

UNITED STATES FOOD AND DRUG ADMINISTRATION
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS
DISEASES

SCIENCE AND REGULATION OF BACTERIOPHAGE
THERAPY

Washington, D.C.

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PARTICIPANTS:

Welcoming Remarks:

MARION GRUBER
CBER

EMILY ERBELDING
NIAID

Workshop Background and Goals:

JANE KNISELY
NIAID

ROGER PLAUT
CBER

Regulatory Framework for the Development and
Use of Bacteriophage Therapy in the U.S.:

CARA FIORE
CBER

***SESSION 1: Choosing and Characterizing
Bacteriophages for Therapy:***

Moderator:

ROGER PLAUT
CBER

Regulatory Considerations for Characterization
of Bacteriophages for Therapy:

ROGER PLAUT
CBER

Bioinformatic Approaches for Determining Phage
Properties:

JASON GILL
Texas A&M University

PARTICIPANTS (CONT'D):
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LUIS MELO
Centre of Biological Engineering, Portugal

The Consequences of Phage Resistance Evolution
for the Virulence Levels of Bacterial
Pathogens:

EDZE R. WESTRA
University of Exeter, UK

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DOMINIC SAUVAGEAU
University of Alberta, Canada

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ROGER PLAUT, Moderator
CBER

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Moderator:

SUSAN LEHMAN
CBER

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SUSAN LEHMAN
CBER

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PARTICIPANTS (CONT'D):

DANISH MALIK
Loughborough University, UK

Manufacturing Aspects of Phage-Based Product
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FRENK SMREKAR
JAFRAL, Slovenia

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Invitris, Germany

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ERICA BIZZELL
NIAID

Focused Session 1:

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Challenges:**

MICHELE WALES
Adaptive Phage Therapeutics

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CARA FIORE
CBER

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ERICA RATERMAN
NIAID

PARTICIPANTS (CONT'D):

ERIN ZEITUNI

NIAID

Room D: Funding for Clinical Trials:

JANE KNISELY

NIAID

RICHARD ALM

CARB-X

ANNA JACOBS

BARDA

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P R O C E E D I N G S

(9:30 a.m.)

DR. PLAUT: Good morning. Welcome to our workshop. My name is Roger Plaut, and I'm a research microbiologist at FDA-CBER. It's my pleasure to introduce two representatives of the sponsoring agencies of this workshop for some welcoming remarks. First, we'll hear from Dr. Marion Gruber, Director of the Office of Vaccines Research and Review at the Center for Biologics Evaluation and Research at FDA. And then we'll hear additional remarks from Emily Erbelding, Director of the Division of Microbiology and Infectious Diseases at the National Institute of Allergy and Infectious Diseases at NIH. Dr. Gruber, you can begin when you're ready.

DR. GRUBER: Good morning. First of all, thank you for having me here to give the welcoming remarks; it's really an honor. And on behalf of the Center for Biologics Evaluation and Research, I would like to welcome everybody to this public workshop on the science and regulation of bacteriophage

therapy that is hosted by the National Institutes of Allergy and Infectious Diseases at the U.S. National Institute of Health and the Center for Biologics Evaluation and Research at the U.S. Food and Drug Administration.

Next slide, please. So, over the next three days, you will hear presentations from experts in government, in industry, and academia on a very diverse range of topics that are related to both the science as well as the regulation of bacteriophage products intended to be used for the treatment of bacterial infections. You'll soon hear more details about the specific goals of this workshop, but I would like to mention today, this morning, that the overarching goal is, the reason we are holding this topic workshop, is to exchange information with the medical and scientific community about the regulatory and scientific issues associated with phage products.

Next slide, please. Now, most of you are probably familiar with the history of

phage therapy and I won't dwell on it, but I do want to point out that the recent resurgence of interest in this topic is due in large to a serious global public health issue, the rise in the prevalence of human infections caused by multidrug-resistant organisms and, specifically, bacteria that are resistant to multiple antibiotics.

According to a 2019 report from the U.S. Center for Disease Control and Prevention on antibiotic threats in the United States, each year over 2.8 million antibiotic-resistant infections occur in the United States alone. And, sadly, more than 35,000 people die as a result. In the U.S., the annual cost of treating infections caused by antibiotic-resistant strains of just six bacterial species of concern is estimated to be over 4.6 billion U.S. dollars.

In addition, according to the WHO, antimicrobial resistance is one of the top ten global health threats. So, there is no doubt that this is a serious public health issue.

Next slide, please. We know that

bacteria will evolve resistance to antibiotics, but it would be helpful if we would have at least more new antibiotics to try. Unfortunately, the development of new antibiotics is stalled for various reasons. But because of the rise in prevalence of resistance to existing antibiotics, and the low likelihood of the development of any antibiotics that employ novel mechanisms of action, many researchers have turned to phage therapy as a possible adjunctive or alternative therapy to traditional small molecule antibiotics.

Next slide, please. Phage therapy has been used in other countries for many years. But for the most part, this has occurred in the absence of evidence from controlled clinical studies demonstrating the efficacy of these treatments. The recent resurgence of interest in phage therapy in this country has led to an influx of applications to the Center for Biologics in which sponsors want to discuss the potential use of phage therapy and also want to conduct

clinical trials. Several such studies are now ongoing under our investigational new drug application process and, if successful, may even ultimately lead to FDA licensure of bacteriophage products.

Next slide, please. During this workshop, you will see presentations and discussions about patients who have been treated with phage under extended access that is also referred to as compassionate use. Now extended access is a mechanism in which a physician is permitted to use a treatment, such as phage therapy, that is not yet approved in the United States to treat a patient who has an immediately life-threatening or serious condition and there are no available satisfactory alternative options available to that patient. At the Center for Biologics, we are committed to permitting such expanded access treatment when they are appropriate. And you can find statistics online regarding the frequency with which we have allowed such treatments to proceed.

But I want to emphasize today that

one of our missions at CBER is to facilitate the development of biologics. And we hope that this workshop is a step in that direction for phage products, through the facilitation of bacteriophage product development, as well as controlled clinical trials that can address the question of whether and in what context phage therapy products may be safe and effective for use against bacterial infections.

I'm glad that all of you are attending this workshop this morning we and hope that you will find the information that you hear today helpful. And, with that, I would like to turn it over to Dr. Emily Erbelding. Thank you.

DR. ERBELDING: Thank you, Marion. Here at NIAID, we are really pleased that we are able to renew our focus in important areas of infectious disease research that aren't COVID-19. I'm pleased for this change of scenery, and I think Marion and her team at CBER is as well. We've recognized for years now that bacteriophage are an important area

in therapeutics for combatting antimicrobial-resistant bacteria in a tailored way, and in a way, perhaps, that could be therapeutically successful without stimulating or promoting broad spectrum resistance.

We realize, too, that our activities in this area have not progressed rapidly, and we need to renew our focus on clearing potential obstacles in phage therapy in order to meet their promise in clinical therapeutics. So, we're excited for this workshop that will address some of these issues in regulatory science and in basic science research. We're hopeful that some of these discussions over the upcoming days will address these gaps and potential obstacles. So, we're excited for the areas of discussion planned over the upcoming days, and at NIAID, we look forward to accelerated progress in the area of bacteriophage therapeutics in the future. So, thank you, and I'm excited for the topics that are upcoming today, tomorrow, and the next day.

DR. PLAUT: Thank you, very much, Dr.

Gruber and Dr. Erbeling. We're just a couple of minutes ahead of schedule, so, I'm just going to pause for one or two minutes, and then we'll proceed. Thanks. All right, so next on our agenda, we will have presentations on the background and goals and of the workshop. I'll begin, and then I'll turn the stage over to Dr. Jane Knisely, Program Officer in the Bacteriology and Mycology Branch of DMID at NIAID.

But, before we get into that, I'm going to go over a few housekeeping items. The first three bullet points on this slide are intended for speakers and moderators. We ask that you please rename your virtual self in Zoom. You can do that by hovering over your name, clicking on more or the three dots, and selecting rename to change it to your name and affiliation. Please mute yourself when you're not speaking. Please turn on camera when you're presenting or during discussion times and turn it off when someone else is presenting. The last two bullet points on this slide are intended for all attendees. If you

have a headset or earbuds with a microphone, please use that for the best audio quality. And please note that this meeting will be recorded and made available temporarily to attendees.

Just some points about questions and answers during this workshop. All attendees will automatically be on mute, and you won't be sharing your video. Please post your questions in the Q&A box. You can submit questions at any time during a presentation. We will try to address your questions during the speakers' presentations if there's time at the end of each presentation. If there's not, we'll be addressing them during the panelist sessions, and if there are still unanswered questions, speakers will try to address them and respond to them using the Chat function.

If you encounter any technical issues during the workshop, you can contact either of the two people who you see listed here, and they should be able to provide some assistance.

Now I'm going to go over the

workshop goals from the perspective of the FDA. Again, my name is Roger Plaut, and I'm a research microbiologist at FDA CBER. Just a disclaimer. My comments are an informal communication and represent my own best judgement. They do not bind or obligate the FDA.

On this slide, I'm just showing three points regarding the history of phage therapy. First, phages were first used to treat bacterial infections over 100 years ago. Their continued use in Georgia, Russia, and Poland—their use continued in those countries but was largely discontinued elsewhere because of the availability of antibiotics and uncertainty about the efficacy of phage therapy. The increasing prevalence of antibiotic-resistant bacteria and a better understanding of phage biology have led to a resurgence of interest as Dr. Gruber mentioned.

In recent years, a couple of phage workshops have been held. In 2015, there was a workshop sponsored by NIAID at NIH. And it was

entitled, Bacteriophage Therapy, an Alternative Strategy to Combat Drug Resistance. Two years later, there was another workshop co-sponsored by CBER at FDA and NIAID, and the title was Bacteriophage Therapy, Scientific and Regulatory Issues.

When these workshops were held, there were fewer people who were doing research in this field, and so, we actually had a little bit of difficulty finding people who could speak on various topics. But that's no longer the case, and I just want to mention that our organizing committee for this workshop—we not able to include everyone who we wanted to in this workshop, so please, don't take it personally if we—don't be offended if you were not asked to present at this workshop.

The workshop that you're a part of right now is called Science and Regulation of Phage Therapy. And, again, it's sponsored by CBER and NIAID. And the workshop goal, as stated on the website, is to exchange information with the medical and scientific

community about the regulatory and scientific issues associated with phage therapy.

I'd just like to elaborate a bit more on what the goals of this workshop are from FDA's perspective. So, in terms of information that we can share, we are holding this workshop so that we can share information that could facilitate development of phage therapy products. And over the coming days, you'll hear presentations regarding regulatory pathways, clinical trials, expanded access, submission structure and procedures, meetings with FDA, what types of meetings are possible and how to prepare for such meetings, and we'll share other resources that are relevant to the phage community.

In addition, you'll hear presentations that go into a bit more detail regarding chemistry, manufacturing, and controls considerations for these products, and clinical considerations. We are also holding this workshop to provide a forum for other stakeholders in the field to share information. In the coming days, you'll hear

presentations regarding product characterization, progress in product development programs, expanded access cases and what has been learned from those, challenges and opportunities in the field, and the latest science by some of the top researchers in the field.

To conclude, FDA CBER welcomes everyone. We welcome your participation in this workshop, and we look forward to an informative and productive workshop. And now, I will turn it over to Dr. Jane Knisely.

DR. KNISELY: Okay. Thank you, Roger. And good morning, everyone. Get my slides up here. Okay. So, I'd just like to reiterate the welcome that others have said. We are so excited to have had such a robust response to this workshop, and we're really thrilled to be able to share the next few days with you all discussing this really exciting science. I'm Jane Knisely. I'm a Program Officer in the Bacteriology and Mycology Branch here at NIAID. And just to set the stage, the mission of the National Institute of Allergy and

Infectious Diseases is to conduct and support research to understand, treat, and prevent infectious, immunologic, and allergic diseases. And among those, as has already been mentioned, is the growing public health threat of antibiotic-resistant bacteria.

NIAID has been working on ways to address this threat for many, many years. What I'm showing you here are two of our most recent strategic documents that guide where we make investments and where we'd like to go in the future. On the left, you see the current National Action Plan for Combatting Antibiotic-Resistant Bacteria. This is a U.S. Government-wide plan that was first released in 2015 and was updated in 2020.

There are five overarching goals, the fourth of which is research to develop new antibiotics, other therapeutics, and that would include phage therapy and vaccines. And is where NIAID has most of its activities related to this CARB National Action Plan. We call it CARB for short.

On the right, you will see NIAID's

own document. This also was first released in 2014 and then updated in 2019. And this is our antibiotic resistance research framework. If you go to this link, you can find examples of basic translational and clinical research to combat antibiotic-resistant bacteria as well as some identified priority areas where we intend to invest in the coming years. And among those is alternatives to antibiotics, and, again, including phage therapy. So, phage are featured in both of our strategic documents.

Shown on this slide is a schematic of the product development pathway from basic research, preclinical development, and clinical evaluation. And mapped along this pathway are some of the activities that NIAID has engaged in to help foster and support research in this field to really provide evidence of whether and when bacteriophage therapy is effective for human use.

In FY16, we had a funding opportunity announcement focused on non-traditional therapeutics that limit

antibacterial resistance. We made seven phage therapy-related awards in that year under this funding opportunity announcement. And that was really focused on the basic to preclinical space to better define the product development for new phage-based therapies.

In FY21, so, just this year, we made 14 new awards under the funding opportunity announcement, Understanding Phage Biology to Support the Development of Bacteriophage Therapy, again, focused on this basic to preclinical juncture. So, these awards are focused on understanding the science of bacteriophage, so that we can better develop these as products.

We've also had, since 2015, an interagency agreement with our colleagues in CBER, characterizing the preclinical assessment of phage therapy for pathogen decolonization. On the preclinical side, we have what are called preclinical services. You'll hear more about these today and also on Wednesday. These are a suite of contract-based resources that are in place to help develop

products along this pathway. They are provided free of charge to external requesters from industry and academia to help advance their products. And we have used these successfully to assist with several different phage products. Again, just this Fiscal Year, we had a small business contract topic on improving technologies to make large-scale, high-titer phage preps to address that research gap. And we're thrilled to be able to make three awards under this announcement.

And, finally, on the clinical end, this is extremely important to conduct rigorous, well-controlled, clinical trials, and one way that we are doing that, and you can hear and learn more about this in the breakout session on clinical trials this afternoon, is through the antibacterial resistance leadership group. They are going to be conducting a phage therapy trial hopefully to begin early next year.

And, as Roger already mentioned, we have sponsored a number of workshops, including today's, and also have given

conference awards to groups such as the Evergreen Conference, to support those over the years.

So, just a word about our current portfolio of phage therapy and its growth over the years. So, a couple of things that I want you to take away from this graph which is showing new grant awards from Fiscal Year '15 to the current Fiscal Year. First, is that the red represents awards that were made under funding opportunity announcements. So, just to show that we have really stimulated the field and made quite a few new investments in a very intentional fashion.

The second is that over the past several years we have seen an increase in successful unsolicited grants. So, it's submitted to our parent funding opportunity announcements, R-21, R-01, those types of things. And we're hopeful that this represents a sense that the phage research community is really coming into its own and will be able to successfully compete in the future. But we are going to carefully keep an eye on this.

So, just to wrap up, the goals of the workshop from NIAID's perspective are simple. First, to provide information on NIAID funding opportunity announcements to workshop attendees to make sure you all understand how we can support you. And second, to stimulate information exchange on research gaps and opportunities to support the field and to inform us as we move forward.

So, once again, thank you very much. I'm leaving you with just a few links for more information, including my contact information. And with that, I'll hand it back to Roger.

DR. PLAUT: Thank you, Dr. Knisely. All right, so we will resume in about five minutes with our next presentation. Thank you.

Okay, we are back. Next, we have a presentation from Dr. Cara Fiore. She's a Senior Regulatory Reviewer and Microbiologist in the Division of Vaccines and Related Product Applications in OVRP at CBER.

DR. FIORE: Hi, how are you? I'm going to attempt to share my screen now. And there we go. Hopefully, everybody can now see

my screen?

DR. PLAUT: Yes.

DR. FIORE: Great. So, I am really excited to be here for the CBER-NIH phage workshop. And, sorry, I'm looking at my picture instead of the slides. So, hopefully everybody's looking my slides. And I'm going to talk over the next few minutes and give you an overview of the regulatory framework for the development and use of bacteriophage therapy in the United States.

So, I'm hoping you're looking at my next slide and my comments are an informal communication and represent my own best judgment. The information here does not bind or obligate the FDA. That's my disclaimer.

Slide 3. So, over the next 20 minutes or so I'm going to give you an overview of the development of biological products. I'm going to discuss regulatory guidelines for a biologics IND submission. And I'm going to talk about regulatory considerations for use of phage therapy products in clinical trials intended to

support licensure. So just to remind everybody that phage therapy products for infectious disease indications is regulated within the Center for Biologics. And reviewed in the Office of Vaccines Research and Review.

So, starting from the top, when in development does FDA get involved. In the U.S., an IND or investigational new drug application is required to conduct clinical investigations of unapproved new drugs. And the definition of a drug is an article intended for the use in the diagnosis, cure, mitigation, treatment, or prevention of a disease in man. And when an IND is in effect, the investigational product is exempt from premarket approval requirements and may be shipped lawfully for the purposes of clinical investigations.

So, what that means is a marketed drug can be shipped legally and a drug under IND can be shipped legally for the purposes of clinical investigations or studies. So, therefore, the first time you use your product, your drug, in humans, you must come

to us for an IND. So, first in-human use. In addition, human studies are conducted under IND, whether or not it's the intention to market or license the product. So, regardless of the intention, you have to conduct your studies under IND. And here I have a guidance about—more information if you need details about that.

So, the FDA's primary objectives in reviewing an IND. So, in all phases of investigation, it is to assure the safety and rights of the subjects in the clinical trials. And so, what you see here below is safety in the red solid bar through clinical development. And in Phase 2 and 3, is to help assure that the quality of the scientific evaluations of the drug is adequate to permit an evaluation of the drug's effectiveness and safety. So, during clinical development, you are going to be fine-tuning your effectiveness and fine-tuning your manufacturing consistency. And that's depicted here by the bar getting more solid toward the right for both effectiveness and manufacturing

consistency. So, manufacturing consistency can be composed of scale-up development of your product. In manufacturing, it could be validation of your assays and process validation.

And further in development, our primary objective is assessment of the scientific quality of the investigations and the likelihood that these investigations will reveal data capable of meeting statutory standards for marketing approval, *i.e.*, could they support licensure?

The next slide is the development of biological products. And, again, as I mentioned, FDA has oversight as soon as you go into people in IND development, and basically, we never go away. So, all the way through IND development into marketing and post marketing, so for the life cycle of the product. You could come to us before this state and that would be products that are not tested in humans. And that would be for preclinical. And then you have also your pre-IND meeting which is when you're preparing go into the clinic.

So, as I mentioned, anytime studies are done in humans, we have oversight on that.

So, how do we do that? We do that with consultations with you, the sponsor, and us, the FDA. We do have formal meetings and milestones during IND development that I am going to go over here. So, before you go in the clinic, we could potentially have communication with you for early review of toxicology protocols, as appropriate. In addition, to that, when you go to start your clinical studies, we recommend that request a pre-IND meeting. And in that pre-IND meeting, you would discuss what you're planning on doing for your Phase 1, your overall clinical development plan, your chemistry, manufacturing, and controls, which is how you're making your product. How you're releasing the product, the lot release, you could discuss that during a pre-IND meeting. And any sort of animal studies. In addition, we have other milestone meetings such as end of Phase 2 and pre-BLA meetings.

So, those are the formal meetings,

milestone meetings that we would have. We also have other meetings, Type C and Type A meetings. In addition, once you get under IND, you would be submitting amends to your IND that the review team comments on and gives you feedback of. So, that also helps you through your clinical evaluations and your product development.

So, starting with the pre-IND meeting, I'm going give you a little bit more detail. First of all, we highly recommend that you come in for a pre-IND meeting with us. It's free and it's on a timeline. So, a pre-IND meeting is a Type B meeting. And we would give you feedback via written responses or, perhaps, a teleconference. Although these days we're giving more written responses.

So, in your pre-IND meeting request, you would have questions for us to answer. On the timeline for that, you would submit a briefing package. And that briefing package represents the data that you expect to be provided in the IND when you file it and in early development. And it could include your

product description, manufacturing and testing, such as the CMC information, supporting data summaries which is product, any sort of preclinical information that you may want us to consider, and clinical information that you may have.

You're going to tell us how you use the product, which is the proposed indication. You're going to give us a rationale for your clinical studies. You might submit a clinical protocol or a summary or a synopsis of your clinical protocol for us to discuss with you. And also, if you give us a little bit of insight as to how you think your future clinical development would be. You would tell us about your plans, future clinical plans.

And, again, in the pre-IND meeting request, and in the pre-IND briefing package, you have to have specific questions for us to answer, and they have to be identical questions. So, you give us a peek at the questions in the brief meeting request, and then come in with the supporting information of your questions in the briefing package. And

there is a formal meetings guidance for types A, B, and C meetings and gives you a little more details about that. And I've cited that below.

So, if you are manufacturing a product and it's going to support clinical studies under IND, but you are not the IND sponsor, you could potentially submit information to us confidentially, in what we call a Type 2 Master File. So, the chemistry, manufacturing, and controls Type 2 Master file is a way of providing confidential information to the FDA, to provide methods used in, perhaps, the characterization, genomic sequencing, manufacturing, processing, packaging, and/or storing a product. It could be any of these things or all of these things in your Master File. It allows parties to reference the material, the CMC material, without disclosing the contents of the Master File to the IND parties.

So, the FDA reviews the technical contents of the Master File in connection with the review of an IND that references them. And

this is a reminder that, if you are going to do phage therapy Master Files, they have to be submitted to Center for Biologics for our review. The Master File holder would provide a letter of authorization to the IND sponsor. And that letter of authorization allows the IND to cross-reference the Master File. So, you have to have a letter in the Master File saying that you want to support a certain IND, and then the IND has to have a letter from the Master File holder allowing us to reference the contents of the Master File.

One Master File can support multiple INDs. And a letter would need to be in every one of these INDs, indicating that we need to reference that specific Master File. So, we do have a guidance on that. And I've cited that below along with the website.

So, this is a very high level, the recommended content and format of INDs. So, if you are a research sponsor, you may be submitting INDs to us via a PDF format. But if you're a commercial entity, you would have to submit it through our electronic gateway in a

common technical document format. But regardless of the actual way you're submitting to us, the information is very similar. So, you have to start out with a cover letter with us and then the forms 1571, 1572, and 3674 for administrative purposes, and it kind of outlines how to get in contact with you, and what you're submitting.

Please include a table of contents and if you're doing PDF format, please paginate your pages. Include your introductory statement and general investigation plan of your IND. And the CMC information in your IND has to be product specific. So, it would be the product that you're using for your clinical study and perhaps if you performed nonclinical studies as well. So, you would include any relevant pharmacology and toxicology information you want us to review and to support your IND. And then include a clinical protocol, also a blank informed consent, IRB approval when it's available and, if applicable, an investigator's brochure.

So, as I mentioned, you can do this

through our electronic gateway, or you can also do it if you are a research sponsor, through a PDF. And you can look at the CFR for more information about content.

So, once you submit the IND to us, what happens? So, for the first 60 days, it's pretty structured. On Day 0, you submit your IND and between Day 0 and Day 30, we assign an IND number to your IND, and we send you an acknowledgement letter saying we have received your submission. And during the first 30 days, we also perform a preliminary review.

On or before Day 30, we contact the sponsor, and we may communicate review issues. On Day 30, we would also tell you whether or not your study may proceed. If we tell you your study may proceed, you are free to start your clinical trials. Or if we have safety concerns, we may put you on clinical hold. If we put you on clinical hold, an official letter of safety concerns for the sponsor to address will be issued. And that would be by Day 60. These clinical hold issues must be submitted to us adequately addressed, reviewed

and concurred with CBER in writing before you may proceed. And we would at that point give you a release hold letter. We also may be communicating items that are not clinical hold items any time after Day 30. For certain INDs, we may do it before Day 30, but usually these non-hold comments are given any time after Day 30, maybe even after Day 60. So, again, if you go on clinical hold, you have to address each of our safety concerns in writing to us, and we would have to review it and give you our acknowledgement and say that you've adequately addressed the concerns before you may proceed.

So, what are the common pitfalls of phage therapy products that we see in IND submissions? So, this is for early development. Basically, it's insufficient information in your IND to assure safety that is equal to a clinical hold. We have two main categories of clinical hold for phage therapy products. It could be clinical concerns that we have, and they could be lack of appropriate screening of subjects for inclusion and exclusion criteria. It may be lack of

individual or study-wide pausing rules. It may be inadequate definition and monitoring of adverse events and serious adverse events or it could be lack of appropriate informed consent. It could many other things too, but at a high level, these are the issues that we see most commonly for phage therapy products.

In terms of your chemistry, manufacturing, and control, that is your product, it may be lack of appropriate product testing, such as testing for endotoxin, exotoxin, or sterility. It could be insufficient genomic analysis on either your phage or the host strain that you're using to propagate your phage, including nucleic acid analysis. It may be lot release specifications that we consider inadequate or testing results that are lacking or insufficient. So that, again, with CMC there could be other issues as well, but these are, at a high level, the issues that we see with phage therapy INDs in early development.

So, in addition to developmental INDs that are going to a product and clinical

development plan, we have a category called Expanded Access. And Dr. Gruber talked about this a little bit as well. And we have three main categories for expanded access. And just to state that the primary purpose of expanded access INDs is to provide access of the investigational or unlicensed product. It is not to collect systematic safety or effectiveness data that you may be collecting in adequate and well-controlled clinical trials. It's mainly about putting an investigational product in the hands when there is no other—in the hands of a treating physician or otherwise when there's no other treatment available. And we do have expanded access guidance for questions and answers available.

Additionally, I will be talking a little more about the Single-Patient Expanded Access IND, which is what we see on the left-hand blue box, and this includes emergency and non-emergency use, in a later focused session. We also have Intermediate-size population INDs and

Treatment INDs that are more widespread use. So, those are our three categories for expanded access.

So, to give you a little bit of flavor of the type of phage therapy INDs that we've seen to date. So, this would be since we first started getting phage therapy INDs and the data cutoff was beginning of August that I looked at this data. So, the majority of our phage therapy INDs are still Emergency Single-Patient Expanded Access INDs. And then coming in as a close second is the Non-Emergency Single Patient INDs. So, that is still the bolus of our INDs for phage therapy. What we do see is this gray box, which is getting larger and larger, which is Controlled Clinical Trials. So, we do see more coming in and we're very excited about phage therapy development.

In terms of the target species, though, what the proposed bacterial strain, the proposed treatment with a phage for the bacterial strain, this is a flavor of what we have—what we've seen in the Office of Vaccines

since, again, the beginning of time until about the beginning of August. So, the majority of the infecting bacterial strains that we see proposed for treatment would be *Pseudomonas*, and that's here in orange. And then coming up close behind it are infecting strains of *Staphylococcus*. We also have *Mycobacterium*, *Acinetobacter*, adherent invasive *E. coli*, and etc. *Achromobacter*, *Klebsiella*, *Enterococcus*, *Burkholderia*. And then we have INDs which are proposed to treat a mixed population of bacteria. And that I've had to lump together here. So, this is the flavor of infecting strains that we see the phage targeting.

DR. PLAUT: Dr. Fiore, you have about two minutes left.

DR. FIORE: Thank you. So, at the end of the day, when you are going to develop a product, it has to be safe, pure, potent, and manufactured consistently. So, this is what we're shooting for, for phage therapy products that are intended at the end of the day for a licensed biologic. So, this is our goal.

So, in summary, phage therapy products are investigational biological products, and clinical evaluations must be conducted under IND. The FDA interacts with sponsors throughout the product in clinical development with formal meetings and comments on the IND submission. The expanded access IND mechanism is available for use phage therapy products, but it's not a substitute for adequate and well-controlled clinical trials intended to support licensure.

So, I'd like to thank my supervisor, Liz Sutkowski and then my colleagues, Laura Gottschalk and Laura Montague, for commenting on my slides. And here are some references that may be helpful. I want to thank everybody.

DR. PLAUT: Thank you very much, Dr. Fiore. I know that we have some questions in the Q&A, but in the interest of time, we're going to hold responding to those questions until the panel at the end of this session. And we are going to move on.

Next on our schedule, we have our

first session, our first official session. And the topic of this session is choosing and characterizing phages for therapy. So, I will be starting us off and I am going to start sharing my slides.

All right. So, again, my name is Roger Plaut. I'm a research microbiologist at FDA-CBER. And the title of my presentation today is Regulatory Considerations for Characterization of Bacteriophages for Therapy. Here's my disclaimer. My comments are an informal communication and represent my own best judgment. They do not bind or obligate the FDA.

I'm going to start off by going through a few common misconceptions that people may have regarding the FDA and phage therapy. First, does FDA have a pre-formed opinion about the safety or effectiveness of phage therapy? The answer is no, we do not. We make our decisions based on science, and we evaluate the applications that we receive on their merits.

Second, are there novel CMC issues

for phage products? Yes, there are some and I will be discussing some of those issues in the coming slides.

Third, are there new and challenging aspects to clinical trial design for phage therapy? Yes, there are some, and you will hear more about design of trials for phage therapy products in the coming days.

Fourth, does CBER-FDA have a history of regulating non-typical products and treatment modalities? Yes, we certainly do, including such products as fecal microbiota transplants, other live biotherapeutic products which you may know as probiotics. And products regulated by the Office of Tissues and Advanced Therapies, such as CAR T-cells and other cellular and gene therapy products.

Next, are clinical trials of phage therapy proceeding under FDA auspices? Yes, they are. There are trials that are ongoing under IND, and I can say that because the sponsors of these products have made that information publicly available. And you can also find trials on clinicaltrials.gov.

Last, does FDA allow compassionate use of phage therapy? And, as Dr. Fiore mentioned, we do, and we prefer to call it expanded access use. That's the official term. And, again, you'll be hearing a little bit more about how that works in the coming days.

Here's an outline of my talk. I'm going to make a brief comment regarding Current Good Manufacturing Practices, or CGMPs. The bulk of my talk will be on regulatory considerations regarding chemistry, manufacturing, and controls for phage therapy. And then, at the end, I'll share some resources.

Dr. Fiore presented a version of this slide, and you'll probably see more versions of it in the coming days. So, here you should be seeing the different phases of IND, Phase 1, Phase 2, and Phase 3. The next step would be a Biologics License Application and then, if that product is approved, then there can be BLA Supplements.

At the bottom, we have different attributes or concepts. And there's a sliding

scale for three of them. But you'll note that safety is important throughout the process. So, we're always concerned about the safety of participants in clinical studies. And, of course, the safety of patients who are receiving treatments that have been approved and licensed.

The sliding scale here is to indicate that, when it comes to effectiveness, there may not be any need to demonstrate effectiveness during early phases. So, Phase 1 is typically focused on safety and perhaps dose-ranging. And, similarly for Phase 2, although sponsors may be interested in looking at effectiveness beginning in Phase 2. But then during Phase 3 is typically where a pivotal trial occurs where a sponsor should be designing a trial that is capable of demonstrating effectiveness of the product. Of course, effectiveness is important for a licensed product as well.

