply in target bacteria.

So, as alternatives, one might consider taking into account, of course, the potential risks and any pre-clinical data that are available, conducting first-in-human studies not in healthy subjects but in relatively healthy subjects who are colonized by target bacteria but who do not have active infections or, to take it even a step further, first-in-human studies in less severely ill patients with active infections caused by the target bacteria.

Once you've selected your patient population, then the question is, well, what is your starting dose? What is your regimen? How do you select these?

Well, one approach would be to rely on data from relevant animal models. If there is prior clinical experience with related phage therapy products, that experience might be informative. Alternatively, do you just go with the maximum achievable titer in preparation? These are all possibilities.

Then, once you've started your trial, how do you optimize the dose and regimen for later development? What data can you collect from this first-in-human study and later studies to arrive at a

dose and regimen that will be the most safe and most effective?

So here are some relevant questions. How informative are pharmacokinetic data for making dose and regimen adjustments? Is clearance from the bloodstream after IV infusion relevant? We've heard an argument that maybe it's not. Is pharmacokinetic data from the site of infection informative? Maybe, maybe not. And how informative are measures of phage activity that are not directly related to morbidity and mortality? For example, does quantitative culture of the target bacteria from the site of infection help you in any way in determining dose and regimen?

Ultimately, the specific data to support initiation of clinical development and the design of early phase studies for phage therapy products will be reviewed by us in the context of IND submissions, and as Scott mentioned, we encourage prospective developers of phage therapy products to request a pre-IND meeting with us to discuss these topics. I have a URL up here on official FDA guidance for requesting formal regulatory meetings.

So let's think a little bit more toward late-stage development and licensure, and the regulatory principles that will be important here are

that labeling requirements are that all indications for a licensed product must be supported by substantial evidence of effectiveness. This substantial evidence must come from demonstration of effectiveness based on adequate and well-controlled clinical studies using a product that is standardized as to identity, strength, quality, purity, and dosage form.

So, as you can imagine, this is going to be challenging enough for defined phage cocktails. It's going to be ever-more challenging for personalized phage therapy. We do have initiatives for development and licensure of personalized medicine products, and so, you know, you can rely on the FDA to exercise regulatory flexibility in its approach to these requirements, but regardless, the intended indication of the phage therapy product will guide the design of the clinical trials to demonstrate safety and effectiveness.

So there are, of course, a number of very important challenges for demonstrating effectiveness of phage therapy products, in particular, those that are intended for use against multidrug-resistant bacterial organisms. Some of the challenges are outlined here.

For example, it can be challenging to recruit adequate numbers of subjects with the relevant disease process and pathogen to the intended use, even in larger multi-center trials. It can be challenging to identify and enroll eligible subjects in a timely manner relative to the course of illness. One related challenge is that the bacteria present at the site of infection at the onset of illness may be very different phenotypically from the bacteria that are present after antibiotic treatment has been ongoing for some time and even after phage therapy has been initiated and ongoing for some time.

And, finally, there is obviously the potential for confounding by non-uniformity of concomitant treatments, such as antibiotics and other therapies, especially for critically ill subjects where you cannot ethically withhold standard of care.

Fortunately, there are potential avenues available to address many of these challenges. One such avenue is the possibility of streamlined and/or adaptive trial designs. Joe Toerner will talk next after me and will discuss in some detail the CBER draft guidance on pathogen-focused antibacterial therapies, parts of which may be relevant to development of phage therapy products.

Supportive efficacy data from relevant animal models may also be important for supporting licensure. And, finally, there's the availability of alternative licensure pathways, for example, accelerated approval in which approval can be based on a surrogate end point that is reasonably likely to predict clinical benefit, with the caveat, of course, that there's a post-licensure requirement to confirm this benefit.

I've heard a lot from people during the breaks about these and other challenges. Oh, my.

(Laughter.)

DR. FINK: Okay. I'll soldier on. So, you know, one suggestion might be that developers who are just starting out on clinical trials for -- is my mike off now? My mike is off.

Okay. So developers who are just starting off on development of phage therapy products might first want to try to minimize the variables that are inherent to the intended use. So, you know, maybe start with disease processes and patient populations where you can minimize those variables. Maybe start with *Staph* infections, *Staph* wound infections, generate data that might be more broadly generalizable to other disease processes and build from there.

So let's spend a few slides talking about personalized phage therapy, and by personalized phage therapy, in case anyone has not yet been hit over the head with it today, is the situation in which one or more phage selected from a library after screening for activity against specific clinical isolates from a specific patient. And then, on top of that, additional phage may be selected during the treatment course if evidence of decreasing effectiveness due to the development of bacterial resistance or immune clearance is found.

So the principal challenge is that the phage library may include a very large number of uncharacterized phage, so how then to provide this as a licensed product while ensuring safety and effectiveness.

Well, it turns out that we've encountered this type of situation in a regulatory manner in the past related to licensure of minimally manipulated allergenic placental or umbilical cord blood for use in specified hematopoietic disorders, and this situation is described in great detail in the FDA guidance cited below.

Just to boil it down to its pure essence, for these cellular products, each lot of the product

is different, but the safety and effectiveness of each lot is ensured by, first of all, an established manufacturing process and, second of all, by specified product characteristics that are used as release criteria for the lot.

Now there's a huge caveat for using this as precedent for phage therapy, and the caveat is that the guidance above was based on a very large docket of data, accumulated over many years, together with advisory committee input on several occasions. So we're talking about a potentially long road ahead to arrive at a similar point for phage therapy products for personalized use.

So it's unclear at this time whether a similar approach would be feasible for personalized phage therapy products. Might be. Might not be. There may be other approaches that are feasible as well. But whether it will be feasible will really depend on this central question: Can safety and effectiveness of an entire phage library be inferred based on specified product characteristics and accumulated experience with a limited subset of phage from that library?

And to break that question down into a couple of different components, first of all, for a

given indication or disease process and usage or route of administration and dosing regimen, is it reasonable to extrapolate safety and effectiveness across different phages? I don't know. Someone out there please tell me.

For a given phage therapy product, is it reasonable to extrapolate effectiveness across different indications or usages? I think that's a higher bar to clear and typically is not the accepted paradigm for licensure of antibiotics.

And so the question that I would ask the field to weigh in on is what variables or product characteristics might be used to predict and prospectively address uncertainties with safety and/or effectiveness, and then how do you apply those predictions to ensuring safety and effectiveness of personalized phage therapy products?

So what does the road ahead look like? Well, right now, after many years of largely anecdotal experience, at least in the modern era, we currently have no licensed phage therapy products available in the U.S., and so while Jason correctly pointed out that at least in the very near term use of phage therapy is likely to be under expanded access, our challenge to you, to the phage therapy field, is to

initiate scientifically rigorous clinical development programs that include adequate and well-controlled clinical trials to support licensure of phage therapy products, and to continue the positive messaging that Scott started before the break, CBER is prepared to assist developers of phage therapy products in addressing this challenge.

Now that doesn't mean that we're going to have ready answers to all of your questions, including many of the big ones, but we will certainly evaluate your proposals and we will help you think about reasonable, feasible, and scientifically sound approaches to address these questions and to develop your products.

So, in the meantime, I'll end the talk with a little bit more information about compassionate use or use under expanded access IND. The regulations for this are outlined in 21 C.F.R. 312, subpart (i), and compassionate use is to facilitate the availability of investigational drugs for patients with serious or immediately life-threatening diseases or conditions. All compassionate use under expanded access is subject to the following requirements.

So, first of all, there is no available comparable or satisfactory alternative. The

enrollment of the patient in a clinical trial, presumably under IND, is not possible. The treating physician should judge that the potential benefit justifies the potential risks and that those potential risks are not unreasonable in the context of the patient's disease or condition. And, finally, providing the investigational drug will not interfere with the clinical development of the product for the expanded access use.

So I cannot overstate that the primary purpose of expanded access use is to provide access to investigational drugs for patients in need. We are happy to do so. However, expanded access use is not intended to facilitate systematic collection of safety or effectiveness data to support licensure, and therefore, expanded access use is not a substitute for adequate and well-controlled clinical trials.

There are several different categories of expanded access, each with its own criteria for initiating use. In general, the level of evidence required increases as the number of individuals to be treated increases. We've heard the most today about individual patient expanded access, which includes emergency use, and for this use, the probable risk from the drug should not be greater than the probable

risk from the disease, as I said a couple slides ago.

