UNITED STATES FOOD AND DRUG ADMINISTRATION

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

SCIENCE AND REGULATION OF LIVE MICROBIOME-BASED PRODUCTS USED TO PREVENT, TREAT, AND CURE DISEASES IN HUMANS

Rockville, Maryland

Friday, April 19, 2019

PARTICIPANTS:

Welcome:

CAROLYN D. DEAL, Ph.D.
Branch Chief, Sexually Transmitted Diseases Branch
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases

Introductory Remarks:

PETER MARKS, MD
Center Director, Center for Biologics Evaluation and Research
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Keynote Address:

Introduction:

PAUL CARLSON, Ph.D.
Principal Investigator
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The Microbiome in Human Health and Disease: A Clinician-Scientist's Perspective:

VINCE YOUNG, MD, Ph.D.
Professor, Department of Internal Medicine/Infectious Diseases Division
Department of Microbiology and Immunology
University of Michigan Medical School

SESSION 1: Regulatory Framework for "Probiotics" and Live Microbiome-Based Products:

Moderator:

PARTICIPANTS (CONT'D):

THERESA FINN, Ph.D.
Associate Director for Regulatory Policy
Office of Vaccines Research and Review
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Dietary Supplements Containing Probiotics:

ROBERT "BOB" DURKIN
Deputy Director, Office of Dietary Supplement Programs
Center for Food Safety and Applied Nutrition
Food and Drug Administration

Live Microbiome-Based Products Used to Prevent, Treat, or Cure Diseases in Humans:

SHEILA DREHER-LESNICK, Ph.D.
Biologist, Division of Bacterial, Parasitic and Allergenic Products
Office of Vaccines Research and Review
Center for Biologics Evaluation and Research
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SESSION 2: Safety and Effectiveness of Live Microbiome-Based Products Used to Prevent, Treat, or Cure Diseases in Humans:

Part 1:

Moderator:

SUSAN MCCUNE, MD
Director, Office of Pediatric Therapeutics
Office of the Commissioner
Food and Drug Administration

Prevention of Necrotizing Enterocolitis
Use of Commercially Available Products to Prevent NBC:

PARTICIPANTS (CONT'D):

JOSEF NEU, MD
Professor of Pediatrics
Director of Neonatology Fellowship Training Program
University of Florida

Prevention of Diarrhea

The Evidence is in for Probiotics to Prevent AAD: What is Holding Up Evidence-Based Use in the USA?:

DANIEL "DAN" MERENSTEIN, MD
Director of Research Family Medicine
Professor of Family Medicine
Georgetown University

Use of Probiotics in Acute Pediatric Gastroenteritis - Two Large North American Clinical Trails:

STEPHEN FREEDMAN, MDCM, MSc
Associate Professor, Department of Paediatrics
University of Calgary

Safety and Effectiveness of Live Microbiome-Based Products Used to Prevent, Treat, or Cure Diseases in Humans:

Part 2:

Moderator:

PAUL CARLSON, Ph.D.
Principal Investigator
Laboratory of Mucosal Pathogens and Cellular Immunology
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PARTICIPANTS (CONT'D):

Prevention of C. difficile Infection

Use of Commercially Available Products to Prevent C. difficile:

A. KRISHNA RAO, MD, MS
Assistant Professor
University of Michigan

Overview of Controlled Studies Using FMT for Prevention of C. difficile infection:

COLLEEN R. KELLY, MD
Associate Professor of Medicine
Alpert Medical School of Brown University

CMC Considerations for Live Microbiome-Based Product Development:

JOHN G. AUNINS
Executive Vice President and Chief Technology Officer
Seres Therapeutics

SESSION 3: Strain Selection for Live Microbiome-Based Products to Prevent, Treat, or Cure Diseases in Humans:

Moderator:

RYAN RANALLO, Ph.D.
Program Officer, Enteric & Hepatic Diseases Branch
National Institute of Allergy and Infectious Diseases
National Institutes of Health

Drugs Based on Rationally Defined Bacterial Consortia:

PARTICIPANTS (CONT'D):

BERNAT OLLE, Ph.D.
Chief Executive Officer
Vedanta Biosciences

Development of Defined Consortia for Recurrent C. difficile Infection:

ELAINE O. PETROF, MD, MSc, FRCPC, AGAF
Department of Medicine/Division of Infectious Diseases
Gastrointestinal Diseases Research Unit
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Finding the Needle in the Haystack: Moving From Consortia to Single Strains:

NEERAJ "NEIL" SURANA
Assistant Professor
Departments of Pediatrics, Molecular Genetics & Microbiology
Duke University

Bacteroides fragilis Used in a Mouse Model of Autism:

GREG BATES, DVM
Senior Vice President
Regulatory Affairs
Axial Biotherapeutics

L. plantarum to Prevent Sepsis: Timing and Strains Matter:

PINAKI PANIGRAHI, MD, Ph.D., FIDSA
Professor of Epidemiology, Pediatrics, and Environmental, Agricultural and Occupational Health
University of Nebraska Medical Center

PARTICIPANTS (CONT'D):

Wrap Up:

CAROLYN D. DEAL, Ph.D.
Branch Chief, Sexually Transmitted Diseases Branch
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases

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P R O C E E D I N G S

MS. DEAL: My name is Carolyn Deal. And on behalf of the National Institute of Allergy and Infectious Diseases, I want to welcome you and thank you all for coming today to this workshop that we're holding jointly with the Food and Drug Administration, the Center for Biologics, on live biotherapeutic products. I think all of us, and certainly by the amount of interest there was in this workshop, we realize that this is a rapidly moving, evolving, and important area. NIAID has supported research in this area for quite a while, mainly in the basic area. And it's exciting to see it evolve from the basic research area into translational work leading to product development. However, we all know this does pose new challenges, questions, but I would say also opportunities. And we hope that these opportunities can lead to new products that will improve public health. For those reasons, NIAID wanted to partner with the FDA to start a discussion with the scientific community and our

manufacturing partners as to how best to approach the need for rigorous clinical studies to evaluate these products. For this, we know there are two requirements. One is well-characterized products, and the other is well-designed clinical studies with defined end- points. These are some of the topics that we hope that we can discuss today, and get your input and thoughts, and see how we can all collectively move forward. We really look forward to this discussion and hope that everyone at the end of the day will come away with some new ideas. And now, it's my great pleasure to introduce Dr. Peter Marx, who's the Director of CBER, who will go into more detail about today's program. Thank you, Peter, for coming.