Manufacturing consistency is also on a sliding scale. So, early on in development, a product's manufacture may be relatively

simple, but then as development proceeds, you'll want to make sure that you're manufacturing a product consistently, and that will need to be demonstrated in the package that's submitted in your BLA.

And, lastly, assay development is also on a sliding scale. When you're first during your assays in Phase 1, you will want to make sure that they are appropriate for their intended use and then you'll want to qualify them and, by the time you get to Phase 3, you really should have validated assays.

I'll just also mention that the inspection process takes place during the BLA application time and BLA supplements can include post-approval changes, new indications, changes to dosing, manufacture, and some changes to facilities and equipment. And, again, these phases are not set in stone. There can be some flexibility here, but these are some general guidelines, what sponsors typically do.

On this slide, I'm showing the first page of a guidance that FDA put out in July

2008 regarding current good manufacturing processes for Phase 1 investigational drugs. And here's an important quote from this guidance. It says, 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 so, what that is really getting at is that for Phase 1, CGMP is not expected to be as extensive as for later phases or for an approved product. For Phase 1, the CGMP should be appropriate for that phase.

And now I'm going to into some detail regarding chemistry, manufacturing, and controls regulatory considerations related to products intended for phage therapy. On this slide, I'm showing a few different aspects, a few different attributes of phages. And I'll be going into some detail regarding these different aspects. So, we have diversity,

specificity, immunogenicity, the fact of phages can mediate genetic transfer, and then it says here that phages are generally assumed not to interact with human cells. That's a bit of a generalization. They can act as immunogens, of course. And there has been some recent work from labs including Jeremy Barr's lab in Australia about how different types of cells take up different phages with what kinetics. But, in general, phages are not thought to cause any detrimental effects on human cells, and, therefore, there's a high expectation of safety, assuming that the products are pure which I'll be discussing in later slides.

But first, to focus on diversity, for most bacterial hosts, there many bacteriophages in the environment that can infect that host. And in quotes we have here an "inexhaustible" supply of natural products to treat infections. Of course, this is going to vary among the species of bacteria, so for some bacteria, there may be many and a diverse set of phages that have been isolated from the

environment. And for other species, the diversity may be more limited. But, in general, it's thought that there are phages out there in the environment that can be isolated.

It's important to note that every phage bacterial host pair is unique. And, therefore, it's not appropriate to draw conclusions, whether good or bad, about the characteristics of other phage host pairs. So, for example, a phage may infect a certain strain of a certain species with certain kinetics. But that phage may have different kinetics or may not even be able to infect another strain. So, in general, you really need to look at each phage and bacterial host pair and examine the kinetics and the details of that interaction each time.

This slide is about the specificity of phages. So, phages will generally be pathogen-specific treatments, in contrast to broad-spectrum antibiotics. And, therefore, you would expect less disruption to the microbiota. But one drawback is that this will

usually require identification of the infectious agent prior to beginning treatment. Generally, specificity of phages for their bacterial hosts is attributed in large part, to receptor interactions. And this is important because sponsors may want to consider this if they are designing cocktails. I'll have a little bit more about that on a future slide. But I do want to point out that there can certainly be other factors that affect the specificity of phages for bacteria. In our own lab, we have some evidence that gene expression in the bacterium can affect whether a phage can infect and also whether the phage can replicate in that host can determine whether that phage will actually be able to lyse and kill that bacterial strain.

Here we're talking about immunogenicity. So, it's likely that a mammalian host will develop an adaptive immune response to phages. And this has been hypothesized to perhaps limit the length of time over which that phage could be used or whether that phage could be reused in that

same patient. Not very many published studies directly address this. We may be hearing some information about this issue in presentations during this workshop.

There are some factors that could moderate the importance of immunogenicity. So, there may be an antibody response, but it may not be neutralizing, so it may not prevent the phage from binding to and killing the bacterial host. The kinetics of the antibody response may allow for a sufficient treatment window, depending on the length of the treatment that is being proposed. And, of course, the likelihood of immunogenicity will vary depending on the route of administration. For an intravenous treatment, you might expect a more likely adaptive immune response than if you were treating topically, for example. Having said all of that, it's unclear at this point what, if any, safety concerns arise from the possibility that humans can develop an immune response to phages.

Bacteriophages can mediate genetic transfer. So, the genes that are transferred

could be part of the phage genome, and here we're talking about lysogenic conversion, where the phage integrates into the bacterial genome, along with accessory genes, and that can confer a selective advantage on the host. For example, if a toxin is part of the phage genome.

And on the right side here, we're talking about transduction. So, when genes that can be transferred by phages are bacterial host genes. And there are two different types of transduction. In generalized transduction, all chromosomal markers can be transduced with equal frequency. In specialized transduction, only chromosomal markers that are near the site of insertion are transduced. I want to point out that if sponsors use non-lysogenic phages, then two of these problems basically go away. So, if you're using non-lysogenic phages, lysogenic conversion is not possible, nor is specialized transduction.

So, having gone through all of those slides, we arrive here at a slide that says

Current Consensus for Characterization of Phages for Therapy. And I think we're on pretty firm ground when we say that. Most people in the field agree that when you're looking at the phage genotypes, that the phages themselves should be free of relevant antibiotic resistance genes. And by relevant, I mean antibiotic resistance genes that confer resistance to antibiotics that could be used in the clinic. And the phages themselves should be free of virulence factors. When we talk about phage phenotypes, it's agreed that phages should be non-lysogenic and non-transducing. And we're going to be hearing later on this morning about whether, perhaps, that information could be gleaned from the sequence of the phages.

In terms of the phage preparations themselves, the phages should be propagated on well-characterized strains. And this is important for several reasons. So, one is so that sponsors are aware of the possibility of contamination of their product with bacterial products. Whether there are prophages in the

bacterial host strain that could contaminate their product. And also whether there are genes that are undesirable that could be transferred via transduction as I mentioned on the previous slide.

The phage preparations should be sterile or low bioburden, depending on the route of administration. And they should be as pure as possible, in terms of endotoxin, exotoxins, and non-product phages. And by non-product phages, I'm referring to phages that could be derived from the bacterial host strains that were used for propagation or possibly, from other phages that are manufactured in the same facility. And, of course, when we say as pure as possible, we're also discussing the excipients that could be of concern that could remain in the product after manufacture.

On this slide, we're discussing the use of cocktails. So, cocktails have been proposed to increase the spectrum of treatment, so that a phage or a group of phages will be able to target a larger number

of strains, different strains of a given species. And the idea is also to use cocktails to avoid the likelihood of resistance developing. This is similar to the idea of using multiple antibiotics to reduce the likelihood of resistance occurring to one antibiotic.

The regulatory implications of using cocktails are, first, each phage should have relevant activity. There should be some reason why a sponsor is proposing to include that phage in the cocktail. The potency test should assess each phage in a cocktail. You want to demonstrate that you know how much of each phage is present. And stability testing should assess each phage in the cocktail so that you know how much phage remains in that cocktail after a given length of time. And future inclusion of additional or replacement phages should be supported by adequate chemistry, manufacturing, and controls information on those phages that you want to add or use to replace phages in the cocktail.

Here, I'm bringing up the idea of

some desirable characteristics that could be used for therapy. These are by no means required, but these are some aspects of phages that have been published and may be desirable for sponsors to consider. The first is reduced clearance of phages from tissues or blood. And the publication I'm showing here is from 1996 from Sankar Adhya's lab, in which a phage was found to have a mutation that caused it to last longer in the bloodstream.

Second is the idea of using a virulence factor as a receptor, so that any bacterial mutants that arrive that are resistant to the phage, would be less virulent. And here I'm showing a publication from 2017 from Minmin Yen and Andrew Camilli in which they used a cocktail of three phages in an animal model of *Vibrio cholerae* infection and strains that were resistant to the phages were found to be less virulent.

And lastly, is the idea of using antibiotic resistance protein as the receptor, so that any phage-resistant mutants would be less antibiotic-resistant. And the paper here

is from 2016 from Paul Turner's lab at Yale.

SPEAKER: All right, Roger, you have about two minutes left, a little more than two minutes left.

DR. PLAUT: All right, thank you. So, on this slide, I'm just going to discuss briefly the idea of using genetically engineered phages. And we do not consider genetically engineered phages to necessarily present more safety concerns than wild-type phages. In each case, we evaluate the specific genetic modification on safety and, again, we make our decisions based on science.

This is my conclusions slide. So, phage products intended for therapy can be regulated and are being regulated under current laws and regulations. There are some novel CMC issues with phage products, which I've just gone through. For early-phase studies, CGMP is expected to be appropriate for that phase. And pre-IND meetings are highly recommended. And I'd just like to make the point here that, if you have a pre-IND meeting, you should certainly address any

comments during that pre-IND process when you submit your original IND submission.

There are some resources here. A guidance on meetings, a guidance on early-phase CGMP and then a website for contacts in CBER.

Finally, I would just like to thank -oh, there's one more slide here. We did put out a paper on this topic a couple of years ago, myself and my supervisor, and the title is Regulatory Considerations for Bacteriophage Therapy Products. And I would like to acknowledge my colleagues at FDA who helped with this presentation.

Thank you very much, and let's see, we have just now run out of time, so I think that it is time for us to move on to our next presenter. Now we're going to hear from Dr. Jason Gill. Dr. Gill is an Associate Professor in the Department of Animal Science at Texas A&M University. Dr. Gill, you can begin when you're ready.

DR. GILL: Yes, there we go. Okay. So, you can see my screen okay, I hope?

DR. PLAUT: Yes.

DR. GILL: Okay, that all works.

Right, so, I'd like to thank the organizers for inviting me to give a talk today early on when people are—everybody's still fresh, hopefully.

So, I'm going to be talking about bioinformatic approaches to—that you can use to predict phage behavior. I am a member of the Center for Phage Technology at Texas A&M. And we do a lot of phage genomics. So, my only conflict of interest, I am on the advisory board of a company called Deerland Enzymes. Just, I think, you can still believe what I tell you.

So, there's a lot of different ways you can analyze a phage genome sequence to get some information out of it. Whole genome sequencing now is pretty routine, I think, for phages. And it really has kind of become a de facto requirement, I think, for therapeutic use. It's just one of the basic pieces of information now that are expected for phage you're planning to use for therapy.

So, you can use that sequence in a lot of different ways. And I can't really cover them all here. So, I'm only going to be talking about ways that you can look at the sequence to determine suitability for behaviors related to safety here. I'm only going to be talking about the lifestyle prediction here for virulent or temperate, and the potential ability to transduce host DNA. Both of which are things which you can get some insight from bioinformatically.

So, here's our favorite phage lifecycle. We've all decided that lysogenic phages, temperate phages are not generally going to be very useful for therapeutic purposes. And that is because they form lysogens, which are immune to infection by the same phage, right? So, you would immediately get this large phage-immune population. And also because temperate phages can often carry virulence factors in their genomes. And this is what Roger was just talking about, lysogenic conversion. You can have toxins or antimicrobial resistance genes can be carried

on the phage genome itself.

So, if you sequence a phage and you know that's all the information you have, a really simple way to try to determine if your phage is temperate or not is just to use a regular BLASTn of your phage against bacterial genomes. And if your phage is temperate, it's quite likely that it will have relatives or very similar phages to that phage, which are already existing as lysogens in other bacterial genomes that have been sequenced in the database. And that database gets bigger all the time.

So, if you just run a regular BLASTn against—with your phage genome against bacterial genomes, and if you have a good alignment, really along, you know, most of the length of the phage and it has a good match, your phage is probably temperate, right there because it has related lysogens in other hosts. So, if you have kind of partial or weak alignments then maybe it means it's related to a phage, to a temperate phage, but it may not necessarily be temperate, then that becomes a

little bit more ambiguous. And you have to really start looking for other signatures and which are associated with temperate phages.

So, this is a map of a temperate phage I worked on some years ago. This is *Burkholderia cenocepacia* phage called BcepIL02. And this was actually isolated. And it behaves virulently because we could not get it to form a lysogen in culture. This will not form a stable lysogen. It was actually used in a preclinical study in a rodent model for lung infection for *Burkholderia*, and it gave us about two-logs reduction. But after doing the genome sequencing, we looked at the genome and it really looks like a temperate phage.

So, and even though this is not able to form stable lysogens, this is a genome map here, it does have a lot of the signature genes or features that you might expect for a temperate phage. Like it has an integrase here. It has a regulatory switch that looks like a temperate phage. It has an *att* site, which is right here. And it also has a potential moron here, which is a good

potential virulence factor, which is just outer membrane proteins, which may make the lysogen more resistant to antimicrobial peptides if it was able to form such a lysogen.

So, if you're looking at a temperate phage genome, you're looking for signals that are associated with the temperate lifestyle. So, they often, and of course, these are never-nothing's every 100 percent in biology, but they often will carry these common genes or signals. So, the integrase can often be recognized by conserved domains or similarity to known integrases, which is represented here by the *int* gene which will be in your genome.

If you want to look closer to your phage sequence, the phage attachment site is usually near the integrase and also has this very kind of characteristic arrangement here. So, if it's going to integrate as a lysogen, the integrase has to recognize a particular site. And depending on whether it's a tyrosine or serine integrase, you'll have different kinds of sites. But generally, you'll have

this kind of perfect or imperfect inverted repeat that flanks the sequence which has identity to regions in the bacterial chromosome and this can look different depending on the on the integrase. But if you look for this, usually it's near the integrase. This would be another indication that the phage is able to form a lysogen.

Also, you can look for lysogenic repressors. And these have common conserved domains and they'll be related to each other. And they're often also arranged in this kind of characteristic, you know, switch arrangement here. You have this divergent transcript where you have the repressor on one strand and then facing away from it, you'll have some other kind of transcriptional regulator facing the other way. And this is a very kind of typical arrangement you'd get for a temperate phage. So, if you see this, this is another indication that it's able to repress itself.

Finally, you can look for morons. Which, moron is a technical scientific term

standing for more DNA. And these are the little gene cassettes that actually accomplish the lysogenic conversion, usually encoding virulence factors. And so, these can be identified as really kind of part of your phage annotation. And you can also do specialty searches against things like the mVirDB or CARD or what have you. If you have own custom database for virulence factors, you can do those searches and look for those in your phage genome.

So, just to sum that up, if you have a clear relationship to existing lysogens, are a good indicator that your phage is temperate. If you have typical signature genes, that can also identify a phage as temperate. But, of course, there are atypical phages, for example, P1, which is a very well-known temperate phage actually does not form a—does not integrate into the chromosome, it exists as a plasmid. So, it doesn't have an integrase. It also has kind of weird-looking lysogenic switch mechanism. So, as in biology, nothing is ever 100 percent.

There are machine learning tools out there which are being developed for predicting the phage lifestyle. It uses things like PHACTS, PhageAI, and BACPHLIP. These tools are still pretty new, and so they could probably be trained with, you know, more and more phages as we find them. And they may have more difficulty with very different phages, which are not like part of the training set.

And really, the gold standard here is still really the formation of a stable lysogen. So, if you can get your phage to make a lysogen in a host, that's still the best way to prove that it's actually temperate. You have to keep in mind that if it doesn't form a lysogen, it doesn't necessarily mean it's virulent. It just means it doesn't form a lysogen in your host. And as I talked about a few slides ago, there are examples of phages which definitely look temperate and they are able to repress themselves, but they will not actually form a stable lysogen in the lab host.

So, another issue is phage

transduction. So, temperate phages are generally regarded as transducing. But there are actually lots of examples of transducing virulent phages. So, just because it's a virulent phage, doesn't necessarily mean it's not going to be able to transduce. And it's represented here by the little red phage here which has packaged some host DNA. And for transduction here, we're really, we're looking at two dimensions here for a therapeutic phage. There's a potential for transduction of DNA from the phage propagation host into the target pathogen when you administer a treatment. And then after you administer the treatment, there's a potential then for transduction between cells in the patient or the environment after you actually administer the phage. So, there's two kinds of transduction here.

So, both of these are controlled by the host range of the phage. The transduction will be limited by the ability of the phage to actually adsorb to and inject its DNA into a cell and the likelihood that the phage is

actually going to be packaging host DNA. And it turns out you can actually predict this somewhat just purely bioinformatically. So, tailed phages, which is really what we're mostly dealing with here, the Caudovirales phages, they package their DNA into an empty preassembled prohead via this terminase complex, which is here.

So, the packaging is initiated by binding of that complex to the concatemeric phage DNA. So, phage generally replicate their DNA in these long concatemers, like multiple copies of the genome. And they'll be a site there which is recognized by the terminase. And that then binds to this and delivers this DNA concatemer to an empty head, which then will engage this large terminase pumping mechanism, which will then pump the head full of DNA and then disengage.

And so, if the phage terminase grabs the wrong piece of DNA at this step, it will take it over to the phage head and then package it into the head as well. And so, this is really where transduction decisions are

kind of made at this step, at this packaging step. So, this packaging initiates at the site recognized by the small subunit terminase. And generally, if this small subunit terminase has a low sequence selectivity, you're going to get higher generalized transduction because it'll just be more likely to pick up random DNA, which is floating around in the cell. And if it's very sequence-specific, you're going to have less generalized transduction.

So, I'm not going to go through all of this in detail. So, these are the four general kinds of DNA packaging mechanisms that phages have. So, you can have this kind of *pac* type where it recognizes, or it grabs a piece of DNA and basically packages it into the phage head until the head is full. That's called the packaging headful mechanism. So, it typically packages more than 100 percent of the chromosome and every molecule of DNA in the phage head will be different. It'll be starting and stopping in different places. And this top one here is the most likely to be transducing because really it has some level

of site specificity to start and then to terminate the packaging process, it just really waits for the head to get full. And so, there's really no sequence specificity there.

So, these other packaging types have more of the higher levels of the sequence specificity, you know, *cos*, for the phage like lambda or P2. And these are generally not good at generalized transduction, and these have site-specific sequences that they recognize for packaging. Phages that have these long or short terminal repeats like T7 or T5 also have a fair amount of sequence specificity. And then there's these phages that will have a terminal protein. And this is like phi29 of *Bacillus*. And these will only package DNA that actually has a covalently linked protein to each end of the DNA here. And so, these would also be very unlikely to transduce.

And so, really, you know, when we're looking at the risk of transduction, the *pac* phages are the highest risk here. And so, we can actually try to predict the packaging just by looking at the sequence. So, if you have a

terminal redundant, or a phage that has a specific packing initiation site, those will tend to be overrepresented in the DNA that's packaged in the phages. So, you'll have more reads then from those regions than the rest of the genome. And so, this is an example here with a phage that has these terminal repeats, which is like here a phage like, for example, a T7 or T5. So, you'll have these, you know, can be very clear breaks in sequence coverage between these two regions of the genome. And this is where the terminal repeat boundary is because this is overrepresented in the genome.

And likewise, if you have a very site-specific packaging system, those are the reads at that site that will be more overrepresented in your sequencing reads. So, you can actually use this then to predict TR boundaries and possibly identify *pac* initiation site.

Another way of doing this is looking at the positions where reads start and stop in the genome. So, if we imagine you have a phage genome here, which has like specific defined

sequence-specific ends here in the little red boxes. And you fragment up this DNA randomly and then you sequence it, you will get, of course, random fragments of DNA shown down here. But because the phage DNA that you put in had specific ends when you started, these ends, the little red boxes here, will be overrepresented in your sequencing reads. So, you'll have a lot more fragments that start or stop at these positions, right? Because this is the phage that you started with. The DNA you started with had these site-specific positions here at the ends. So, then these would be overrepresented. And you can look for this as well to try to identify, for example, *cos* sites or potential *pac* sites or terminal repeat boundaries.

So, there is a tool called PhageTerm, which does this for you now. We and other groups had various kinds of home-brewed methods. But this group made this tool called PhageTerm, which is a command-line tool. And it does both kinds of analysis. It looks for coverage and read start stop-start and stop

frequency. And it also generates like a nice PDF report for you when you're done. So, this is an example of the report that you get. It'll show you this is an obvious coverage, discontinuity here. And it predicts that this phage here, for example, it has a short terminal repeat, which would be then less likely to be transducing.

So, this method works well, except for the time that it doesn't work, like a lot of methods. So, this approach can sometimes give you kind of an inconclusive result. And it's pretty sensitive to sequencing in coverage depth. It's also really affected by how sequencing libraries are prepared. So, it really assumes you have like a perfectly random fragmentation of DNA to do the statistics. So, a lot of the newer sequencing methods that are used strictly for Illumina, that are used in these tagmentation-based methods are not actually perfectly random because they're enzymatic. And so, there will be some slight biases in where the fragments or boundaries occur.

And so, you'll get a slightly biased library, and it makes it so it can kind of cover up the signal that you might get from either coverage or read start-stop boundaries because of that bias. That's something to think about when you're sequencing phages. If you want to stick with the more laborious but possibly more accurate physical shearing of the DNA rather than using the tagmentation method, which is a lot easier.

You can also predict the termini by the large terminase sequence. So, phage DNA packaging is conducted, the actual motor is the TerL protein. And it looks like there's really clades of TerL proteins that are predictive of the packaging type. So, there's no real explicit cutoffs here, but you can do phylogenetic approaches here. This is a tree from a paper from some years ago where Sherwood Casjens had aligned and made a tree of phage terminases. And you can see that the terminases really do kind of sort out into clades based on how they package their DNA.

And so, again, if you're worried

about, for example, *pac* phages, like, for example, these guys right here or these here which are headful packagers, then, you know, you can look for that signature in the terminase. And we've repeated this analysis more recently with a larger set. I don't expect you to be able to see this, but the phage terminases do still sort out into clades, you know, that can be associated with packaging type. Although you do have some edge cases here shown in white, which don't really fall into any obvious packaging type and so, obviously there's more validation here that needs to be used if you want to use this to predict packaging.

So, finally I'll talk about host DNA degradation. This is another aspect of transduction. So, many virulent phages degrade the host chromosome as part of their infection process. So, if you understand how this mechanism works, you might be able to use this actually to predict transduction. So, this is not really well understood outside of a few paradigm phages. In T4, the genes that

accomplish this host chromosome degradation are known.

But a lot of other phages, even in some paradigm phages, it is not really well understood how exactly the host chromosome is degraded. But if they do this, then they're unlikely to be very good transducers regardless of how they package their DNA because there's no DNA left to transduce by the time you start packaging if you dissolve the host chromosome into nucleotides. So, phages such as T4 and K and are known to be inefficient transducers. And so, then it's possible for you to understand this mechanism better in different phages, you might actually be able to, you know, predict this bioinformatically as well to know that, for example, your phage degrades the host chromosomes early in infection and is therefore to be unlikely to transduce.

So, just to kind of sum up on that. For predicting phage transduction, you can determine the phage packaging type by either looking at the sequence reads or

classification of the phage terminase. And that'll give you some idea of the phage's likelihood to be transducing with *pac* phages being more likely.

You can look for degradation of the host chromosome. So, if we have a better understanding of this, you can maybe catalogue the proteins that are involved in the various pathways. Or maybe there's room here to develop some quick empirical methods to look for host DNA degradation. Like when this was done classically, you know, it was done using radiolabeled nucleotides, you know, and solubilization of the chromosome and then looking at that on a scintillation counter, which is not so easy today. So, if there's better methods for that, that might be helpful.

And really there's still needs to be empirical determination of transduction to validate all this stuff.

MR. PLAUT: Dr. Gill, you have about two minutes left, two minutes.

DR. GILL: Okay. I'm just about done.

So, the classic here is, you know, movement of selectable markers between hosts, which gives you an actual quantitative number for transduction, but if you ever actually had to do it, it's a bit of an arduous procedure. It's also possible to use things like deep sequencing or qPCR to enumerate transducing particles if you're just trying to find like the amount of host DNA packed into your phage head. But this method requires very stringent controls because you have to get rid of all of the host contaminating DNA that's just floating around in your phage prep.

And then finally there's a related question here for then let's say if you find out your phage does or doesn't transduce, so, what is the actual limit for transduction? Does it need to be zero or, you know, below 10 to the minus 12 particles? Or is there some number which, you know, for example, if it's 10 to the minus 8 or 10 to the minus 6 , is that really then not much more than just the natural gene transfer that's happening in the environment anyway? And so, maybe less of a

concern.

So, measuring phage transduction is, I think, a useful safety measure, but then you have to figure out what really is your target for transduction. And that's kind of a risk-benefit type of analysis.

So, I want to wrap up by giving a plug. This is for our phage Galaxy, which we use for a lot of these kinds of analyses. We have PhageTerm on here, so, you don't have to deal with the command line. And so, this is a public, you know, infrastructure here which is available for phage annotation and analysis. So, you can go to this link here at the bottom.

And I'd just like to wrap up by thanking everybody and people who have funded our work over the years. And the CPT group who have contributed over the years also to everything I've talked about there. So, with that I will wrap up.

DR. PLAUT: Thank you very much, Dr. Gill. Just as a reminder, we know that there are questions in the Q&A box, and we'll try to

address them during the panel session later this morning. And if we don't have time in the panel session, then we'll ask the speakers to try to address them on their own also in the Q&A box. But for now, we're going to take a 10-minute break, and we will resume at 11:10 Eastern. Thanks.

Our next speaker is Dr. Luis Melo, a Junior Researcher at the Center of Biological Engineering at the University of Minho in Braga, Portugal. Dr. Melo, please begin when you're ready.

DR. MELO: Thank you, Roger. Good morning, afternoon, or evening, depending on where you are seeing this. I will talk today about the interactions and the complexity in biofilm communities. This presentation will be divided in three different moments.

And first, I'll talk a little bit about biofilms. Biofilms are ubiquitous in nature, and they exist in all habitats or at least are described to exist in all habitats and mainly in these five big habitats. But the take home message from this slide is that what

is described is that 40 to 80 percent of the microbial biomass present in our climate is in the form of a biofilm. And that impacts everything that I will say later on.

So, biofilms, or in this context, bacterial biofilms are communities that are attached to a surface and surrounded by a self-produced polymeric matrix. And they are so complex that there are several aspects that should be taken into consideration when we talk about their interaction with phages.

For example, some bacteria, as it was described before, have prophages and they are described to impact biofilm formation, for example. And to favor phage diffusion throughout biofilms, biofilms have water channels that allow phages to access the bottom layers of the biofilms. Also, some phages are composed by depolymerases, that is at least some of them are described to be able to degrade the biofilm matrix.

But, for example, biofilms are composed by several factors that also can limit phage predation. For example, the matrix

is described to be a barrier for also phage diffusion. The presence of other vesicles can also be described as a decoy for phages. And the last thing that that will refer in this slide is the presence of dormant, the cells that have low metabolism, or even the phage-resistant cells that when phage is present in the biofilm, these cells can dominate the biofilm hours after phage predation.

Regarding their clinical implications, biofilms, as I said before, are present everywhere, including in the majority of the infections or bacterial infections caused in our human bodies. They can be subdivided in two main groups. So, the device-related infections, for example, urinary catheters or central vascular catheters, or tissue-related infections that can be in every tissue of our body.

Regarding the interest in using phages to control biofilms, the huge interest became or increased significantly around 10 years ago where the number of publications using phages to control biofilms increased

dramatically. So, there is a lot of information that we need to track.

Regarding the work in our group, one of the first phages that were isolated in our group was for *Pseudomonas fluorescens* and this, I would say, amazing phage led to the reduction of six logs in very mature biofilms from 72 to 168 hours. And reduced the number of viable cells and also the bacterial biomass. So, this was almost one of the starting points of working with phages in our biofilm group.

But then I started my PhD. And when I started my PhD, I isolated one of the first phages regarding *Staphylococcus epidermidis* that is one of the main causative agents of device-related infections. And we observed that when we tested this phage, a Myovirus, against biofilms, it did not cause significant reductions on the cell counts nor on the biomass.

So, we wanted to understand why some phages were active against biofilms, why some phages were not active against biofilms, and

we followed that study. So, in this case, we wanted to understand if the reasons for this biofilm inefficacy were a consequence of the dormant population that resides in the biofilm or if the biofilm matrix can act as a barrier.

So, to see if the problem was the dormancy, we started to test the phage on planktonic exponential-phase cells. And we observed that the phage could reduce significantly the number of viable cells. And we also tested the phage on stationary-phase cells. And although it takes a little bit longer to make an effect, this phage was shown to be very effective against stationary-phase cells.

And as this was shown, at least at the moment, to be a rare feature, we wanted give more insights on what's happening. So, we developed a flow cytometry protocol regarding the use of live dead, and we observed that on stationary phage cells, five minutes after infection, we already have an increase on the mean fluorescence intensity of the SYBR. Which means that something is happening on the

replication, the amount of DNA or RNA are increasing and for sure are increasing their fluorescence. And this increase was constant, or not constant, increased significantly every time until the 45 minutes of infection. They were stable after 60 minutes of infection.

And 150 minutes of infection, we see three things. We see an increase on the number of dead cells, which are propidium iodide-positive. We see an increase in amount of debris here. And we see, in total, that probably you cannot see here, but we see less cell counts. So, this shows that the phage really reduced the number of cells. So, these phage show to infect stationary phage cells, as we saw on the phage counts, but we see a very quick response of the bacterial cells to phage predation. And then we wanted to understand what happened in the transcriptomics part. And we did some RT-PCR assays on exponential and stationary phage cells, having in the count phage genes and the host genes.

Regarding the exponential-phase

cells, we see that as it is described on these Myoviruses, we have a modular transcription, we see that, so, as expected. So, early genes being highly expressed before middle genes, and those are highly expressed before late genes.

Regarding the host RNA polymerase, we did not see significant responses on the host machinery. In opposition, on stationary-phase cells, we see, immediately after five minutes after infection with this phage, an overexpression of the RNA polymerase, which even increased in the minutes afterwards. And this we also see that we did not see so modular expression of the phage genes on stationary-phase cells, but you see an expression of the phage genes in almost every time when it's tested. So, these studies will be pursued further on in a larger picture, but it's what we have so far.

So, these results showed us that the dormant population of phage cells was not affecting the phage efficacy. So, then we wanted to understand if this was a question of

the biofilm matrix. For that, we used two different assays. First, we form the biofilm and then lightly scrape them, not a complete disruption; we just lightly scrape them with the tip. And just to disrupt a little bit the structure without compromising all the biofilm architecture. And just by doing that, the phage was started to being effective against the scraped biofilms. So, this suggested that the structure was really affecting the phage efficacy.

Then, we developed a FISH procedure using DNA mimics in combination with a confocal microscope, and using a triple staining with DAPI, WGA for the matrix, and this probe was developed to be red, we observed that the phage-infected cells, which are the ones -- or one for which the probe was designed, was predominantly located in the regions that has low PNAG which is the main component of the *Staph epidermidis* matrix. So, this suggests that the biofilm matrix can impair this phage efficacy against biofilms. And not to corroborate this, but to

corroborate what I'm saying, two different recent papers came out showing that the biofilm architecture can prevent the viral predation.