The next step up would be an intermediate size population expanded access IND. Here, there needs to be evidence that the drug is safe at the dose and duration proposed for use to justify a clinical trial of approximately the same size as the number of patients intended to be treated. There should also be preliminary evidence of effectiveness.

I'd really like to steer the field away from intermediate-size expanded access use. I think whenever possible the use should be in the context of controlled clinical trials because that's where the most useful data is going to be generated.

The last category of expanded access which I'll just mention very briefly is treatment protocol or widespread use. Here, this use generally requires clinical data from Phase II or III trials, and usually there needs to be active pursuit of marketing approval for the investigational drug.

So here are the procedures for requesting expanded access use of a phage therapy product or any investigational drug for that matter. The request can be made as a new IND submission or as a new protocol in the context of an existing IND. The request needs to include applicable administrative CMC, pharm/tox,

and clinical information, as outlined in the regulations.

The information that we would require for single patient emergency use is going to be much more limited than for more widespread expanded access use. Ideally, this information would include a clinical history and treatment plan and CMC information about the phage source and preparation, endotoxin content, and sterility, and activity against a clinical isolate.

As you saw from the examples this morning, these are not absolute requirements, and, you know, obviously, it would depend on the clinical status of the patient and the degree of the need. All expanded access use requires documentation of informed consent and IRB approval or, for emergency use, IRB notification after the fact.

CBER can authorize emergency use expanded access for single patients based on informal communication, for example, by telephone communication, oftentimes within hours. The authorization is given for a single treatment course. So what does that mean, single treatment course?

Well, ideally, this would be defined with respect to the duration and the number of doses, but

we recognize that for phage therapy this is not going to be possible a lot of the time, and there may even be some change to the product administered, as you saw in Chip Schooley's example as emergence of resistance develops over time.

So we'll work with you, you know. Come with a proposal, we'll work with you. If emergency expanded access use is authorized, a formal submission is required within 15 days after this authorization.

I have below some contact information for physicians who are considering emergency use expanded access of phage therapy for single patients. There are phone numbers for contacting our office during business hours as well as, after hours, the emergency line, or you can, if you prefer to contact us by email, there's a general address, and then Cara Fiore, who was mentioned in several of the morning talks, has graciously agreed to have her email address made public. She's really the focal point of phage therapy regulation in our office.

Okay. So I'll end with a couple of summary points. Clinical evaluation and use of unlicensed phage therapy products in the U.S. must be conducted under IND. Development and licensure of phage therapy products will depend on product characteristics and

intended use. CBER is, of course, prepared to assist the phage therapy field in addressing scientific and regulatory challenges. And, finally, the expanded access IND mechanism is available for compassionate use of phage therapy products but is not a substitute for adequate and well-controlled clinical trials.

So I'd like to thank everyone at CBER who helped with preparation of this talk, and if we have time for any questions, I'm happy to take them.

(Applause.)

DR. CARLSON: So we have just a little bit of time. We can take maybe two questions.

AUDIENCE MEMBER: Yeah, thank you for that talk. I'm just kind of curious. I work at CDC and there's a real interest there, of course, in antimicrobial resistance and particularly in, you know, the use of fecal microbial transplants and probiotics in potentially treating -- you know, preventing antimicrobial resistance in the gut, in the gut flora or microbiome.

I'm kind of just curious. Do you see any sort of corollaries between your view of how you're going to deal with these issues with using probiotics and regulating those and phage?

DR. FINK: Right. Well, I guess the biggest

parallel is that, you know, while probiotics may be defined organisms, once you get into the realm of microbiota-based products, you're dealing with a, you know, largely uncharacterized product, and so that may vary from, you know, from batch to batch and lot to lot. And so there you're kind of running into the same questions about, you know, how do you ensure that a given lot of product is going to be safe and effective, you know, based on whatever data you've accumulated with that product to date.

So there are parallels and, of course, we have been working to address those very questions with microbiome-based products as well.

MR. OUSTEROUT: Hi. Dave Ousterout from Locus Biosciences. Just curious what your thoughts are on making smaller data sets in terms of Phase II and, you know, the extreme is Animal Rule and how that might be more applicable in phage therapy, particularly for MDR.

DR. FINK: Right. So, you know, we do recognize that, you know, powering trials for, you know, MDRO-related indications is going to be difficult. There are, you know, various ways that clinical trials can be structured that might be able to take advantage of smaller sample sizes while still

providing substantial evidence of effectiveness. Joe might touch on that a little bit in his talk as well.

The issue of Animal Rule is an entirely different issue, you know, altogether.

DR. CARLSON: Okay.

AUDIENCE MEMBER: Hi, I'm just wondering about the safety of phage therapy. As you mentioned, you know, we have a lot of confidence on the safety of phages and your concern you highlighted will be the impurity and also endotoxin level.

So, if we follow the instruction and do the testing, eventual testing, do we still need to do the standard package for toxicity?

DR. FINK: So, by toxicity are you referring to GLP?

AUDIENCE MEMBER: Yeah.

DR. FINK: General toxicology studies?

AUDIENCE MEMBER: Yeah.

DR. FINK: Right. So, you know, our position at this time is that based on the available data we do not see a requirement for GLP general toxicology studies for phage therapy products.

Now, there may be certain safety concerns that arise on a product-by-product basis that might be addressed with more focused safety studies that could

be conducted in animals, but we don't at this time have a requirement for general GLP toxicology studies.

AUDIENCE MEMBER: Okay, thank you. Can I ask another one? Okay.

DR. CARLSON: Yes, go ahead.

AUDIENCE MEMBER: You know, for the phage therapy you are dealing with the bacteria-resistance infections, so therefore it's not ethical to choose negative control, placebo control. And then if you choose the current, the standard treatment, now, if it's actually resistance do you think it's -- what's your suggestion like to choose the current standard when we know probably it's already resistant to it?

DR. FINK: Right. So, I think Joe Toerner may have, you know, more to say about, you know, the control arm for some of these trials in his talk coming up. But generally you'd be looking to, you know, demonstrate statistical superiority of the combination of phage therapy plus whatever standard of care treatment is being given, whether it's actually effective or not in your trial.

DR. CARLSON: So that is a good lead-in to introducing the next talk. So, next we're going to have Joe Toerner from the Center for Drugs who is going to talk to us about development of single

species antibacterial drugs.

DR. TOERNER: Hi. Good afternoon. Thank you.

I spent six enjoyable years in the Division of Vaccines and Related Product Applications in CBER, and so I really appreciate being here and working and presenting again with friends and colleagues from CBER.

About 10 years ago I transferred to CDER in the Office of Antimicrobial Products, and it occurred to me putting this talk together that in the next half an hour I'm going to describe for you what was probably a decade of work in advancement of regulatory science to arrive at recommendations for sponsors interested in antibacterial drug development to have an achievable clinical development program, yet still falls within our statutory requirement that we establish safety and effectiveness of new drugs, and as part of the work that we've done we did include single species antibacterial drug development.

And so we recognized over the past couple of years that sponsors are more interested in clinical development programs in areas of unmet medical need, for example, patients with highly resistant bacterial infections. So, we did issue a draft guidance

document in 2013, and that draft guidance is a guidance for unmet medical need more generally. I know Doran had mentioned a guidance that we have. It doesn't pertain specifically to single species drug development but the concepts in that guidance apply to single species antibacterial drug development.

Clinical trials have been completed and in fact some antibacterial drugs have been approved using the approaches in this draft guidance document. For example, ceftazidime-avibactam was an approval on the basis of some of the concepts that were described in this draft guidance document to help streamline antibacterial drugs for unmet medical need.

The types of drugs that we're seeing who are interested in this area of unmet medical need are generally drugs that have activity against Gram-negative bacterial, generally *Enterobacteriaceae* that -- and some of which have anti-Pseudomonal activity, and the link below is the direct link to the draft guidance document.

So, in our guidance document we describe some clinical trial design options, and in the guidance we provided a discussion that a single trial in this area of unmet medical need can be adequate evidence of safety and effectiveness.