DR. MARX: And so, good morning. I want to welcome all of you in the room and on the webinar to this workshop on the Science and Regulation of Live Microbiome-Based Products used to prevent, treat, or cure disease in humans. Before I go further, I want to thank colleagues at the National Institute of Allergy and Infectious

Diseases, and of the Food and Drug Administration for putting together such a stimulating program. We really have a group of presenters assembled today that's highly qualified to discuss the relevant issues. And I hope you'll find all the presentations, panels, and interactive dialogue informative and engaging. Just to orient you to the day, we'll start off with the key-note address by Dr. Vince Young of the University of Michigan. And this will be followed by two presentations on the regulatory framework for probiotics and live microbiome-based products. After the morning break, we'll first hear part of presentations on the safety and effectiveness of live microbiome-based products used to treat, prevent, or cure disease in humans. And these presentations and the discussions will continue after lunch. And then following the afternoon break, we'll hear presentations and a discussion of strain selection for live microbiome-based products to prevent, treat, or cure disease in humans. Now it's certainly true that over the

past two decades the relevance of the human microbiome to maintain health and to prevent the occurrence of disease has never been more greatly appreciated. And I think, the following, which is quoted from the science journalist, Michael Specter, summarizes this all quite nicely. I think his words are much better than mine could be. "We inherit everyone of our genes, but we leave the womb without a single microbe. As we pass through our mother's birth canal, we begin to attract entire colonies of bacteria. By the time a child can crawl she or he has blanketed by an enormous unseen cloud of microorganisms -- a hundred trillion or more. They're bacteria mostly, but also viruses and fungi, including a variety of yeast. And they come to us from all directions. Other people, food, furniture, clothing, cars, buildings, trees, pets, and even the air we breathe. They congregate in our digestive systems and our mouths, fill the space between our teeth, cover our skin, and line our throats. We're inhabited by as many as 10,000

bacterial species, and those cells outnumber those which we consider our own by 10-to-1 and weigh -- all told -- about three pounds, the same as our brain. Together they're referred to as a microbiome and they play such a critical role in our lives that scientist's have begun to reconsider what it means to be human." So it's my sincere hope today that you'll find the presentations stimulating and the dialogue will provoke questions that will help define where additional work is needed, to fully realize the potential of microbiome-based products to prevent, treat, or cure disease in humans. And with that, I wish you all a wonderful day engaging on this topic, and I think we're actually about on time. So thanks very much.

SPEAKER: Thanks, Peter. So with that I'll introduce our first speaker. Our keynote address today is by Dr. Vince Young. Vince got his bachelor's degree from MIT. And then went on to Stanford for his M.D. and Ph.D. before starting his first faculty position at Michigan State

University. In 2007 Vince moved to the University of Michigan which is where I met him, and we've interacted quite a bit since then. He is currently the William Henry Fitzbulter Professor in the Department of Internal Medicine and Infectious Diseases. He has a joint appointment in the Department of Microbiology and Immunology. And I think most of you probably know Vince. Vince has been on the cutting edge of the microbiome field and also C. difficile -- both in the context of the microbiome and beyond. So with that, I will turn it over to Vince who's going to give us an overview of the microbiome from his perspective as a commissioned scientist.

DR. YOUNG: Thanks to Paul, thanks to the FDA, and NIAID for giving me the opportunity to speak today. I want to tell you a bit about the microbiome. And I know people have varying expertise and everything, so I apologize for those people who've heard me talk before, and I'm going over things again. But I wanted to kind of set the stage for the day. We're going to have a lot

of discussions about the microbiome. And I think it might be useful -- since I am a clinician, and I've had the opportunity to kind of think about how we might use this in clinical medicine -- to kind of set a framework for this. And first of all, my disclosures, yeah, I've done some consultantships, but I won't be talking about any of that work and I won't have any discussions of off-label use or any FDA-approved therapies, and I've retired from football (laughter).

Microbiome, right. We all hear about it. This was from Saturday on the airplane, back from San Diego, like what's the latest count when you put microbiome into compartments -- they're up to 45,937 papers as of Saturday. And finally, we actually have more primary literature than reviews. There was a time where we kind of were the other way around. There was like three times as many reviews on the word microbiome than there was data. And you can kind of see some of the ones that come up with best matches there. It's kind of interesting. So I published this a year

ago in BMJ, because I think clinicians are very interested in the microbiome. And I was actually at the American College of Physicians in May at their national meeting. And I was speaking to a group -- there were probably about a 1000 practicing internal medicine physicians at the ACP. And I asked them, "Who's heard of the microbiome?" Everyone laughs, every hand went up. And I said, "Who has had patients that have asked them questions about the microbiome?" And about 70 percent of the hands in the room went up. And then I asked, "Who has had patients bring in microbiome service that they've gotten through various commercial," -- I won't name any of those entities right now -- but places that you can get a microbiome survey done. About 30 percent of the practicing internal medicine clinicians in the room raised their hand. And then the final question was, "Okay, who knew what to do with these?" And there were no hands up. And I said, "Yeah, you notice my hand isn't up either. Because I'm not sure what to do either." Because,

you know, this is something that we encounter all the time. On Saturday, I'll also end up doing the Google News search, you know, look at what we're talking about with the microbiome. It's the usual thing. Is your microbiome making you sick? This one, with regards to today, they took a couple of the papers that were published from the group out of Israel a couple of weeks ago -- and kind of saying that, oh yeah, the probiotics don't do anything. I don't, you know, I made sure to read those self-papers. They didn't come out and say that, but that's how it was interpreted in the news. So it's out there. There are a lot of people interested in the microbiome. So for the purposes of my talk -- and I know other people use different definitions -- but when I refer to the microbiome, I am talking about the microbes, but I'm also talking about the environment they inhabit. In other words, the soil of the human body. And this is important for me. Because when I refer to the gut microbiome, this is the organisms, these are the compounds that are being