So, now moving on to the second part of my talk. So, I told you that biofilms can difficult phage predation and now we are seeing what can we do to overcome these biofilm challenges? We proposed four different approaches that can be used. And I will detail some of them.

So, one possible way to do it is using matrix-degrading enzymes or mechanical debridement, so something to affect the matrix as I show in the case *Staph epidermidis*, it was a problem. The use of phage cocktails can be a solution if the case of the biofilm is to increase. For example, the emergence of resistant phenotypes, and in this case, we can use the phage cocktails to reduce the development of phage resistance variant's development. Phage genome engineering can be used for a lot of things, and one of them is to increase the efficacy against biofilm. And

one that I will detail a bit more is the combined therapies, so combining phage with other antimicrobial agents.

Regarding the mechanical debridement just to show what I showed you before. So, if we do a mechanical debridement, for example, on a wound and there are *in vivo* studies corroborating that, phages have a better efficacy against biofilms. So, at least for possible topical biofilm applications, this can be a solution to increase phage efficacy against biofilms.

Regarding the combined therapies, we can use a combination, for example, with antibiotics. It is described the phage antibiotic synergism, it is a well-studied effect. And in this case, we used for, this study was performed in *Pseudomonas aeruginosa*, and we treated the cells with phage and gentamicin, and they were applied sequentially or simultaneously. And after six hours, we observed that the cells are stable. But, when they were added sequentially, in this case, phage in the first six hours and then the

antibiotic was added, then the optical density was almost, was highly reduced.

So, we did some confocal microscopy on biofilms treated like this, so treated with gentamicin, treated with just the phage, and treated with simultaneous or sequential treatment. And what we see is that when the sequential treatment was applied, it almost led to the biofilm eradication. So, for example, if a patient is in the hospital, sometimes is under administration of antibiotics. So, is nice to know what's happening on the combination of phages and antibiotics. Of course, there are antibiotics that are antagonistic with the phage efficacy, so, this should be also more studied.

Regarding phage engineering, I will not show any results regarding my work. I am doing some modifications to increase their efficacy against biofilms. But there several studies coming out showing that phages can be used for targeting biofilms. For example, one of the first studies of Tim Lu's group was to engineer T7 with dispersin B.

Regarding for biofilms, what can we do? We can add enzymes, for example, dispersin as I said, or depolymerases, or add some endolysin that is active against biofilms, on the tail, for example. There are several things that are being done at the moment, and phage engineering opened a really a huge amount of possibilities.

Now, I'm moving to the third part of my talk, which is something that also should be considered in this case more adequate to the purpose of this workshop. That is, the methods that are being used to study biofilms and to analyze their interactions with the host. I will focus more on the methods for biofilm formation and how can they impact the results that are coming out for these several studies.

For example, in our group using the same conditions for biofilm formation, the same culture media, the same microtiter plates, in this case it was microtiter plates. We just changed one thing, we put one microtiter plate at 37 degrees in static

conditions and the other was going through rotation.

DR. PLAUT: Dr. Melo, you have about two minutes left.

DR. MELO: Okay, thank you. And we observed that the dynamic conditions made the biofilm more homogenous in opposition to the static conditions in which the biofilm is more heterogenous. And regarding the phage addition, what we observed is that on static conditions, the phage reduced less the amount of biofilm cells. But it's more stable than what's happening in dynamic conditions in which after the two hours in which you have a huge reduction, at 8 and 24 hours we have a prevalence of resistant phenotypes.

Also, in collaboration with Ghent University, we did two *in vivo*-like models, one simulating epithelial cell model, another lung model, and we observed that in the 3D lung model, there is also more similar to what we obtained *in vitro*. So, almost eradication of the biofilm when we use the sequential application. But in the artificial lung model,

although we observed a huge reduction, this was far from biofilm eradication.

So, to conclude, there are a lot of future directions in which we should study the phage biofilm interactions and how to target them clinically. But also, one thing that should be included in the future is more standardized methods to form the biofilms and to analyze them in order for us to have a better comparison. I showed you that changing a little bit the model it will reflect a lot of things on our results which makes very difficult to compare everything that is happening in the data.

So, I want to thank everybody involved in this study. And, to finish, I just want to invite you to, next year to attend the Viruses of Microbes meeting that we'll occur in person in Guimarães, Portugal. And thank you for your attention.

DR. PLAUT: Great. Thank you very much, Dr. Melo. We're going to move on to our next speaker who is Dr. Edze Westra. He's a Professor of Microbiology at the University of

Exeter in Cornwall in the United Kingdom. Dr. Westra, when you're ready.

DR. WESTRA: Thank you, very much. Can you all see my slides?

DR. PLAUT: Yes.

DR. WESTRA: Excellent. Okay, well, thank you very much for this wonderful workshop and for allowing me to be part of it. So, I will be talking a bit about work that is carried out in my lab which focuses on how bacteria evolve phage resistance, which of course, is a concern in the context of phage therapy.

And of course, we all know this sort of simple life cycle where a phage, is talking to a bacterial cell, it uses a phage receptor to do so and injects in its genome. And of course, what is desirable in the context of phage therapy is that this leads to lysis of the bacterial cell in order to decrease their numbers. And so, virulent phages are the phage of choice in these clinical contexts.

Now, what could happen, of course, is that the bacteria can mutate the phage

receptor, and these bacteria, the mutants, they then are totally refractory to phage infection and are resistant to the phage. What work from amongst others, Paul Turner has shown and which will be discussed later in this conference and which was also mentioned earlier today already, is that we can actually take advantage of this process if we make sure that the phage is using something that is important for bacterial virulence. For example, an important virulence determinate once it gets mutated would lead to an attenuated pathogen that can no longer cause infections as efficiently as the ancestral strain.

So, this is something that also we became interested in and the study system that we use is *Pseudomonas aeruginosa* strain PA14. And in our case, we use a mutant of a temperate phage which is called DMS3. So, this mutant, DMS3vir carries a deletion in its C repressor gene, but it is locked in this lytic cycle. And it enables us to just explore some of these conceptual questions about the

evolution of phage resistance and how it trades off with bacterial virulence.

When we run infection assays, there are, in principle two outcomes. Either the phage is going to lyse the cell, or alternatively, the bacteria may lose its phage receptor which in our case is Type IV pilus, and this may be associated with attenuated virulence because we know that Type IV pilus is important, for example, for biofilm formation. However, for this bacterial strain, there is a third option as well, which is that it would acquire CRISPR immunity. This bacterium carries a CRISPR-Cas immune system on its genome which is shown here at the bottom of the slide. So, there are six genes, and two of these CRISPR arrays that collectively enable a bacterium to acquire adaptive immunity to phage infection.

So, CRISPR, you're probably all familiar with it. It is an adaptive immune system. The acronym is for Clustered Regularly Interspaced Short Palindromic Repeats. Basically, what this system does is that it

enables a bacterium to fight off a phage infection after the infection has already taken place. It's a post-infection immune system, as opposed to this receptor mutation which is effectively a pre-infection defense.

And it consists of two stages, I'll just briefly talk you through it. So, first of all, there is this acquisition stage where the bacterium acquires immunity. What happens there is that the CRISPR-Cas machinery is capturing a small piece of the viral genome and integrates it into the CRISPR array on the bacterial genome. So, this CRISPR array is effectively a database of viral sequences and plasmid sequences, and bacteria can accumulate these sequences in order to extend their resistance profile.

And then upon reinfection, the bacterium can use this genetic information to launch an immune system. So, basically, it produces these Cas proteins that associate with transcripts of this CRISPR RNA and are processed into small CRISPR RNAs. And this ribonucleoprotein complex carries the RNA

molecule and can basically base-pair with the phage genome and then cleave the phage genome, leading to resistance.

So, what we wanted to know is whether or not it would make a difference in terms of the virulence tradeoffs, whether the bacterium would acquire CRISPR immunity versus surface-based resistance. And to do that, we carried out these infection experiments in wax moth larvae. So, we inject bacteria that are either phage-sensitive or that acquired CRISPR immunity or surface phage resistance into these larvae and we measure how long it takes before these larvae die from the infection.

What we see is that the time it takes is shortest for the ancestral strain. But equally short for the CRISPR immune bacteria. There's no significant difference between those two. Whereas, the surface resistant mutants that lost the Type IV pilus take significantly longer to kill the wax moth larvae. So, in other words, they have attenuated virulence compared to the wild-type or the CRISPR immune bacteria.

So, that means that if we think about the evolution of phage resistance, it's really important that we are able to steer this process towards the evolution of surface-based resistance and avoid the evolution of CRISPR-based immunity.

So, what determines which of these two defenses evolve? That's a question that my lab has been studying over the last several years. And there's a range of different ecological factors. I want to discuss two examples of work that we've done today, one of which focuses on the microbial community composition which turned out to be very important. And the second one focuses on the use of antibiotics which, of course, in the clinic, is something that is often going hand in hand with the application of phage.

So, first about the biotic complexity, the microbial community composition, one of my PhD students was interested in understanding how this might affect the evolution of phage resistance. So, the type of experiments that she carried out

is shown here schematically, so basically, she takes the wild-type *Pseudomonas aeruginosa*, mixes it with phage in either the presence or the absence of a competitor species. And then after three days, she determined how many of the bacteria acquired CRISPR-based immunity and how many acquired surface-based resistance.

And what she found is that in isolation, in monoculture, if she just runs an infection experiment with *Pseudomonas aeruginosa*, the vast majority of bacteria under those conditions would evolve surface-based resistance. So, they lose the Type IV pilus. And only a very small fraction is actually using the CRISPR-Cas immune system.

But then when she added additional competitor species to these evolution experiments, she saw that the fraction of bacteria that would acquire CRISPR-based immunity would increase. And the extent to which it would increase depended on the species identity of the competitor. So,

Acinetobacter baumannii, in particular, was a very strong promoter of the evolution of CRISPR immunity. And also in mixed community where all these species are coexisting, such as *Staphylococcus aureus*, *Burkholderia cenocepacia*, and *Acinetobacter baumannii*, would trigger a lot of CRISPR immunity within those *Pseudomonas aeruginosa* populations.

And further analysis showed that this was because the fitness tradeoffs that are associated with mutation of the phage receptor are amplified in the presence of these competitor species. So, what we see here are the results of competition experiments. And we measure the relative fitness of bacteria with CRISPR immunity, the relative fitness compared to the surface mutants. And if that value is one, it means it to have equal fitness, and everything greater than one means that the CRISPR immune bacteria are outcompeting the surface mutants.

So, what you can see is that in monoculture, the CRISPR immune clones are slightly fitter than the surface mutants. But

this is amplified in the presence of *Burkholderia cenocepacia* and *Acinetobacter baumannii* as well as in the presence of the mix of all the different species, so the mixed community. So, that explains why CRISPR immunity is favored in the presence of these competitors.

Okay, now I want to tell you about a study that we put on bioRxiv recently, which looks at the effects of antibiotics and, again, the experimental setup is very similar. We run these evolution experiments and observe the effects of different antibiotics on the evolution of phage resistance. And we decided to explore the effects of a wide range of different antibiotics that act on different targets, but that also have different effects on bacterial survival versus having a bacteriostatic effect.

So, we have four antibiotics here that are bactericidal and four antibiotics that are bacteriostatic. At first, again, I want to show you the results of what happens in the absence of antibiotics. In this case,

Tatiana Dimitriu monitored the evolution of phage resistance on a daily basis, on Day 1, 2, and 3. And this is in the absence of antibiotics, so what we, again, see is that most bacteria are evolving surface-based resistance and only a small proportion evolves CRISPR-based immunity in those experiments.

And then she carried out the same experiment in the presence of a low dose of chloramphenicol. So, we are looking at sub-MIC levels that have a slight effect on bacterial growth, but very minimal. And what she sees is that under these conditions, the vast majority of bacteria are evolving CRISPR-based immunity.

And she then tested how dependent this response was on the concentration of chloramphenicol. And it turns out that this effect is observed across a really wide range, whether she is doing experiments above MIC or really tiny amounts, she consistently observes that the vast majority of bacteria evolve CRISPR-based immunity in the presence of chloramphenicol. But if she uses a clone that

is already chloramphenicol-resistant, so it carries a chloramphenicol resistance gene, these effects completely disappear. So, this is not something that has to do with a chemical effect of chloramphenicol per se, but it's something to do with the effect that chloramphenicol has on the physiology of the bacterial host.

And then she explored the effect of the total diversity of antibiotics that we decided to cover here, so that's eight different antibiotics. Four of those are bacteriostatic and four of them are bactericidal. It's all the bacteriostatic ones that trigger these effects. And we don't see any signature of a shared molecular target, some of the ones that act on translation, for example, are bactericidal as well as some that are bacteriostatic. So, it doesn't really seem to be related to whatever the antibiotics are targeting. Instead, it really seems to be related to the effect that the antibiotics have on bacterial growth. All the ones that slow down bacterial growth and are

bacteriostatic promote the evolution of CRISPR immunity.

And one hypothesis that we came up with was that this may be because phage are replicating very quickly, and that gives a CRISPR-Cas immune system limited time to clear the infection. So, if there is something that can slow down bacterial growth and, as a consequence, potentially also slow down phage amplification that could give the CRISPR-Cas immune system more time to detect and destroy phage genomes.

And to test that, Tatiana performed these one-step growth curves where she examined phage amplification in the presence and absence of these different antibiotics, and what she found is that in black, in the absence of—so, the black lines show phage amplification in the absence of antibiotics. When she was adding these bacteriostatic antibiotics, she would always see this delay in phage amplification. But in the presence of these bactericidal antibiotics, she would sometimes see no effect, for example, with

ciprofloxacin and carbenicillin. And with streptomycin and gentamicin, she would see that there was a total inhibition of phage amplification. So, there's something going on there that makes it very hard for phage to amplify altogether. But we don't see the delays that are so typical of these bacteriostatic antibiotics.

So, that led us to think that probably, you know, the reduction in bacterial growth rate and the associated reduction of phage amplification rates, could provide more time for the CRISPR-Cas immune system to acquire immunity and to then use these immune memories. So, to test that, Tatiana performed very short-term infection experiments where she measured over just three hours of infection, the proportion of bacteria that acquired novel immune memories in their CRISPR arrays. And what she saw is, again, that all the bacteriostatic antibiotics were associated with significantly elevated rates of acquisition of CRISPR immunity. So, it really seems to act on this particular stage of the

CRISPR-Cas immune response, that first stage where memories are being acquired, if bacterial cell growth is reduced and phage amplification rates are reduced, then the acquisition of novel memories is much more efficient.

Okay, so that leads to this model where we have a CRISPR immune system that is quite involved. It consists of three different stages, adaptation, then everything needs to be expressed, and then these ribonucleoprotein complexes can detect and destroy incoming phage. If we have a fast-growing bacterium where phage can also amplify very quickly, then the phage may be outpacing the bacterial immune system. And CRISPR may just be too slow in many of those instances.

However, if bacterial growth rates are reduced and phage amplification rates are also reduced, then CRISPR-Cas can actually be quick enough and can deal with those infections in a timely manner. And we actually confirmed this also by changing growth conditions using carbon sources that also slow

down bacterial growth and, again, we find that these slow growth conditions favor the evolution of CRISPR immunity.

So, I just want to summarize the findings. So, the evolution of CRISPR immunity is associated with no detectable virulence tradeoffs in *Pseudomonas aeruginosa*, unlike the evolution of surface-based resistance, loss of the Type IV pilus is associated with attenuated virulence. The acquisition of CRISPR immunity depends on a range of biotic and abiotic variables, and I've just been discussing two of those today, which are the presence of competitor species and the presence of bacteriostatic antibiotics, both of which are relevant to the clinic, and both of which promote the evolution of CRISPR immunity. And that may have implications for how we may be able to shape the evolutionary responses of the bacterial pathogens in the clinic.

Of course, I should also point out that all this work is done with a system that is a model system really to study bacterial

phage coevolution and the role of CRISPR. It never was our intention to study, you know, the therapeutic application, although the projects have moved in that direction. These are maybe not the phages that one would choose for clinical applications, so, it will be very interesting to see whether or not these same effects will also be observed with virulent phages that may be candidates for the use in therapy.

All right, I want to acknowledge, in particular, Tatiana Dimitriu, a post-doc in my lab, and Ellinor Alseth, who did her PhD with me and is now writing up her thesis and will soon be moving to Georgia Tech on a fellowship. I would like to acknowledge the funders, ERC, NERC, Leverhulme Trust, BBSRC, Wellcome Trust, and the Marie Curie Actions. The paper on the effects of bacteriostatic antibiotics is on bioRxiv. I also want to acknowledge the collaborators who took part in these studies and, of course, everyone in the lab. And thank you very much for your attention. I'm happy to take any questions

later. Thank you.

DR. PLAUT: Thank you, very much, Dr. Westra. We're going to move on to our next speaker and, again, we do plan to address the questions in the panel session to the extent possible. Our next speaker is Dr. Dominic Sauvageau, who is an assistant professor in the Department of Chemical and Materials Engineering at the University of Alberta in Edmonton, in Canada. Dr. Sauvageau, you can start when you're ready.

DR. SAUVAGEAU: Thank you very much for the invitation to talk today. And I will talk today about the method that we developed to assess virulence and phages. So, the previous talk was talking about bacterial virulence and in the case here, we're talking much more about the virulence of the phage against its host.

So, the first thing is, I have to acknowledge the great contribution from my group. This was a pre-pandemic picture so some people have moved on and new people have been added since then. But our group is working at

many things, but amongst them is doing a lot of work on the development of phage-based technologies and also in phage production.

And in that course, like basically it may seem like a very applied thing, but at the core of everything that we do, we need to understand that interactions between phages and hosts. And it's in this context that we developed the method that I'll be talking about today.

So why are looking at potentially standardizing phage characterizations? Well, standards are very important, I'm sure we all understand that. They're important to understand phage properties properly, so comparisons between phages, reliability of a phage or reliability of the behavior of a phage in different conditions.

Also, we may want to demonstrate manufacturing consistency, and overall, standards are used to set up a proper regulatory framework. So, in the standardized method, our data should be informative, of course, reliable, measurable, so quantitative

data, and then also comparable between situations. So that's the mindset that we had in approaching this.

Of course, when we characterize phages, we're looking a lot at morphology, the genetics, the host range, perhaps the absorption. But there's also a very important factor which is the ability to kill its host. And that's defined in many different ways. We talk about virulence, we talk about infectivity, we may talk about phage fitness, and its replication. But all of these things are related to rates. Phages, phage infections are dynamic systems, so a lot of my thought process goes into rates, how fast things happen in relation to one another.

So, what we would want in developing a method that can assess virulence, we wanted it to be amenable to phage, phage products, or phage cocktails. We wanted to be cheap, rapid, easy, high throughput perhaps, to use in an automated system or manual so that even if you don't have robots, you can still do the method. We want it to be standardized, of

course, reproducible, and versatile.

But most importantly, what we want out of virulence, out of a virulence metric is for it to reflect the dynamics of the infection and to provide us with ideally a number, because numbers are good. They help us compare, order, list, etc. So, what would a method like this be used for. Well, we could screen phages to select the phage for a given application. We could compare between variants of phages, compare performance between media and conditions, is our phage just as efficient at different temperatures, for example.

We want to test between sites. When we ship a phage over to someone else's lab, we would like it to be tested the same way. We can test the efficacy of a product, the stability of a product. We can use this in a QC/QA system, and maybe we want to develop formulations as well. So, in this context, also the idea of assessing synergistic and competitive interactions is very important. And as we just saw in the previous talk, the investigation of the rise of resistance also

can be very important.

So, what are the factors impacting virulence? Well, that's a very complex question. You know, there are multiple factors, and I guess that's why it becomes a very big task to try to access every single one of them individually. So, on the phage side, we can think about the adsorption rate and adsorption efficiency. So, the recognition of the host by the phage. The replication rate, the burst size, the eclipse, the lysis time, etc. So, all of these factors will play into how well or how quickly a phage is able to kill its host.

But there's also factors on the host side. So, how fast is the bacteria growing, what phenotype is expressed. What's the density of the receptors on the surface of the cells because that will have an effect on adsorption, for example? At which point in the life cycle is the host cell, what are the growth conditions, etc.

And to add to all of this, there are also factors that come from the environment.

So, are there cofactors that help for the adsorption, are for their competitors, inhibitors, etc. So, we end up having a dynamic system that's quite complex.

I will just give you a small example. There's a study that we're preparing that we did a few years ago where we used phage B1 and phage B2 which are phages of *Lactobacillus plantarum*. And then we looked at their different parameters, put them together in a competitive infection environment. And if you look at the parameters for these phages, the adsorption rate constant is much faster for B2, but the adsorption efficiency is lower. So that's going to have an impact on how the competition will take place. Also, the lysis time is much shorter for B2, but the burst size is much smaller.

So, if you're looking at these two phages and then you put them into a system with just these parameters, it's very difficult to know which phage will outcompete the other and by how much. And when we did this, we saw that phage B1 which is the solid

line here was actually, although this graph is on different scales. So, what's important to see here is that phage B1, although it has a much larger burst size was actually outcompeted by B2 by two orders of magnitude, which is non-negligible. And this is not something that we could have expected just by looking at these parameters.

The other thing is that there is the reality that dynamics, they're dynamic systems, and when we talk about dynamics, we're not just talking about time, they're dynamic genetically as well. So, there's more and more evidence that shows that, you know, there's hypermutable regions in phages. So that even when we have a single round of replication, we have a diversity in the genetic population, which has the impact that even when you pick a plaque, although you have a dominant sequence, you're not necessarily looking at a clone of phages, you're looking at already a population that's quite diverse.

So that in essence brings us to the point where, well, if we have these kinds of

dynamic systems, what are the important factors when assessing a phage product that's heading towards clinical studies or therapeutic use. And one thing that is important is what's the efficacy of the products, right. So, we can handle some diversity in the genetics as long as our product is still behaving the same.

So, just typical method that anyone working with phages will be familiar with. But, you know, we already tried to characterize phages in many ways. Some of the methods include titer assessment which is, you know, a measurement of the active phages essentially, but it's a static measurement. It doesn't give us any information about the dynamics of the infection. The efficiency of plating is the same thing.

One-step growth experiments provide some useful information, but to be honest, they're tedious and time-consuming and they're very difficult to automate and even standardize between groups, let alone between students.

Another approach that a lot of people use is bacterial reduction curves. So, this idea that you're comparing the optical density of cultures that are infected, and this is great. This provides information on the dynamics of the infection, but there's really a lack of standardization to what, like, how do we measure things out of this. Some groups have used visual inspection to just see the level of lysis in a qualitative matter.

Other groups have used an OD at different time points, which has limitations as well because it doesn't tell you about the history of the infection or how the infection is proceeding. And other groups have used area under the curve, which is the basis for the method that we'll be talking about today. So, the advantage of the area under the curve is that it provides you with information on the equivalent of a person-hour equivalent. Like a bacteria-hour equivalent during the infection process, and I will be talking about that in a second.

So, with this premise, we developed the virulence index method. As I say, it's based on the bacterial reduction curve. So, this is just a schematic of the reduction curve at a given MOI. And what we're interested in, as I said, is the area under the curve because that tells you essentially how quickly the infection is overtaking the bacterial population.

So, it's based on a 96-well plate assay, but it can be done in other systems, and I'll just take you through this very quickly. On the 96-well plate at the bottom left, you can see a control which is just the medium, that's your medium reference. Then above it is the control or reference with the host population alone. So, there's no phage in the system. And this essentially is, as I said, a reference. You're comparing infections to this, to these cultures, so that you can assess the virulence in terms of a relative impact on the host growth, right.

It also ensures that you're performing all your assessments against a host

that's growing under the exact same condition that your infection is taking place at. So, if your conditions lead to a slower-growing host, that doesn't mean that your phage is less virulent because the infection takes more time. Again, it's a comparison against the kinetics of the growth of the host.

So, in the rest of the plate, there is the exact same number of hosts in every single well. That's our graph. Then we move on to, each column will have a different range of MOI starting from an MOI of 1 to 10^{-7} in dilutions. So, that range is pretty important because that's your dynamic range for the evaluation of the virulence. And also, it's low enough that in most cases, you avoid factors like lysis inhibition, lysis from without, that has a big impact on the infection process, or the length of the infection process.

The other thing is our lowest MOI is 10^{-7} , we never go below that because essentially, we're looking at a Poisson probability distribution. So, in the lower

wells, you want to have at the very least one phage per well. So, going below that dilution would be too many wells having no phages at all. So again, that MOI range is your dynamic range for the evaluation of virulence.

The other thing also is why are we looking at a range of MOIs and not just one MOI. Well, it can account for eccentric OD patterns during lysis and also can give you more information on the efficacy of the phage against a host. So, just to recap, in the 96-well plate assay in the first column, you've got some host growth and some medium, wells containing only medium.

In column two, you have phage at different dilutions to provide you MOIs from 1 to 10^{-7} . And generally, what we do is we took three columns as replicates so that we're able to account for variations in the results. So, when you do this, if we're looking at the growth of the host alone, we're getting just a normal growth curve and again, that's our point of reference. But there is one thing that is very important here is we will use

this to establish a limit of integration to show where exactly we'll integrate.

So, then we're looking at our three replicates at an MOI of 1, and then we can get a first curve, and we're going to move on to our replicates of an MOI of 0.1, get a different curve and so on. So, once I have all of this data, I can establish a limit of integration at the flattening of the growth of the host. So basically, when we are well into stationary phase when we reach a plateau, this is our limit of integration.

And even though lysis is not necessarily finished in all cultures, in fact you'll see in further data that some cultures have not lysed completely at all, that's not important because what I want to compare is how the dynamics of the infections are taking place over this time period, and that information is provided.

So, I use my control reference. I do area under the curve. For those of you who are not familiar, you can use some very simple algorithms like the trapezoid rule, so that

allows you to integrate and get the area under the curve. Once you get it done a few times, it's not a problem. It's something that's fairly simple and can be set up as a macro or as a program, if not done by hand.

Then if I'm using the MOI of 0.01 as an example, I'm going to do the integration or the area under the curve with the same limit. Once I have these two areas, I can get a first parameter that's called the local virulence. So the local virulence tells me that at an MOI of 0.01 or 10^{-2} , I'm going to get an index that goes between zero and 1, zero being that the phage is not able to infect the host at all, and 1 being absolute virulence which would be that all cells are lysed at time zero. So, I'm getting this scale from 0 to 1, and closer to 1 being more virulent. So, it's 1 minus the ratio between these areas, area 10^{-2} over area of the reference.

And I can do this for each MOI. And then I plot the log of the MOI versus the local virulence for each of the MOI tested, and I get this curve. And that curve is the

virulence curve, and because we love integrals and areas under the curve, this information, the shape of the curve itself is providing you with a lot of information already. So, that's already a characteristic of the phage against a host under some conditions.

But if I want to get a metric out of that, a general metric that spans our whole dynamic range, I can do the area under the curve using the same algorithms, the same method, and I get an area of the phage. And then my virulence index at a given temperature in a given medium for a given phage is going to be this area over the maximum area of possible which is 7. So that's calculated for you if you're using the proper range.

So again, for a phage that would not infect at all, I would have a flat line at 0 and my virulence index would be 0. And for a phage that would have infinite virulence, I would have all points at 1 and then my virulence index would be 1. So, for any phage that's not imaginary and ideal, you'll get a virulence index somewhere between 0 and 1.

So, there's another metric also that can be very useful and that's the MV50. It's similar to LD50 in toxicology, for example. So, this is the MOI that provides you, or that leads to a local virulence of 0.5. So, on the graph here, it's fairly simple. You go from 0.5, you catch your line and then you go and get the MOI.

DR. PLAUT: Dr. Sauvageau, you have about two minutes.

DR. SAUVAGEAU: Oh, I'm going to go quickly then. So, we have three metrics. The local virulence between 0 and 1, the virulence index again, between 0 and 1 but over the whole dynamic range, and the MV50. And all of these depend on the environmental condition so that's why the reporting temperature and medium are important.

So, I'm just going to go quickly, comparing phages. So, comparing T4, T5, and T7, we can run on a single plate the assessment of phage T4 virulence index of 0.6, T5 will have again, you can see in the curve here how at low MOIs T5 is not very efficient.

And then at T7 we get much higher values. So, our virulence index is 0.84.

And then we can play around with media and temperatures. So here we have brain heart infusion at 30, 37 and TSB medium at 37, and then we can get these metrics that are allowing us to get very quick comparison of the different phages.

We can also use this in phage cocktails. So, here's a study that was done with a group in Leicester where we used local virulence with different phages, and you keep the individual MOIs constant. You can look at different formulations. So, here's a heat map, which is darker colored means combinations that have higher virulence.

And from that data, you can compare. So, these are spider plots, and very quickly just to show because I think this is pretty useful. So, in orange is the data for an individual phage. So, for JK08 it was very low. When I combine it with 113, already I'm getting a higher virulence index. So, that means that there's synergistic effects between

these phages. And then when I put all three phages together it's the red dots, and again, that shows the efficacy of the phage product or the cocktail. So, you can use virulence index for a wealth of applications. So, phage variants, the impact of genetic modifications on a phage, phage stability, rise in resistance, etc.

And then finally, I just want to point this out. This is not a perfect proxy for infections of bacteria in human and animals. So, it's not always a good predictor but it does provide a clear basis for comparison to head into those systems. And finally, the method can be applied for biofilms although it hasn't been done yet. But theoretically, it's possible.

And then future applications would be integration in QC/QA practices and also integration of artificial intelligence based on both the killing curves and the virulence curves. So, I want to thank all the students and collaborators and the funding, and I guess if there are questions, I can answer them in

the panel.

DR. PLAUT: Great, thank you so much, Dr. Sauvageau. All right, we're now going to begin our panel discussion for this session. So, if our panelists could please share your cameras then we can begin. All right, great. Okay, so I am actually going to begin sharing because I wanted to kick off our discussion with a slide that I presented, so hopefully we can all see this.

So, this was a slide that I presented on the current consensus as we see it for characterization of phages for therapy. And so, I just kind of wanted to open the floor to anyone on this panel. Do you have any comments on the attributes that are on this slide? Is there anything that you want to add to what I've said or clarify?

I mean, I know that Dr. Gill addressed whether we could assess the possibility that the phages could be lysogenic or transducing based on their sequence. So, I think that was a great summary that he provided. Anybody have any other thoughts? If

not, I'll just go to some of the questions that we've gotten from the Q&A.

Okay, that's fine. I will go directly to the questions then. So, we had a question in the chat regarding INTERACT meetings. So, I wonder if Dr. Fiore could just briefly describe what an INTERACT meeting is and the circumstances where it's appropriate.

DR. FIORE: Sure. So, INTERACT meetings are available. They're intended for novel products that introduce regulatory, unique regulatory challenges due to unknown safety profiles that are the result of complex manufacturing technologies and issues. They could also incorporate innovate devices and the use of cutting-edge testing methodologies.

The pre-IND meeting that I spoke about is comprehensive, and so depending on your product and if your product falls into those guidelines, a pre-IND meeting may fit the bill better because it is more comprehensive but that is what INTERACT meetings are.

DR. PLAUT: Okay, thank you, Dr.