For a more traditional development program we usually require two adequate and well-controlled trials, but a single non-inferiority trial in a body site of infection can be used as evidence of efficacy, and we have a number of different indication-specific guidance documents that clearly describe the end points and the justification for the non-inferiority margin to be used in those guidance documents, and of course a finding of superiority is always readily interpretable. But we have said that you can pool across different body sites of infection for a finding of superiority in a single trial, and to discuss with us what the end points would be for such a trial for superiority.

We also described what was a part of an Infectious Disease Society of America White Paper on drug development, and that's the nested trial design where from the beginning a trial was designed for non-inferiority, but as with any patient who enrolls into a clinical trial you subsequently obtain the *in vitro* susceptibility results and a patient in clinical practice as well may inadvertently have a bacterial infection that's resistant to the control antibacterial drug in such a time, and while you would never design a clinical trial where the comparator

group would be ineffective therapy, there does exist at least a potential for a few patients to have received ineffective therapy with a control drug, and it provides an opportunity then to pull that subgroup out and do a separate superiority analysis. So, we describe that type of trial design in the guidance.

And what's not specifically in the guidance but we have -- we have done this with the approval of ceftazidime-avibactam is for new beta-lactamase inhibitors where they're pairing with an approved beta-lactam drug, we can rely on our previous findings of safety and effectiveness from the approved antibacterial drug that's paired with a new beta-lactamase inhibitor and show a safety profile of the combination as well as providing evidence that the beta-lactamase is reversing the resistance.

And then, of course, a superiority trial design with adjunctive therapy plus standard of care showing superiority over standard of care.

So, these are some of the trial design options that we discussed in our draft guidance document, and they -- some of them are applicable to single species-specific drugs, but there is an increasing interest in this area, in particular, drugs that have activity against *Pseudomonas aeruginosa* or

Acinetobacter baumannii, and designing scientifically sound and feasible development programs has been the focus of workshops and advisory committee discussion.

We do acknowledge that there are challenges with products that target a single species. They're not commonly identified in any one particular infection type. These patients are generally very ill, often in an intensive care unit setting, and you need to start effective therapy immediately, and the therapy should be empiric therapy because there's often diagnostic uncertainty at the time of presentation, and it's very difficult to identify patients in advance to even approach them for potential enrollment in a trial, and there's difficulty in maintaining a registry of such patients, but we do recognize that there is potential clinical utility of antibacterial drugs that target single species of bacteria, and we want to find feasible solutions to develop these products.

And so for the rest of my talk I'm going to be summarizing our discussions at two public workshops and then an advisory committee meeting that we held recently.

So, about this time last year we held a two-day workshop on facilitating antibacterial drug

development for patients with unmet need, and we also discussed antibacterial drugs that target a single species of bacteria.

We then held another workshop in March of this year on animal model development; in particular, animal models for *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, and then we brought all of this information that we gathered from these workshops and presented before an advisory committee meeting in a public discussion.

So, the workshop last year was a two-day workshop. The first day was on developing drugs for patients with unmet medical need in general. The second day was devoted to drugs that target a single bacterial species.

So, for the first day we did discuss the trial design considerations that were outlined in our unmet need guidance document, and an important issue emerged in that workshop was that there are truly significant challenges in pre-specifying a trial that's designed to show superiority in patients with multidrug-resistant bacteria.

And what was also emphasized at that workshop was the importance of obtaining good pharmacokinetic data in the target population of

patients in the intensive care unit to ensure that you're offering patients the correct dose.

And again I provided the link for the meeting documents and the meeting transcript.

So, the second day was devoted to drugs that target a single bacterial species, and it was acknowledged that there are difficulties in conducting trials. We did provide a hypothetical case scenario of a drug that had antibacterial activity limited only to *Pseudomonas aeruginosa*, and so there were several clinical trial designs and topics that were discussed and all of them have challenges and limitations, and in the next four slides I'll go through each of these potential trial designs and clinical development considerations.

So, the first consideration was the non-inferiority clinical trial design. As I had mentioned, we have a number of indication-specific guidance documents that describe the end points and the justification for the non-inferiority margin, the treatment effect of a control antibacterial drug, and it was acknowledged that you can enroll in a single trial patients who have hospital-acquired pneumonia or ventilator-associated pneumonia, HABP/VABP.

You can enroll them in the same trial and

include patients that have bacteremia regardless of the source of infection because those patients with bacteremia and multidrug-resistant organisms have the same mortality outcomes as patients with HABP and VABP, so you can enroll them in the same trial.

At the workshop discussion, the participants thought this could be a feasible option if we were to consider greater certainty in the efficacy findings.

So, for example, if we were to entertain a wider non-inferiority margin than what we describe for a traditional drug development program that you perhaps could have a smaller sample size and these would -- these non-inferiority trials could be a feasible option.

Enrollment wouldn't need to be limited to patients who have broadly-resistant organisms. You could enroll all comers, if you will, with these particular types of infections, and the availability of a rapid diagnostic would obviously help identify patients for enrollment but they wouldn't change the frequency with which these infections occur.

It was also acknowledged in any non-inferiority trial that you're going to have confounding by concomitant therapies and -- that are often used in this very sick patient population.

The second option was superiority trials and, again, this is an obvious finding of efficacy, and here you would want to try to enroll patients who have evidence of broad resistance to available therapies, but these may be very difficult to enroll and identify into a trial. And you could enroll patients with one or more body sites of infection as we've outlined in our draft guidance document for unmet need, but the determination of superiority is difficult. And furthermore, to show superiority may be time-limited because it just depends on the available therapy and whether or not that therapy is considered to be sub-optimal, because once new therapies become available then your ability to demonstrate superiority becomes difficult.

So, the third option was to conduct a study in patients with a higher likelihood of having infections due to *Pseudomonas aeruginosa* such as patients with cystic fibrosis, and you'd need to clearly identify the clinical condition that you were treating whether it be, you know, pulmonary exacerbations caused by *Pseudomonas aeruginosa*, and then extrapolating the findings from a patient population with cystic fibrosis to a general population with other infections may be challenging.

And then the fourth option was approval under the Animal Rule, so this is a setting where efficacy data is obtained from animal models of infection and this is generally done in settings where efficacy trials are not ethical and in the situation that we were considering that efficacy trials may not be feasible to conduct, and we acknowledged that animal efficacy data would likely be supported by at least some clinical data from patients with a variety of infections caused by the single species of bacteria.

So, it was this last option that led us to consider another workshop that we held in March. This was our animal models workshop, and we wanted to discuss in greater detail the current state of animal models of serious infections caused by *Acinetobacter baumannii* and *Pseudomonas aeruginosa* and future directions in this area.

We did have participation from academia, industry, and other government agencies, and sponsors came to present their proposals for clinical development of two products. One had activity against, only against *Acinetobacter baumannii*. The other sponsor's product had activity only against *Pseudomonas aeruginosa*.

So, the key topics that were discussed was an overview of the use of the Animal Rule to support approval for treatment of plague and treatment of anthrax. We discussed the current role of animal models, their attributes and shortcomings, and given the urgent need for these unmet medical need therapies we entertained what role the animal models would have that would accompany the limited clinical data that we would see in a clinical development program.

So, we approached this workshop with sort of some general achievable considerations that you could obtain at least some clinical data but it would be very limited. There would be evidence of activity and perhaps evidence of efficacy in a relevant animal model of infection. There would be robust pharmacokinetic and pharmacodynamic data that would be included in a clinical development program, and an acknowledgement that there would be limited human safety information, and of course we would have the required non-clinical safety data as any drug development program would have.

And so what was discussed at this workshop, again, was the concept of the non-inferiority trial, could this be done, and is this feasible, and, again, the use of prior and concomitant effective therapies

could confound the assessment of the treatment effect of the investigational drug.

But using a wide NI margin could potentially be feasible, and just to provide an example for HABP/VABP we allow, and we describe in our guidance document a non-inferiority margin of 10 percent for standard development programs, but for products that have and can address an unmet medical need, we consider a wider non-inferiority margin of 12.5 percent that has the effect of reducing the sample size in a trial that could be completed in a sooner period of time.

But in this -- in this discussion we did entertain the possibility of considering a non-inferiority margin that's even wider, more equal to the estimate of the treatment effect, and I think an entire talk could be designed on just how we approached and defined the non-inferiority margin for HABP/VABP. But as just a general consideration, and to try to summarize it as best as I can promptly, it took a number of published clinical trials over years to ascertain that ineffective therapy has probably the best mortality rate of about 60 percent.