produced in there. And what's very important with regards to the later, that is actually due to the metabolism of the host and the microbe. So it's actually the biome. That's the root of the word. That it's this environment there. And then when I use the word microbiota, I'm going to just be referring to the microbes. So, you know, we've all seen various pictures like this. This idea that it's a forgotten organ. We have a lot of different species in there. As we go through the GI tract, as you go through the lungs, as we go through the skin, as we go to the GU tract -- there are microbes in and on us. Okay? And they can be very important in terms of what we're doing. And they can be important for two ways. They can be important both in terms of anatomy -- when we're talking about the microbiota, we can just be wanting to know who's there? What's the anatomy? Taking census. Doing 16S surveys to say what are the microbes that are there? Doing fungal surveys. Doing sequencing so that we can look at the viruses that are there, you know. But

the physiology -- as a physician -- it's important not to know just the anatomy, but we also want to know what they're doing. In other words, what can they do, but actually what are they doing at any given time? And this is just kind of modified from a review, where we kind of looked at the kind of plethora of different techniques that people use to study indigenous microbiota and the microbiome itself -- as we look at proteomics, metabolomics, you know. Cultivation is still important. We do a lot of 16S surveys. And if we're going to try to come up with a biotherapeutic, I can't imagine that we're going to ever treat someone with a 200-base para-snippet of their 16S gene, but we might treat them with an organism that contains that 16S gene. So what do mean by anatomy? Well if we look at the human anatomy, we do note that there are different organisms that are on different parts of the body. And they're fairly characteristic, but there's a lot of individual variation. We knew this from the human-microbiome project -- that everyone's

sort of individual. And so the anatomy can vary. But what we are finding a little bit more is that the physiology -- the functions of these communities seem to be relatively stable in individuals that we would consider "healthy". And they carry out a lot of different conversions. They can break down compounds. We hear a lot of about how fermentation of resistant starch can give rise to short-chain fatty acids. Which may influence how obese we are or how much inflammation we have in our gut. We can actually take xenobiotics -- drugs and toxins. We can convert them in multiple, multiple ways. I'll discuss that a little bit later with some examples. The microbes themselves can just synthesize things that are useful to us. And there is a lot of signaling back and forth between the host and the microbes through the epithelium, through the immune system. And so this microbiome here, as you can see it, this is all related to the host and the microbes. And it has a pretty dramatic and very complicated physiology -- and

what can we learn about it? Well I'm going to pick a couple of examples. I'm going to start out with a little bit pharmacology, you know, the FDA is sponsoring, so I will talk about drugs and microbes. But I am an infectious-disease physician, and we are here at NIAID, so we'll end up on that. And I know that there are a number of people who are giving talks on C. diff, and they have shorter talks. So feel free to just kind of skip over some of your intro slides as you need to. As I'm going to kind of cover C. difficile in some detail here. But drug metabolism, I was saying that the microbes can do all sorts of things. And they can metabolize, you know, biotics which includes drugs. And Digoxin's a classic example. In medical school I was taught, oh, Digoxin's a great cardiac, I mean in glycoside, it's good for arrhythmias, et cetera. Except for the fact that it has this narrow therapeutic index. The amount of Digoxin that you give to a person between helping them and becoming toxic is very, very narrow. And even more tricky

is the fact that some patients you can give the tiniest whiff of Digoxin and they go to toxic levels. Other patients you can keep on can keep on upping and upping the dose before you get therapy. And they don't seem to have toxicity. Well a while ago, it was reported that this particular bacteria -- Eggerthella lenta -- could map metabolized Digoxin. And that kind of just stayed there for about 20 years, until Peter Turnbaugh decided to revisit it and actually figure out exactly how did E. lenta inactivate cardiac glycosides. And could that could that actually be used to predict the ability of a person to actually get toxic or actually have a good therapeutic effect. And what he found out -- as a good microbiologist -- he kind of got different strains of E. lenta. and found that not all of them had the ability to reduce the drug. So it actually varied. And that's one thing, you know, that's very important. That's actually why it's important not to just grab a snippet of 16S and say, oh, you have E. lenta there. Depending

on which strain you have, it might be able to reduce cardiac glycosides like Digoxin, but there's others that don't. And then he looked very carefully to see what was happening. He took patients that were these reducers versus not. And he showed, yeah, okay fine, they could reduce -- the microbiota itself can convert. And E. lenta itself could convert Digoxin. But there seemed to be some sort of interactions between this organism -- E. lenta that has this particular gene cluster that he found out that was very important for this bioconversion -- and there was interaction with the microbiota. Okay, so here are the two strains of E. Lenta -- this one is very good at reducing Digoxin, this one that can't. And he took a patient who did not have the ability in their microbiota to reduce Digoxin, and when he added the type strain, sure, he actually got good reduction. And in fact, even more reduction based on the number of organisms than E. lenta alone. Where the gut microbiota did not enhance the ability of the organism that didn't have this

ability. So there's not just the bug, it's the bug and all the other microbes that are there. And so sometimes we try to reduce things too much. Oh, does the person have this organism? Or does this person have a microbiota? But it's more complicated than that. When you actually put human genetics on here, now you're really building up this idea that this is a very complex system. How about outcome a little bit more modern in terms of therapy? Cancer immunotherapy. It's being advertised on TV now, right? You know, this so and so's place that does all of this anti-tumor therapy based on the host immune system. There are a number of drugs that have come out. And about three-years ago, I was being invited by some of my old residency and med-school classmates -- who are all in (inaudible). I said, "You guys like these papers that came out in science, didn't you? You want someone to start?" Finally, after wondering like, what are you doing studying this microbiome thing? They said they all wanted me to come and talk, because there were two papers that