Fiore. Let's see, we had a question about, there were a couple of questions that I can address. So, one was about environmental assessments. So, the question came up because I had a slide about genetically engineered phages and I pointed out that genetically engineered phages are not necessarily considered to be more of a safety concern than so-called natural phages. And then the question is well what about the environmental impact of engineered phages.

So, I'm going to post in the Q&A a link to a guidance document on this topic. The FDA requires an assessment of environmental impact of a biologic and so, let me just pull up what I want to say here. So, when you submit your IND original submission, you need to include either an environmental analysis or a claim for categorical exclusion from the need to conduct an environmental analysis.

So, generally when a product is being studied under IND, because it's a relatively small number of people that are being treated with that product, most INDs are

eligible for categorical exclusion, there are some exceptions. So, I would just direct you to that guidance and remind you again that we make our decisions based on the information that we receive in each application and that we make our decisions based on science. So that's the best response I can give you to that question.

Let's see, so I wanted to bring up the issue of using antibiotics in combination with phages because that came up in a couple of presentations. And we're going to be hearing more about that in the coming days. So, Dr. Melo, you discussed how the biofilms were more easily eradicated if phages and antibiotics were used in combination. And Dr. Westra, you described how with some antibiotics, like with bacteriostatic antibiotics, that CRISPR-based immunity is more likely to occur. So, I just sort of wanted to discuss how you would sort of reconcile those two ideas and also if anybody else wants to chime in about the idea of using antibiotics and phages sequentially or using

them at the same time, please do so.

DR. WESTRA: Yeah, maybe I can just make one comment about this. Something that I perhaps didn't point out clearly enough in my presentation, which is that CRISPR-Cas immune systems are not universal. I think probably the strain that Luis used, they don't have CRISPR- Cas, I imagine.

DR. MELO: It was *Pseudomonas*.

DR. WESTRA: Which strain was it?

DR. MELO: It was PA01.

DR. WESTRA: Yeah, so it doesn't have a CRISPR-Cas immune system either. You know, that may explain some of those differences. I think, you know, it's usually an important question obviously to think about the impact of these antibiotics, different classes of antibiotics. We did some further experiments I didn't show today that also show that in the vast majority of cases where we combine antibiotics and phage at the same time, we co-administer the two *in vitro*, they seem to interact antagonistically. So, it's quite rare for them to actually act synergistically or at

least in short term. So, the dynamics is going to be quite complex but it's not again in that combination of the two will lead to better outcomes.

DR. MELO: I can add to what Dr. Westra has said. Yeah, our best results were actually on the sequential application of both, not the simultaneous application. In some cases, we see some synergistic effect when they were applied simultaneously but we always get better results when they're applied sequentially. But we are just trying also to figure out what happened, what's happening in a patient that can be hospitalized. So, he's probably under antibiotics and if you think on applying phages we probably want to see what we are dealing with.

DR. PLAUT: Sure and, you know, in terms of clinical trials, it's important that the participants in the clinical trial not be delayed the standard of care unreasonably. So, you know, if you're proposing to treat with a product that is not approved, you want to make sure that you're not preventing someone from

getting the treatment that they need.

So, you know, it's pretty typical for sponsors, you know, it makes sense for some sponsors to propose using their phage product in combination with antibiotics. And so, I just wanted to again, bring up the issue of sequential or simultaneous administration. I don't know, Dr. Gill, do you have any thoughts about, you know, whether it would make sense to use phages before you use antibiotics so that the phages have a chance to replicate and then treating with antibiotics or alternating? What do you think about that?

DR. GILL: Well, I think the interaction is pretty complicated, as was mentioned. It's not, universal that phage-resistant mutants will become more sensitive to antibiotics. It can go only go either way. We've seen that with *Klebsiella pneumoniae* that in some cases, the MIC can go up when it becomes resistant to phage.

So, it really depends on the drug and on the phage, on the host and on a lot of

things. There's no kind of universal principle. I think it helps, if you actually understood the underlying principles for why, for example, you had synergism then you could select the phages that promote that because that could be done bioinformatically. But you'd have to have some understanding of the underlying mechanisms first which is still, I think, pretty opaque at this point in most cases.

DR. PLAUT: Okay, great, thank you for that. There's another question that I think I can address, and it has to do with exotoxin levels. So, there was a specific question, what does the FDA consider an acceptable level of exotoxins in a product. I'm going to say again that we make our decisions based on the information that's presented to us.

So, as I mentioned before, if you can grow your phages on a strain that doesn't produce exotoxins then that's ideal because then we wouldn't be worried about contamination with anything in particular. But

if you are growing your phages on a strain that does produce an exotoxin, then in your submission you'll just need to propose a level that you think is appropriate, describe the method that you're using and, you know, justify that level that you think is appropriate and we will evaluate it on its merits.

And, of course, not all exotoxins are the same, so some are extremely toxic, extremely potent. And so again, we'll be making those evaluations on a case-by-case basis.

DR. FIORE: I just wanted to add, and Roger was very thorough. This was a question you can ask us during a pre-IND meeting and get our feedback. This could be a very important question, and I would recommend that if you're concerned about it, to ask us during, in the context of a pre-IND meeting.

DR. PLAUT: Exactly. Thank you, Dr. Fiore. Let's see, Dr. Westra, there was a question about whether you saw the delay in CRISPR-based immunity in strains that were

already resistant to antibiotics. Can you address that question?

DR. WESTRA: Yeah, I just typed an answer to that. So, I interpreted this question as being about the speed of phage replication which became slower when bacteria were exposed to bacteriostatic antibiotics. It's a great idea to also do that with an antibiotic-resistant strain, which we haven't done. So, it's a very good suggestion. Of course, we carried out evolution experiments with antibiotic-resistant strains and we saw that those strains were not affected by the presence or absence of antibiotics.

And so, the prediction from those data would be that the phage replication phage would also not be affected in the resistant strains, but we didn't carry out the experiments. It's a good suggestion, thank you very much.

DR. PLAUT: Okay, great. Dr. Melo, there's a question. Given Dr. Melo's results, would you expect formulations with antimicrobial preservatives to be less

effective than those without due to slowing of bacterial growth and therefore propagation of the phage. Actually, I think that maybe well, I'm not sure whether that was intended for Dr. Westra. Talking about slowing down bacterial growth.

DR. MELO: Is it really for me?

DR. PLAUT: It's not clear. They addressed it to you, but you can take a stab at it if you want.

DR. MELO: I'm not sure. It depends on the preservative I would say and the chemical interactions with the material. Because we don't know if also, they are universal or if they slow bacterial growth or if you can think about further because we did not do that.

DR. PLAUT: Okay. Dr. Westra.

DR. WESTRA: Yeah, I don't see this question, sorry. I'm scrolling through the list of questions.

DR. PLAUT: Oh, that's okay. So, it just says would you expect formulations with antimicrobial preservatives to be less

effective than those without due to slowing of bacterial growth and therefore propagation of the phage. So, this is again, getting at the idea of timing.

DR. WESTRA: Yeah, yeah. So, this is in our hands, you know, a very important factor. If we add antibiotics, we often see antagonism between the antibiotic and the phage due to population dynamics effects that the antibiotics have. Rarely we see synergism where the antibiotics are actually, you know, make the phage more effective.

So yes, in general, I think that's the expectation. I also would like to add to that, that a lot of that knowledge is based on experiments in test tubes and, you know, the dynamics will be so different in more realistic environments when we, you know, add some more ecological realism to our experiments we often get very different outcomes. So, we should be pretty careful extrapolating from test tube experiments, I think.

DR. PLAUT: Okay. There was another

question that I would like to address. So, there was a question about the fact that some phages can bind to either virulence factors or antibiotic resistance proteins, and that therefore strains that would become resistant to the phages would be more susceptible to antibiotics or would be less virulent.

So, the idea is that that would be something that you would try to determine either *in vitro* or in an animal model. And then the question was well, what about the strain that you're growing your phages on when you're producing your product. And for that, you would not necessarily need to use a strain that has the—you would not need to demonstrate that effect in that strain necessarily. It would need to have the receptor that you think—it would need to have the same receptor as a strain that you would be treating a patient, you know, that a patient would have.

But the fact that you are doing that in, you know, as part of your manufacturing process, you don't need to demonstrate it with that strain necessarily. I hope that sort of

answered the question that was in the chat.

And another question about using strains that are well-characterized to manufacture your phages, there was a question about well, wouldn't that necessarily mean that those phages would be less effective against a strain infecting a patient. And that is really an open question. So, there's this idea that you need to train phages, it's called, where you try to adapt them more optimally to a strain that you're trying to treat. And so, that is possible to do but it's not necessarily something that you absolutely have to do.

So, I think that that needs to be evaluated on a case-by-case basis whether growing a phage on a well-characterized strain that perhaps possess fewer virulence factors, whether that would reduce the effectiveness of your phage versus a strain that's actually infecting someone. So, I don't think we can draw—make any generalizations about that.

And again, if you're submitting an IND, we would want to see what you're

proposing to do and justify why you're using a particular strain or strains that you're using for your manufacture, and we'll discuss it. And again, as Dr. Fiore mentioned, that's the kind of discussion that we can have during a pre-IND meeting, and hopefully those issues, those possible concerns can be resolved. So, when you submit your original submission for your IND, you'll know pretty much what to expect our response to be.

DR. WESTRA: Could I add something to this? I think that's a very interesting point what the, you know, the host strain should be when amplifying the phage. And I think as we are gathering a lot of insight into the mechanisms that bacteria use to defend themselves against phage, and CRISPR is just one example. As many of you know, over the last several years, dozens and dozens of previously unknown defense systems have been identified, and, in many cases, they were based on epigenetic, self/non-self discrimination mechanisms.

So, I think once we have a better

understanding of how that process works exactly for all these normal defense systems, we can also use that knowledge to optimize the production of phage, so that they carry genetic modifications that enable them to bypass known defense systems and perhaps even use genetic information of pathogens from a patient, in order to make sure that the phages are produced accordingly, sort of a personalized medicine-based approach.

DR. SAUVAGEAU: If I may, I also wanted to add that that can be addressed pretty quickly if you're looking at post-production assessment. So, virulence is an example of this where if you have a good characterization in the host, you do a production and then you go back and run, for example, a virulence index analysis. You can see if the dynamics of the infections are remaining the same on the target host even after production in the secondary host. So that's the kind of things that can be done. Even if you don't understand the whole processes of the genetics terms but it can be

done actually in terms of product efficacy.

DR. PLAUT: Yeah, that's a great point, thank you. I see a question in the chat for Dr. Gill. The question is, you make the point that we need to think about what level of transduction presents a concern. Do you have any thoughts about how to address that question, taking into account different infectious contexts?

DR. GILL: Well, it's a complicated question, actually, because the—so let's say the phage is able to transduce, and if you're looking at a gut or a wound community, there's other organisms there. And there's going to be some background level of genetic transfer, right, through the phages that are already there, and also, and actually, the rate of phage transduction might be relatively minor compared to, for example, the rate of conjugal transfer of plasmids or the ability to acquire, you know, DNA just from the environment through naturally competent organisms.

So, the ability that of a phage to

move things around actually might be relatively limited compared to just natural transformation and conjugation because, you know, transduction is also limited by the host range of the phage. The phage actually has to be able to infect the cell to transduce into it, so that actually might make it more limited than for example plasmids or natural transformation mechanisms.

But my understanding of these systems also is that we just really don't know how much natural transformation happens. Like what frequency DNA moves around between cells in these contexts. And so, I think you need to have some idea about what is the acceptable background level of natural horizontal gene transfer and then that the phages just aren't increasing that to some large extent.

And also, that the phages aren't able to specifically mobilize things like virulence determinants or antibiotic resistance determinants, which has been seen in some cases. Either the phages themselves, or something we haven't really talked about is

sometimes phages can activate elements that are already in the bacteria like SaPIs or PICIs they're called now, which will also transfer these elements. So, you have to make sure you're not triggering these things.

So, I mean to me, the ability of the phage actually to degrade the host chromosome is really important because then there is nothing left, a PICI can't work. There's no DNA for it to package and the phage can't transduce if there's no chromosomal DNA left. So, that seems to be something that's worth looking at in the phage life cycle.

DR. PLAUT: Okay, thank you, Dr. Gill. There's a question in the chat for Dr. Sauvageau. How does the virulence index method deal with late occurrence of insensitivity? For example, if the OD drops at first and then one hour later starts recovering and eventually recovers completely, but the maximum OD of the culture with no phage occurred earlier than that at 45 minutes for example, how would this index account for this?

DR. SAUVAGEAU: Right. Well, this is where, for example, the range of MOIs is important because you're going to see slope or if you have a rise of resistance or something like that it will occur earlier and in higher MOI. So, this is where, for example, your trend of the virulence index can start to show a maximum.

So, at the MOI, you can have a reduction in your virulence because you're going to see the occasion of resistance which will have an impact on the integral that we were talking about in that method. So, there are ways that you can do that.

I also mentioned briefly that you can adapt a method to actually investigate virulence. I actually want to actively look at it. So, this is where your limit of integration can be much further down into the infection process and then you can look and actually rate the increase of virulence. So, you can look at things like the slope or how, again, the integral is increasing faster in different cases.

So, you can definitely account for this. I also mentioned briefly about the eccentric lysis patterns where we don't see the full collapse of the culture, you're seeing more of the flattening. Again, from a relative comparison perspective, that's not a problem. Because these trends will be consequential with the different MOIs that you're investigating.

DR. PLAUT: Okay, thank you. There's a comment in the chat from Andrew Camilli. He writes, antagonism between phages and antibiotics doesn't necessarily mean the combination won't be more effective in humans or animals. This often happens when testing antibiotics or antivirals *in vitro* but the combination turns out to be superior in humans.

So, this is getting to this question of, you know, if we see antagonism or synergism between antibiotics and phages *in vitro* in a particular method, what can we really conclude about what to expect in animals and should we always be testing those

combinations in animals? Anybody want to jump in on that?

DR. WESTRA: Well, it kind of relates to the comment I made earlier as well that we have to be careful about how we interpret data from test tube experiments. I completely agree with this statement that, you know, you can't just extrapolate these findings necessarily. I mean most, you know, environments in a patient are much more heterogeneous both in terms of phage exposure and antibiotic concentration perhaps. It's very difficult to predict exactly what's happening in an environment like that and the interaction with the immune system as well.

So, I think moving towards perhaps, well, *in vitro*, *ex vivo* systems and then definitely also *in vivo* models to explore these questions is very important. I also would like to add that the patterns of antagonism and synergy at least in our experiments, they tend to be quite transient. There may be initially an antagonistic interaction, and then in the longer term that

may turn into synergistic interaction. So, trying to cover that sort of dynamic change over time is I think also very important and will help to understand maybe what goes on in a patient.

DR. PLAUT: All right, Dr. Fiore, there was a question about the difference between the information needed for a single-patient IND versus a so-called regular IND. Complex question, can you address it?

DR. FIORE: Sure. So, the bare bones is going to be the same. You have to have product information, clinical information, and if you want to provide some sort of animal data, that is fine too. The level of detail will depend on the benefit-risk of what you're doing. So, for a single patient it's going to be usually much less detail than if you're doing a clinical trial with thousands of patients, obviously. But the categories are all the same, it just depends on the risk-benefit to the patient population that you're treating, whether it's one patient or a thousand.

DR. PLAUT: Yes, thank you, Dr. Fiore. I'll just add that the risk versus benefit calculation is something that we do consider all the time but particularly in single-patient IND cases, it's really on a case-by-case basis. So, every patient is different, and the options that are available to that patient are different and, you know, possibly even the risks of any experimental treatment could be different too. So, those need to be evaluated very carefully.

All right, we're coming to the end of our panel discussion here, and I'd just like to remind the panelists that if you are able to address the questions that are in the Q&A section, please do so, and thank you very much for your participation. It is now time for our lunch break, so we have a 30-minute lunch break and we'll be back at 1:10 p.m. Eastern Time. Thank you.

(Recess)

DR. LEHMAN: Welcome back, everyone, from our break, whether that was lunch or dinner or something else, depending on where

you are joining us from. We have four talks this afternoon in this session, and then we will switch to the interactive breakout sessions. If you're joining us for the first time today, please use the Q&A box to submit questions for the speakers. We do not have a panel discussion at the end of this session as you will see from the slide. So, hopefully, each speaker will have time to answer one or two questions verbally, and we'll encourage them to answer remaining questions afterwards. The questions submitted through the Q&A box won't be visible to everyone but answered questions should become publicly visible. And with that, we will move to the first speaker, which is me.

So, today, I will be talking a little bit about some of the scientific factors that can affect the choice in development of assay methods that are used during product development.

DR. PLAUT: Dr. Lehman, we don't see your slides.

DR. LEHMAN: Oh, shoot. Thank you,

very much, Roger. All right, so my comments represent my best judgment as a scientist and do not bind or obligate the FDA. I'm going to talk a little bit this afternoon about some of the roles of QA and QC assays in product development, and some of the general considerations for selection and design of those assays. Mostly, I want to focus on two specific examples, host cell protein and next generation sequencing, as a means to discuss some specific scientific considerations that can affect how assays are chosen and used.

I want to be very clear, I am not advocating for one method over another, nor am I attempting to define specific requirements for any one product or stage of development. The purpose of this talk is to discuss some of the scientific factors that can influence assay selection and development.

So, what are the roles QA and QC assays in a development program? Direct testing, whether it is conducted on cell banks, phage banks, drug substance, drug products, that is one part of managing product

quality, and it is far from the only part of managing product quality. Testing occurs at different stages with different purposes. Release testing focuses on critical attributes of drug substance and drug product. So, things like identity, potency, sterility; these tests have acceptance limits that have to be met in order for that material to be released for further use. There's also in-process testing, which generally monitors some aspect of the manufacturing process. Maybe, it's predictive of process success or failure. And these generally have action limits, some value above or below which some action should be taken to manage that manufacturing process.

And additionally, there is characterization testing. Some of this was discussed in detail this morning by several of our speakers. Things that look at the intrinsic properties of specific stages, whether they are lytic, non-transducing. But there is also general characterization testing that might be intended to inform process development. So, things that aren't product

release testing but maybe, you're characterizing certain aspects of your final product in order to understand more about manufacturing consistency. Maybe, this is going to help you choose something to use as the release testing later on, maybe it is going to help you refine your production methods as your product development continues.

The expectations of assay development as a whole are related to the expectations for product development. So, as Roger was discussing this morning, safety is a primary consideration at all stages of product development. And then, assay development progresses as product development does. So, in early stages of development, it's most important that assays support product safety. And then, as development continues, assays have to support these other aspects of product development, and to do so in an increasingly robust way. Some assays may be used very early in development and then replaced by something that's better. They may also continue to be developed themselves and eventually, validated

for Phase 3.

What are some general considerations for picking or designing assays? You know, for a number of things, there may be a compendial assay available. So, this is always worth spending some time looking for. These are also sometimes called pharmacopeial assays. I've listed a few of the U.S. Pharmacopeial Standards here for sterility and endotoxin. Other national bodies define pharmacopeial standards as well, the European Pharmacopoeia, for example. And many of those have been harmonized with the U.S. Pharmacopoeia for international exchangeability. There may also be an FDA-cleared test that is available and that is suitable for use with your product. So, if those exist, those can be some great questions to have answers for and help you choose your assay.

If you'll be developing your own assay, or adapting a commercially available kit, spend some time thinking about a few key questions. What is the purpose of the assay? Is it release testing, or something more

exploratory? What's the material being tested? Are you looking at drug substance, drug product, or process intermediate? These may affect how much data you want and how you go about generating it. And ultimately, be very clear what you want to be able to say about the particular material, based on your assay results. And really spend some time asking whether your assay design and the supporting data provide scientific justification for the conclusions that you want to draw.

And those two questions, you know, what is the purpose of the assay? And what are the conclusions that you want to draw? Are going to shape—so, I guess, those are the main themes of the two examples that I am about to work through.

So, the first example is host cell protein. In general, phages are grown on a bacterial host, and then they are processed to remove residual culture medium and various host components. Things like exotoxins, and host-derived proteins, and nucleic acids. Host cell proteins like exotoxins potentially pose

a direct safety risk to the patient. And even if there aren't specific toxins that are a concern for your bacterial species, residual HCP can lead to unintended immune responses that may also be a concern.

So, what are some of the components of risk mitigation? There are a number of strategies that can be incorporated into this kind of a plan, and they may be useful at different stages of development. One of the most basic is understanding what the high-risk toxins are for the bacterial species of interest. If it's *Pseudomonas aeruginosa*, exotoxin A is a fairly potent toxin. Understanding whether those specific toxins that are relevant to your species of interest are actually encoded by the bacterial host being used. So, good genome sequences of a host can be valuable in addressing this question.

You may also be able to evaluate whether the purification process itself is good at removing substances that have similar properties to either a specific toxin or the

host cell protein for your bacterial species in general. And of course, there is direct testing for residual host cell protein in the drug substance, drug product, or various intermediates. And these are different components, as I mentioned, that come together to create package of information and as I mentioned, can be used at different points in development.

I want to break this down a little bit into some specific considerations for evaluating a purification process, and some considerations for direct testing. If you're going to look at a purification process, one common approach is to try a spiking study. So, this is an experiment in which a known quantity of either a specific toxin or some other indicator protein gets added to unprocessed lysate, and then, the removal of that protein is monitored over the course of the purification process. In that approach, it's important to understand whether you can assay your toxin or your indicator protein reliably. And maybe that question actually

influences whether you're going to use a specific toxin or another indicator protein. How well can you assay that substance? If using an indicator protein, it's good to understand how that protein relates to the toxin that you're trying to sort of model, in terms of the specific parameters that are relevant to your process. If your process is based on size and separating substances of different sizes, is the size of an indicator protein relevant to removal of your toxin of interest? If the purification process is based on charge or hydrophobicity, is the indicator protein similar to the protein you are trying to model in those respects?

If instead, you're looking at direct testing, or if in addition to purification process characterization, you are looking at direct testing, are you interested in total host cell protein? Are you interested in a specific protein? Or are you interested in both? Is there an established or commercially available detection method? In some cases, there are commercial ELISA kits available for

host cell proteins in general for a specific bacterial species. Or there may be an ELISA of course, for a specific toxin.

But regardless of whether you are considering a commercial or custom detection method, it's important to understand how the assays are formed with your specific host strain. Something that's good for most *E. coli* strains may not work for the specific strain that you are looking at. So, it's good to consider that question as well. And also, whether the assay performs as you expect in the presence of the phages that are in your product.

And I also want to mention, if you do plan to use one assay in early development and transition to another assay later, it can be useful to think early on about a plan to generate bridging data. The data that will show that even though you've switched assays, you were you know, your new assay is at least as good as your old one was at detecting some of the same things, because you still want to be able to link safety data that you generated

with your early product, to the safety data that you are generating now that you are using this new assay.

And my second example is next generation sequencing. This can be a very powerful tool for asking in a very broad way, what's in my sample? And you know, whereas PCR can only ask whether the, you know, the target that that primer set binds to is present in that sample, NGS asks the question in a very broad way. And as it has become less expensive, it's been discussed as a way to characterize product identity impurity. Looking for things like host nucleic acids, other phages in the facility, and this can include both other phages in your product, or, if you are using a contract manufacturer, it can also potentially ask whether there is any cross-contamination from other phages in the facility itself. Maybe you're interested in looking for the presence of host-derived prophages or phage-inducible chromosomal islands, transducing particles, things like that.

At the same time, NGS generates a lot of data that needs to be dealt with, and it takes time to run. So, it's worth spending some time really thinking about what you want to get out of this type of data and what is the best way to go about it.

I mentioned interest in using NGS to investigate both product identity and product purity. If the attribute that's of interest is identity, how are you planning to use it? If the aim is to compare a sequencing result to a reference sequence, what threshold for percent identity makes sense, and why? You know, can the method differentiate between two related phages in your product or production facility? Maybe that's a basis for determining an identity threshold. If you're interested in looking at product purity, the specific thing that you're looking for can really affect how you go about setting up the assay. For example, if you're looking for DNA that is present in phage capsids for example, host-derived prophages, you may want to treat, pretreat that sample with a nuclease to remove

free DNA. However, if you do that, now your sequencing results can't be used to look at residual host DNA because the nuclease has digested that free DNA. So, just because NGS can look at many different things, it doesn't necessarily mean it you can look at all of those things in the same sample. So, you really want to think about what you are trying to do and how you want to set up the assay to do that.

Another question in with purity is what's the sensitivity of the method? So, if you want to be able to speak to a certain sensitivity, again, spiking studies can be useful. And then, it's important to understand what parameters of sample preparation and gene analysis need to be standardized in order to consistently achieve that sensitivity once you have demonstrated it.

So, at the end of the day, with all of these kinds of things, you know, really keep your goal in mind. Because if you're aiming to use this type of assay as a release test, the requirements that you have to

generate supporting data to really feel confident in the statement that you are making about your assay and about its results, may differ quite a bit from how much data and the type of data that you want to generate if your goal is to learn more about your product and process and just inform, continue to inform development.

So, to wrap up, overall, there is a lot of freedom to choose the best assay methods for each product and for each development program. And the questions, some of the big questions to really spend time considering are to make sure that you are clear about the purpose of your assay, what material you are testing, and what you want to be able to say about the material based on its results. And whether your assay design and supporting data provide scientific justification for the conclusions that you want to draw. And then, that will shape, you know, whether your assay is suitable for its intended use, how long you want to use that particular assay, and so on.

I wanted to add in here, there is a guidance for analytical procedure and method validation for drugs and biologics. I've put the link up here. This guidance focuses on applications for licensure, not INDs, so the amount of information that is needed in an IND will depend on a variety of factors, things like product development stage, and the like. But even though this guidance is focused on applications for licensure, it can still provide useful recommendations for your thought process, and how you plan to develop things over time. It also discusses suitability testing for compendial methods, and then, a validation process for non-compendial methods. And so, suitability testing, you know, gets into whether this works for your specific product and the conditions under which you're using it with your product. So, this can be a useful document, even though, as I mentioned, it is focused on licensure application and not specifically on INDs leading up to that place.

Now, I want to take a moment to

thank some of my colleagues who provided input on this presentation. And if there is time, Roger, for a few questions?

DR. PLAUT: Yeah, there was one question in the chat regarding whether next-generation sequencing is appropriate to test for sterility of a phage preparation.

DR. LEHMAN: I think it would, I guess, my question would be, why look for something that is different from the USP, the established compendial method for sterility? USP71 is pretty well established for that, and I think quite a bit simpler than developing a new NGS-based assay for that.

DR. PLAUT: Okay, I think we can move on.

DR. LEHMAN: Great, thank you. Thank you, our next speaker is Danish Malik, from Loughborough University, who will be presenting about metrology tools for use in phage manufacturing and formulation. Danish, if you can activate your video and share your screen, please?

DR. MALIK: Hello, well, I hope you

can see my screen and you can hear me?

DR. LEHMAN: Yes, you're good.

DR. MALIK: Great stuff. So, firstly, thanks very much for inviting me to give this talk. My name is Danish Malik. I'm a Reader in industrial biotechnology here at Loughborough University in the U.K., and the focus of my work is really, working with industrial partners to do preclinical development of phages, both from a phage therapeutic development, formulation, manufacturing, metrology tools to look at better physicochemical characterization of the phages, but also, for biocontrol purposes. And therefore, looking at scalable manufacturing of phages and phage-derived biotherapeutics is the focus of my research group.

I think the lead-in from Susan and discussions earlier this morning very much focuses on, from a CMC point of view, consistent manufacturing of phages that are sufficiently well-characterized, that are safe, efficacious drug substances and formulated as drug products. And so, my talk

really is divided into firstly, looking and presenting to you upstream manufacturing of phages, and evaluating some new sensors that can be used to give eyes in the reactor in terms of looking at particular process parameters that can improve a phage, tighter yields. Also, provide confidence in terms of the design space in which the reactors can be operated. And I will show you results which have been carried out for its a model T3 *E. coli* phage that allowed us to increase titer, reduce batch times. And then, looking at how that can also be transferred to produce phages in a continuous fashion, by decoupling host propagation from phage predation. So, that is something that I will talk about.

And on the right-hand side here, it's just your typical process flow sheet, where you've got your bioreactor. Here, I'll look at two sensors. An infrared sensor that measures biomass in real time at line. And carbon dioxide and oxygen emission rates that give us some indication of host physiology that can be linked to phage production. I'll

talk a little bit about purification using tangential flow filtration to reduce host cell proteins and endotoxin. A monolith system, a mixed-mode monolith for reducing endotoxin. And then also, using some tools that can be used at line, for example, the AKTA chromatography can have a light scattering detector but, in my case, I'll show you some offline data where we characterized phage aggregation and linked that to development of liquid formulations using design of experiment approaches. And I'll very briefly talk about spray drying phages to improve phage stability.

So, that's sort of what I will go through. In terms of phage production, I will show data for an instrumented stir tank bioreactor. These are available as single-use bioreactors which are really important in terms of a contract manufacturer manufacturing phages for multiple clients and the challenge of sterilization of blast reactors, is a problem there. So, single-use stir tank reactors are available at different scales

that can properly instrumented with standardized instrumentation that allows scale up; scale up of stir tank bioreactors is well known in terms of mass transfer rates, mixing times, power to volume rates, and so on.

So, the two sensors we are going to be looking at are an infrared sensor for biomass, and a blue sense sensor for measurement of CO₂ and oxygen. I'll also talk about coupling the upstream reactor where the bacteria are being grown continuously and a second reactor, where the phage are being produced continuously. And there, just to, I published this a few years ago in the paper, in the journal *Viruses*, you can look up the details. But just to understand, there is a whole liter of working volume for a five-liter reactor, and you are operating that at one liter per hour. Then, the dilution rate is something that I'll call upon. So, one over four would mean a dilution rate of 0.25 or a four-hour residence time. So, something that you have to just get your head around as I talk about how you can control continuous

production and look at steady-state production of phages and the advantages that gives you in terms of control, in terms of process intensification.

So, the graph on the left simply shows a calibration curve. The x-axis represents concentration units of the Fundalux sensor, and you have two y-axes, so there is a linear correlation, the black squares that shows the OD versus CU. So, you can see that typically, you know, the batch production, you might inoculate your bacteria when the OD gets to about 0.2, which corresponds to about 10^7 , 10^8 CFU/ml. You might add phage at different MOIs. So, you can see the red circles represent the CFU/ml and the black squares are the corresponding ODs. So, the idea is to use the in-line, real-time infrared sensor to be able to look at what is happening in the reactor to evaluate when the point comes at which the biomass concentration is sufficient to add the phage at the correct MOI, and then to evaluate when the amplification is over so that the batch can be harvested and then we

can do downstream verification.

So, in this particular case, we added, we had our in-line CU sensor which are the blue triangles. And at about 165 minutes, we took an offline measurement of the OD, and we were working, this was just to demonstrate that you know, taking samples, taking them offline, measuring the OD, and then, finding when the OD is appropriate for the phage to be added can be a hit-and-miss thing. But if you have your sensor in-line, you can very nicely control the time at which the phage is infected. You can see that the bacteria rose and then, the OD values fell and so did the CU values. Residual cells in the sampling line can be a problem, but the CU values give an early indication of when the host organism concentration fell, and the phage had amplified. So, this shows the phage amplification, the green circles represent what happened. So, you've got your OD, we've got our CU values and you can see that the phage titer rose between 10^{11} and 10^{12} PFU/ml. And we harvested the cells at 300 minutes.