Effective therapy has probably a worst mortality rate of about 30 percent. So that treatment

difference then is a 30 percent difference of mortality.

So, that is a -- and because we're looking at older studies, there are cross-study comparisons, some are observational studies. So, in order to discount some of that uncertainty we define a treatment effect of approximately 20 percent, and so while for unmet need programs we're willing to go to 12.5 percent. Perhaps for single species product development we could consider a non-inferiority margin that approaches more towards 20 percent that would further reduce the sample size. And what Doran was mentioning could -- you know, could we work within the fact that we will have a limited trial size and could this potentially be feasible then for a sponsor to pre-specify this as a non-inferiority margin to move forward with clinical development.

Again, we discussed superiority trials, and as I had mentioned it's a time-sensitive approach. As new standard of care therapies become available it's not going to be possible to show superiority of an investigational drug, and so sponsors generally aren't willing to pre-specify a finding of superiority when they're planning their efficacy trials.

And so you'll see on this slide a lot of

this language is very similar to language that's in the Animal Rule, but when considering even evidence of activity in an animal model we would want to know that the effect is demonstrated in more than one animal species, and that it's expected to react with the response predicted for humans; that the animal model infection is relevant to the clinical condition being studied in humans; and that the end point in the animal model is actually a -- is similar to the desired benefit in humans, which is generally survival or prevention of major morbidity.

And so, you know, at the conclusion of the two workshops that we had we thought, well, what are -- what are potential outcomes of these types of programs that we talked about, and the best scenario is the first one, that we have a successful clinical trial with a finding of superiority or non-inferiority, acknowledging the limitations, and there are no major safety concerns.

The second possibility is that we just -- there's just no evidence to support a meaningful benefit, and similarly, the fourth scenario that the safety concerns do not allow a favorable risk/benefit assessment. You know, those are situations we don't like to see sponsors be in, but those would be more

clear scenarios of the findings of a clinical data package.

We have an interest in this third potential scenario which is that you can't really discern efficacy in the completed clinical trial that's small due to multiple confounders and to what extent then can we rely on the animal models of infection in such a scenario?

And just so I won't forget to mention, in our guidance document we do allow a very limited population at the dose and duration of therapy, approximately 300 patients is what we describe in our guidance document.

So, we took all of this information from the workshops and we presented summary information to our April 13th advisory committee meeting. We also presented information that was discussed in the public from the two sponsors who presented their proposals for clinical development scenarios, and the two key topics were development programs for single species-specific antibacterial drugs where the bacterial species is not commonly identified, and should a clinical development program not be feasible or the clinical data are not interpretable, what is the role of the animal models of infection.

And so here on the slide is just a summary of our meeting -- of the discussion by our advisory committee, and the committee agreed there is an unmet medical need and that species-specific products are important for continued development.

But the next two bullet points were important for us to hear: that trials in humans can be conducted. They're complicated, they're difficult to do, but they can be conducted. And the third bullet point that there are limitations in the current animal models of infection and that the results of animal model studies should not be used as the sole source of efficacy. So, those two bullet points were important for us to hear.

They did find some interest in the presentations of the clinical development strategies in favor of the non-inferiority clinical trial design. For example, the investigational drug that has activity against *Pseudomonas aeruginosa*, if you pair that with ertapenem, that has a notable lack of activity against *Pseudomonas aeruginosa* but has broad coverage for many other bacterial pathogens that you'll be worried about empirically when starting therapy, you could design a non-inferiority trial with this as your test arm, compare that to a drug that has

efficacy against *Pseudomonas aeruginosa* and that would enable you then to show non-inferiority of the investigational drug for *Pseudomonas* in the patient population that has *Pseudomonas aeruginosa*.

Other comments from our Committee members were global clinical trials networks and it's noteworthy that in last week's New England Journal of Medicine Drs. Woodcock and LaVange from CDER described the concepts of having platform clinical trials, and described the antibacterial drug development as a potential area where having a platform clinical trial could -- could help industry, academia, and regulatory authorities to work together, and thought that rapid diagnostics would help enrollment in a clinical trial.

The Committee also talked about some post-marketing strategies. Is there a possibility for a drug distribution network? That was a question raised by our Committee members. Can we limit the indication to "salvage," for use only as a last option? And then our Committee members reminded us that we now have an operational Sentinel system where we can evaluate post-marketing safety and to make use of that.

And so I included the link here too for the Advisory Committee presentations and transcripts.

So, the punchline is that in CEDR we are working with sponsors to design clinical trials that will establish safety and effectiveness of single species drugs, but we're willing to exercise flexibility and show greater uncertainty in that clinical development program that would allow a smaller clinical trial to be conducted.

We want sponsors to conduct robust pharmacokinetic analyses in the patient population that would use these drugs, and we're still interested -- because of the uncertainty in the clinical development program, we're still interested in establishing animal models having greater certainty, greater understanding of the results of the animal models, so we're still interested in that component because that could still be supportive of the clinical trial findings in an overall data package.

So, I thank you for your attention and happy to answer any questions.

(Applause.)

DR. CARLSON: Given the fact that we're a little bit behind time we're going to go to the panel in just a couple of minutes, I think, since I see one of our panelists. Brian, did you have a question?

We're just going to go to the panel.

AUDIENCE MEMBER: I have a question. On the panel? Okay, I follow you.

DR. CARLSON: Yes, we'll just start the panel discussion now.

AUDIENCE MEMBER: Okay.

DR. CARLSON: And since you're going to be up here you can ask your question. So, I'll invite all the speakers and our two extra panelists to come up. The additional panelists are Cara Fiore who is a primary reviewer in CBER. You've seen her name on the screen a few times today, and Marion Gruber, the director of the Office of Vaccines.

And I'm told to remind all the panelists to speak directly into the microphones so that you are heard by the people in the overflow rooms.

So, we can go ahead and get started with your question.

DR. GRUBER: Okay. Well, thank you. So, I had a question for Joe. Actually, I thought it was a very intriguing discussion here and I think we can -- we should really benefit from having further discussion with that division and to see, you know, if we can borrow from some of the approaches that they have mapped out.

In that regard I wanted to ask Joe. So, you talked a bit about, you know, the value of doing non-inferiority or superiority trials for these, you know, single species, drugs or therapies. What I wanted to know is a bit in these clinical trial designs, the non-inferiority as well as superiority trials, the end points that you would be looking at. So, that's one question.

And the second perhaps related to this, you mentioned clinical trials and you mentioned Animal Rule approval, but you didn't really discuss the accelerated approval provisions that are also available to us, so I wondered if you could comment on that a bit.

DR. TOERNER: Sure. Thanks, Marion, for the question.

So, we have -- we've done quite a bit of work to establish the end points for our indication-specific guidances, and many of the end points are different. So, for example, for HABP/VABP we found a strong treatment effect on the end point of all-cause mortality. So, clinical trials are being designed and conducted in HABP/VABP, and the primary efficacy end point is an end point of survival. And so that's one example.

Another example is complicated urinary tract infection where we found a strong treatment effect on an end point of -- it's a responder end point where patients have to have microbiologic eradication on a urine culture after treatment, and they have to show evidence that their symptoms of urinary tract infection are gone, are resolved. And so that responder end point was found to have a very strong treatment effect and, you know, a third example is complicated intra-abdominal infection where we expect 28 days after completion of -- 28 days after enrollment we expect the patient to be free of symptoms from their complicated intra-abdominal infection, and so those are three very different types of end points.

And so if you're entertaining a clinical trial where you're enrolling lots of different infections, that's where we say in the guidance come and talk to us about how to approach this, and we've already had a strong discussion that allows patients with bacteremia at any site, any body site infection because their survival rate is identical to the survival rate in HABP/VABP, you can enroll those patients in the same trial and have the end point of survival.

But how to approach a clinical trial where you're enrolling patients with complicated urinary tract infection, complicated intra-abdominal infection, HABP/VABP, you know, what's the end point to be used. We think for a finding of superiority you could probably use a combination of end points, but it may take some work to sort out how to approach this. Are there statistical concerns that we have to think about? Should we give more weight to survival end point and give less weight to a complicated urinary tract infection end point? You know, is there a way to weight the different end points in the patients?