came out on animal studies where they showed the efficacy of cancer immunotherapy was modified by the microbiota. I'm going to go over these two papers briefly -- not so much that I want you to have the details -- but I want you to understand how we can actually look pre-clinically to study the effects of the microbiota. So in this first paper, where they were looking at ipilimumab -- and they showed that the microbiota was necessary for anti-CTLA4 therapy. And what they did is, okay, so here's the therapy. You know, they put tumors in some mice and if they used basically an isotype-control antibody, these tumors get bigger and bigger and bigger. But if they give one that's related to ipilimumab, anti-CTLA4, the tumors shrink. Okay? Or don't grow as fast -- they don't necessary shrink -- but they grow as fast when they're transplanted into these mice. Now they did something interesting, you can raise mice without any microbes. And if you take these germ-free mice and inject the tumor -- and it doesn't matter now if you give the anti-CTLA4

antibody, the tumors grow just the same as if they got isotype-control antibody. And they could also kind of replicate this by taking animals that do have an intact microbiota, but kind of suppressing it somewhat by giving an antibiotic cocktail. And once again, instead of seeing the anti-tumor effect, they've eliminated the anti-tumor effect by changing the microbiota. And they did some other studies we won't go into here. It's not all antibiotics -- it depends what the spectrum of activity is. So there's certain elements of the microbiota that are responsible for mediating this anti-CTLA4 response. So they did the same thing with anti-PD- L1 therapy. And they did a different kind of study. Again, don't worry about the details or what the message is -- but here's another way to study it -- okay, once again they were taking mice. And people used to say, oh yeah, get a black-six mouse. Wild-type mouse. Doesn't matter where you get it from as long as they're genetically identical, you should have the same results. Not true. If you buy your mice

from Taconic or Jackson Labs -- two of the major vendors -- you had different responses in genetically-identical mice. So again, you didn't have as good of response to the anti-PD-L1 antibodies if you buy your mice from Taconic as opposed to if you buy your mice from Jackson. So the differences that people might see in their studies depends where they buy their "genetically-identical mice". Okay? We did some immunology here, we'll kind of skip that a little bit. What they did show though, if you house the mice together, before you start treating them -- and mice are very convenient, they like to give each other fecal transplants. They'll pick up their neighbor's feces and they'll eat it. And so you kind of "normalize" or at least, I don't know, neutralize the affects you have of the different microbiota. At that point, if you house Jackson and Taconic mice, now you have the same response in both. Okay? And they kind of worked a little bit further on this to kind of figure out that, yeah, there's certain elements of the microbiota

that might be important. Well, that was all kind of fun. It was in mice. That was 2015. This is an example of how fast things can move -- just in January of this year three papers came out in science. And these are studies now in humans that are showing that the microbiota actually has some sort of influence on anti-PD-L1 therapy. Again, for epithelial terms, melanoma. Now the interesting thing about these papers is that they got the same results as far as, you know, the microbiota being able to help or influence a response. But there were some differences as to what they found as the microbes that are "important". Or at least associated with these kinds of affects. Showing again, not everything's the same. It's not just an individual organism that you need to find -- okay, let's find this organism, if you have it or you don't. It's a little bit more complicated than that. And I think that's why there's some frustration in the field. And we'll be hearing some talks about people who are using similar-strain probiotics,

looking for communities, looking for combinations, and perhaps trying to tailor the therapy based on the patient. And let's move to my favorite topic -- infectious diseases, okay? So for 100 years we've been associating microbes with disease -- using things like Koch's postulates. Or finding an organism and giving it to a medical student or a mouse, re-creating the disease, pulling it out again and, you know, saying, well, okay. This is how we can get pathogens. But there's a classic case that we would find. So this was the case that was first presented to me about 30- years ago when I was a med student. So you have a patient that has chronic-lung problems. He comes in, he has an exacerbation of his chronic bronchitis. He's given "broad- spectrum antibiotics". This is more of a modern kind of therapy as opposed to what we might have given when I was a med student. And he gets better from the pulmonary standpoint. But three days into hospitalization, he develops abdominal pain, diarrhea, hypertension, actually has to get transferred to the intensive-care unit.

You know, what happened? You were trying to treat a person with pulmonary infection with antibiotics -- and now he gets GI distress? Maybe he didn't -- hopefully our foods clean. He didn't develop the gastroenteritis in the hospital. What's going on? Well, this is C. difficile. A lot of people know about C. Difficile. And it was sort of even said at that time by one of my Ph.D. advisors, (Stan Faul). He said, "Well, we disrupted the normal," he referred to it as flora at the time. You know, the normal gut flora was disrupted by the antibiotics and somehow this allowed C. difficile to come in. And so the paradigm is that people have a normal microbiota, it has this magical property of colonization resistance. Able to keep away certain pathogens from growing in. But when you alter the community with antibiotics you create a more susceptible microbiota -- whatever that means. And C. Difficile is a spore flora. And interestingly enough, the spores are unfortunately, all over the hospital. And you see the alcohol dispensers in the hospital. They

don't get rid of the spores, they just help you spread them around, perhaps, a little bit more. But when the spores encounter the right environment -- we'll talk about that a little bit -- of this susceptible microbiota -- the spores germinate, you have the vegetative form that produces a very potent toxin that causes all the damage in the intestinal tract. That's when you get disease. And depending on who you are, what the strain is, perhaps what the microbiota are, you might have mild disease. Even asymptomatic colonization -- or you might actually have a more severe fulminant disease. And we don't know all of the aspects of the microbiota -- the pathogen and the host -- that determine all that. But there are a number of us who are studying that quite intently. But as an infectious disease doc, even if you got in trouble with antibiotics, hopefully monitored or recorded antibiotics will get you out of trouble. So you treat the C. difficile. Hopefully when you stop all the antibiotics, the microbiota goes back to normal.