The X-gas sensor gives information on the amount of carbon dioxide that is being emitted in the reactor, and the oxygen uptake rate because the reactors are instrumented with mass flow controllers, so we can work out in real time, and link this with scatter systems for data acquisition, so we can look at what is happening batch by batch, and we can drive the process and run our batches in a more consistent manner. So, the off-gas analysis also shows us here that as the phage started amplifying, the metabolic activity of the bacteria rises to about 200 minutes and then falls away as the phage titer reaches 10^{12} PFU/ml.

So, the next batch was run not relying on OD measurements but relying on the CU of the infrared sensor. So, we inoculated earlier, at 127 minutes after the start of the culture when the CU value was 0.1, the calibration told us that the corresponding OD would be 0.2, and we were able to amplify the phages earlier so that the cycle came to an end much earlier. So, we were able to harvest

our phage at 7×10^{11} PFU/ml within 195 minutes. That's a third of the time saved off the batch.

And here, you can also see the corresponding, and let me see if I can just move this out of the way. So, you can see that the carbon dioxide emission rate goes up. And as the phage titer starts rising to high levels of 10^{11} , the bacteria are attenuated, and obviously, the CU values are falling, and we can pick up the point at which we harvest our phage.

So, the next thing is operating the two reactors now in series where Reactor 1 is a host propagation reactor, it is 4 liters, it is operated at about 1.75 liters an hour, which is a dilution rate of 0.43 hours. We start the reactor, and you can see that within three hours, the first reactor reaches steady state, the CU values indicate to us the cell concentration in the reactor, which of course, we also take in—we measure it, so we have cells going into the second reactor at 10^9 to 10^{10} CFU/ml. And we can see that the bacteria

are in very good metabolic activity producing high levels of carbon dioxide, consuming oxygen, and everything you can see is controlled at steady state. This steady-state operation also allows us to look at dynamic responses of the system. How are our phage-host interactions? How stable they are. And you can do lots of interesting things in terms of process development.

And you can see that in the second reactor, the phage concentration starts rising. This is continuously producing phage, and within a few hours, the phage titer grows to over 10^{12} PFU/ml. And then, we can carry on continuously producing phage. So, over a 24-hour period, you know, we can produce tens of liters of production, working volume of only 4 liters of our batch reactor. The carbon dioxide emission results for the second reactor show us that the bacteria in the second reactor were metabolizing and producing carbon dioxide. And then, as the phage titer starts rising, there is—obviously, the bacteria are now being killed off and high

titers of phage are being produced.

We can take samples from the reactor as the phage are being produced. And we can use dynamic light scattering, and that shows us that our phage which has a size about 60 nanometers, it's a Podovirus, and we can measure that at line by simply diluting the sample 1 in 10 in Tris buffer, and within a minute, we've got a measurement of what is happening in our reactor.

So, hopefully, I have given you some indication of how we can use these at-line sensors to give real-time control, and how one can optimize phage production upstream. And by thinking about scalability, we're thinking about the critical process parameters both upstream as well as downstream. So, upstream, we're controlling the oxygenation rates, we're controlling the pH, the temperature, and so on, and so forth, and we can optimize those to maximize host physiology and the bacterial physiology to produce maximum amount of phage and produce high titers of phage. But also, we can run the reactors upstream but the amount

of contamination in the reactor reduced bottle necks downstream. For example, for the downstream microfiltration and ultrafiltration systems. So, here we are running a diafiltration TFF system using a Repligen hollow-fiber system at 300 kilodalton membrane in order to buffer swapping and reduce host-cell proteins, in this case measured with a BCA assay. But there is an online UV sensor also coupled to the Repligen TFF system. And we can reduce endotoxin by between 1 to 3 logs, just by the TFF system itself.

The phage sample can then be further purified as a polishing step. And we looked at two different monoliths that were made available to us through BIA Separation. So, this is in batch mode, the monolith was a 1-ml monolith. Column operated at a flow rate of one column volume per minute using gradient elution starting at 150 millimolar Tris and increasing that over 20 column volumes to 2 molar sodium chloride. And you can see that the phage are coming out where the salt concentration gets to 1 molar sodium chloride.

A hundred percent recovery of the phage. This is with a QA-1 column, which is a quaternary amine column. And then, I compared that with a Prima S column, which is mixed mode, it's got both quaternary amine and OH functionality. Again, we got 100 percent yield and recovery of the phage. But the amount of endotoxin in the sample was considerably different. So, in the crude lysate the endotoxin measurement was between 10^6 and 10^7 EU/ml measured with the LAL assay. After TFF, this could be reduced by at least a log, if not—it varies from phage to phage. But the quaternary amine column, although we were able to recover all the phage, the endotoxin levels remained the same as we had previously. But with the Prima S, we got 100 percent recovery but a 2-log reduction down to sub—a few hundred endotoxin units per ml.

The phage titers are important in this respect. We also look at the effect of purification on the charge properties of the phage. The phage is negatively charged, which you can see in terms of the zeta potential.

And that could be part of the QC aspects of the drug substance and can also be related to the stability of the phage formulations which I will talk about in a minute. So, the phage, after Prima S, you can see gives a beautiful, single peak there showing that the phage are mono-dispersed and have a size of about 17 nanometers here.

But when we look at the universality, so one of the things, I am interested in is the universality of these methods, we find that different phages, these are nine different phages, at different concentrations, you can see that the endotoxin levels can be very variable. So, there is no universal method to be able to reduce endotoxins levels to very low levels using the system that I have spoken about. And so, one of the things that I am working on is how can we improve and better understand the downstream purification of phage substances?

Susan, are you there? Is that 2 minutes call, or?

DR. LEHMAN: Yes, it is.

DR. MALIK: Yes, okay. So, I'll just very quickly talk about liquid formulations. So, one of the things that I am looking at is how we can you know, improve the stability of phages and liquid formulations using some of the tools that I've already mentioned. We use a lot of dynamic light scattering after purifying the phages to look at phage stability, by addition of different recipients controlling—addition of antioxidants, looking at freeze-thaw cycles and temperature stability, and so on. So, here, you can see that the phage has aggregated, which is why you see two peaks, almost 60% of the substance is now at 400 nanometers, compared to the original size of 100 nanometers.

We have looked at the effect of charge on phage stability. So, the ionic strength of the formulation has an impact on charge. Low charge is related to phage aggregation, particularly at higher temperatures. We use design of experiments approaches to do screening in orthogonal design, so we can look at different levels of

factors, and therefore, we can screen and improve phage formulations that can be stable. Looking at the effect of accelerated temperatures on phage stability. And here, you can see that the design of experiments is showing us that factors A and D are significant. We can design orthogonal screening matrices to see whether there are combinations of factors at different levels that might improve phage stability.

And you know, given time is passing by, I'll probably not talk much more about that. I'll probably discuss spray drying and other things later. So, I'll probably, you know, draw my talk by making a few concluding comments. There's a lot still to be done in terms of better understanding of the phage bioprocessing unit operations, the critical process parameters by which we can link the critical quality attributes of the phage with the critical process parameters of the various unit operations. And then, lots of new metrology tools can be brought to the fore to improve phage characterization and link those

in terms of formulation, development, and stability tests. And so, I will stop there.

There is one paper that I recently published on some of the things, some of which I didn't cover, in Current Opinion in Biotechnology, so you might want to have a look at that. My email is up there, and I'd like to thank Sartorius and BIA for supporting the work on the sensors and supplying the model maps. And of course, the funders who funded the work. Thanks, very much.

DR. LEHMAN: Thank you. I am going to see if we can squeeze in one quick question here. There were some questions early on about choosing the most appropriate optical density or CU value at which to add phages for best results. And I am wondering if you know, across the experiments that you have run, is there a lot of various—is there a lot of trial and error with the results being different for each phage? Or have there been some patterns across, one method is more consistently indicative than the other?

DR. MALIK: It is very easy to do

quick 96-well screening experiments that can bring quickly on a small scale, get the sweet spot. And then, you can scale that up using an Amber 250-type system that is a small-scale system. And then, going you know, to a 5-liter vessel to give a further-get confidence that actually, you know, you are on the right zone for the upstream manufacturing side of things.

DR. LEHMAN: Great. Thank you very much. You do have several questions in the Q&A if you have a chance.

DR. MALIK: I can answer those. Thank you much, everyone. Take care.

DR. LEHMAN: Thank you. Our next speaker is Frank Smrekar. Frank, if you could, go ahead and share your screen?

DR. SMREKAR: Thank you, Susan.

DR. LEHMAN: Great, we can see and hear you, Frank.

DR. SMREKAR: Can you hear me? Yeah? Okay.

DR. LEHMAN: Yes, you're good.

DR. SMREKAR: Okay, thanks. So, yeah, I'm Frank Smrekar, CEO of JAFRAL. I'll speak

today about manufacturing aspects of phage-based product development from early steps to production of clinical trial material. So, and before I start, so, thanks to the organizers to inviting me, or us, to speak on the CMC topics. So, okay. Maybe just first, a few words about our company which I think it is important, so that you understand so, about the topic that we are speaking. So, JAFRAL was founded in 2011, it is independent and privately-owned biotech company. And we are focused on research and development manufacturing of phage-based products. We are based in Ljubljana, Slovenia, so for us, in the center of Europe, and with some few very nice spots close to us, so when the COVID is past, you are all welcome to visit us.

So, JAFRAL to our knowledge is the only CMO/CRO which primarily focus is development and production of phage-based products, GMP or non-GMP applications from human therapeutics and up to the agriculture and yeah, we are classical CMO, so, we don't have our own products where we are just

helping to bring our customers, bring the products to the market, or to the clinical trials.

About our capabilities, I think it's -this is important from-it's one of our features, but it's also important from what was mentioned before, and also, the speak from Susan, so we have two facilities and that actually, helps us to do work in two different locations that we can work on different projects in parallel, and this is important, because with this, this is one step to prevent cross-contamination because working with phages is having really separate facilities, separate HVACs is really important to assure the good quality. Then having two facilities also addressing possible contingencies, so, clean rooms have to work 24/7, so, whatever goes wrong, it's always-can be some delays, so working in two facilities that mitigates the risk. And the third one is actually, once the material is produced, we always want to have the material split and saved in two different locations, so even though there are, like,

backup system and generators, it's—also, we try to separate in the cell banks or the phage banks to two different locations. Or, maybe also, the drug substances and drug products. So, we have a GMP certificate for the cell banks, phage banks, drug substances, and drug product, also the placebo. According to EU laws, we have a grade from B to A, and according to the U.S., that means from ISO 8 to ISO 5, which means also, possible to produce the sterile products. From the track record, we produced, or we are producing phages for different applications from human therapeutics to cosmetics, different route of administrations, and development of from early development stage, up to the clinical trial material. Worked in past with different phages for the different bacteria and then phages to achieve different process capabilities, and qualities.

And so far, where—I think it's important that we gained a lot of experience, the good or the bad, we learned through the way, so, so far, produced more than 40 phage

or viral banks, bacterial banks, and more than 30 APIs that were then used for clinical trial material and also, 7 drug products that have been successfully used in clinical trials.

So, going into the let's say more technical than the basic stuff for the production of clinical trial material. So, sometimes when we are seeing the CMC part, a lot of people understand the more just the "M" part, so manufacturing. But it is actually also chemistry and control, and also documentation, so what we see in order to prepare the GMP product, all three parts have to be in place. So, manufacturing and then the control and analytics, and also, the third part, so everything that is done has to be well-documented, so the quality assurance has to be there, through the whole path from the beginning until end, and at the end of the day, when that is produced, everything has to be submitted to the regulatory. So, all the work has to be in line with the regulatory requirements. So, this collaboration between the different departments, or different

workflows is very important through the whole process.

Here is just an example of the product production scheme of, for example, one phage product. That's an example, that's an example for the maybe natural phages, also GMO phages where the phage bank or phage seed is needed. Where we start is what we call as non-GMP level to produce the research cell bank, RCB, or research phage seed, RPS, or some call it research phage or viral seed. This is something—it's not that that would be just a bank or a vial. Already, that has to be at a certain point, recorded and characterized because this material is then starting material for the GMP material, so the master cell bank, master phage seed. And if you want to release this to basic starting material, cell bank and phage seed, it has to be released against something. So, again, the COA of, for example, research cell bank and research phage seed.

And then, the next one is usually, the preferred way is to do the two-tiered

system. So, that means that after getting master cell bank, that also, working cell bank is done, as well as the phage seed. But there are also cases when at the early stages maybe, only one tier is done and then, the second level is done later. But at the end of the day, to minimize the—also, the business risk, because these banks have to last until the end of the clinical trials, also commercial. It's really advisable to have this thing sorted out and well-established.

Then production of the drug substances and drug product, again, here is just an example that there was like four drug substances, it can be one, two, or maybe, 10. So, for each of them, we need a cell bank and phage seed. There can be more cell—so, there can be cell bank for each drug substance separately, or maybe, only one. It can depend on how the phage cocktail is designed. And then, once the drug substance is produced in the right quality and quantity, then this is mixed to the drug product. Where, even though there is only mixture, this step is of

critical importance though, because here you are putting all the drug substances together, and no mistakes are advisable at this stage. So, also, this kind of maybe relatively simple step, it's advisable to test at early stages.

Then if—now, we will go through how we see as development manufacturing process and then also, the analytical method through the whole path. So, everything starts with let's say bacterial infection or the panel of bacteria that phage product wants to be developed. So, then it's phage selection, isolation, phage design, then the next one is small-scale process development. So, at this stage, you want to understand whether phage is actually possible to produce. Is it possible to produce to a certain quality and quantity? So, it's not that we get final answer for that, but you are getting at least insight, like a feasibility test whether that is even possible. So, with some optimization you'll know better, but that's initial testing. And with this process it's also the product, it's produced in small-scale, which can then be

used for *in vitro* testing or *in vivo* testing and then, put on stability. Then, the next step is to do the scale-up. And advisable put again on stability, and then, the last next step is, at least for the clinical trials, is produce the GMP material. So, first usually, in our case, that we are doing the engineering runs or testing runs. And then, actually, the clinical trial material. As it can be seen here, there is a lot of stability here. This is something that I always repeat, it is really advisable to do as much as possible stability, too, also at early stage because even if the product is really good, if it's not stable, it's not so usable, or it's really difficult to use. And this is one thing which cannot be speeded up. So, the stability factor here again, we advise to have as much as possible.

Then here are a few considerations or questions to be answered during the development of the process, or production of clinical trial material. So, this talk or this presentation is not long enough to address

everything, but it's at least something to put in your mind so at least some questions that need to be asked, so, while you are designing the process. So, first if you are just starting with selecting production hosts. So, as it was already mentioned, so in the talk before so, are there any undesirable genes for that first criteria? And what are the production capabilities of the host cell? Can you deliver the high titers or at least the titers that you need for the production of clinical trial material? Then the question, have one or more hosts? So, from the production point of view or cost point of view, it's actually great if there is only one manufacturing host, but that can actually influence then the host range, because producing all the phage on one host can influence the host range of other phages? It's usually some kind of compromise has to be made at this stage.

Then the next one is selecting phage candidates. Again, any undesirable genes there? Yes, or no. And then, production

capabilities, similar to production host. And quite commonly, that one is quite a tricky question because whether to know how much material you need, you actually have to know what are the clinical needs. So, actually, you have to know something which usually in the early stage is quite difficult, but at least some estimates need to be done at this stage. So, then, it's again stability, at early stage as possible, as mentioned before, it's also, in the talks, the phage-host pair, critical. So, whether, if you change the host, it is not the same phage anymore, or phage product. So, at least some testing has to be done.

And then, the number of phages, of candidates. So, also in the past, there were clinical trials who were testing, so, have three-phage cocktails or ten-phage cocktails from the production point of view of course, it's easier to have, like, less. On the other side, whether you want to compromise with the host range, because with more phages in the cocktail, it's actually better to have more of them, but you have to have in mind that if

there are more, then you have to do more production, more stability. Also, determine the potency of each candidate in the cocktail and then on stability, so, the question is, what if you have ten, and one drops and others not, so a lot of things have to be discussed or decided already at the early stages.

Then, from the upstream process and downstream process, point of view, they are similar, the questions. So, again, quantities needed, or quality needed and recoveries that can be achieved. Then, we do really advise that raw materials to be used already, at the beginning that are acceptable at GMP levels. So, because usually, whether you exchange them later, it can influence the process, sometimes a little or a lot. Or even if not, we have to, some kind of justify why you think that that doesn't change a lot the process.

Then, it needs to be considered a critical process parameter so, because the processes are not needed to be validated at this stage, Phase I, Phase II, it is of critical importance that you know the process

parameters, so, what does influence critically that you can achieve the quality and the quantity of the—that is needed. And then, furthermore, once you do that so, whether the process is robust and whether the process is scalable. So, all this stuff usually, needs to be done on small scale and then, when you do the scale-up or CTM, it just needs to be confirmed, and all this information has to be included in the IND, so the agency sees that you control or understand the process and you can repeat it anytime.

Then, at the end is, of course, formulation. So, question, what is the final phage concentration? Which excipient are allowed and so, that is of course linked with which buffers improve the stability of the product? So, even some excipients, and quite typically, sometimes it comes that there is some excipient which is animal-derived, which, unfortunately, doesn't go through, so, but it's good for stability on the other side. So, it's the things that you have to consider at the beginning, and maybe save you some time

that you don't test something that then you cannot use.

And yeah, what is the desired shelf life? Of course, as long as possible, but there is also some kind of minimum just to— that enables you actually, to execute not only the clinical trial, but maybe also later to have the commercial product. And also, what is the preferred containment closure, the volumes, and all of that stuff have to be addressed.

Then from the analytical point of view, we start with analytical method of development including critical agents, then methods have to be qualified, validated, and furthermore, they can be used either for the release testing, in-process testing, or stability. So, this is as intended use. And then, there are different methods as already mentioned before by Susan. So, there is...

DR. LEHMAN: You have three minutes, Frenk.

DR. SMREKAR: Yeah, okay. So, for every method, you need to understand why you

are using, and sometimes its exact value that you need from that method, but on the other side, it's also that you have—just collect data that can be usable for later stages. Then, from analytical point of view also when you are developing the method or when you implement, some things to consider, whether the method is already USP or European Pharmacopoeia, or it's product-specific, whether you have the controls, whether they are applicable for you, and while you qualify the methods, what is the vulnerabilities? Selectivity, precision, linearity, and at the end of the day, also reproducibility and turnaround time. So, if you need the result tomorrow or in one week so, whether that method, whether you have the method that delivers that or not.

So, at the end of the day, you have certain COA, which has to address the critical quality parameters that you believe, or the agency then also believes they are sufficient. At the end, stability and formulation development. So, it can be liquid formulation,

dry powder, usually this is tested under time-real conditions and then also the accelerated conditions. And these are just a few examples, so two phages, four different buffers. For one, Phage A, if you look only at the left column, so 2 to 8, it's stable, where for the Phage B, only one buffer works for this phage. So, if you mix this together, it's—we know which buffer to use but yet, this is advisable to use in advance.

And the last slide, the third factor is a sort of production quality control and also assurance documentation, so all the documentation has to follow GMP guidances. So, certified facility, vendors, suppliers, methods, and then everything really needs to be captured in the Module 3, so, reporting, qualifying, and characterization of the host, development and analytics, and also, reporting compatibility of container closures with the products. So, thank you for your attention. I hope it was useful, and if there is any questions, I am happy to answer also happy to type. So, thanks.

DR. LEHMAN: So, we have one question for you. As a contract manufacturer, you work with a number of different customers, each of whom is going to have their own phages, their own processes, their own assays. When a customer is trying to budget the time that it takes to transfer these to a contract facility, what kind of time should they be thinking about and are there one or two things that they can do to really help that process along?

DR. SMREKAR: Usually, it's tough to generalize about what kind of time, it can be from a few weeks to a few months, two to three, but it's really how well they already have the setup and their system. So, and that comes to how well they define the critical process parameters. So, if they have really good reproducible process, then it is easier to transfer it. If it's not defined, then it can take longer. And that is based on the discussion that we have, documentation that we get, that is something we can figure out.

DR. LEHMAN: Okay, thank you very

much. And our final speaker for this section is Dr. Kilian Vogele from Invitris. If you can, turn on your video and share your screen?

DR. VOGELE: Is it working?

DR. LEHMAN: Yes, we can see your screen, and there you go, you're in presentation mode.

DR. VOGELE: Okay, perfect, thanks a lot. Yes, and thanks a lot for the you know, for the chance to speak here. And I am coming from Invitris, so we are kind of a project who is aiming for to produce bacteriophages *in vitro*. And yeah, so, basically, I think you all know this slide on bacteriophages, are already quite used for therapeutic purposes. And due to yeah, so, there are a lot of, at least in European countries, a lot of cases where they already worked. But so, for clinical trials are, a little bit lacking those. And the thing is, one thing we believe which is really important for bacteriophage therapy is personalization. So, because phages are highly target-specific and they co-evolve also, to find new mechanisms to overcome

resistant bacteria. And as they have a really high diversity, there's always a chance to find a new bacteriophage against a new pathogen. And therefore, we really think that it is really important that you do really personalized phage therapy.

But one struggle with personalized bacteriophage therapy is that bacteriophages are really, you really need the host bacteria for the production. So, therefore, currently, when you want to use bacteriophages for personalized therapy, you always have to search for a safe strain to produce the bacteriophages, which is sometimes also not possible. Also, from the host bacteria you use for production, you get some impurities, which can also cause problems later. And also, this process is quite tricky for parallelization, because if you do personalized treatment, you need a lot of different phages and that's a little bit tricky to have this all on an assembly line.

And due to that, we try to come up with a solution for this. And we used an *in*

vitro system which is able to produce bacteriophages always with the same kind of platform. And with that, we are able to eliminate the dependency of the host bacteria, and with that you have a system where you always use more of the same system.

And this one is highly characterized, therefore also you can create a better purity because as you get out what you put in, you don't have any prophages present, or where you don't have to care about—you can reduce the endotoxins from the beginning, and with that it's also as in an *in vitro* system, you can also personalize it more rapidly. And therefore, hopefully, produce bacteriophages, scalable and for personalized therapy on demand.

And the thing is actually, as you can see here, usually, you need for each bacteriophage you want to produce, a corresponding host bacteria. And then you produce them and then you will have, if for example, you wanted to make a cocktail out of these three bacteriophages, you have to do

this process three times. And with our system, we created an *in vitro* system which is able to produce bacteriophages in a kind of a test tube. So, here you just have always a cell-free expression system. You add the DNA, and with that, you can produce bacteriophages kind of on demand. And at the heart of our *in vitro* expression system, it's a so-called cell-free transcription translation system. And you can imagine it like if you have an *E. coli* cell, you remove the cell wall, you remove the DNA and with what you are left, is the expression machinery of the bacteria.

So, here we used a well-characterized lab strain, where you already know really what is the genome, that it's free of prophages, and actually to produce the cell-free system, you first cultivate the *E. coli* in a big batch. Then you lyse them, you do some processes like centrifugation, dialysis, and similar steps. And then you come to a protein solution which is containing the ribosome and all the proteins which are necessary for expression.

This system, you add some amino acids, DNA, and some cofactors, and when you do this with the right encoding DNA, you get transcription as well as translation, and with that, you are able to produce protein of interest, but what is even for me, at the first place it was quite surprising that you can do it for GFP which is part rather simple. But if you add the cell-free system, a complete phage DNA, in this case the T7 *E. coli* phage. You get a fully phage assembled, and with this huge genome, it was for me, quite surprising that it worked at all, to be honest. But also the titers are quite satisfaction. So, like here, for this T7 phage, you can get titers up to 10^{11} , in just a test tube by mixing some expression system and some DNA and then you get this phage.

And also, it was quite interesting from the scientific level, all the—a lot of mechanisms also work in the cell-free system. So, here, we looked in the DNA replication of the T7 phage. And here so there's all the required proteins in the cell-free system, and

also the other ones are expressed by the phage. And if you put this all together and look at the DNA level at time T0, you don't see the phage genome, which would be up here. And when you wait four hours, the genome gets quite replicated, and you can see here a band is emerging. So, also when you measure the concentration by qPCR, you see that you get a 90-fold increase of the T7 genome. So, you have DNA replication, you have protein expression, and you have transcription, and with that, you can produce bacteriophages.

So, the only thing which you can produce, so usually, we focus on lytic phages, as there is no membrane present, you cannot express, for example, phages which are membrane-bound, like this M13 phage. But the lytic phages we were interested in so far were quite good. And what is also really nice is, if you imagine you produce *E. coli* phage and an *E. coli* extract that works rather nicely, but what about phages for other host bacteria? So, then we got away from these *E. coli* phages. Here are some—we tried also some

bacteriophages against some pathogenic *E. coli*, some EHEC, and with those phages we were also able to produce. And then, of course, we thought, we got away more and more from *E. coli*. And with the help of some really nice collaboration with the AG Bugert of the military in Munich, we were also able to test our system with some pathogens, and you see here now, for example, with *Yersinia pestis*, *Klebsiella pneumoniae*, *Pseudomonas*, we are able to produce these bacteriophages with this *E. coli* cell-free system. And of course, the question is, can you also produce bacteriophages which are really far away from the phylum of *E. coli*? And this is also working. Here we have for example two *Bacillus subtilis* phages, and we modified our system a little. So, we are adding a plasmid which is encoding some host factor. And this host factor is also expressed in the cell-free system to get a—that can then actuate the expression of the phage DNA. And then you have transcription, translation, the self-assembly of the phage, and in the end, you get a

complete phage. So, that was rather nice, so you can produce a quite big range of bacteriophages. And of course, the next thing is, we want to go more to medical conditions and therefore, also, and important question is the endotoxins as well as the sterility of the system.

And for cell-free systems, we just used a normal phage-host pair, and measured the endotoxin level after the production. Compared it to the cell-free production and you already see, the endotoxins levels are lower in the cell-free system. And then we thought, what is the main component of the endotoxins in the cell-free system? Which was, in our case, lipid A. And then we produced from our production strain, for example, a knockout strain, which then produces, lacking this lipid A. And with that we get a further reduction of the endotoxins level in our cell-free expressed phages. And that's before any purification. So, we aim that our initial product has less endotoxins. And then, further purification on the line is hopefully then

also easier, more easily done. We also check for the sterility of our cell extract, and we just found that there were a few bacteria left in a milliliter of cell-free reaction. As we get also quite high titers, for further dilution it should be no big major problem. And this is also before any sterile filtration of the cell-free system.

So, next, what I also wanted to show you which is interesting is, as the cell-free system, it's basically just a test tube where you can pipette in things, you can also do some manipulations. So, with the cell-free system together with the phage DNA and some modified proteins, we were able to show that we can produce bacteriophages with some modified capsid structure. So, for that we choose the T7 phage, we expressed the T7 phage in the cell-free system, but together with the normal T7 phage, we also produce added a plasmid which is also encoding for a fusion protein. Which is able to self-assemble, also go into the capsid of the phage, and therefore, produce a modified bacteriophage.

Our first trial was to see if we can, in this case we added like our purification tag and a luciferase, to see whether we can modify this capsid. And to show the modification of the capsid without modifying the genome. We produced this phage, we made here a wild-type phage, and here the modified phage, then put it on our His-tag column. And then purified with several washing steps our phages, and we saw that the phages which were expressed with this had the titers higher compared to the wild-type phages which got washed off more. So, with that, we were able to show that we can add some purification tags for our bacteriophages, and what we were also able to show, this is kind of the same experiment, that the bacteriophages also lose their modifications after one replication round. So, that we showed that we made a transient modification. So, we had not only this purification tag, we also had this luciferase here, and with that, we measured the luminescence of the bacteriophage before replication and then we let it replicate to

full clearance of the bacterial culture. Used these phages and then check for the luminescence again, and we saw that those—the luciferase wasn't present anymore there in our experiment.

So, with that like, we are able to produce bacteriophages with the cell-free system, which is also quite controllable. Then, we also showed that we can modify it, and then we can also produce a high range of bacteriophages. And our long-term hope to help with that is that we can produce bacteriophages for personalized therapy, so that in principle, when you have a patient with a resistant infection that you can diagnose this patient, and then, if you have the diagnosis that you get from this adaptive phage library, just adding from this DNA library, the DNA to the cell-free system, then produce on demand the bacteriophages to get them a personalized product and then hopefully, treat the patient successfully.

DR. LEHMAN: You have three minutes.

DR. VOGELE: Okay, I think it's all

almost done. So, the team slide is obviously always the last. Of course, I want to thank my colleagues, Sophie von Schönberg and Franzi, they helped me a lot in the lab, and so those also.

DR. LEHMAN: And that's great, because we do have a couple of questions.

DR. VOGELE: Oh, yeah, for sure.

DR. LEHMAN: One is, given that phages adhere to the cell membrane during assembly, is the cell-free system perhaps, less efficient? Or is there something in your cell-free matrix that helps address that?

DR. VOGELE: So, bacteriophages which need the cell membrane for production, for some certain phage? Is that what you are...?

DR. LEHMAN: I think the question was referring to, you know, capsids assembling at the cell membrane with an anchor point. And whether that spatial structure within the cell you know, helps the assembly? And whether that affects the underlying efficiency of a cell-free system? Or if there are components of your cell-free extract that seem to address

that?

DR. VOGELE: So far, we have rather focused on bacteriophages which not necessarily need this effect so much. So, that's why.

DR. LEHMAN: Another question about whether you have tried your system with phages that rely on protein-primed genome replication?

DR. VOGELE: The DNA replication, we have basically focused on the T7 phage so far. For all the other phages we supplied a larger amount of DNA already in the extract, so that it's not necessarily required that they replicate their genome. So that already, all the DNA is present, can already be expressed and packed into the phages.

DR. LEHMAN: Oh, and one last question I think that we can take is, how scalable you think the process is, ultimately?

DR. VOGELE: So, we hope quite scalable because the bacteriophage production and the cell-free production is scalable, and it's just pipetting in a test tube, so we hope

that we don't have too big constraints there, and we are optimizing our product host quality, too. Get rid of all the steps where it's hindering scalability.

DR. LEHMAN: Thank you very much. I would like to thank all of this afternoon's speakers and all of the speakers today for your time, and also, for helping us keep on schedule. Erica, if you are available, I will turn things over to you to brief everyone on the breakout sessions.

DR. BIZZELL: Yes, thanks, Susan. I will—can everyone hear me?

Dr. PLAUT: Yes.

DR. BIZZELL: Great. I will go ahead and let us out for the breakout sessions. But first, I wanted to thank everyone for joining us for the first day of this science and regulation of bacteriophage therapy workshop. Before we transition to our focus breakout sessions, I would like to also thank the speakers and moderators for the stimulating presentations and discussions today. And thanks to everyone who contributed questions

to the discussion. This main Zoom link will remain open until 2:45 p.m. Eastern Daylight Time for speakers to address questions that may not have been answered in the Q&A. At 2:40, we will begin our first group of focused breakout sessions. These sessions will provide opportunities for attendees to learn more in-depth information from individuals with experience in topics today, such as funding, preclinical services, and patenting, and the IND process surrounding phage therapy. Each session will be held in separate Zoom rooms which are included in the agenda which was previously sent to all registrants. That agenda will also be included in the chat area for you to see those links if you don't have access to it now. Please note, the room designation for the focus session that you would like to join and click the link for that room at the end of the agenda.