So, those are some considerations that we have. We have thought about accelerated approval and in fact there is a brief paragraph about it in our draft guidance document, but because our clinical trial end points always occur within a couple of days or weeks with therapy, you know, the course of therapy is short, your clinical benefit is achieved -- is known in a very short period of time, we're finding it very difficult to apply the principles of accelerated approval where you have a surrogate end point.

But in the case of, you know, these infections there's really not a need for a surrogate. You know the clinical outcome at a very short period

of time during or after completion of therapy. So, we've found it challenging to apply the principles of accelerated approval to antibacterial drug development.

DR. YOUNG: I don't have a question but I do have a suggestion for Doran and Scott. I'm getting inundated by phone calls and emails requesting information about clinical trials for phage therapy. And so I would hope that you would consider taking a subset of those beautiful slides the two of you showed and putting together a website at the FDA that we could send people to explain there are no clinical trials in the United States, and outline the other procedures that are open to them.

For example, having their physician explore eIND and the mechanism, because this is going to get worse and the publicity is increasing and this is a very unusual situation as somebody pointed out; it's something that we -- you know, lots of people think it's going to work but it's years away from any type of clinical approval. Just a suggestion.

DR. FIORE: I actually have a question for Joe. In terms of the platform approach could you, for those of us who are not familiar with the platform clinical trial approach could you tell us what that

would look like?

DR. TOERNER: No, it's actually I would -- I'd refer you to the July 6 New England Journal of Medicine. There's a review article by Dr. Woodcock and Dr. LaVange, and as you know Dr. Woodcock is center director and Dr. LaVange is the office director for biostatistics. And in their review they discuss, it's mainly trials in cancer research where they have platform trials where you're enrolling -- it's just a way to -- it's just a way to enhance clinical development in cancer therapies.

One example is the I-SPY trial and another example is the Lung-MAP trial, but you're enrolling patients with different phenotypes of cancer because drug -- you know, oncology is getting more focused on, you know, what -- focused direct development that pertains to the expression of, you know, tumor expression factors, and so they want to capture a large number of potential patients into a trial, and so it's a way of having one trial, and so it's actually -- master protocol, is the title of the -- so, having a continuously running functional master protocol it's -- you know, you continue to enroll patients in a protocol, and if you don't have an antibacterial drug ready to go you're gathering data

on patients enrolled in the protocol on standard of care therapies, and then once you get an investigational drug that's ready to go you plug that into the master protocol, have it randomized controlled.

You can rely on -- I mean, to some extent it's a -- it's a historical control but when you plug in the new investigational drug into a master protocol you then can randomize so you have a component of a randomized concurrent control study, but you can also rely on some of the information you've obtained from your previously enrolled patients who have standard of care therapy and you can consider a three-to-one or four-to-one randomization, and it's just a way of efficiently doing a clinical development and multiple sponsors can then use the master protocol, so you could have two or three different investigational drugs that are entering and exiting the master protocol.

DR. GABARD: Maybe a couple of comments. For all these products, phage therapy products that are going to target a single bacterial species when we are going to do comparisons with the standard of care and antibiotic, and if you want to show superiority and non-inferiority, the only segment where we can

really show that, because most of these antibiotics are fairly efficient, is on a subgroup of bacteria which are resistant to the antibiotics.

And if the subgroup is the only choice to show the superiority or the non-inferiority then you need to recruit forever because you know that these cases are not so frequent, and then the level of recruitment is so low that it will take you maybe five years to get the proper number of patients to show the superiority. What can we do to avoid this problem?

DR. TOERNER: I guess that question is -- I mean, it is a question of antibacterial drug resistance, and you are -- in a rough analogy you are comparing this to the Infectious Diseases Society of America and their nested clinical trial design. It just depends on how you set up your clinical trial. If what the Infectious Diseases Society of America is -- and our guidance -- what we're saying is you set up your trial for non-inferiority and you seek out to establish non-inferiority.

It's only at the end of the day that you come to recognize that some of the patients may a have resistance phenotype. You can then pull those patients out and do a superiority, but you still have the clinical trial to show evidence of efficacy by

non-inferiority in the patients who have fully susceptible bacterial pathogens.

DR. GABARD: Have you been thinking about expanding the therapeutic area to several therapeutic areas with one treatment? So, in other words, would it be possible to, instead of treating only with infection with a single product which is targeting one bacterial species, that you take in account in a trial several therapeutic areas. For instance, with infection and maybe ulcers and maybe something that are fairly comparable so that we expand the number of cases where we can provide the treatment to patients and then at the same time expand the number of cases where you might have resistance to antibiotics and then the frequency of the cases.

DR. FINK: I think, Joe, you mentioned, you know, a strategy similar to that where you have enrollment of patients with multiple disease processes in the same trial, although in your scenario the unifying principle is that all patients get the same antibiotic against the same bacteria, so that would have to translate.

I think, going back to your first question, I think we do acknowledge, you know, for phage therapy there is an added complexity or challenge with respect

to demonstrating even non-inferiority which is that, you know, unless you're willing to remove all concomitant antibacterial therapy with activity against the organism of interest like you might do with the, you know, investigational product plus your ertapenem strategy, then you really can't demonstrate non-inferiority.

And so I don't think that the phage therapy field is at a stage yet where we have the confidence of, you know, going it alone with phages for, you know, an infection of interest in covering, you know, everything else with empiric antibiotics, but I think you have to definitely think about that more.

DR. LEHMAN: This is not a broad answer to that because this is a special case, but one of the things that -- one of the scenarios where that -- where we might not have some of the same problems. We found with chronic rhinosinusitis the patient population that really has that unmet medical need is a population that has already been through rhinoplasty and multiple rounds of antibiotics, and they still are experiencing symptoms that are not life threatening, but make their lives fairly miserable, and it's an unusual situation. That's why I say this is not a broad answer.

But it is one indication where we may have an easier time collecting some of that data because, you know, standard of care for the patient population that we used in our clinical trial was a sinus wash. It's a saline wash. It relives some symptoms, but doesn't provide a long-term decolonization or eradication of the infection, and that's a scenario where there is an option to look at a placebo-controlled situation where the standard of care is not that great because it's not life threatening, not dealing with that same problem.

AUDIENCE MEMBER: So, we've talked a great deal about sort of the clinical development side. I was wondering if we could take a moment for the non-clinical. So, I guess the question is what are the additional considerations or perhaps notable exceptions for non-clinical data and in particular just to get you to an IND, and in light of some of the things you may run into in the clinic, a more robust IND package?

DR. FINK: So, you know, proof of principle in a relevant animal model is always nice. I don't know that it's an absolute requirement to initiate, you know, clinical trials, but it's certainly nice to have. PK data, to the extent that it might be useful

and, you know, that's an open-ended question, is -- you know, it's non-clinical information that could help guide and support the initial clinical, you know, trial design.

I don't know if there is any, you know, *in vitro* information that --

AUDIENCE MEMBER: I think you're kind of nailing it in the sense that these parameters aren't exactly well defined as they are for a small molecule brethren, right. So, you know, your tox study, is that done in an infected animal or is a healthy animal okay? What does that signal really mean? You know, it's these types of situations for phage therapy as an active therapy that don't seem that clear.

MR. STIBITZ: So, my view of pre-clinical data prior to Phase I trials is -- I mean, we would only be talking about safety studies. I think we are not surprised, that we kind of expect sponsors to do proof of concept, to do studies that convince them that proceeding with this makes sense, but in terms of what's actually required to go into the first human study we're really looking at safety, and I don't know if it's been clearly --

DR. GRUBER: No, I mean, I just wanted to add to what Scott was saying but I think this is such

a new area that we are actually, and this is part of the reason why we are having this workshop, because we would like to hear from you, too, you know, what makes sense, you know; what should be, you know, recommended. We are not having any requirements right now. I think this was already clearly stated by Doran to say that we're not asking for the typical GLP repeat dose toxicity studies that we have been asking for other products the Office of Vaccine regulates; and that we are also right now, we don't borrow from the small molecule drug development paradigm, but -- and it was also mentioned if there are some directed safety studies that we feel would be needed, you know, when we have the discussions with you when you come and propose a clinical trial that is something that we can then further elaborate on, but at this point this is a fairly new area and field for us, and we're really, you know, trying to map out a non-clinical/clinical development program that makes sense and that is feasible and scientifically defensible, and that's actually one reason why we're having this workshop because we also would like to hear from you, you know, what does make scientific sense.