Everything's back to normal and you don't have disease. But a lot of patients, unfortunately, when you stop the antibiotics -- about 20 percent depending on the series after the initial treatment -- will develop recurrent disease. You stop antibiotics, even though they got better when you were treating their C. difficile -- they have disease, they're toxin positive again, and you have C. difficile infection going around. And you can treat them with more antibiotic's, and you can go through this recurrent cycle. And we're going to hear about some of the approaches that people have for breaking this. But one of them that has a lot of interest is this idea of fecal transplant. My younger son is a freshman at the University of Michigan. He's taking freshman biology. And in the second lecture they were talking about fecal transplants from C. difficile. He actually texted me with the slide of the professor -- and kind of giving me the thumbs up. And interesting enough, he happened to be sitting next to a friend of his from high school -- who's

the son of a friend of mine who's the gastroenterologist who started the fecal transplant program at Michigan. So I actually kind of wrote a quick e-mail to the professor. He had at least two people who were pretty amused by that, so. It goes back a long ways. You know, they're talking about Pliny the Elder, and we can go to ancient Greece about him using fermented milk products and perhaps fecal transplants. And in China there is this talk of having yellow soup -- which is basically, you take feces, you mix it up, you let the thick part settle, you take the kind of liquor from the top, and that can be used to treat a variety of illnesses. I mean, that's kind of fun, you know. I don't know. If you ever see yellow soup on the menu, I don't know (laughter). You can decide what you want to do with that. Really, the modern age of FMT came from our surgical colleagues in 1958. So it was after people started using antibiotics, they noticed that there was this pseudomembranous enterocolitis that could arise. And actually,

it's interesting to read this article, because a lot of the stuff that's said here -- you know, we're 60 years on -- we're still sort of saying the same thing. We assume that it has something to do with antibiotics -- adjusting the microbiome. And you know, they had a case series of giving basically fecal enemas to rescue these patients that would normally have had to have their colon taken out. And of course, a lot of people are very familiar -- when this paper came out, we're going to hear updates to this. Our colleagues are going to talk about, you know, really much more. And this is based on a total of 16 patients that everyone, you know, if you just take this paper at its face value, that's the reason to use fecal transplants. But we have a lot of other data that we'll hear about using feces to treat recurrent C. difficile. But how does this work? What's going on? So I'm going to take a somewhat older paper from my lab. This is on a C. Cath, and as of January, so he'll be an assistant professor at Clemson, continuing to work

on the role of the microbiota and C. Difficile infection. But when she started as a post-op -- we actually had some fecal specimens that we had gotten from a number of investigators in Minnesota -- who actually had been treating patients with fecal transplantation for a number of years for recurrent C. difficile. But they saw me at a meeting, and they wanted to say, well, what does this do to the microbiota? And so you have Bakken, the former president of the Infectious Disease Society in America, Charles Gesser -- who's now retired, but has done a lot of fecal transplantation -- asked us, what do you need? I said, well, I want the fecal specimens before you transplant the patients and after you transplant. And I also wanted the donors. And these were patients who had a lot of C. diffs. This is the time that they got their fecal transplantation and the circles were the positive -- these are times they had positive tests for C. difficile, and then the colors of the various treatments that they had had -- with regards to antibiotics to try to treat

C. difficile. These patients had a lot of recurrence up to the time they had their fecal transplantation. And interestingly enough, they did what we're not supposed do -- they tested for cures. So some people were still positive, but of these patients, all but two responded to the initial FMT -- some of them an additional FMT and they subsequently responded there. But I'm not telling you this because FMT works for C. difficile -- but this is what we did. This is kind of to show a little bit of the example of one of the many, many techniques to look at the microbiota. And this is sequencing amplicons of the 16S gene that encodes for the small subunit of the ribosome RNA. Because it's conserved in life, you can have kind of near universal or basically group-specific broad-range PCR. And because of these stem-loop structures, there's variability. We use these sort of, you know, people refer to them as bar codes for specific bacteria. This is how we can kind of get an idea of who might be present. Not what they're doing, not what their

functional capacity, but what organisms might be there. And you read microbiome papers and you see all of these different kinds of analyses that people are doing either for this or metagenomic sequencing -- you hear about all these diversity indices, and your eyes kind of glaze over and you're, what do you do with all these data? You know. But I want to take you through some of these, just to show that it's not rocket science. One of the simple things you can do is you can try to classify what organisms might be present. And so all the patients here are organized in that you have their pre-FMT sample, the post-FMT sample, and when we got it -- a couple of them we missed it -- what does the donor look like? Okay. And who cares what the organisms are being classified. Because sometimes you can actually get fooled. For example, C. difficile gets classified as Clostridium Group XI. You know, and if you're not familiar that it might be in there -- who cares? But then your kind of, oh wait, that's C. Difficile itself. But if you just look at the

communities -- let's look at patient number one. You see the pre and post -- doesn't matter what they are -- the compositions quite different. And interestingly enough, the post looks more like the donor. And this is two weeks after transplantation. You can see this over and over again around here. So this is one way to look at things. This is kind of simple. You only have a handful patients -- this is okay. But what if you have a study with a 1,000 patient's seeing all these stack-bar charts might -- you know, it's hard to make sense of it, what are you going to do with it? Well one of the things you can do is, you can let the data speak for themselves. Now these are all the different types of bacteria -- based on the 16S -- arranged here. They're kind of clustered taxonomically. But now we're looking at the communities, and we're using one of these various clustering techniques to see -- okay all of the samples, how do they cluster? Which samples are more similar to the other? And what you see, there's two main groups here. And even

if you just look from afar, and you can notice that, hey there seems to be fewer bacteria in this left-hand cluster than there is in the right-hand cluster. Okay? This is more diverse -- this is less diverse. And you can even look, that this has a lot of things related to E. coli over here -- not C. difficile -- related to E. coli. That's something we see over and over again. And then if you look to see what the samples were -- you find out that the pre-FMT samples are in this low-diversity group. And then all the donors and most, but not all, of the post -- in green FMT samples -- they're also over here in this diversity group. I told you that two patients didn't respond. When I saw this, I said, "Oh, oh, Anna, please tell me that these two samples were from the two patients who didn't respond." She goes, "No, that's not true." (Laughter). So you can't use -- as much as you can get broad generalities from looking at groups of patients, perhaps we don't have enough resolution in ideas for this technique to be able to look at an

individual-fecal specimen from an individual patient and make any sort of predictions at this time, okay? That's the lesson there. One last thing is, we kind of showed that this idea of lower diversity -- this is actually the first paper I published on C. diff back in 2008. And again, we were just kind of looking. I was just learning how to use these techniques. And yeah, patients with recurrent disease had lower diversity than patients had an initial episode that responded or healthy controls. Okay? And that can be seen again when you treat these patients -- you go from pre-FMT -- and it doesn't matter what kind of diversity in the mix -- you don't have to worry about the details here. But the pre and the post -- you basically increase it. You don't get quite to where the donors are -- but in general, you increase the diversity. So diversity in and of itself doesn't predicts things -- but it's sort of associated with a more healthy microbiota. But I think we have to go down to the details of, really, who's there and what are they