A few housekeeping notes for the focused sessions are to please, keep your camera off and mute yourself during the presentation as this will be a separate Zoom

link where you will have access to cameras and microphones, and please, type your questions into the chat. Also, submit your questions much like earlier in the general session today, at any time during the presentations. The first day is now complete, and we won't reconvene after the focused sessions. So, I will release you and the next time we will reconvene at this general link will be tomorrow at 10:00 a.m. So, thank you very much, again, for joining. And I hope that you all enjoy the rest of your day.

(Whereupon, at 2:34 p.m., the
PROCEEDINGS were adjourned.)

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Breakout Session:

**Room A: Patentability of Phage Therapeutics—IP
Challenges**

DR. WALES: Hello, everybody, this is a breakout session for the patentability of phage therapeutics. My name is Michele Wales, and I am the chief legal officer at Adaptive Phage Therapeutics. I just joined them about a month ago after representing them for a good five years, and I should say that before that, before joining APT, I had my own firm dealing with a lot of gene-based clients and inventions. And before that, I was at Human Genome Sciences, where I ran the IP and litigation department. And we also dealt with— if you don't know HGS, we dealt with a lot of human genetic sequences and the patenting on that. So, I've been in this field of patenting natural products, essentially, for a good 20 years. So today what I'd like to do is just talk to you about how attorneys and, you know, how we are trying to protect these new types of therapeutics. If you have any questions,

please don't hesitate to ask. You can ask during the presentation. I have plenty of time to address questions as well as sometimes, these topics are a bit complicated, so it might help just to ask them at the time I'm discussing the topic. So, the first thing I want to show you, if I can change my slide. There we go. So, the first thing I want to show you is, I was interested in this, and I did a really quick search. And what I looked at were how many applications were filed recently with the word "phage" in either the title abstract or claim and compare that to how many patents actually issued in the United States. And what you see is that there is a huge increase in the number of applications that have been filed, although the number of patents that are actually issued are very small. I was actually pretty surprised by this. And when I looked at what exactly was getting issued in the United States, now these are just patents in the United States, for 2021, we have about 21 or so different patents and they can be broken up into different

buckets. And notice that the traditional ways to protect a therapeutic protein, you can do it usually by compositions of matter, or methods of treating. Well, we have those types of claims issuing for phage. But we also have methods of screening, methods of making phage. And that would be a really-phage, you know, directed to the synthetic phage that people are using to treat patients. We have methods of detecting, and a large number of applications that are directed to using phage to express proteins or using phage proteins in their bacterial vector. So, for our purposes today, we are just going to focus on the traditional way to protect products. And that's by composition of matter, and methods of treating.

So, what are the big problems? Why is it difficult to obtain claims to therapeutic phage products? And really there are three major hurdles in the United States that we deal with, and then I want to compare that at the end to the ex-U.S. considerations. Okay. So, let's see.

First, let's deal with written description. So, if you're not aware of this, you can file a patent on your invention, but in order to get it allowed or granted by the patent office, you have to meet a number of hurdles. And the main hurdle that I believe phage companies will be addressing and dealing with in the United States is this written description. And this written description hurdle requires us to describe the invention with particularity. Now, as you can imagine, lawyers debate, what does particularity mean? How specific is particularity? How much information do you need to describe? And I believe that for phage, for protecting phage, I believe the case law on antibodies will be very relevant, and let me explain to you why. In the past there were two ways in which to protect antibodies. The first and the traditional way is to describe in your patent application the specific antibody sequence that you have isolated and obtained. But clearly, in order to define the sequence, you actually need to have made the antibody. The

other way in the United States that we were allowed to protect antibodies was by describing the antigen to which the antibody binds. So, if you were a scientist, you found a new DNA sequence, you predicted its translated amino acid sequence, you could draft a patent application that claimed not only those DNA sequences—I'm sorry, the protein sequences, but you could also get a claim to all antibodies that bind to that protein. This standard is called the "well-characterized antigen standard." And we, the United States, established the standard way back in the '80s and it really led, not only the antibody industry in the United States, but it set the pace and the tone for the rest of the world, and the rest of the world followed us. Unfortunately, for the antibody companies, is that this standard was eliminated in 2017 by a case called Amgen v. Sanofi. And what the court basically said is, for antibodies, we no longer will let you protect it by just isolating the protein to which the antibody binds. Well, this, believe

it or not, is a huge deal for the antibody industry, because just last week, the standard was once again validated when the federal circuit, which is the highest patent court besides the Supreme Court, of course, the federal circuit said that a patent application that was granted based on this well-characterized antigen standard is no longer valid. And it eliminated this huge win by BMS and, it basically—a huge settlement—I mean, they had almost a 30 percent royalty granted to them, has been eliminated. The reason why we're focusing on antibodies is because if you look at phage, the claims are very similar. So, if you wanted to say, let's say our antibody claim was all antibodies that bound to a particular bacteria. The same thing, if you had a very well-characterized bacterial strain, you could not, I believe, cannot get a claim that says any phage that can bind to that bacteria. All right? So. What does that mean? It means that in my opinion, the patents in the United States, if you want a patent to a phage, in the United States, I believe you

will need to recite both—you need to do it by describing the specific phage that you are claiming. So, I went back and looked in this past year to see what patents—if this, my opinion was consistent with what has been happening, and yes, here is an example of an issued composition of matter claim, and what you see here is that the phage are described by sequence. Similarly—and that's in the claim. Another way to approach this is to name the phage specifically. In this case, it's phage 241. Name that phage specifically in the claim, and then in your application, you have the deposit of that phage, or you have the specific sequence of that phage. And notice, this phage—describing the specific phage occurs in both a composition of matter claim and it also occurs in your method claim. So, I believe that written description for therapeutic phage claims will be the highest barrier, and that in the United States, we will need to refer to the phage by sequence or by name. And what that practically means, though, is that when you file your phage

application, it will occur after you actually have the phage in hand. You can't file an application that's hypothetical. So, essentially, I claim all phage that inhibit this particular bacterial strain. I don't believe you're going to be able to get that claim. Now, the reason why this is important is because by describing your phage, by sequence, for infringement purposes, if somebody changes just one nucleotide of that sequence, they potentially have now designed around your claim, and they will escape what is called literal infringement. So, if you have a claim that says a phage comprising sequence ID2 and let's say sequence ID2 is your full genomic sequence, a single nucleotide will escape literal infringement. So, that means then you want to do something and get some variation such as a genomic sequence at least 90 percent identical. But again, you know, your scientists are probably going to be very smart and are going to be able to identify regions in the genome that can be altered and escape that 10 percent

change. So, if I was the patent owner and I want to go after claims, I want to go after the 30 percent claims. Unfortunately, I doubt the patent office will grant those, but you know, you have to balance the specificity by which you are claiming your phage sequence with the ability to prevent others from designing around.

DR. LEHMAN: Michele, you mentioned on this slide, sequence and/or deposit of the phage. Could you talk a little bit about what deposit means in the context of patenting?

DR. WALES: Absolutely. So again, the United States was the leader in this. Early in the '90s, when everybody was trying to claim sequences, the U.S. had a case that made it very clear that if you deposit your construct, whatever it is, your biological sample with a public depository such as the ATCC, that will be sufficient for written description. All you have to do is refer to that deposit in your application. And so, what people do is very often they'll put, you know, a construct or something with the ATCC and just refer to it.

The problem is, is that each time you deposit something, it's about \$2000 or \$2400. So, this was done really prior to the very method for sequencing DNA sequences. So, when it was harder to sequence, people relied on deposits. Now, most people just sequence what they're trying to do and describe the sequence and the application is filed. Okay? So, then let's talk about—so that's written description. Probably what everybody has heard about, though, is the fact that you can't patent phage because they're found in nature. And this is what is often called as patentable eligibility standard or natural products. You can't patent something found in nature, and that is true. In the United States, we have a very famous case and a series of cases, where the Supreme Court disagreed with the highest patent court and said, it doesn't matter that even though our Constitution states that we can cover patents that are discoveries, the Supreme Court said no. Discoveries alone is not enough, that even though you may find something so very important and changing, if

it can be found in nature, it can't be patented. So, initially, when you think about this, this is pretty daunting because it appears that you can't patent anything found in nature. And theoretically that's true. The patent office applies this incredibly broadly. They basically say, anything found in nature is a natural product and is not patentable. However, the thing is, is that you are able to overcome this, and I have found that the standard isn't that difficult to overcome, because what you need to focus on is any difference between your product and what could be found in nature. And so, if you are creating and using as a therapeutic product, let's say a synthetic phage or parts of phages, or something that you derive in your laboratory, that alone will be sufficient to overcome this argument, this rejection. And let me tell you, it really is just focused on any difference. The other thing—so focused on any sequence difference, for example. But the other thing is, if you can make a reasonable argument that your product cannot be found in

nature or is not readily found in nature, that also will work. So, let me give you a couple of examples. We look at, again, another patent that just granted in the United States. Here, the claim is again a composition, and it claims three different bacteriophages. Notice, again, with the written description requirement, these three bacteriophages are referred to by name. ICP-1, -2, and -3. And the spec does in fact describe what those are. But the reason why this claim cannot be found in nature and was patentable is because the attorneys will argue, these three specific bacteriophage are not found in nature. We found them not together as a composition in nature. They may be found in three different locations in the world, but not together as one composition. That alone will be a sufficient argument for overcoming this natural product rejection.

Similarly, here's another patent. Okay, so this again, patent issued this year. This is the one we saw earlier where they referred to the bacteriophage by sequence ID

numbers, but again, their argument for not being found in nature is that they're claiming at least two of these seven specific bacteriophage, were claiming two of them, at least two of them and the argument is, they're not found in nature. The other argument could have been an excipient or carrier comprising a preservative. So, the patent office traditionally—unless you have the preservative recited by name, the patent office will often read this claim broadly and say, oh well, sucrose is a preservative, and it is likely that this phage is found in nature with sucrose. So, that's not sufficient. So, I guess my point is, I don't think this last part about the excipient or carrier is specific enough to get them out of what is found in nature. I'm sure they relied on the fact that it's at least two bacteriophages. But again, this is a way to overcome this rejection by specifying the preservative and then making the argument, hey, this preservative wouldn't be found with these specific seven phage. Okay?

So, the bottom line is, we've dealt with written description. We have talked about natural products. The next question is, in the United States, is it obvious to treat a bacterial infection with a phage? And I think most examiners would say yes. We've known this to be a potential therapeutic for years and years and years. So how do we overcome that? Well, in looking at—it's almost the exact same way that we overcome the natural products, is again, if this claim didn't recite the specific phage, phage 241, but instead, that preparing the food item by contacting the food item with a phage in the amount effective to lyse, the patent office would not allow it. They would have said it's obvious. However, when you specify the phage, the combination of phage, or a specific phage, the U.S. Patent Office will say, any newly isolated phage is not obvious. We have this concept in the U.S. that a new sequence is not obvious, since you wouldn't know where to make the changes. So, in the U.S., if you want to get a claim for, let's say, a method of

treatment, if you can specify the phage, you will get that claim in the U.S. Here's another one. Again, we're going—it's the same theme. For the U.S., if you have two bacteriophage, the U.S. will treat those as not obvious. Now, let's talk about how that compares to ex-U.S. and in particular, the EPO. So, it's really interesting because in the EPO or outside of the U.S., they still rely on the well-characterized antigen test, and they do not prohibit natural products. Let me tell you, the way the U.S. has gone, the rest of the world has called us "off the rails," in these case laws, I mean is really pretty surprising. But essentially, if you have a new bacterial strain that is unique and really important, if you can, you know, if you can overcome obviousness, which I'll explain in a second, you don't need to describe all the phage that bound to that bacteria. The problem is, in Europe is, they take the position that another antibody, let's say, to a protein is obvious. It's just another antibody. Similarly, if you come up with a phage or if you come up with

another bacterial strain and you want to claim all phage that would bind and inhibit that bacterial strain, the EPO would say, that's just another phage. It's obvious. Finding more phage, finding more bacteria, is obvious. And therefore, to actually overcome this—and they call it inventive step in Europe—what they say is, you have to come up with something unexpected about this particular group of phage. And that unexpected property must be disclosed in the spec as filed. So, again, you know, this could be a relatively low hurdle because finding synergy with a group of phage is pretty common. You know, we saw that today during some of the presentations, that they were able to show synergistic activity *in vitro*. But the problem is, once again, to find this unexpected property, you are going to need to have the phage in hand.

So. The summary, is, I believe that whether you are in the United States, or in Europe or ex- U.S., I believe to get claims to therapeutic compositions or methods of treatment, you are going to actually have to

have characterized the phage that are in your pool. And you really should think about the combinations or the activities that are not found in nature, the synergistic activities, and those all lead you to these unexpected properties. So that whether you're filing in U.S. or the rest of the world, these three factors, you know, the sequence, combination, and unexpected properties, are really the information that you want to include in your application as filed, so that you can obtain issued claim. With that, I am done, and I think, I'm done and if I have any questions I can easily go back and talk about them.

DR. LEHMAN: Hi Michelle, you don't have questions that have popped up in the chat box. So, I think I would encourage people to unmute themselves and just ask.

DR. RESCH: This is Gregory Resch, speaking from Switzerland. I have a question regarding the phage sequences. Is it a problem if you in the past previously have disclosed the sequence in a database, and then you want to patent something with this phage?

DR. WALES: Yeah, so yes, it is a problem. There is a grace period that many patent offices provide you. So, some countries provide you six months. Some countries provide you 12 months. So, if you put your phage sequences in a public database, it's considered a publication of that information. And so, I would urge you not to put them in the public databases until you have filed a patent application on them. Once you have filed a patent application on those sequences, they don't publish for 18 months. And so, if you have put them in GenBank, for example, or the EPO—I'm blanking on the name right this second. If you put them in GenBank for example, you want to get them on file quickly.

MR. RESCH: Yeah, thank you. Thank you very much.

DR. WALES: You're welcome.

MS. PASTRANA: This is Diana Pastrana from the NIH, and I had a follow-up question. If you're discovering a new phage, how different does the sequence have to be to be able to patent it?

DR. WALES: Uh-hmm. So, in the United States, it's wonderful. So, in the United States, a single nucleotide difference will allow you to get a patent on it, because the U.S. has a doctrine that says, any-it's called structural non-obvious, but it's basically, a single nucleotide difference will allow you to get a claim. So, the U.S. is great. Europe, on the other hand, will say, that's just another phage. It's not a big deal.

MS. PASTRANA: Thank you.

DR. WALES: Uh-hmm. Now, I just want to add one thing. Let's assume, however, that your single nucleotide difference, your claim will have to be to that particular sequence. Because you have other sequences out there that are so similar, the scope, the breadth that you are trying to get for claim will be much narrower and it's going to be that particular sequence.

DR. ALEXANDER: I have a question, a follow-up. This is Bill Alexander from NIAID. Can you try to run an experiment to broaden your protection by mutating your phage and

showing it still works but now the variability of your phage is, say, 30 percent difference of your sequence? And you could still kill the cell. So, to try to avoid that one base change, say you could mutate it, come up with a gemisch of different phages, sequence them all, show that they still killed the target bacteria and now make your claim to 30 percent difference in sequence.

DR. WALES: Yeah. So, the U.S.—so, do I think you can do that? Yes. Let me tell how I think it has to be done. The U.S. examiners do not like to go down for percent identity. They're uncomfortable with percentage identity that is lower than 70 percent. In Europe, for example, they're okay with 30 percent and it still kills the bacteria. They would be fine with that because you're still having the function tied to it. But the U.S. examiners just have kind of an unwritten rule that they don't like anything below 70 percent. However, so, I think that if you were trying to get something as low as 30 percent, you would need to try, let's say a hundred phage that have

the range from 100 percent to 30 percent, 30, 40, 50, 60, so let's say you would have to provide 50 different examples and show that it would still work. And then you'd have to fight. Because they just don't like 30 percent.

MR. ALEXANDER: Yeah, I'm with you. I was thinking it was still 70 percent the same, 30 percent difference. I'm a former examiner.

DR. WALES: Oh. [Laughs.] Well, then I'm consistent with what you would do.

MR. ALEXANDER: What about the obviousness to treat. Can you get around that by saying a long-felt need in the art, with people still being killed by this bacteria after decades? It's obvious to treat. It's obvious to try, but it's not obvious to succeed.

DR. WALES: Yeah. I would—I mean, I think there could be something there. The only problem is, have there really been real attempts at solving it with phage. Well, I'd have to think about that. Can I think about that?

MR. ALEXANDER: Sure, go ahead. I just like to figure out ways to get around. should have some pretty solid protection when you reduce it to practice, not just one base.

DR. WALES: Yeah. Yeah.

DR. LEHMAN: There are a couple of questions that have been submitted through the chat box. One is, do unexpected properties have to do with the behavior of a unique phage product? Or are there other aspects of, you know, whether it's a single phage or combined, or are there other aspects of, you know, having unexpected properties that -- that might be available to claim?

DR. WALES: So, usually the unexpected property would be tied to what composition you are trying--is tied to the composition, and so if you had--let's just pretend that the claim is a phage plus a 10 percent sucrose solution, and what you're saying is, well, when I combine phage plus sucrose at this very specific composition, I get this unbelievably great activity. Then I would think your unexpected property is really

going toward the formulation. And so, I mean, that can work as well, but usually your unexpected property is tied to the composition.

DR. LEHMAN: Oh, I think we lost her.

MR. PINSON: Yes, she did disconnect. If you have her contact, Susan, you can reach out to her again, but she did drop off. I apologize.

DR. LEHMAN: I actually don't. At least, not a text number, that would be rapid. I'm sure she knows she's been disconnected.

MR. PINSON: Okay. I'll try to reach out on my end on my back channels, but stand by. Michele, you are back but you're muted right now. If you could please unmute, sorry.

DR. WALES: That was weird. Sorry about that, everyone. Go ahead.

DR. LEHMAN: I was going to say, the second, the other aspect of the question that you were just answering when you froze had to do with, you know, sequential application or method of application, whether there are unexpected properties associated with that.

DR. WALES: I'm sorry, how?

DR. LEHMAN: You were talking about unexpected properties, how examiners treat unexpected property claims with respect to composition and the—I'm inferring a little bit from your answer and the question whether the question might have also meant things like unexpected properties arising from manner of application, for example, order of treatment or something like that.

DR. WALES: Oh, sure, sure. So yeah, you're absolutely right. Unexpected properties are really anything. You can rely on improved activity, improved stability, improved or, you know, longer life in the blood, you know, I mean, it can be really anything. It does not need to be scientifically earth-shattering. It's really, again, it's kind of take your science, see what you have there and try to highlight something surprising. And then you just write that in your application.

DR. LEHMAN: Thank you. And it sounds like you had seen the Nagoya protocol question so that was --

DR. WALES: Yeah, the Nagoya protocol is, for those of you who don't know what that is, it's a policy that is—people are trying to implement. I know that Europe has addressed it and accepted it. U.S. is still back and forth on it, but the bottom line is that if you obtain products from nature, from countries, you are supposed to place a very clear statement in your patent application, and you're supposed to make a very clear tracing of the source of those materials, because the country may decide to come back and ask you to provide them with royalties, essentially. You know, what often happens is that those products that are isolated in one country could very well just as easily have been isolated here. But, you know, when you are doing phage hunting, you should think about how to—you should have essentially a plan as to how you plan on dealing with your Nagoya protocol. Okay, I have another one. Does not even need to be a functional genome, is that the case? Okay. So, the next question, I don't know if everybody's seeing it, just to circle

back, I understand that a single nucleotide change does not need to be in a functional gene. You're absolutely right. In the United States, if you have a single nucleotide change, if it's in a nonessential gene area, then it's fine. Then it should be patentable, theoretically. I would caution you, though, if you are making changes in just the non-coding region of your phage sequence, your claim will be to that sequence. So, if I was a competitor, I would then make additional changes in that exact same region that you found to be not as essential, and I'd make the same changes and again, I would be outside your claim. The scope of your claim. Does that make sense?

DR. SAUVAGEAU: Absolutely.

DR. WALES: So, I mean you're kind of, quite honestly, between a rock and a hard place. If you make all the changes in the non-coding sequences, somebody else could do the exact same thing and escape literal infringement.

DR. SAUVAGEAU: And the other

question I had at the same time is that around that 70 percent mark that we were discussing on different sequences, what's surprising is that that's well beyond what seems to be becoming the target for species differentiation?

DR. WALES: Meaning its species differentiation is more of like 80 percent or 90 percent or below that?

DR. SAUVAGEAU: Exactly. Yeah. In the 90s.

DR. WALES: And is that limited to the entire genome or just the coding sequences?

DR. SAUVAGEAU: The entire genome, yeah.

DR. WALES: I mean you could even think about, if you wanted to, you could, you know, codon-optimize three of your phage sequences or three of the genes in the phage, that codon optimization would likely get you out of, let's say, a 90 percent claim, but you're essentially, you know, producing the same proteins.

DR. SAUVAGEAU: Yeah, no, it's very interesting.

DR. WALES: You are absolutely correct. It is a very interesting, like, patent problem, you know, it's kind of a puzzle as to how to negotiate through all of this.

DR. LEHMAN: We are a couple of minutes from our scheduled end, and I am wondering if maybe you wanted to expand on that last comment a little to talk about how things play out in terms of patent submission and an examiner's review versus how they play out—how they may play out—I mean, this is somewhat hypothetical for this field, down the road. You've alluded to challenges from competitors and at the beginning of your talk, you talked about how case law can change over time, and so maybe that's a convenient point on which to kind of tie the beginning—tie us back to where you started your talk.

DR. WALES: You know, actually I think that's like a great—Brian, are those slides still up?

MR. PINSON: Yes, I'll go ahead and bring them back up. Stand by.

DR. WALES: So, let me show you—let's go back to like the third slide once they're up. And you know, for this whole talk, I focused on the compositions of matter and the methods of treatment. But you know, it was also very interesting to me that—thank you, Brian—that that, you know, the methods of making phage—I was—in these phage protein use and expression systems—I almost wonder if I went back and looked at these a little bit more carefully, you know, there are a lot of companies out there where they are describing how to make the synthetic phage. And they have a number of steps involved, you know, first you take your phage, then let's say you evolve the phage, and then you put some sort of selective pressure, or you put some sort of gene in there. You know, and I found it very interesting. I mean, if you think about it, there's like about 12, 13 different patents that have issued on this in the last year. And initially I thought, wow, this would be a

really great way to protect these phage. The problem is, is that the steps, you know, to get a method claim, you have to list your steps and to escape infringement, what you can do then is—to escape infringement you can just omit or reorder the steps, or you know, do something a bit differently. And so, I do think another approach could be, you know, going after these methods of making the synthetic phage, but again, I think it's a little bit of a problem or a legal challenge to really make sure your claim isn't so narrow that somebody can design around it very easily.

DR. WALES: Any other questions?

Well, then, I really want to thank all of you for participating. I appreciate the attention and the great conversation that we had. And if you have any follow-up questions, feel free to, you know, drop me a line and, you know, have a great rest of the conference. It's very interesting.

DR. LEHMAN: Thank you.

MR. PINSON: All right. This NIAID

Meet. It was a pleasure, Dr. Wales. If anybody
doesn't have any further questions, feel free
to close out or leave and enjoy the rest of
your day.

* * * * *

Breakout Session:

Room B: Single-Patient INDs—Step By Step

DR. FIORE: Hi this is Cara Fiore, I was hoping that people could see my, the first slide of my presentation.

DR. REINDEL: Can't see it, Cara.

DR. FIORE: Can't see it—huh. It says I'm sharing.

SPEAKER: You just had it up, currently you're sharing your current screen. So, if you click on the presentation on PowerPoint button at the bottom, it should pop back up.

DR. FIORE: Like that?

SPEAKER: Yep.

DR. FIORE: Okay great. I'll give it a few minutes and then we'll start.

SPEAKER: Hey, Cara?

DR. FIORE: Yes.

SPEAKER: I think it would be better if you shared just the presentation instead of your screen. So, I can walk you through that.

DR. FIORE: Okay.

SPEAKER: If you go back to Zoom, and then you hit "share screen," it should give you the option to pick the PowerPoint slide, yep that one there, and you can hit "share."

DR. FIORE: Is that better?

SPEAKER: Yes, way better, thank you.

DR. FIORE: What did it look like before?

SPEAKER: No, it looked the same, but this time we'll only get the presentation and not the chat messages and other stuff on your screen.

DR. FIORE: Okay, thank you.

SPEAKER: No problem.

DR. FIORE: Hi, this is Cara Fiore, and I am sharing my screen. I'm not currently sharing my video to save a little bit of bandwidth on my end, I'll share the video after, once we have a discussion. The way I believe this session is going to work with all the focused sessions is I will give a brief presentation and then open it up to questions, so I'm going to talk the next few minutes about Single Patient Expanded Access IND

Process or SPIND 101. So, in order to give this some sort of clinical—some sort of context I'm going to have to repeat a few slides from this morning, but it's going to be in the context of Single Patient INDs. So, for those of you that tuned in this morning you will see a couple repeat slides. So, my comments are an informal communication and represent my own best judgment. The information presented here does not bind or obligate the FDA, so that is my disclaimer.

Over the next few minutes, I'm going to outline why request a Single-Patient Expanded Access IND or SPIND. I'm going to chat about our experience in the Office of Vaccines with SPINDs for phage therapy and what information is recommended for SPIND requests currently. So, just as a reminder, phage therapy for infectious diseases in patients is regulated by Office of Vaccines Research and Review within the Center for Biologics. So, this is where you're sending inquiries and SPIND requests.

So, when in the process does the FDA

get involved? This is a repeat slide, but for context, just so you know, in the US an investigational new drug application is required to conduct clinical investigations of unapproved new drugs. This includes Expanded Access Single-Patient INDs. I am having, don't know if other people see it, but I have something on my screen which I didn't have before.

SPEAKER: No, you're fine.

DR. FIORE: Well, it's covering up part of my screen, this is weird. Okay well, sorry, this is, there it is, never mind, there it goes. Okay, so you need an IND for Single-Patient Expanded Access INDs. And you need it because it's an unapproved drug, and the definition of a drug is an article intended for use in the diagnosis, cure, mitigation treatment, or prevention of a disease in man. And the IND, when an IND is in effect, it allows exemption from premarket approval requirements, and the product may be lawfully shipped for purposes of clinical investigation. So, basically, when we issue an

IND number, that makes it legal for you to ship your investigational product, which in this case is a phage therapy product for the Single-Patient IND. Human studies are conducted under an IND, whether or not the intention is to market the product or not, so therefore Single-Patient INDs will not go, necessarily support marketing, but you still need an IND number. And here I have cited a guidance document which may be helpful about it.

So, Expanded Access INDs. So, the purpose of expanded access is to provide access of the investigational or unlicensed product. It's not to collect systematic safety or effectiveness data, which would be data from adequate and well-controlled clinical trials intended to support licensure. It's not a product development pathway. The CFR does define this, this Code of Federal Regulations, and expanded access INDs can be issued when a patient has a serious or immediately life-threatening disease or condition, as defined in the CFR. A serious disease or

condition means a disease or condition associated with morbidity that has a substantial impact on day-to-day functioning. This is determined by the treating physician, and the rationale is provided to us when we evaluate your request. The information that you must include is outlined in the CFR, is administrative, which is evaluated in the 3926 form, CMC, chemistry, manufacturing and controls, that's how you're making and testing your product. The clinical information, which would include your treatment plan for your single patient, and any sort of pharm/tox information that you want us to review as applicable to your product. So, as I outlined this morning, there are three categories of Expanded Access INDs, the Single-Patient IND is here on the left in the brownish box, and the Single-Patient IND includes both emergency and non-emergency Single-Patient INDs, so that is what we're going to focus on in the next few minutes.

So, what are we looking to evaluate in a Single-Patient IND, a Single-Patient

Expanded Access IND request? First of all, we're looking that the probable risk to the person from the investigational product, which is going to be your phage therapy product, is not greater than the probable risk from the disease that the patient has. You also have to provide information to us that there's no satisfactory alternative therapy available, and that you've exhausted all other licensed treatments. So, the patient cannot receive this investigational product through any other existing clinical trials or expanded access protocols, in other words, this is the only way that this patient can get this product. And also, by granting a Single-Patient Expanded Access IND it would not interfere with other investigational trials that could support a product's development, or marketing approval. So, an emergency use Expanded Access Single-Patient IND can be issued in the situation that requires a patient to be treated before a written submission can be made. So, at the end of the day, the information that we are going to look at is

going to be similar, it just depends on when we look at it. If it's truly an emergency, we may require less information up front. If you want to inquire about a non-emergency Single-Patient IND, you can do it through our industry.biologics email address, and I'm going to tell you over the next few slides how to actually make the request directly to us if you're ready to actually make the request.

So, before I get into that, I just want to tell you about our experience with Single-Patient INDs. This morning I presented all INDs, but this focuses on just the Single-Patient INDs, both emergency and non-emergency obviously. And the majority of our requests are still emergency Single-Patient INDs. So, you see that slightly edges out the non-emergency Single-Patient INDs that we have granted, but they're almost equal at this point in time. And I've listed here the top six target strains that the phage product is targeting, so this would be the bacterial infection, and at the top of the list for the Single-Patient INDs are *Pseudomonas*, followed

by *Staphylococcus*, and then we have these other four categories, *Mycobacterium*, *Acinetobacter*, *Klebsiella*, and extended spectrum beta-lactamase *E. coli*. So, these are sort of the most common strains that we see phage therapy products trying to treat for Single-Patient INDs.

So, in terms of requesting a Single-Patient IND, as I said earlier, it always has to include administrative information, product information, clinical information, pharm/tox as applicable. You can make a submission directly through email for non-emergency Single-Patient INDs using this email address. When you do this, you would include all the information I've outlined here in PDF format, along with the 3926 form, or if it's an emergency, you can phone us directly and I will give you those numbers in a second. The initial information may be more limited for requests for a Single-Patient Emergency IND, though I did refer to that earlier. But all INDs require documentation, informed consent, and IRB approval or IRB notification for

emergency use. So, you still have to have the critical information that you need for INDs. And here is a website, we have a pretty robust website on expanded access if you'd like to check it out.