I think you've heard, you know,

proof-of-concept studies, you've heard, you know, characterization data that were outlined in Scott's talk, you know, *in vitro* studies as applicable, and that's where we are right now. But, again, I mean, we invite comments from the audience on these -- on these questions.

DR. CARLSON: And I should have started the panel off by saying what we're looking for is really a discussion between the regulators and interested parties to try and figure these things out in some instances.

DR. FIORE: So, I just want to add that the pre-clinical/non-clinical studies are often very important for you to inform your development plan, and if you have those studies, you know, we'd love to see them, but they could be more important for you in some cases than they are for us.

AUDIENCE MEMBER: Fair enough. Thank you.

AUDIENCE MEMBER: I have a question for colleagues from FDA, and this topic is about the phage substitution or phage addition in the approved cocktail, for example, and it has different, of course, subtopics like CMC and clinical efficacy.

The question is how do you think the industry and regulators would initiate the

discussions? What would it take to replace the phage in the approved cocktail from the CMC standpoint and from the clinical efficacy standpoint?

For example, stability data, we cannot generate let's say two years real-time real condition stability or if you're talking about clinical efficacy if the requirement would be go for Phase II, Phase III again this would make this impossible. Would you please elaborate on these a little bit? Where do we start to discuss this?

DR. FIORE: For myself and my colleagues here do you mind defining when -- are you talking about coming in with a defined cocktail and then switching out or are you talking about a panel of phages?

AUDIENCE MEMBER: The defined one.

DR. FIORE: Defined one, okay.

DR. STIBITZ: Well, again I'll ask a question back to you. Are we talking about a licensed product?

AUDIENCE MEMBER: Yes.

DR. STIBITZ: And then you want to change the phage makeup.

AUDIENCE MEMBER: Right, yes, to replace or to add an additional one, for example.

DR. STIBITZ: Right. So, my understanding, and my colleagues can correct me if I'm wrong, is that you could submit a BLA supplement to make those changes to the product.

Now, exactly how at some point it could be different enough that we consider it to be a new product, but I think the devil as always is in the detail. So, are we talking about a similar phage from a genetic perspective and it's a variant? Is it a brand new phage that, you know, you just isolated?

I think -- I mean, we can talk about, you know, exactly how we want to pursue that in the structure of our regulatory process. In other words, is it a new BLA? Is it an amendment and so forth? But I think in general it will be possible with, you know, the same CMC information and enough information about the applicability of that phage to have it included.

I know that's not terribly precise but I think it's the best --

AUDIENCE MEMBER: This is going to be treated in a case by case depending on the data available, right?

DR. FIORE: I just want to clarify because what I heard you say is Phase II and Phase III, but

Scott's referring to a marketed product. When you use the word "license" we mean already approved and marketed, and out there for clinicians to use.

So, during your IND development you would submit that to your IND, or if you had a master file, which I am a huge proponent of, which would include all your CMC information, you would submit it to your master file or IND with the same type of information that you would submit with your other phage products.

After licensing it's a little bit different. It may be slightly more complicated and more expensive, but nonetheless it would go through the process that Scott was talking about.

DR. GRUBER: Yeah, I just wanted to add that it may be a little bit premature to discuss the type of product characterization data that's required after you have licensed a defined, you know, phage cocktail, because we have to actually see first what really makes sense, what product characterization data would be required, you know, all the way -- and if clinical data even would be required. I'm not saying that this would be the case but I think we are a little bit ahead of ourselves. I think the criteria or the type of information requested to support a supplement to a license for a defined phage cocktail is something that

we need to discuss, but once out there I think we would have a set, you know, of required information. I would not think that this would be case by case at that point in time, but I think right now we need to get some clarity first if you -- you know, let's say you have a defined cocktail that is not licensed or you were to swap phages, you know, what type of studies, what type of data would be needed. I think this is something that we would need to start in order to really define what is requested once these products are licensed.

AUDIENCE MEMBER: Okay, thank you.

AUDIENCE MEMBER: I understand there is a general assumption of safety for the most part, but I was curious if there are specific safety concerns. For example, I saw some recent findings about impact of phage therapies on the microbiome. So, I'm just curious your thoughts on that or if there are specific safety concerns that you may have going forward because I -- not that it was sort of brushed aside but I do understand in the field there is a general assumption of safety.

DR. FINK: So, I presented a couple of, you know, safety considerations that one might think about in my talk that are related either to, you know,

direct effect of the phage material on human tissues or indirect effects such as microbiome changes. I don't know if anyone else has any more specific --

DR. GILL: Do you want to go first? You go ahead.

DR. STIBITZ: All right. This will be short. I mean, I think the problem -- we have the tools to look at changes in microbiome that might be associated. We don't have the knowledge to interpret what those changes mean. So, in many ways we're in the same region that we're in with FMT and live biotherapeutic products to some extent.

And what was the other -- oh, and the other thing is just, I mean, certainly there will be some changes to the microbiome, but I think we all think that those will be more acceptable than the massive changes you get with wide spectrum antibiotics.

AUDIENCE MEMBER: A couple of small observations. Any decisions --

DR. CARLSON: We had a little more feedback on the last question here. Just a second.

DR. GILL: The other thing that we've talked about is, you know, the possibility of horizontal gene transfer made by phages and that can be screened for. We're looking for transducing phages. And so as was

brought up earlier some phages they degrade the host chromosome early in the infection cycle, and so if you use only phages that do that they're unlikely to transduce.

And another thing is that it depends on how the phage is packaged, their DNA into the head. So, some phages are quite permissive and others are very site-specific, and so if you have a phage that doesn't necessarily degrade the host chromosome, but if its DNA packaging is very, very specific to its own DNA then I think we were looking -- it's not that it will never ever transduce, but as long as the transduction is lower than what you normally get, you know, just from the normal traffic of DNA in that ecosystem, then I think it should be okay.

DR. CARLSON: Just to follow up briefly on the question of microbiome damage. Joe, is this something you guys consider in terms of antibiotics, even single-species antibiotics? Is that something that's looked at as a safety signal?

DR. TOERNER: That's a good question and we have not specifically looked at that issue. There are a number of concerns with it. How are -- you know, how are the cultures ascertained; what's the -- you know, how do you go about knowing what the microbiome

-- it's such a dynamic -- yeah, how would you begin to characterize what's considered to be normal and what's considered to be not normal.

AUDIENCE MEMBER: Okay. I was just going to say there are quite a few steady state late stage infections, chronic area infection, possibly Randy Fish's work on toe ulcers where there is no standard of care, and those can provide a way in. I'll say if you want to know about that I'll tell you afterwards, happily.

The second thing is, of course, in regard to the last question doses in phage therapy can be very tiny indeed, microgram, nanogram, even down in one study to picogram doses. So, input toxicity is an issue that needs to take that into account.

But my actual question was for Scott, and it was -- you made a very interesting comment that a GM product, it will be about what was added and how you're changing the GM agent which is then introduced. So, how would then would be regarded a zero residue removal? For example, taking out a lysogeny cassette from a phage where there are no lytic phages as with *Clostridium difficile*. If you did a zero residue removal of the lysogeny cassette, how would that be regarded?

DR. STIBITZ: Well, that's the only kind of removal we do in my lab, but you're talking about a completely clean deletion, for example, in-frame in a repressor gene, correct?

AUDIENCE MEMBER: Yes.

DR. STIBITZ: So, I mean, I think the answer is really the same. I think phages that have been genetically modified or genetically engineered are not viewed really any differently than wild type phage with the exception that we know a change has been introduced and therefore we will want to understand the results of that change.

So, I think when you're adding something there are perhaps more questions than when you're simply removing the repressor.

AUDIENCE MEMBER: I have one question and second one is like a suggestion. The first question is, is there any chance to use historic safety data for phage therapy for this type of, you know, approval process?

DR. STIBITZ: You're talking about historic controls for a clinical trial?

AUDIENCE MEMBER: Right, historic safety data. Historic phage --

DR. STIBITZ: What the occurrence would have

been without the intervention, is that correct?

AUDIENCE MEMBER: Right.