doing. But how do we study what's going on? Okay, so you're making these observations. How do we get at mechanism? Because if we're going to come up with drugs, we need to know the mechanism. So we do have model systems -- the hamster's one model system, mice are the other. And the mouse work -- actually, around the time -- whoops, sorry, this is blurry, don't worry about it. In 2008 Karin Kelly and his group in Boston, revisited the mouse model and showed -- and that's actually nice that it's blurry -- it doesn't matter what antibiotics you're giving. You can give a whole set of antibiotics here, and then if you infect with C. difficile, you can take a mouse that has a normal colon and you can create C. difficile. You get a lot of edema, a lot of destruction of the epithelium. And we've played with this for about the past ten years in a lot of different ways. We can model recurrence, we can model varying severity -- depending on the microbiota varying severity -- based on host factors varying severity -- based on the C.

difficile strain. And we actually have a systems biology grant where we're trying to look at all these. So look at -- can we get an idea of what the host and the microbiota are doing specifically to try to interfere with C. difficile? And there are a lot of potential mechanisms here. And let's go over one of them -- and this came from Casey Theriot from when she was a post-op in my lab. She's now an assistant professor at NC State. She's now in Atlanta hiding the storm. She was actually wanting to look at -- how do we look at functions? Well let's looking at the metabolites. I mentioned the microbiome. Let's look at the metabolites. What could be going on in C. difficile infection? And she used one of the models where you can take mice with a normal microbiota -- she used a single drug at this time, cefoperazone -- the animals become susceptible to C. difficile, they develop very bad disease. And from another post-op showed that after giving this antibiotic, if you take them off the antibiotics -- keep them on sterile food and water -- six

weeks later they're microbiota goes back to a different state -- not the original state, but Casey showed that this secondary state is still resistant to C. difficile infection. So what Casey did is, first she looked at who's there? And she showed that when you become susceptible -- again these are the microbes along here clustering -- based on the types of organisms that present in the community -- the susceptible state is quite different than the animals here and here, that never saw antibiotics -- either at right away or eight weeks later. Their microbiota is pretty stable. But this altered community had different community structure -- the population of organisms. The community was different. Even though it had the same function -- that is resistance to C. difficile. And when she looked, she looked at a lot of metabolites, she looked at thousands of metabolites. She kind of focused on bile acids. And she saw that regardless of what the community looked like, the panel of bile acids seemed to be very similar in all the animals --

resistant versus those that were susceptible. And what might this have to do? I told you that C. difficile comes in as spores. Certain of the bile acids -- in particular the conjugated-bile acids -- the ones that are secreted in our liver -- are very good at triggering sporulation of C. difficile. Where other forms of bile acids -- such as deoxycholate -- were actually very toxic to vegetative C. difficile. And what's important here -- this is the idea of co-metabolism -- sure our liver has these glycine and taurine-conjugated-bile acids. But there are microbes that will take off -- through bile salt hydrolysis take off those amino acids. And there's still other microbes that will do these conversions. Like 7-dehydroxylation that can produce these toxic -- at least toxic to C. difficile -- toxic bile acid specimens. So that you assume that if you disrupt the microbiota-mediated metabolism of bile acids, you might change your susceptible to C. difficile. And Joe Sorg actually posited this about eight

years ago when he was looking at this particular organism -- C. scindens -- that was able to take bile acids and convert it to deoxycholate. And actually Eric Pamer through a separate set of experiments came across the same thing a number of years later and showed that this particular organism -- Clostridium scindens -- because of its 7-dehydroxylate assay in an experimental model could restore bile acid mediated-resistance to C. difficile in a mouse model. So again, this idea that it's the host and the microbes working together. Final story I want to tell you is -- it's not all about bile acids. So my friend and colleague at Michigan, Pat Schloss -- two of our grad students were working together, Matthew Jenior in Pat's lab and John C. Lesley in my lab -- we were trying to look a little bit more at how altering the structure and metabolism function of the microbiome could actually promote sustained colonization by C. Diff or actually just make C. difficile change its physiology. Well what do we mean by that? Well what Matt did is, he took

three different antibiotic regimens. He used cefoperazone, which he used before, streptomycin and clindamycin. He took genetically identical -- and this case mice would be exact same microbiota. I actually have a breeding colony of wild- type mice. And people ask me, why do I have that? You can always buy them. I said, but you can't always buy the same microbiota. Which is why I've been breeding these animals for almost 20 years now. What he did is he created three different environments for C. difficile by giving three different antibiotic treatments to these mice. And then to look to see what C. difficile was doing -- they did RNAC, basically purified C. difficile right from the community and basically looked at the transcription response to the pathogen. To see, how is it behaving in mice treated with cefoperazone versus mice treated with streptomycin versus clindamycin. Then he also constructed some metabolic networks computationally based on the genome of the infected strain and the response that he saw. And

so here's the transcriptional response. So basically, what he did is he looked at all of the different things that C. difficile was doing -- in terms of transcription -- under the various conditions: Clindamycin treatment, cefoperazone treatment, streptomycin treatment. And he found that C. difficile actually had a different transcriptional response depending on which kind of environment it was. It wasn't behaving the same. Certain genes were turned up in cefoperazone-treated mice, versus clindamycin-treated mice, versus streptomycin. And he focused on the fact that a lot of them had to do with core metabolism of the pathogen -- the sugars they were using, monosaccharides, disaccharides, proteins that they do, transporters for nutrients. So when he did this, he was able to kind of predict modeling the metabolic network. What kinds of sugars would C. difficile utilize under the different conditions? Or if he used the shared under all conditions. But then he looked specifically for strep, cefoperazone, or clinda.