So, looking at just the emergency Single-Patient INDs, how we issue them. Generally, the requester is the treating physician, we call this the IND sponsor, and the treating physician contacts the FDA with an emergency Single-Patient IND request. As I mentioned, they can do it by phone during business hours, it's this 1-800 number, if it's outside of normal business hours, holidays or weekends, they will call our emergency call center number, which is there as well. And this information is on the website that I just referred to. When we get the request, we review it, if it's appropriate, we will authorize it, and assign an IND number to the application. If emergency use is authorized over the phone, the treatment may begin immediately. We may initially have questions back and forth with

the treating physician or the IND sponsor, but once we authorize that IND number, you can begin treatment. The treating physician or the IND sponsor should provide this IND number to the phage supplier, so that's whoever is making your phage, so the product can be shipped legally. So, if they need to ship the product, the IND number, because it's an investigational product, needs to be on the packing, the packing label and the slip. For emergency INDs, it's required that you notify your IRB within five days, and the IND sponsor must submit the required information to the FDA within 15 days. So, this is if you get your IND number over the phone, so if it's an emergency IND you've requested over the phone, you have 15 days to submit the information that we request from you to your IND. You can do this by email, and you can email it directly. All amendments can be emailed directly to the CBERSPIND email address that I have there. So, in that email correspondence, we ask that you always cite your IND number and include a 3926 form with every amendment.

So, what you would do is you would get your IND number over the phone potentially, and then you would submit all the information directly to the IND via email with your 3926 form filled out. And like I said the IND number should be in the subject line and also in the body of the email.

So, what happens for the non-emergency Single-Patient INDs in the first 60 days? This is not unlike a regular IND, it's on the same type of review clock. On day zero is when you would submit the non-emergency Single-Patient IND, and you can do this by email to the same exact email address, so this would be for an initial submission of a non-emergency Single-Patient IND. Between day zero and day 30, we assign an IND number, and we send you an acknowledgement letter saying that we have received your request. During these 30 days we also perform a preliminary review. By day 30 or before, we contact you, and we may communicate review issues. We could do this any time during the 30 days, but by day 30 we would do this. And

on day 30 we would tell you whether or not your study may proceed. In this case it would be for the single patient. And if your study can proceed, you would provide that IND number to the manufacturer, or on day 30 if we have safety concerns, your IND could go on Clinical Hold. If your IND goes on Clinical Hold, between day 30 and day 60 we would send you an official letter outlining our safety concerns that you would have to address prior to using the investigational product. So, you address these issues, whatever we tell you in this official correspondence, by submitting the information to your IND using that email address I have up front, saying you're responding to a Clinical Hold. You have to be very clear about this, and there is guidance about how to do this. Again, this is not different than a regular IND at this point. So, we have to review it, and we notify you in writing about whether or not we release you from clinical hold or we had continued safety concerns. At any time during this process, we may also notify you about non-hold items. We

do recommend that you address non-hold items in a timely fashion.

So that's the first 60 days, very similar to a regular IND that's going through clinical evaluation. So, there are responsibilities for the treating physician or the IND sponsor, and we have recommended information that we request you to consider when you request a Single-Patient IND for phage therapy. On the left-hand side I have the product information, so you need to provide a unique phage identifier for every phage in your treatment, whether you have a monophage or a cocktail, you have to have a unique identifier for each phage. You would have to provide us with a certificate analysis. On the C of A, we would expect to see the name of the product, the date it was manufactured, who manufactured it, potency, that's usually measured in plaque-forming units per mL, sterility testing, we recommend you use USP 71, and potentially endotoxin content. We ask that you give us a description of your manufacturing process that was made to

manufacture the phage. We would like to see phage lytic activity against the infecting bacterial strain that you're trying to treat, any sort of characterization of the phage and the host strain that you're using to propagate the phage that would be relevant, we would like to review that, if there was exotoxin content in your product, we would like to know how you're mitigating that, and any sort of exotoxins that perhaps your host strain or your phage may have, we need to know how you're going to mitigate that when you're treating the patient, usually via the manufacturing process. If you are the manufacturer and you are not the IND holder, and you want to submit the information to us confidentially, this morning I outlined how to submit a Type 2 Master File. So, you can submit a Type 2 Master File to us that would contain all the manufacturing information on the phage, and you would also submit a letter of authorization from the manufacturer to the Master File that allows us to review the Single-Patient IND in the context of your

product. This is confidential, we don't reveal, we don't reveal the contents to the IND holder, but it would have to support the IND that's being proposed. You submit this Master File information to the Center for Biologics, because that's who's reviewing the IND. An individual Master File can be used to support multiple INDs if it's the same product and you're making it the same way. So, if you have half a dozen, for instance, a half a dozen Single-Patient INDs that are all treating *Pseudomonas*, and you have one phage and you're supplying these to various treating physicians, you can use the same Master File. So, it may be helpful to manufacturers that are supplying multiple products to INDs. So, on the clinical side, there is the 3926 form that I alluded to earlier. On that form, you need to put the patient, the disease the patient has, the clinical history of the patient, any interventions that have been tried, the current patient status, so is the patient inpatient, outpatient, etc., the weight of the patient, the sex, some sort of

identifying initials, and any sort of relevant patient test results that you want us to consider. Keeping in mind this is Expanded Access INDs and I outlined our criteria earlier. In the clinical information, and you can attach this, usually the 3926 form is not long enough, doesn't give you enough room. You should include a treatment plan, this is sort of a protocol of sorts for your single patient, that treatment plan should include the proposed treatment in terms of a dose that you're planning on administering to the patient, the length of time, are you doing it for a week, two weeks, are you doing it multiple times a day, the route of administration, are you giving an IV, topically, inhalational, etc., what type of safety monitoring you're planning on conducting on the patient, the follow-up plan after your active treatment amount, and then collection of adverse events, and if you see under footnote two, I've cited the CFR for expedited reporting of adverse events. You should include a toxicity grading scale, and

footnote number three I've cited an example of one. Please give us criteria for discontinuation of therapy, any sort of other concomitant therapies that the patient is having at the time, this would include antibiotics and any sort of other therapies, and how you're assessing the treatment. So, also please include a blank Informed Consent Form that we can comment on, and please include the IRB approval when available. After you've finished the treatment and the follow-up period, please submit a summary of the clinical outcome to your IND and withdraw the IND at the end of treatment. All this again can be done via email, to the email address that I posted earlier.

So, in terms of retreatment of a Single-Patient Expanded Access IND. So, this is when you're a treating physician or an entity, and you've treated a sick patient, and you've gotten an IND number, but you want to retreat the patient for whatever clinical reason. So, how we decide the regulatory process depends on what you're doing to the

patient. So, on the product side, if you have a new product, that would be a new phage, and you're targeting a different bacteria, so the patient now has another infection, a different strain, you would start the process all over again. You would request a new Expanded Access Single-Patient IND. If you have a new product, but it's the same target bacteria that you've been targeting before, and it's a new manufacturer, or it's just someone new that's making it, again we would ask that you start the process again with a new, requesting a new Single-Patient Expanded Access IND. If you have a new target, and it's a new phage, but it's the same target bacteria and it's the same manufacturer, we would recommend that you send an amendment to your existing IND with a certificate analysis of the new product. You will also want to include a full product description as you did before for the first product, and outline the manufacturing, etc., in addition to the certificate of analysis. If you have the same product that you used before, and it's the same exact lot number,

and there's just more of the product available, we may need to look at updated stability information depending on the length of time that has gone by since the product was made initially. And that updated stability information may contain potency information, or other data as needed. If you're using a new lot of the same product, so it's the same product, same phage, but has been manufactured again by the same manufacturer, we would like to see that new certificate of analysis, and we may ask for other data as needed. So, that's the product side. On the clinical side, if you want to retreat a Single-Patient Expanded Access IND, in order to start the process in addition to the product information depending on the route you're going, we would need a summary of the previous treatment and the outcome. So, what happened the first time around to that patient. We would want to see a rationale for retreatment, we would want to see updated phage susceptibility data, and a new treatment plan or protocol as you had done previously. So, it's a new treatment, so you

have to provide a new protocol. You also may have to re-consent the patient, and the Informed Consent Form may need modifications based on new treatment. And we would like to see that Informed Consent Form, a blank copy please. And then basically the same as before, where at the end of the day, a summary of the final outcome of the IND and then withdraw the IND when you complete it. Again, you can do all of this by email.

So, in summary, Single-Patient INDs are regulated under 21 CFR 312 and must include enough information, both clinical and product, to assure the safety of the patient. If the IND is put on Clinical Hold, then the sponsor cannot proceed until CBER has indicated in writing that all Clinical Hold comments have been adequately addressed. Non-hold clinical comments are advised to be addressed as timely as possible. The sponsor, and usually this is the treating physician, has regulatory responsibilities for IND submission, maintenance, and withdrawal at the IND, of the IND at the end of the day.

So, I'd like to thank my supervisor, Liz Sutkowski, and my colleagues, Laura Gottschalk and Laura Montague for commenting on my slides. Here are some references, and at this point I'll open it up to comments. So, let's see, that means I have to manage it somehow. So, let me look at the comments.

DR. PLAUT: Cara, this is Roger Plaut. There was one comment that I responded to because it was about CMC.

DR. FIORE: Okay.

DR. PLAUT: But I do have one additional comment of my own to make, and this was about the letter of authorization, so it's somewhat related to the comment that's in the chat. So the 3926 form does have a spot where it says, "letter of authorization from the manufacturer," and sometimes we have sponsors who don't really know whether or not they need to fill that out or not, and as you explained, Cara, the reason to fill that out is if the manufacturer either has a Master File with us or has a previous IND with us, and then they can provide that letter of authorization to

the sponsor of this Single-Patient IND, and they'll submit it to us. But if there is no existing Master File or previous IND describing the manufacturing information, then there is no letter of authorization, that only applies if there is a Master File or existing IND. So sometimes sponsors get confused about that, it's not really explained on the form. So, if it's a new product, you know, it's just been made for this purpose, then there's no need to include any sort of letter from the manufacturer.

DR. FIORE: Thank you Roger. I actually don't see any chats, does anyone, I don't know if I can un-mute individuals, but --

DR. PLAUT: They should be able to un-mute themselves.

DR. FIORE: Okay, so if you would like to have a question, or discussion, you can unmute yourself and go ahead and make sure your volume is up adequately and let us know what your question or comment is. So, I see the question here, "Is the addition of one

more phage to a cocktail, would it require a new SPIND?" So, I went through, I'm going to go back to that slide, because I did go through a lot of different scenarios on that slide, and I'm hoping everybody can still see it. So, it would depend, so if you have a cocktail and you're using the same manufacturer, it would actually be a different product, but we, at this point, I think that we would look for an amendment to your existing IND assuming you're treating obviously the same patient, and it's the same manufacturer. And the same target bacteria. So, we do, I will give the caveat, and you can see in the title of this slide, where regulatory considerations current practice, I do, I will say and my colleagues will agree that this is done on a case-by-case basis, but generally that's the current practices that we're following. So that was directed at David Jensen I believe. Well, this could be a very short talk if no one else has any comments.

DR. HATFULL: Cara can I, this is Graham Hatfull here, can I ask just a quick

question?

DR. FIORE: Absolutely.

DR. HATFULL: I, this is just pertinent to essentially the source of phages and to what extent it may make a difference in the review process, specifically, one potential source of phages are phages which were identified as prophages genomically, and then have been propagated lytically, engineered so that they don't form lysogens and to satisfy the other requirements. Is it okay to assume that it would be treated similarly to a phage that did not come from a prophage, but came from the environment as a lytically growing phage, if that makes sense?

DR. FIORE: I'm going to defer to Roger, before putting my foot in my mouth or anything. Roger, do you have a comment on this?

DR. PLAUT: Sure. So, Dr. Hatfull, you're describing a situation where a phage was initially determined to be lysogenic, and was engineered to no longer be lysogenic, and you're asking if that could be used in a

Single-Patient IND?

DR. HATFULL: No, it's a, it's a sort of subtly different question, as you may or may not know we've dealt with a number of engineered temperate phages that have been authorized through the IND process. This is a slightly different question, this is using not a phage that was isolated say from the environment, but a phage that was identified solely as a prophage as part of a bacterial genome, but which was then essentially enabled to grow lytically, right? And so, it not only has the genes you need to engineer so that it becomes lytic but you know, prophage genes that could influence you know, the physiology, or potentially the virulence, so, I guess the question is does the source of the phage really make any difference to the approval process?

DR. PLAUT: Okay, so, this session is about Single-Patient INDs, so in that case I don't think that it would be reviewed any differently from any other phage, as we've been saying all along, each Single-Patient IND

is evaluated separately based on the risk-benefits profile. And what you're describing to me just on the face of it doesn't sound like it would represent a safety risk, so I wouldn't see why that would raise any particular concern with us.

DR. HATFULL: Okay, no, that's very helpful, thank you.

DR. FIORE: Are there any other comments, or questions, or discussions that folks on the line want to have?

DR. PLAUT: There's a question in the chat, Cara.

DR. FIORE: Okay. So, this question says, "We are at a small medical foundation hospital, and I'm wondering if there are examples of an SPIND that could be used to help educate our IRB and other physicians about using phage therapy as we move forward with our research." So, that is a great question. We don't actually have examples, but on the webpage that I referred to, it does go through whether you're a patient, whether you're a treating physician, it does walk you

through, it's a pretty good website. I would refer to that website, we can certainly answer questions, you know, patient- and product-specific, but I would refer you to the website, it's a general Expanded Access Single-Patient website, it has information about how to do it and when it's, you know, when you should request one. But we don't have an actual example. One of the reasons why, and I'm going to go up one slide, one of the reasons why I have this information here is because what we have found over the last few years is that there has been a struggle of what to, what to give us for our review of this, whether it's emergency or non-emergency, so we've put together this list of, of high level, pretty much must-haves I guess. So, in terms of phage therapy itself, we don't really have an educational avenue, but in terms of the information providing to potentially administer phage therapy, this is, you know, what I have on the screen now is high level what we would like to see when it comes in. And I will say, and, that we have numerous, if

you actually look through the literature, there are quite a few now, of US-based phage therapy papers of individual phage therapy papers, several sponsors have put together journal articles that sort of encompass several different phage therapies that they've done that might be sort of educational for a foundation such as yourself.

MS. MCCALLIN: Cara, I have, this is Shawna McCallin here, I have a, just kind of a general comment, I'm looking more just to see about your opinion on something. So, there's definitely been an increase in the number of these single-patient cases, both in the United States and elsewhere, so there is an initiative to start an international database, or sort of patient registry where these cases are going to get either retrospectively entered, or prospectively entered, depending on the center, you know, overall, do you think it's a good idea, do you think there's certain things that should be included in that database? I mean, we're almost done with the initial version with input from about 20

different researchers, and I think we cover a lot of the things that you have on here, but do you think there's potential value for that?

DR. FIORE: I do think there's potential value, I think keeping in mind that Expanded Access is really not meant to support clinical development or go toward data that would support licensure of a product, but I do think there's value in seeing what other physicians and entities that are treating patients with Single-Patient INDs are doing. You know, what I think we would like within, within Office of Vaccines, is we would like to see these patients enrolled in controlled clinical trials and less Single-Patient INDs, because we think that way you would get more meaningful data, and potentially move phage therapy closer toward a product that was meaningful, and useful and potentially licensed, although I wouldn't deter from collecting Single-Patient IND information, I think the, what we want to see is development of a phage therapy product and putting it in controlled clinical trials to really get the

most meaningful data that you can out of the investigational product in the patient. So, I think that's a little bit of a soapbox for me, but the Single-Patient INDs are fairly labor-intensive for us, but we do have them for a reason obviously, and you know, it is available for treating physicians and other entities to use. But moving toward controlled clinical trials helps phage therapy move forward in development, so that's sort of where we would like these to go. Less Single-Patient INDs and more controlled clinical trials, and I think this is sort of a naturally occurring process, but it's just been a little bit slow to get going in the phage therapy community.

MS. MCCALLIN: Yeah, I definitely agree with you, and I mean, the idea behind the database is not to take away from any effort or momentum to do trials, but more so eventually to see if there's any type of signal or things that we could use to actually build better trials, but thank you for your input on that.

DR. FIORE: Sure, thank you. Okay, so we have a question here, "Not sure if this is the right place to ask, but I am curious about the issue of NGS versus USP 71 sterility results in development of new phage for therapy. NGS would be done anyway in order to characterize, to verify the phage, so there is some replication of information, although I understand that each has their validation standards." This is more in the weeds of a CMC issue, and I would, if Roger is still on the line, I would ask him to take a stab at this.

DR. PLAUT: Sure, sure. So, I guess what, Anca, if you want to unmute yourself and respond that's fine, but I guess what you're asking is, if you're using next generation sequencing anyway to determine the sequence of your phage, why would that not be enough to show that your product is sterile? And it's an interesting question, but the way that we look at this is, okay, you have a phage preparation that you have made, and since we're talking about using this for a single patient, then your question is actually a little bit more

relevant. If this were a product that was intended for development, then I would say, well that's just one, one bottle, one preparation that you've made, and you know, you're not really demonstrating much about sterility, if that's just the one that you're sampling. But if you're talking about, you've made a preparation and you want to use that very same preparation to treat a patient, the problem is that the sterility test, USP 71, has been validated and we know what that detection limit is, we know how it works, we know what organisms it can detect. And next generation sequencing, although it's very powerful, we don't know very much about what you can and cannot use it for, and what the detection limit is. So, we know that if you do USP 71 properly, you should be able to detect very, very, very low numbers of organisms. And we don't know enough about the next generation sequencing to know whether or not you'd be able to detect one organism, that you know could be a sporulating organism that could potentially be dangerous if it was

administered intravenously. So, it's just a matter of making sure that the assay is properly validated and that we know the limit of detection and we know what it can and can't do.

ANCA: I guess the question would be one of saving time, because sometimes time is a big issue, and the USP 71 test can take almost a month, I guess. That's the issue in addition to cost, but I don't think cost is the biggest issue here.

DR. PLAUT: Sure, so USP 71, you know, the method itself takes 14 or 15 days, so I can see what you mean by that. So again, if we're talking about an emergency situation, then the physician would determine whether the benefits outweigh the risks, of you know, whether they would really want to proceed with a product that they weren't sure was sterile, and you know, we'd have to discuss that at great length, depending on how seriously ill the patient was. If it's not an emergency, you know, in that timeline, we have 30 days to review, so there would be plenty of time to

get the results of a USP 71 test. So, it kind of depends on a case-by-case basis, and we do discuss these sorts of issues with Single-Patient IND sponsors.

DR. FIORE: Thank you, Anca for that question. Are there any other questions or comments? With Single-Patient Expanded Access use, whether it's emergency or non-emergency? Okay, I don't see anything coming through. I'll give another minute or two, I'll go to the end slide, I don't have my email address up. There's my—if you have questions, you are free to email me and I will try to provide as good an answer as possible in terms of the context that you give me, whether it's for phage therapy INDs in general I'm happy to try to answer questions. So, unless anyone else has questions, we'll close the session. I really appreciate everyone calling into this, today has been very impressive for this workshop, and I'm excited that there's so many people attending it in general. So, any parting remarks before we close? Okay so if you have any questions, feel free to email me,

if not, tune in tomorrow. Thank you so much.

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Breakout Session:

Room C: Preclinical Services at NIAID:

DR. ZEITUNI: What do you think, Erica? Okay, let's do it. I'm going to share my screen here. All right. And you're able to see the slides?

SPEAKER: Yes.

DR. ZEITUNI: Wonderful. Thank you. All right. So, welcome everyone to the Breakout Session covering Preclinical Services, which are available at the National Institute for Allergies and Infectious Diseases or NIAID. My name is Erin Zeituni, and I'm co-hosting this Breakout Session with Dr. Erica Raterman. We both serve as preclinical services program managers in the Bacteriology and Mycology Branch here at NIAID. And again, if you have any questions throughout the talk, please submit them to our chat, and we will go over them at the end of the slides.

So, for product development, we have multiple sources of support for different

projects from basic through to clinical evaluation. And so, here at the top of the slide, we have what we call our product development arrow, which covers basic research going through preclinical development on through clinical evaluation.

And at NIAID, where we cover a variety of different human pathogens, we support product development for many different types such as small molecules, vaccines, monoclonal antibodies, host-targeted therapies, and phages, which will be of particular interest to this group.

Most people are familiar with our grants and contract mechanisms, which are shown here in yellow going for both basic, translational, and clinical research in the case of grants such as our R01 and R21 mechanisms. And preclinical development and clinical evaluation can be supported by our product development contracts. And this provides direct funding to an institution or investigator.

In addition to these sources of

direct funding, we also have a variety of free resources and services that are shown here in blue at the bottom of the slide. These include our resources for researchers, preclinical services, which we will be largely talking about today, and our phase one clinical trial units.

So, just briefly on one slide, I do want to cover our free resources for researchers, which provide a variety of reagents and data to product developers and research communities. Many of you would be familiar with our BEI Resources, which is a central repository containing a variety of organisms and reagents that are freely available. And you can access these through our online catalogue. There's a screenshot of that catalogue shown here on the slide. And these resources and reagents are free, although domestic investigators do need to pay for shipping.

Other centers that are funded and managed by NIAID program staff include our Structural Genomics Centers for Infectious

Diseases. These provide 3D atomic structures of proteins and molecules that are of interest to microbiology researchers and product developers. And so, if there's a particular target of your phage or protein or molecule that is of interest for you to have that 3D structure to inform your program, this could be a good resource for you to take a look at. And the link is provided here.

Finally, we also have Bioinformatics Resource Center, which is funded and supported by NIAID program staff. And this is a resource center that provides platforms for data sharing and access to make sure that there's interoperability between the datasets and provides access to computational tools. Again, the link is provided here.

So, now that we've gotten through these, I just briefly want to give you an overview of what we're going to be talking about for preclinical services. First, we'll go into what these services are, who is eligible, how you access the services, and then Erica will be telling you all about some

specific examples of services that we have provided for phage developers, and open questions around the best types of services that would be available to phage developers to help fill gaps in their programs.

So, NIAID's preclinical services are a suite of contracts that we manage to provide free studies to support anti-infective product development. Unlike grants and contracts, which can provide multiple phases and multiple studies for a product development program, these services are intended to be gap-filling, so we're hoping that product developers will come to us after identifying a particular gap in their understanding that they're having a hard time getting over in order to continue moving their program forward. The intention is that these services would lower the risk and advance promising discoveries through the product development pathway.

So, if this sounds interesting, I'm sure you're wondering who's eligible. Investigators and product developers from academia, non-profit organizations, industry,

and government are all eligible. Domestic and foreign institutions are eligible, and you don't need to have NIH funding.

Additionally, I just want to note that it is a simplified request process to access these free services, so you can make a request year-round. There's no specific due date for requests. And the intention there is for it to fit more in line with the timelines for product development, which can be pretty rapid requirements for business and research decisions.

So, if this is sounding like something interesting, I'm sure you're wondering how to access preclinical services. And it starts, as I mentioned, with the product developer identifying a gap in their program and a need for additional data to fill that gap and move forward, and in the cases of phage, Erica and I wanted to sort of define what a phage product would look like and what data package we would hope it would have before you would come and talk to us. Because at this chat box here, you see the starting

point is that you need data, but pretty much everyone needs data from basic through translational and clinical researchers, so we want to make sure that we're looking at a phage product that is somewhat on its way to a preclinical program targeting clinical application.

And so, Dr. Roger Plaut did a great job this morning outlining some phage characteristics that are ideal for clinical application, and I'll just reiterate a few of those here and add a little bit more flavor to it. So, we would hope that you have a non-lysogenic, non-transducing, well-characterized, fully-sequenced phage that doesn't have any of those sticky bits that are going to make it difficult for clinical use, such as virulence factors or antimicrobial resistance genes or segmented genome. And if you have a phage cocktail, we would hope that you would have an understanding of how each of the elements of that cocktail is providing activity to the cocktail as a whole, as well as an understanding of the individual elements

and their activity individually.

For preliminary data, we'd like to have you have an understanding of the spectrum of activity of your phage, and we'd like to see a pretty good representation of activity against a panel of clinical isolates, something along the lines of hitting 70 percent of those isolates for a pathogen that your phage is targeting.

And since we're talking about the pathogen that you're targeting, and how you would be thinking about your phage product is pretty important to us, so we'd like to understand your target product profile, what is the clinical indication that you're aiming for, and what sort of characteristics of your phage and its use do you think are going to help it go towards that particular clinical indication. Okay. So, you have a phage, and you have an understanding of the characteristics of that phage. You have a little bit of preliminary data about its activity. You're thinking about what clinical indication you might use it for based on its

activity to date, but you still need data.
That's a great time to come and talk to us.

And a classic example of one of those data gaps is when you're talking either to venture capitalists, or you put in a grant application, and you receive your summary statement back. And in either case, they say, you know, we'd really like to see some proof of concept animal efficacy data just to give us the idea that this is working *in vivo* as expected before we would throw in our hat for support. But maybe you don't have access to an animal model, or you don't have the funding to support an animal model, that would be a great example of a time to come and talk to Erica and I.

And so, it does start with email. At the bottom of this slide, I have listed a link to a list of all of the preclinical services program managers for our division. That list is delineated by pathogen and indication. So, if you're looking at ESKAPE pathogens, then Erica and I are the people to talk to. For some other indications or groups, it's all

listed there. But if you have any concerns or confusion, we would be very happy to point you in the right direction. Don't hesitate to reach out to us.

So, once we get in touch, we would invite you to give a talk to help us understand your phage program and your preliminary data package and to understand what sort of request you're looking for. That allows us to verify that we have task orders that could support the particular request that you have.

And so, if it does seem like there's a good match, we would then invite you to submit your request to our online management system. And Erica and I then bring your request to our product development team for discussion, where we will approve or deny the request.

And if approved, we then put you in touch with our contractors to start a three-way conversation around the study design for your request. You have full input into that study design and the ability to ask

questions of our contractors. We do have constraints around some specifics, like animal numbers and infection routes and that sort of thing based on the task order contract, but you do have input and the ability to approve the study design.

So, once it is approved and finalized, we then move forward. The contractor will ask you to send your phage, and then the contractor would start the study.

Once the study is completed, they would submit a draft report for all parties to review and approve, and then in the end, you receive that final report. And that data is yours to do with whatever you like. You can publish it. You can use it in a grant application. You can use it to garner additional funding from other sources. You can use it in your IND application. However you want to use it. At that point, NIAID backs away and you go forth with your data package.

However, sometimes questions do come up, or years down the road, additional gaps come up in your program, and at that point, we

can enter back into this loop of discussion about receiving requests for your program. And we'll always be asking you for the rationale and justification behind the request and looking at our current capabilities at any given time.

And so, at this point, I'm going to be kicking it over to Erica to give you more information about the specific types of services we've provided for phage groups, and to also start us thinking about what types of studies are ideal for phage developers and any kind of gaps that currently exist in different preclinical packages and animal models and things like that. So, Erica, go ahead. Take it away.

DR. RATERMAN: Thanks, Erin. So, as Erin mentioned—can you advance the slide, Erin? So, as Erin mentioned, we just wanted to give you a taste of these sorts of services, and particularly the animal models that we have available throughout the year.

So, what I'm listing here is the small animal. So, these are all mouse models

that we offer throughout the year. These are for the ESKAPE pathogens. So, as Erin mentioned, for any of the other pathogens, we would refer you to our other branches.

We do run a 24-hour mouse thigh and lung model, as well as 120-hour *Pseudomonas aeruginosa* mouse lung model. I'll mention that all three of these models are in neutropenic mice. So, that's something to keep in mind.

We also have seven-day bacteremia and peritonitis models available as well. So, these would give you a survival and a CFU burden readout. And these are also in immunocompetent mice. So, a little bit different if, you know, having intact immune system is important for your product.

And then we also offer a seven-day mouse UTI model. So, in this model, it's immunocompetent mice, although we can make them diabetic through streptozotocin treatment. And this model is only available for *E. coli* and *Klebsiella*.

So, I'll mention for all these models, these were developed with small

molecules in mind. That's the product they were originally developed for. So, just keeping in mind that we might consider, you know, making modifications in the future from feedback from the community of anything that might be more useful for assessing a phage product versus a small molecule.

We also typically offer *in vivo* PK and PD experiments, although I'd say that we're in the very beginning phases of being able to offer that for phage products. So, typically, again, this would be for a small molecule, traditional product. We can also perform IND-enabling GLP studies.

And then in addition, we can also help you with your product development plan. We can help you with the IND documentation for your submission to the FDA. So, those are all services that are available through us.

So, going to the next slide, Erin. So, these were the different variables we had in mind when we're considering models specifically for phage products. So, some of the model parameters we've considered are

impact of the immune system. So, do you really want to do—would there be a difference between doing these experiments in neutropenic mice versus immunocompetent mice? How long should the infection model be? So, it might be that that 24-hour model that works really well for a small molecule doesn't work well for a phage product. You might need longer than the 24-hour period to really see an effect. Endpoints, do you want to see survival or CFU counts or both? And whether or not you want to combine your phage product with an antibiotic.

Some of the phage parameters are cocktails versus single phages. The dosing routes, so which administration route might be ideal? And that might depend from phage to phage or the type of infection. How often you should dose the phages, and for how many days to see an effect? And then another is the phage kinetics and distribution, so, depending on the administration group, where does the phage go in the animal, and how long does it stay there?

So, I'd say that these are all gaps

that we've identified in PCS, and that we're currently working towards trying to get some answers to some of these questions. So, in particular, that question about the phage kinetics and distribution, we have set up a new task order to take a look at this question. So, this is going to be as close as we can get to sort of like a PK/PD model for a phage product where that we'll be looking at different administration routes for a particular phage cocktail, see where it goes in the animal, how long it stays there, and then also pairing that with the efficacy data from a variety of those animal models that were in the last slide.

And just as a note. So, we have performed these different infection models with phage products in the past. Dr. Erica Bizzell will be giving a talk at 10 a.m. on Wednesday in this workshop that she'll go over some of that data. And she'll also go into some more of the details about that phage kinetics task order that we're kicking off soon.

Next slide, Erin. And so, now we come to where we're going to ask you if you have any questions about NIAID preclinical services. And then we'd also really like to hear from you, the community, of what you see is most challenging gaps for phage product developers. And then are any of those animal models of interest to you? Or would you propose any modifications to those models? We're open to suggestions. So, we'll open it up to questions now.

Okay. I'm not seeing any questions.

DR. ZEITUNI: I have to say that we're very thorough. (Laughter)

DR. RATERMAN: Yeah. I mean, if there are no questions or no one has any comments --

DR. SEGALL: I --

DR. RATERMAN: Oh, go ahead.

DR. SEGALL: I actually have a question. Hi, this is Anca Segall from San Diego State University. Sorry for the background noise. I can't do much about it. I had a question if there's any--so, you mentioned that some of the preclinical

services you have like animal models or even tissue culture models you have for the ESKAPE pathogens. I would be interested in model systems for *Achromobacter*. And I'm wondering if there's an easy way to know what you may have available or what could be developed.

DR. ZEITUNI: So, that's a great question. I'm not familiar with models against *Achromobacter*, but we could put you in touch with the branch that covers that pathogen, and then you can start conversations with them.

DR. SEGALL: That would be great.

DR. ZEITUNI: Sure. So, at the bottom of this slide --

DR. SEGALL: Yeah.

DR. ZEITUNI: -- either you can reach out to us--and I'm going to throw up our emails in the chat box here, if anyone wants to email us questions or get in touch with us. But at the bottom of this slide, there's also a link to the preclinical services contacts, and you should be able to go on there and identify the branch that would be most likely to--I don't know off the top of my head where it would

most likely be. But in the chat, in the meantime, I'm going to put our emails so that you can send your question to us, and we can get you in touch with the right folks.