DR. FINK: Or are you talking about historical safety data?

AUDIENCE MEMBER: Historical safety data, mainly historical safety data.

DR. FINK: Yeah, I don't know that we would really consider that. I guess it would depend on exactly what the nature of the data is.

AUDIENCE MEMBER: Okay.

DR. FINK: Is it with the same product? Is it with a closely related product? How closely related? How long ago? How similar were the, you know, monitoring procedures to the procedures that, you know, we would typically require to determine safety? All of these are questions that, I think, you know, kind of stack the deck against, you know, relying on historical safety data.

So, I don't want to come out and say under no circumstances absolutely, but it does seem a little bit unlikely for, you know, any particular given phage product what historical safety data might contribute to supporting licensure.

AUDIENCE MEMBER: Okay.

DR. STIBITZ: Do you have a particular

example in mind?

AUDIENCE MEMBER: Yes. One thing was like 1931 or sometimes they did a *Staph* or a -- I think it was a *Staphylococcus*, the clinical trial in USA. Yeah, so they did a trial and they showed that 31 percent cases they are successful and there is not much adverse effect, something like that it was -- so, this type of data, can you mine this type of data, mining, and can, you know, produce to FDA to find out that what they think about it, you know.

DR. GRUBER: I don't have a lot add to what Doran just stated. I think it would really, really depend. So, let's say if a sponsor would come and propose that safety information to us as supportive or pivotal demonstration of safety for a product in a given target population against a specific condition in 2017, I think we would, of course, look at that data, but I don't think, you know, we can give you an answer here on the podium to say yes, that would be acceptable or no, it would not be acceptable. I mean, it really would depend.

But I have to agree with Doran. I think it's rather unlikely.

AUDIENCE MEMBER: So my second point is this. I am hearing a lot of -- about the problem with

the transductions. With this transduction things is happening in the environment, you know, millions and millions of time, and not only that, the plant biologists use phage randomly to hose down the trees and other things and they don't, you know, check all of their phage or phage composition, you know, for transduction ability.

So, why are you worried so much about that, you know, little transduction? What is going to happen when they inject some phage, you know, in human body?

DR. STIBITZ: So, this is the way that I think of it and I think I've convinced my colleagues to think about it. It's sort of a belt and suspenders approach.

If you're using strains to propagate your phage for therapy that are completely free of any troublesome genetic material, it's probably not as important. But it seems more and more likely as we're talking about isolating phage from nature for a particular patient isolate, that maybe -- and then perhaps adapting that phage to that isolate, it seems more and more likely that we will be growing the phage on virulent strains, and in that case I think it becomes essential to make sure that you're not

delivering to the infection site, you know, additional little care packages with weapons in them because this is not a theoretical concern at this point. You can measure the degree, the number of transducing particles in a lysate.

AUDIENCE MEMBER: So then why EPA doesn't control it? Because in environment if you release this type of transducable phage it can cause problem to transfer the antibiotic genes and other things.

So, my point is that why they don't control it and why when we come to this type of, you know, clinical treatment at that time we consider it so much. Environmental biologists are using phage, lots of phage. They don't do all those type of study and they release this phage for farm and also for poultry and industry, and they are using it, you know, to clean the poultry housings.

DR. STIBITZ: I'm not positive I understand your point, but I think what we're getting at is perhaps adding 10 to the 9th, 10 to the 10th phage particles into an existing infection with what 10 to the 5th, 10 to the 6th, 10 to the 7th, transducing particles if it's a transducing phage.

So, I mean, I think -- I believe you're making the argument, and correct me if I

misunderstood, that this is happening in nature all the time.

AUDIENCE MEMBER: Yes.

DR. STIBITZ: And so I think it's largely a numbers game to some extent.

DR. LEHMAN: I'd also posit that the risk assessment for that is a little bit different when you have a human patient in front of you than in an environmental setting.

AUDIENCE MEMBER: (Away from microphone.)

DR. LEHMAN: It could easily be asked in the other direction as well. If the human therapy field finds it important, should the animal side of things and the environmental side of things also find it important. There are two directions in which to ask that question, and I know that at least -- my knowledge of the food animal portion of this is somewhat limited, but I know that in at least some cases the phages are intentionally applied after the animals have basically been removed. They've been removed from interaction with the rest of the herd or the flock.

I'm not saying that that's happening in all cases but I know some of the people who are working on that do care about that because they are concerned

about confining the effect to just the treated population so as not to just have broad environmental exposure.

DR. LEHMAN: The comment was that EPA is asking these questions now.

DR. CARLSON: Go ahead.

AUDIENCE MEMBER: I have two easy questions and one comment. I know that a number of guidance you have new antibiotic development for a range of indications. I understand from your talk that we can reference those guidance for phage development, right? That's one.

The second one is a comment. The comment is that when we choose the standard treatment and we use the data actually for any drug where it's actually developed, when it's new and at that time it's no resistant, it’s actually sensitive bacteria, therefore the data usually it's generated when it's -- everything, it's sensitive, no resistance. But when the time you compare with it actually it's very high resistance, so the data in the literature usually not reflect the situation. So, that's my comment.

Actually whether it's non-inferiority or superiority, we would choose the marginal use at that time, sometime can be difficult.

DR. TOERNER: Thanks for the comment. Also our guidance documents clarify that when you are doing the non-inferiority analysis you have to ensure that the control drug has activity and is shown in *in vitro* susceptibility to be susceptible to the control drug in order to establish non-inferiority to the investigational drug. And so that is part of the population that's used for the efficacy analysis in a non-inferiority trial.

AUDIENCE MEMBER: The last one I ask that you opinion on the definition of the standard of care treatment. So, what is it? Is it the most commonly used drug or it's a drug you find in the society guideline?

DR. TOERNER: We define standard of care therapy and there's a definition we provide in the guidance documents, and it's a drug that's approved for the treatment, but we recognize in some cases it may not have that specific approval yet standard of care guidelines provide the recommendation for its use, and so we say if there's enough data you can provide to us a rationale for why you want to use a particular comparison drug, and there may be very good reasons for doing that.

AUDIENCE MEMBER: Yeah.

DR. TOERNER: You may want to have a blinded trial, and the only comparison drug that's administered twice daily, maybe one that doesn't have that specific indication. Yet it's recommended in treatment guidelines, or that the twice daily dosing isn't in the FDA labeling, but there are other data that support uses of twice-a-day administration. So, we are willing to be flexible and you just -- you know, sponsors can just provide a strong rationale in the use of the comparator drug and why it's felt to be effective.

AUDIENCE MEMBER: That's good because I do come across all these situations. I do see society guidelines recommend a drug which is not approved in the country. I do see recommended drug in the guidance it's not the most prescribed drug either.

DR. TOERNER: And it's important to recognize too, clinical trials are global, and so there are some drugs that are available in other countries that -- and they're available here but they may not have that specific indication that they have in the other countries, and we recognize that and look to professional societies for their guidelines as well.

AUDIENCE MEMBER: Thank you.

DR. FIORE: I would like to add to what Joe said to address your first comment about the guidances.

So, Center for Drugs and Center for Biologics we do have some shared guidances and we also have separate guidances, so just to keep that in mind, and a draft guidance is a draft because we're still accepting comments on them whereas the final guidance is final.

DR. FINK: And just to add on to that in case it isn't already clear. We don't have a guidance that is, you know, specific for -- that specifically covers phage therapy at this time. So, while Joe went over a number of CEDR guidances for antimicrobial products that, you know, portions of which may be relevant to development of phage therapy products those guidances were not written with phage therapy in mind, so just a caveat.

AUDIENCE MEMBER: Thank you. That was the best possible lead-in to my question.

So, we've talked about sort of two different arenas today. One is phage therapy and development of phage therapies and one is development of products, antimicrobials that meet an unmet need. And to your point exactly, there's a little bit of a disconnect in

my mind between what is most important to FDA on the unmet need side versus on the phage side, and I think that taking a step back we can sort of anticipate that phage are going to be, at least initially, in the clinic used in areas of unmet need, maybe areas of MDR or maybe areas where there is a second or third line defense rather than adopted out of the gate as a first line use. Therefore, this kind of puts them, we can predict, into an unmet need use kind of situation.