And he saw that different sugars were preferentially going to be used by the pathogen under these settings. He tested to make sure that in vitro -- that C. diff could utilize all these -- it's the so called pregnant-source -- and he did and that was true. But then he also used untargeted metabolomics. And he showed that, yeah, under the different situations, different of the sugars were being not only generated on infected animals -- when he infected with C. Difficile, those sugars were dropped. Suggesting -- not directly testing -- but in indirectly suggesting that the C. Difficile was utilizing those sugars. So this is how we can get an idea at how changing the metabolic landscape present in the gut can influence not only the host, not only the indigenous microbes, but a potential pathogen. Where do we go? You know, there's a lot of things here. There's a lot of things that are going on. We have to consider -- not just host, not just pathogen -- but now we have to really consider these hundreds of thousands of different species

of micromes present in the gut -- in all sorts of setting of health and disease. So, you know, I hope that some of the work that I'm showing you -- we're just trying to go away from the association. Oh, this microbiota is different in patients with disease versus patients without. And we begin to get a causation. And we being to understand how this altered -- and people sometimes use the word dysbiotic microbiota -- what's different in terms of the function of that community? And therefore, could we then try to intentionally manipulate the microbiota to "improve health, prevent disease, treat disease" -- what's the FDA statement? Yeah, we all know it. Actually all the things I saw this morning when I had CNN on -- the FDA has not evaluated these statements. These are not intended to do all these things (laughter). It is kind of funny. So what could the future look like? You know, this is something that I would like, perhaps. You know, we talk about precision medicine. There is this "all of us" that NIH has started. That they're going to try to get --

what? I think it's a million. I think it's a million individuals. They're going to look at their genomes to try to predict from their genomes: How is the host going to respond to drugs, how susceptible are they to developing certain types of diseases, what happens if they're in different environments? You know, can we predict adverse reactions to drugs? Like I was saying with digoxin. But I would like to say that maybe there should also be a microbe-sensored microbiota, you know, focused-precision medicine. Interestingly enough, I hope no one's here from the NIH -- who's responsible for this -- they're probably listening. Microbes are not a part of this. And I think that was a conscious decision for whatever reason. And that's fine. But I would like to think that perhaps we also need to consider what the microbiota would do. Because if we assess the microbiota, there might be deleterious organisms and there might be beneficial organisms. The things that we predict from the host genome, might be influenced by the

microbes that are there. We already saw that with a couple of examples I gave -- with immunotherapy, with response to relatively simple small-molecule drugs. And perhaps if we can do all of this data analysis of both the microbe and the host -- then we can come up with customized therapy that's based on genetics and predisposition. So I hope I gave you the proper overview of this idea that this indigenous microbiota is part of a balanced eco-system. But health reflects the balance between us and the microbes that live in and on us. And we have evidence from the past almost 20 years now that disturbances in this balance can lead to the pathogenesis of multiple conditions. We haven't talked about autism, we haven't talked about inflammatory bowel disease, we haven't talked about depression, we haven't talked about alopecia areata. There's a number of things where there are associations between the microbiota. But I would really like to stress that it's going to take teams of people working together to understand the dynamics of the system, what is the

function of the system, and more importantly -- for the clinicians in the room -- how are we going to be able to manipulate this complex system to prevent or treat diseases. So let's stay on time, Paul. Okay. I'd like to thank a lot of my collaborators. Again this is team science. This is just a small handful of the people I've worked with at a number of institutions. And we come from all sorts of backgrounds. Bacteria, Pathogenesis, Immunology, Clinical Microbiology, Machine Learning, Computer Engineering, et cetera. Microfluidics, of course all the people in the lab who actually do work. And I'd like to thank NAIAID, also NIDDK from previous awards to study the microbiome and health and disease. I'd be happy to take any questions at this point, thanks (applause).

SPEAKER: We have plenty of time for questions. I just want to ask that if you have a question you come to the microphone and give your name and affiliation prior to your question.

DR. YOUNG: Have time. And I know a lot

of the questions will -- yeah, if you can come to the microphone. I know a lot of these questions might be best addressed by some of the subsequent speakers. So if a question comes up and one of the speakers says, I'll get to that, the speaker could raise their hand and say, I'll get to that, and I won't try to flail around and answer (laughter). Go ahead.

MR. LILLIS: Hi. I'm Christian Lillis from the Peggy Lillis Foundation. In addition to antibiotics that we put into our systems, have you guys looked at anything in terms of how the environment itself might be impacting us? Like the overuse of antimicrobial soaps and different things that we're kind of putting in and on our bodies?

DR. YOUNG: Yes.

MR. LOWES: Because I've always wondered about that.

DR. YOUNG: Yes. So the question is about, you know, how does the environment -- outside of drugs that we use -- in particular

antibiotics. Antibiotic residues in food. There have been a number of papers that have tried to associate that. Triclosan, that's a lot of studies on Triclosan and what that may do to the microbiota. And you also raised the idea of -- there is a whole field of so- called, the microbiome of the built environment. There are people who are looking at how microbiodes that we could get exposed to in our cars, in our houses, and restaurants, in the health care systems can influence as well. And trying to assess that out. So the long and short is, almost any time you do kind of a study to compare two groups, you will find that there are differences in the microbiota. But a lot of times we don't know if there's a significance there. Because many times we're not necessarily looking at function, and we're not looking at how it impacts health directly, so.

MS. EUNIS: Thank you very much, for your talk, Dr. Young.

DR. YOUNG: Thank you.

MS. EUNIS: I'm Jessica Eunis with the

IPA.

DR. YOUNG: Thank you.

MS. EUNIS: (Radicus incision). My question is about sex dimorphism. I really appreciate the work you guys are doing with the mouse models, but can you maybe make a comment on that? Especially in light of the topic of abortion?

DR. YOUNG: Right. So sexual dimorphism in microbiota responses is something that should be looked at. I know my program officers here, we write a section on that right now. And we make sure that we always look. And in our studies, we do stratify by sex. And in our studies -- and probably because we're giving antibiotics, which really overwhelm the microbiota -- we haven't seen any sex differences in responses. But I know there are papers -- when they're using more subtle perturbations in the microbiota -- where there are distinct -- in terms of the response of the microbiota and also the response of the whole system -- in other words whatever the health

outcomes are based on sex. And so that's some we have to keep in mind. But in the human microbiome project, we did not see -- other than the obvious -- that the vaginal microbiota is only seen in women. For example, we didn't see anything say with skin and gut and other things with sexual differences. Again, looking at a very crude high-level bi-16s in metagenomics -- we haven't seen that. But it something that needs to be kept in mind. Yes, thanks.