DR. SEGALL: Thank you.

DR. ZEITUNI: Sure.

DR. CAMPBELL: Erin and Erica, this is Joe from NIAID. In the absence of more questions, I might mention that there's one other thing which we have done for at least some phage developers, and that is come up with product development plans.

One of the things that Erin mentioned that we consider when we're deciding whether or not to offer our services is that you have a fairly clearly defined target clinical indication. And product development plans can help you understand what steps you'll need to get, and that could help you identify the gaps that we might be able to fill for you.

And we have another—I'll try to put it in the chat. We have another contract, which is for the development of

biopharmaceutical products, and I'll try to paste that into the chat.

I don't know, Erin and Erica, do you want to—I mean, you gave a nice discussion of most of the issues that you consider, but I just—I know I was sitting in some of your product development meetings, but what is usually sort of the—or is there, you know, a few pain points that make you decide whether or not the services are going to be offered?

DR. ZEITUNI: Sure, absolutely. And then Dr. Segall, I see you also went off mute, so we'll get to you after answering this question.

So, one of the main driving questions that we have when we are discussing a request is the rationale for the request and how the data will be used. So, we really want a clear understanding of how this is a gap for you that you have tried to get this service elsewhere but unsuccessfully and then how it would be used—how the data would be used to move your program forward or to serve as a go/no-go decision point.

And the second, I would say, most critical bit of information that my team wants to understand is the support that a group has for their program. So, what sort of grant fundings have you had in the past, do you currently have? Do you have collaborators who have brought a phage product to clinic before or any product to clinic before and an understanding of the product development path requirements and where you are going to get support to keep moving things forward.

Other areas, Erica?

DR. RATERMAN: I will just add what you had mentioned earlier in your presentation about they generally want to see, you know, a lead product or at least a handful of leads. So, if you're still really in the screening phase, that's probably where you're going to get the "no," that we just have a limit to how many products we could actually put through our assays for you, so we wouldn't be able to do a large screen of various phages for you.

DR. ZEITUNI: Okay. I see a question in the chat from Joelle Woolston. "Could you

point us to some papers where the models were used? Any model involving *Serratia*, *Enterobacter*, *E. coli*, *Staphylococci* would be of interest."

We could absolutely try to point you to some places where our models have been used. I would say the one difficulty is that we can't drive someone to publish their results, and so we are waiting for folks to publish their results, but I do have some recent news that some of these phage product results are on their way to being published or have been published. So, *Serratia*, I don't think we have any models covering that, but—nor *Enterobacter*, actually. But *E. coli* and *Staph*, I would be happy to try to send some publications your way. Please reach out to me using the email in the chat to send them to you.

And again, some of the information around more specifics of the studies that we've run for phage are going to be presented on Wednesday by Dr. Erica Bizzell. Oh, there goes Joe saying the same thing.

But because our studies are confidential, unless something is published, we de-identify the data. So, you'll be able to see the results of the studies, and the study design is included on the slides, but the name of the PI or the institution will be blinded, unfortunately, unless published.

So, I'm seeing another question coming in from Dong Lee. "Do you have baseline tissue pathology in your models? The infections themselves will most likely cause underlying histopath." And he's curious if these could impact the mechanism of action of the candidates.

That is a great question. And it depends on the model. So, for our multi-day lung infection models, we do include tissue pathology—or we did. In a previous iteration of that task order, tissue pathology was included. And we did see when we ran phage in those models that there was a positive impact in the tissue pathology numbers. So, it's a very interesting question around the lung pathology.

Recently, we reissued that task order, and we used two different strains of *Pseudomonas aeruginosa* in those task orders. And while we were validating the 120-hour lung infection model, we included tissue pathology as one of the readouts for us to validate. And what we found was, surprisingly, we didn't see a significant scoring in our tissue pathology.

So, in that case, it's not a desirable readout for a model, because it would be difficult for someone to show a significant impact of their product if the untreated score is already low. So, in that case, while we wanted to include histopathology as one of the readouts, we ended up dropping that as one of the readouts for that model. So, now, it is solely survival and CFUs. But I absolutely agree that it is something interesting to look at, particularly in those lung infection models, where you can see some really interesting results, I guess, depending on the pathogen and the course of disease.

Erica, can you think of any other

cases?

DR. RATERMAN: Yeah, I can't think of any of the other models. At least the ones that we offer that we include histopath as one of the readouts.

DR. ZEITUNI: Yeah. Some of those 24-hour models, it's just too short really. And the intention there is to look at the reduction in CFUs as the main readout. Great question, though. Thank you.

So, maybe rather than asking for a question, if-it would be helpful for us to hear of the models that you're using as sort of your workhorse models in your labs to take a look at your phages and their activity, if you feel comfortable sharing that in the chat, I think that could be a very interesting place for us to take a look at what you all are using now, and if there's any gaps that you have or qualities in a model that you're particularly interested in.

DR. RATERMAN: Okay. So, it looks like we have a comment. "A lot of models seem acute, but human infection especially that are

reoccurring are chronic. Any thoughts on translation?"

So, I think you're right that these are all acute models. So, you know the—how far you are going to be able to extrapolate to a human disease is going to be limited. I would agree with that. But I think that we have seen success at least in a few cases with phage products in these models, so I think that it can at least give you that primary indication of whether or not your phage is going to be active in vivo, which would be valuable to have, but I agree that it would be beneficial if we could also have more chronic models to really look at those long-term infections, especially one that you're going to see maybe much more of, you know, damage from the immune system or those sorts of factors coming into play.

Erin, anything to add to that?

DR. ZEITUNI: No. I absolutely agree. Yeah, I know --

DR. RATERMAN: I'm so sorry. I would just like—we unfortunately don't have any of

those models developed or available right now.

DR. ZEITUNI: Yeah. We do make attempts to address some of the chronic models. We have an ongoing effort to develop a cystic fibrosis model, for example, but there are so many challenges in validating, developing, and making them—make sure it's reproducible. There's a reason why those models tend not to be easily accessible in the community or through CRO. So, it's definitely something of interest to us. We tried to bridge some of that with our 120-hour models. But like Erica said, in the end it's just a surrogate, and you're hoping to see activity that would then be indicative that it could be translatable to a clinical case.

DR. CAMPBELL: Maybe—I don't know if you got—this is Joe. I think it might be useful for the others as well as re-edifying me as to what kind of UTI models are available. Some of those I think are a little bit longer, or am I misremembering?

DR. RATERMAN: So, you're correct, Joe. So, the UTI model that we currently offer

is a seven-day model. I don't know that I would go so far as to call it a chronic model, though. It's longer, but I wouldn't say quite chronic. I would still say in the acute realm. But we can do those. So, if we induce diabetes in the mice with streptozotocin, they do tend to hold on to that infection longer. So, I'm not quite sure how far out time-wise that you could go with that induction, diabetic induction. Maybe you could get something eventually that looks much more like a chronic model. But to my knowledge right now, I don't know that that chronic UTI model exists without having to do something like, you know, surgical implantation of a catheter or something like that.

DR. CAMPBELL: And I don't know, Erin and Erica, you might want to comment on the fact that we're also starting out and we're right now piloting it with the traditional therapeutics, hollow fiber *in vitro* models, which, you know, have the disadvantage of not being in a whole animal, but the advantage that you can make them last sort of as long as

the cartridges last, which I think is a long time. You know, it has all the disadvantages of the fact that it's not in an animal, and it doesn't bring in the immune system, and it doesn't do all the things that animals do and people do. But it is a chance to do a longer duration. And depending on what issues are of interest to you the most, that might be—as I say, we will probably know better once we get a little experience with using those models for traditional therapeutics, what will and won't be possible.

And I guess—sorry, I'm just talking too much. But we do in addition to the animal models, we do have—and we haven't used them, but we do have a variety of *in vitro* services. And one of the things that we can do in that certainly is come up with panels of strains. And you can also get panels of strains from BEI to help you start addressing, you know, some of the questions that Erin mentioned about what kind of coverage you have of, you know, relevant clinical isolates and stuff like that.

DR. ZEITUNI: Yes, and thank you for that BEI plug, Joe. We did just recently have a deposit go live in BEI, which is a 100-strain panel from WRAIR, Walter Reed Army Institute of Research, I believe, that's for *Acinetobacter*, and a 100-strain panel for *Pseudomonas*. They're very well curated, whole genome sequenced, representative of particular clinical resistance phenotypes of interest. So, if you wanted a well-characterized, validated panel of strains, that could be a great place to start for those particular priority pathogens.

And there's also I will say the—if you're looking for strains, the CDC-FDA Clinical Antimicrobial Resistance Bank—so, if you just Google CDC-FDA AR Bank, it will come up. They have panels of strains available for particular resistance profiles of interest or pathogens of interest.

DR. RATERMAN: Okay. So, I'm thinking, you know, if there are no further questions or comments or any input from the community, then I think that we could go ahead

and log off and give everyone eight minutes of their day back. Agreed, Erin? You're on mute, Erin. (Laughter)

DR. ZEITUNI: I agree. That sounds great. But I was saying I think it's only three minutes, because I think we're supposed to end --

DR. RATERMAN: Only three?

DR. ZEITUNI: -- at 3:25.

DR. RATERMAN: Oh, 3:25. Okay. Never mind then. All right.

DR. ZEITUNI: But I thank you all for your attendance. And please do reach out to us with any questions.

DR. RATERMAN: All right. Thanks to everyone for joining.

SPEAKER: Thank you.

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Breakout Session:

Room D: Funding for Clinical Trials:

DR. KNISELY: Okay. Good afternoon, everyone. Let's go ahead and get started with our focused session on Funding for Clinical Trials. I'm Jane Knisely. I'm a program officer here at the Division of Microbiology and Infectious Diseases at NIAID. And I'm joined by two colleagues, Dr. Richard Alm from CARB-X and Dr. Anna Jacobs from BARDA. And what we'll do is each of the three of us will go through and tell you about funding opportunities available at each of our institutions. And then I hope that we'll have plenty of time for questions and discussion about funding clinical trials.

So, with that, I am going to go ahead and share my screen. Please let me know if you cannot see my slides.

MS. JACOBS: It looks good, Jane.

DR. KNISELY: Great, thanks. So, I'm going to go through several different mechanisms that we have available. And the

first is our Investigator Initiated Clinical Trial grants. So, these kind of fall under the category of things that people think about when they think about NIH funding, things like R01s or R21s and RU01s for direct funding to investigators to conduct clinical trials.

If you would like to conduct a clinical trial under grant funding from NIAID, you need to apply to specific funding opportunity announcements that allow clinical trials. And I will detail those funding opportunity announcements on the next slide.

For applications that are over \$500,000 per year in total cost, you need to request prior consultation with NIAID program staff at least 10 weeks before the application due date. That's a little bit longer if you are seeking to do an extended clinical trial. And just a note is you may want to consider applying for an R34, which is a Clinical Trial Planning grant. So, this allows just one year of funding, \$150,000, to allow you to assemble your team and get the documentation in place that you will need, write your protocol to

make the transition to your clinical trial more seamless for follow-on funding from whatever source is available.

So here are the funding opportunity announcements as I mentioned, and you can find them all at the link below or by putting "NIAID investigator-initiated clinical trials" in your favorite search engine. The parent R01 that allows for clinical trials can be used for trials that are not considered high risk. So, these would be things like an approved intervention or approved indications where you may be comparing different strategies that are all using approved indications. Something of that effect might be an example of that.

There's also an extended version of that funding opportunity announcement that allows for seven instead of five years of funding. The R21 Exploratory and Developmental Research Grant Program can be used for mechanistic or investigative studies that are not high risk. So, these are, as you know, a smaller award. For trials that are high risk, we recommend the U01 mechanism, which is a

cooperative agreement. And so there will be substantial NIAID programmatic input and involvement. And finally, if you are from a small company, you may utilize the SBIR Phase II Clinical Trial Implementation Cooperative Agreement, a U44 mechanism, which is specifically designed for small businesses.

And I just want to point out that these grants do come in and are reviewed either by CSR or by NIAID Special Emphasis Panels. And they are funded under our standard R01 pay line. However, we do have some examples of successes of grants that were successfully funded under Investigator Initiated Clinical Trials. And I'm showing a press release from Intralytix for their *Shigella* phage product here which was funded last year. So, it's possible to do this.

The next mechanism I'll tell you about is the Antibacterial Resistance Leadership Group. This is a large, diverse, clinical trials network housed at Duke University, but with collaborations across the country and around the globe. They have an

online submission portal for study ideas. They also are available to consult with small and large companies on clinical trial designs. And they also have a variety of training opportunities, early-stage investigator seed grants, and the like. So, there's lots of information that can be found on the different opportunities that they have on their website which is just arlg.org.

They do have priorities on resistant Gram-negatives and on innovative products, including phage therapy, and, as I mentioned, mentoring and diversity. They are planning a clinical trial of a phage product for cystic fibrosis patients who are stably colonized with *Pseudomonas aeruginosa* and we're hoping to begin that trial early next year.

Two other funding mechanisms and then I'll pass it over to my colleagues. Our Infectious Disease Clinical Research Consortium, or IDCRC, is another leadership group-based funding mechanism network, which is comprised of the Infectious Disease Leadership Group and ten Vaccine and Treatment

Evaluation Unit clinical trial sites who are very experienced in conducting clinical trials with our division.

So, this group really serves our entire division. So, they have a very broad focus in terms of pathogens and have been very focused on our COVID-19 response. However, I know they're very interested in focusing on other priorities as they are able. And they have some expert working groups that would be relevant to people working on phage for different indications, including the Emerging Infectious Disease Working Group, respiratory infections, enteric infections, and sexually transmitted infections. So once again, there's a lot of information available on their website, which I'm showing here. And they also have an online concept submission portal.

The last mechanism that I will mention will be established in early 2022, and that will be called our Early Phase Clinical Trial Units. This is replacing our Phase I Unit of Therapeutics which is currently in existence and currently is focused only on

therapeutic trials in healthy volunteers. With this new solicitation, we're establishing a new contract tool to conduct early phase clinical trials to include Phase I and Phase II clinical trials of therapeutics and vaccines in both healthy and sick populations.

And so, I think there's more opportunity here in the future to support additional clinical trials for phage therapy. So, feel free to reach out to us in 2022 and see when that might be available.

So, with that, that is all I have. Feel free to reach out to me with any questions that don't get addressed during the panel. With that, I will turn it over to Richard. Thank you.

DR. ALM: Thanks, Jane. Yeah, let me work out how to do this again. So good afternoon, evening everybody. My name is Richard Alm. I'm an alliance director at CARB-X. And for those that don't know CARB-X, we are a public-private partnership that funds non-dilutively programs aimed at both the prevention, the diagnosis, and the treatment

of bacterial infections caused by drug-resistant bacteria.

We fund a little bit earlier than this session only covers. We fund from "Hit-to-Lead" through to the end of "First-in-Human" testing. So, we do do Phase I testing, we support that. But we do take programs that come a little bit earlier. Unlike just grants that give straight funding, we also provide strategic, technical, and business support to our funded programs. A lot of our companies are smaller companies and so we wrap around some business support and some strategic support for them. And we also design and execute what we call Cross-Project studies that overcome barriers to progress that many companies in their portfolio may be finding they're coming across. And so, we will do a Cross-Project opportunity where we will try to answer those questions and then feed the information back to multiple companies.

CARB-X has a strong commitment to innovation and nontraditional products. It now represents just over one-third of our entire

portfolio. And within that nontraditional product portfolio, we have four programs that are based on bacteriophage, plus one phage lysin program. The phage programs that we support currently explore both the prevention and treatment of infections. And I'll go through the portfolio briefly at the end.

In terms of application, it is a competitive application process. The call for funding rounds will typically have some fixed scope. This will be with respect to the pathogen spectrum or the modality.

Importantly, applicants can enter the program at any stage within the funding scope. So, if you're currently about to go into Phase I, you can come in and apply for a Phase I spot, or if you're early in the programs, you can come in and apply for Hit-to-Lead or Lead Optimization.

We will support groups with no geographical boundary at all. We support them all over the world, and in fact, our four phage companies come from four different countries.

So why did CARB-X get into phage?

Well, I think, as everyone here knows, the phage world has really exploded in the last, I would say, decade in terms of trying to understand what the role of phage can be to effectively fight AMR-based infections. What CARB-X aimed to do was to establish a broad portfolio where we can explore bacteriophage products through support of these companies to understand some of the key questions. And we built our portfolio with programs that target different patient populations, different pathogen spectrums, different stage of intervention be it prevention or treatment, different technologies, and also different team approaches.

And CARB-X maintains a commitment through this funding to help answer some key questions. And some of the questions that we're interested in is, is there improved effectiveness with bacteriophage against certain bacterial species or certain clinical indications or routes of administration? Is it possible to actually use bacteriophage

products as first-line treatments? And then if so, because of their specificity, what is the role of diagnostics?

We also support the development of predictable and clinical translatable preclinical animal models to help understand doses and dose range finding as we go forward into human testing, and also phage biodistribution. And we'd like to determine and/or influence the clinical trial design for their ability to be a form, so their doability, and the potential to support necessary regulatory label.

So briefly, as I said, we have four programs in our phage portfolio. The first one is Eligo, based out of France and they are looking a preventative decolonization of specific resistant *E. coli* and *Klebsiella* lineages with engineered CRISPR-based phage in patients that are undergoing organ transplants. And so that's a very vulnerable population, and so they're looking at a decolonization approach to prevent breakthrough infections.

We also have Locus Biosciences, which is based in the U.S., and they are looking at CRISPR-based phage that are targeting as a treatment serious, complicated, and recurrent urinary tract infections caused by *Klebsiella*. Most people will know that they have an *E. coli* product further in development. This a follow-on program aimed at *Klebsiella*.

Our third company is PHICO Therapeutics, and they are looking at engineered phage to deliver small acid soluble spore proteins to precisely target *Pseudomonas* infections that are causing ventilator-associated pneumonia. So that's a treatment program.

And our last program is SNIPRBIOME, which is a prevention program, and they are looking at microbiome-sparing CRISPR phage to target breakthrough *E. coli* bloodstream infections in patients with hematological malignancies and oncology patients.

So, there are our four programs at the moment. We are excited about progressing

them through their different stages towards the clinic. Some of them are further along than others. And as I said, we fund all the way from Hit-to-Lead through to First-in-Human testing. And hopefully, we'll be able to bring in some more phage programs into our portfolio in the near future.

That's all I have, I think. Jane?

DR. KNISELY: Okay. Thanks, Richard. Anna? We see you, but you're muted.

MS. JACOBS: Every time. I do that every time. Let's see if I can figure out if I can correctly share my screen now that I've unmuted myself. Okay. All right. So, you guys can see the BARDA website, yes?

DR. KNISELY: Yes.

MS. JACOBS: Okay, great. Thank you. So hello, everyone. I'm Anna Jacobs. I'm a project officer in the antibacterials group at BARDA. And so, I don't actually have any slides to share today, but I am going to sort of show you a few things on the website and give you a little background on what BARDA does that will hopefully be helpful for you

guys if you're considering advanced development of your products.

So, BARDA does support advanced research and development of medical countermeasures. So that includes preventatives, diagnostics, and treatments, so very similar to CARB-X. But at BARDA, we're addressing multiple threat areas. So, it's not just biological such as bacterial infections, but also chemical, radiological, nuclear, pandemic, flu, emerging infections. And we do this through public-private partnerships that rely on flexible authorities, non-dilutive multiyear funding, and then we also do include subject matter expertise and core services.

So, we can assist with clinical, nonclinical, manufacturing in terms of giving advice and helping companies. And sort of all of that combined we hope accelerates product development versus just handing money over to these companies. And so, we typically support products that already have an IND in place, and we start at Phase II clinical trials and can support all the way through post-FDA

approval activities.

So, historically, BARDA has invested in the antibacterial space for two reasons. Initially, it was to support products that can address threats to national security, so any sort of biothreats such as anthrax, the plague. But then our second reason is that within BARDA's larger mission space where we are trying to address public health emergencies, whether it's a CVRN threat, we've been doing a lot of things related to COVID-19, as I'm sure you know. We also need antibiotics or antimicrobials that will treat opportunistic and secondary infections that are likely to occur during the course of treating patients for these other issues.

So, in that sense, antibiotics are a necessary medical countermeasure for all the threats on BARDA's mission space. And one of our main focuses right now is sort of new, innovative, and novel products, so that would include phage. We're specifically looking for novel mechanisms of action, things that can address unmet clinical needs. And currently we

are supporting three—within the last year, we've started supporting three nontraditional antimicrobials.

So, one of those is a company called Vedanta, who actually is developing a microbiome bacterial product. So that's not phage-related, but then we also have ContraFect, who is developing a lysin against *Staph aureus*. And then Richard sort of stole my thunder a little bit, but we also have Locus, and they're developing a CRISPR-Cas3 phage product against *E. coli*.

So just, if we actually look at the website here that I'm sharing, so if you want to learn more about BARDA, you can go to medicalcountermeasures.gov/barda and find out much more detailed information about what we do and the kinds of things that we support. I do want to tell you that we currently have a broad agency announcement that is open for companies to apply to. Typically, our system involves a whitepaper submission that is reviewed, and then if you're invited to do a full proposal submission, that would be the

next step, and then we would negotiate to award a contract.

If you do get far enough on the website, you can also—one thing that we recommend before just submitting a whitepaper is to request a tech watch. A tech watch is essentially a one-hour meeting to give you an opportunity to present what you're developing and ask BARDA any questions that you have before you spend the time to write an entire whitepaper or an entire full proposal just to make sure that we're all on the same page about what BARDA is looking for, what you're developing.

I will say that if you look at the Medical Countermeasures website, which I have up right now, it does say that it's limited to the COVID-19 response only. That is not entirely accurate. If you do submit straight to the website, you probably will get bumped. COVID-19 has been a very big weight on BARDA in the last year and a half, but we have—specifically, the Antibacterials Group are doing tech watches and having meetings with

companies that aren't developing COVID-19 products.

What I can recommend is if that is something you are interested in, you should reach out directly to someone in the group. So, you can reach out to me, if you're familiar with Mark Albrecht, he's my boss, you can reach out to him. Let's see if I can pull up—I don't have slides, but I did type my name and my email address on this slide, or on this Word document, so please feel free to email me directly to answer more questions or to set up any sort of meeting.

I think that is mostly what I had.
Jane?

DR. KNISELY: All right, great. So, yeah, so we've given you a flavor for the types of funding opportunities that are available from NIH, BARDA, and CARB-X. Certainly, we're not the only funders out there, there are others. But how long do you want to make your panel, I guess? So please, if there are any questions that we can answer, feel free to put them in the chat or, you

know, this is a relatively small group. If you would like to come off mute and ask a question, please feel free.

Okay. I see a few coming in in the chat. This is a really good question, I don't know the answer. So, the question is, a lot of companies are selling services to help businesses to apply to grants you're speaking about. Do you have statistics on how often awarded grant applications have been written solely to the investigator company—by the investigator company versus got help from such companies?

That's a great question, I don't know the answer. I doubt that we have such statistics. I would love to know the answer. If anyone else does, feel free, or I don't know if Richard or others have experience.

DR. ALM: Yeah, the only thing I would add, Jane, is that CARB-X is a multi-stage application process because of the confidentiality clause that come in as we ask more information. And CARB-X has a set of accelerators. And so, companies are offered to

go to an accelerator to help them prepare for their final application and their presentation to the advisory board. So, I don't have exact numbers on the top of my head how many take advantage of that, but they're certainly—every applicant is offered that if they get to that stage.

DR. KNISELY: Yes. Okay. The next question is for you, Richard. Do we have a timeline to next round of CARB-X applications?

DR. ALM: Yeah, so CARB-X is in the process of sourcing new funding. I think everyone probably knows that the BARDA BAA was announced back in May, I think, the application was due in June. So, we are waiting to hear back from BARDA. It was a competitive application so there will be—BARDA is committed to another biopharmaceutical accelerator, whether that's the Boston University Group or someone else, we don't know yet. But as soon—if it is us and if we are lucky enough to get more funding, then we will go into another round of applications. But I can't give you a timeline just yet

because we're still waiting to sort out the funding and—either from the U.S. Government or others.

DR. KNISELY: Thank you. Okay. The next question is about appropriate controls in preclinical models. So, it's a long question.

DR. ALM: Yeah, I'm reading it.

DR. KNISELY: You're reading it, too. Yeah, I'll be interested in your thoughts. Will funding agencies require preclinical data or clinical trial controls addressing verification of the proposed issue of mechanism of action of phages, lysis of the targeted microbe? Let me think about this one. I mean, I think for all of the funding mechanisms that I mentioned, certainly preliminary data is—some degree of preliminary data is required. And for the investigator-initiated clinical trials, it's peer reviewed. So, it's really the peer reviewers who are making the determination of the adequacy of those controls.

For things like our preclinical services, which is not the top focus of this

discussion, or some of the other mechanisms that I mentioned, NIAID may be a little bit more involved in evaluating. And we do ask for, you know, depending on what is being requested, so if someone is coming in and asking for a clinical trial, we do ask for a lot of data obviously. So as for the adequacy of the controls, I don't know if, Richard, you would like to, if you have any thoughts on that.

DR. ALM: Yeah. I mean, typically within a CARB-X program, a certain set of experiments are suggested and budgeted for and then we work with the company through those. We encourage all of our companies to have early regulatory interactions. And so, they would go to either pre-IND or an INTERACT meeting to discuss with the agency what controls are needed. I will say that I think it is useful to run, in terms of the immunity side of the question, I think it's very useful to run animal models in both immunocompetent and neutropenic animals to understand the role of the immune system.

And so, I think that there's a lot that is not known about phage and phage biology in an animal model. And I think that's one of the areas that CARB-X is very interested in trying to work out and then—with our companies, and then have that information made available to the greater ecosystem.

DR. KNISELY: Thanks. Okay. Now we have a good question. If the company, if a company wanted to pursue funding from one of your organizations, what is the typical timeframe the company should plan on from the start of an initial request of funding? So, for us, it's going to be a little bit different whether you are talking about an investigator-initiated grant application. So those timeframes can be, you know, a little on the long side. The receipt dates are—there are two to three receipt dates per year. And then depending on when you submit, it can be a year before you know the outcome.

So, you definitely want to plan the— with significant lead times. Some of the other mechanisms can definitely be more rapid. It

really just depends. So, I'm going to pass it over to you guys to address the same question.

MS. JACOBS: Yes. So, at BARDA, I will say that the application process is rolling. So even-if you look on the website for our BAA, it does have an end date, but a new BAA will be put in place, and people can pretty much reply whenever they want to and we're not going to wait until that end date before we start looking at the applications, the proposals that we've received.

For a full proposal, I would say we typically try and do a turnaround of a month or two to review it and give feedback to the company. And then assuming that we're going to enter into negotiations, depending on how long those take, I'm going to say from the point where you submit to receiving funding, if we have the funds available, is probably six months. That would be longer with a whitepaper because obviously we would review the whitepaper just as quickly as a full proposal, but then you would have to-the company would have to then prepare the full proposal which

could take some time. But I would say six months to a year is typical.

DR. ALM: Yeah, and for CARB-X, once an application round opens, typically you would get notified. I would say on average you'd get notified of a successful application to enter into negotiations with us in about maybe six to seven months. And then it varies beyond that because once a company has been accepted, because it's a cost share reimbursement-based grant program, we enter into negotiations to come up with a set of defined milestones that both sides agree on. And that can depend on the company and how quickly they turn around the paperwork. But from then it can take anything as short as six weeks to four months after that.

DR. KNISELY: Thank you. The next question is can academics, *i.e.*, PIs at universities' medical schools apply to CARB-X and BARDA, or are we restricted to NIH?

DR. ALM: So, I can address that from CARB-X. So, CARB-X funds product development, it doesn't fund the raw underpinning science.

And so, if you are a PI at a university or a medical school and you have product development goals, and we do have some universities in our portfolio. All of them have some sort of tech transfer or business development support from the university. We will fund that. So, we're not restricted to a company. We will fund universities and medical schools if they have the appropriate focus on their product development and some business support from their university.

MS. JACOBS: I think that's also true for BARDA. Actually, I'm trying to think. I don't know if we have any specific universities in our portfolio or if they're all companies. I don't see why not if they have the capability, but don't quote me on that. And I can try—the person who asked that question, if you email me directly, I can try and confirm that for you.

DR. KNISELY: Thank you. The next question is about an application that kind of sits at the intersection of three different NIH Institutes: NICHD, NHLBI, and NIAID. So,

two questions here. Is the R34 where we should be going? I think you—it could be one way to help you sort of get all the planning for your application done and do some more—get you some funding to do that planning, because it can take a lot of time and resources. So, it could be something to explore. Feel free to reach out to me and I can definitely put you in touch with the appropriate folks at NIAID even though you may have already spoken with them.

And I like this question, is there a phage central administration at NIH? Sadly, no. So, yes, we're not the only institute that funds phage research. I think we probably have the largest portfolio with maybe the exception of NIGMS, though theirs might tend to be less clinically focused. But that's an interesting suggestion. Thank you.

Is the U44 direct to Phase II? I believe the answer is yes. Verify that on the website that I shared, but I'm pretty sure it is a direct to Phase II and you do not need a Phase I SBIR to get that funding. Okay.

DR. ALM: Actually, Jane, I have a

follow-up question to that. Is there a fast track as well process within the phage thing? Can you go—like SBIR, you can do a fast track, you can do Phase I, Phase II fast track. Is there such a mechanism within the phage, within U44's?

DR. KNISELY: Within the U44?

DR. ALM: Yeah.

DR. KNISELY: So, I believe it's a direct to Phase II.

DR. ALM: Okay.

DR. KNISELY: So, instead of the fast track where you combine the two, you just --

DR. ALM: You just go straight?

DR. KNISELY: You come in with the data already. Right?

DR. ALM: Okay.

DR. KNISELY: Yeah. And I believe that is the way it works for the U44. They're not super common, we don't see a whole lot of them, but—because I think it's a relative new funding mechanism, only been around for a few years. And so probably not a lot of people know about it. And we just don't have a ton of

experience, but it is there.

Okay. Any other questions? Let's see. Thank you so much, great advice. Yeah, okay. Okay. Because phages do not primarily interact with mammalian cells, please discuss your organizations expectations for nonclinical studies beyond the standard GLP tox.

I guess my response to that is we really defer to the FDA. And so, as Richard says, we really—if we're at the stage of a clinical trial, we're going to want FDA's input on that and the suitability of the preclinical data for proceeding into the clinic.

DR. ALM: Yeah, I will say that the FDA has been fairly generous with companies to date about not requiring GLP tox studies like you do for small molecules with phage products. CBER will give you that advice when you go for your regular free interaction with them before submission. But up to date, they haven't absolutely required it to my knowledge.

DR. KNISELY: Yeah. Yeah, that's been my impression as well.

DR. ALM: But they will definitely take it on a case-by-case basis, so you have to go and make a case. And it's better to do that before than to submit an IND without it and then be asked for it.

DR. KNISELY: Yeah.

MS. JACOBS: One hundred percent agree, Richard.

DR. ALM: Yeah, go and speak to them first.

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