What I'm hearing is that for the antimicrobials that address an unmet need there's a lot of emphasis on PK and pre-clinical data whereas it's kind of the opposite for phage, where the PK situation is very hard to nail down because of the self-replicating nature of phage, not as concerned about the pre-clinical animal models, not even looking at the toxicology necessarily, and sort of moving straight into the later phases.

So, how do you synthesize the conversation that you all have had around phage development versus the conversation around products that address an unmet need when truly what we're talking about is a product in the Venn diagram of both of those things that overlaps both?

DR. FINK: So, can I take this first stab?

Okay.

I think the point that I took away from Joe's talk is that for these products that are intended to address an unmet need, and phage therapy, as you've said, certainly, you know, would fall into that category for certain uses, it's going to be challenging to accumulate clinical data, clinical trial data of the type that would usually support licensure for antimicrobial products.

And so what CDER has decided and what their advisory committee has agreed with them on is that some, you know, less robust package of clinical data could conceivably be supported with animal model data as well as PK data because those PK data are very useful. Now, for phage therapy products PK data may or may not be useful.

And so if we were to, you know, go along a similar path, you know, we might say that licensure or demonstration of effectiveness could be supported by some, you know, package of clinical data that's feasible to achieve, plus some animal model data where the animal models are reasonably relevant, plus whatever other non-clinical or *in vitro* data might help to inform the effectiveness of the product. And so, you know, it may not turn out to be PK data, it

may turn out to be something else.

I would, you know, love to hear from the audience out there what -- what do you think those data should be.

DR. GRUBER: I just wanted to make one additional comment before we let you answer, and that is, you know, I think we're not really looking at this point to reconcile what, you know, is asked in the world of, you know, anti-infectives and, you know, phage therapy.

I think what was interesting is, you know, the paradigm that Joe's division worked through to see, you know, how can clinical trials for these type of products be conducted to support development and licensure, and we, you know, invited Joe today to really, you know, explain this to us to see how they approach this very complex field and to see can we borrow, are there some common, you know, themes or elements, but I don't think we are at the point yet that we can say okay, you know, this is sort of the paradigm that we would follow for phage therapy clinical trials, yet there are some interesting approaches and we would love to really discuss those further, and again hear your perspective on that.

DR. FIORE: I'm sorry, if I could just add

to that.

One of the elements that could also possibly be added to in a package is something that I haven't heard mentioned although I did see, I think, maybe on Joe's slide, is post-marketing, and then also some element of our expedited programs which is a guidance document. Thank you.

AUDIENCE MEMBER: And I had one more comment to follow up as well, and I apologize I neglected to introduce myself at the beginning. I'm Lucia Mokres. I'm the chief medical officer of EpiBiome, which is a small company working on bacteriophage therapies.

And I want to disagree with the comment that it's too soon to think about post-market manufacturing and changes to manufacturing. Early stage companies are really at the forefront at a lot of this development. A lot of big pharma companies are not willing to take on the enormous risk that it would take to get a phage therapy progressed through a clinical program.

As such, we're really contingent or dependent on the investment of venture capitalists. There is no grant -- amount of grant funding and non-diluted funding in the world that will bring a product all the way through the market, and one of the

questions that we always get from potential investors is what happens when resistance develops or a new strain emerges or, you know, like is this going to be like the flu vaccine that gets updated. And if we can't answer that they won't invest.

So, I'd like to encourage everybody in the room to kind of not be afraid to have those conversations early because they do matter and early stage companies do need to grapple with them, at least have an idea of what that might look like earlier than one might think if one had a continuous revenue stream and could just kind of cross that bridge later.

DR. GRUBER: Yeah, the point is well taken and perhaps I was misunderstood. What I was trying to convey here is that we're right now trying to really get our arms around, together with interested product developments, to see what are the criteria about which we can characterize, you know, a new phage to be introduced in a defined cocktail. And as long as we don't really have that clear and mapped out under the IND, you know, how can we provide guidance here, you know, for something that may be approved in the future?

But you're point is well taken. I mean, this is -- you know, clinical development strategies,

what would be required, you know, overall thinking about this, you know, how -- how the tests and methodologies apply then to a licensed product, I mean, is something that's, of course, part of the discussions to be having with the product developer during the IND stages. Thank you.

DR. FIORE: And just to add what Dr. Gruber said, it's going to be possible. We just can't give you a concrete answer. It's not like it's unfathomable. It's going to be possible. We just can't give you an exact concrete answer exactly how you're going to do it at this point in time.

DR. CARLSON: We can do one or maybe two more questions. We're pretty much out of time for the day.

AUDIENCE MEMBER: I'm JeShaune Jackson from EpiBiome as well. Promise we didn't plan or practice the synergies there but, you know, but great presentations individually and collectively a ton of knowledge so far on this panel.

My question goes to another kind of question that we get asked sometimes, too, and that's if you're treating sometimes like non-life-threatening diseases, where we talked about a single bacteria and, you know, *Pseudomonas* and all these other ones, but if it's non-

life-threatening, what is the potential option for doing like over-the-counter or off-the-shelf or, you know, or even like a nutraceuticals route as a -- like at what point does the FDA have to step in and regulate that for phage therapy?

DR. FIORE: If you're planning to use a product to cure, treat, mitigate or prevent a disease you need an IND. It doesn't have to be life-threatening. In fact, we have many products that luckily aren't used for life-threatening situations, but you need an IND and you go through the IND process, and we certainly can help you with that.

DR. FINK: The requirement -- one of the requirements for expanded access use, and I'm thinking about particularly emergency use for single patients, is that the product has to be intended to treat a serious or life-threatening disease or condition. So, it can be serious or life-threatening.

What does serious mean? Well, there's a -- we typically draw on our guidance for expedited development of drugs to treat serious diseases or conditions, and under that guidance serious is defined as it causes a substantial impact on day-to-day function.

So, if the patient is suffering substantial

impact on day-to-day function from their disease or condition then that would be considered serious and would qualify for expanded access use.

DR. FIORE: I apologize. I thought you said non-serious.

AUDIENCE MEMBER: I'm saying non-serious, like, you know, acne, uncomplicated UTIs or like skin care, women's health.

DR. FIORE: So, if you're trying to treat you would need an IND and we would help you through that. So, the IND process is for any drug development. So, we don't -- it wouldn't be -- if it came to us it wouldn't be a nutraceutical or anything like that.

DR. GABARD: Maybe a couple of ideas to fuel the discussion. From our own experience with three different regulatory agencies I can provide some information to you.

Regarding the kinetics of the phages, what we have been agreeing with the three agencies is that we would test the concentration of phages at a thousand-fold -- one hundred to a thousand-fold above what was expected to be administered to the patients in healthy mice and in healthy pigs.

So, the mice got one hundred the times of

phage that we provided to the patients, and the pigs got one thousand-fold times the amount of phages that would be provided to the patients. Those animals were healthy without any bacterial infections, and we followed the course of the disappearance of the phages in organs and in fluids, and that was agreed by the agencies.

Concerning the effect of the phages in infected organisms, during the course of the Phagoburn studies we also have been following the concentration of the phages day after day each day of the treatment during 14 days to see what was happening to the amount of phages as the bacterial infection was disappearing, and that was agreed also by the authorities.

AUDIENCE MEMBER: Thank you.

DR. CARLSON: I think at this point unless anyone on the panel has anything else to say we're going to have to end the discussion for now, but I'm sure everyone is willing to stick around for a little while if you have more questions for them, and we'll continue again tomorrow starting at 8:30.

Roger, do we have any announcements or anything for tomorrow? No. Okay. Don't forget to bring your I.D. badges back tomorrow or it will be difficult to get into the building.

Thanks, everybody. We'll see you tomorrow.

(Applause.)

(Whereupon, at 5:00 p.m., the workshop in the above-entitled matter was adjourned, to reconvene at 8:30 a.m. the following day, Tuesday, July 11, 2017.)

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REPORTER'S CERTIFICATE

DOCKET NO.: N/A

CASE TITLE: Bacteriophage Therapy Workshop

HEARING DATE: July 10, 2017

LOCATION: Rockville, Maryland

I hereby certify that the proceedings and evidence are contained fully and accurately on the tapes and notes reported by me at the hearing in the above case before the Department of Health & Human Services, U.S. Food and Drug Administration.

Date: July 10, 2017

/s/

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