MR. RAY: You talk about --

DR. YOUNG: Could you give your name quickly and your association?

MR. RAY: Emmond Ray. You talk about cardiac glycoside, digoxin, metabolism in the --

DR. YOUNG: Uh-huh.

MR. RAY: -- microbiome. The metabolite is still effective? This would be more broad than the actual (inaudible)?

DR. YOUNG: I'll have to remember the '83 paper. I believe that actually some of the metabolites were no longer active but were still

toxic in some ways. They had less antiarrhythmic. And I can't remember which ones were inactivating versus the primary response or not. But that's something that's seen over and over. In some cases you create new compounds that have differing activities. In some cases you create compounds that are just different structurally but maintain the parent activity. And that's something that we've seen -- like for example with the bile acids. You can shift function with some of these. But specifically with the digoxin, I'd have to go back to that paper to figure out which of the metabolites still had toxicity versus therapeutic affect on that. But it was looked at in both of those studies, so it's in there. Other questions? Comments? Have we solved it all? Are we ready to (laughter) -- are we ready to go out and treat patients? Send their fecal specimens or whatever specimens to whatever diagnostic lab? I think there's something very important. I mentioned the two papers in my introduction that came out from Erin Ellanoff's group. They looked at some things

that were very important. Things that we had looked at -- but finally they published. We use feces a lot to kind of serve the microbiota. They also do colonoscopy -- both prepped and unprepped. And there are differences between the microbiota in the mucosal surface, the microbiota in the lumen, and those were quite different from feces. So we have to also figure out how are we going to most properly assess a patient's microbiota. I think some of the things in feces -- like most of the things in the GI tract eventually end up in feces, but the relative abundance that you find is going to be altered in feces as opposed to what it might be more proximal in the GI tract. And I had gotten into a number of, shall we say heated comments, about people talked about the relative efficacy of stool. Because I said, yes, it may or may not. But I think now that these have been published, I think people will be a little bit circumspect that stool is not the only analyte that you can look at. Or if we look at it, there's certain caveats that we have to have when

we look at feces as a marker for what's going on as far as the microbiota. Yes.

SPEAKER: I'm Euan First, Alan Capital. I wonder what thoughts you've given and what research you might be aware of -- just generally speaking -- going on identifying the impact from maybe changes in soil. Given that that affects our food supplies. And one would expect that, you know, maybe there's some changes going on there.

DR. YOUNG: Yes, so it's interesting. I think his name was on there -- Tom Schmidt. He's the person who taught me how to do microbiota when I was up at MSU. And his research at the time was looking at different agricultural practices --till, no till, amended, again whether or not they had fertilizer in the soil -- on the effect of the microbiota of the soil itself. And how actually that could change the flux of greenhouse gases to those soils. And this was done at the Kellogg Biological Station up in Michigan. Where they had different plots all in the same area where they had these different treatments. And he

saw that it was quite dramatic effects on the microbes that are present in the soil and their ability to take and fix nitrogen. And basically to take CO2 from the atmosphere and fix it back into plant material. And so that's where I actually got interested in it -- and I said, hey, would you want to look at another community? I actually have a different community of microbes. I'm very interested in their function. And that's how we started looking into that. So no one's tied together those changes that you see clearly in the soil with happens to people who eat crops grown under those different measures. That actually hasn't been done. I don't know how much I would expect that to change. But really, this idea of an integrated-earth microbiome -- that it's not just the ones in us, you know. For example, the microbiota in cows and how much methane they can produce. You can actually have cows that produce more or less nothing -- speaking of greenhouse gases. So everything is interrelated in this world. And the microbes may

be actually the link between a lot of them. So these people have been looking at soil microbes for like 30 years. And there was an article published in the New York Times about five years ago that I showed to Tom. It was talking about people who were looking at crop microbiome. And it says, "Taking clues from people who are looking at the human microbiome, soil scientists are now," -- and I said, "Hey, here's a little bit of revisionist history." (Laughter) I said, "I learned from you. But supposedly you're learning from me now." So, you know, you have to kind of take that broad view. I think I only bring up that anecdote -- it's important -- and that's how I was trained -- doing reductionist, mechanistic-based science. But some of these questions are so complex that you sometimes have to back out a little bit and try to look at the big picture. Move back down, and zoom in on mechanism, so. That's the approach. Yeah.

MR. FORRY: Sam Forry, NIST. I wanted to ask about sampling recommendations on test of

human samples. I know with mouse models -- where they produce fecal material more often -- we can track and see dyno cycles. In humans -- where people often have a single bowel movement a day -- it's much harder to pull that out. And I'm wondering what you do in the context of -- as a clinician in your research -- how you go about acquiring fecal samples and what the best practices are to try to amass that or control point.

DR. YOUNG: Yes. That's actually a very important question when you're (inaudible). And I already brought up that stool only gives you one kind of view. So invasive sampling gives you a different view. We've actually sampled the upper GI tract using an FDA-approved device. That actually has four lumens -- 2 meters long, goes down -- and we're looking at drug dissolution studies. And we've looked at the microbiota through there. And over time in these individuals who are fast and fed -- you see all of these changes. We didn't monitor them long enough to

look at dyno variation in a fasting subject -- but these are all these things that you have to take in account. You want to sample as much as possible. For example, one of the areas that a number of investigations -- you'll hear more about necrotizing enterocolitis. As you collect all the feces that comes out an infant -- if you can and see how that changes. But know that it dwells there for a while. You're right. Some people only have one bowel movement a day. I mentioned Stanley Falco. He had this saying that, "One man's constipation is another man's diarrhea." Right? You know, is one sample a day enough on a person who has four bowel movements a day, or only has a bowel movement every three days, you know. These are the considerations that we have. And we don't have best practices. And I know other people from NIST have been at some of these conversations. And it's kind of daunting to figure out, how do you standardize this? I think a couple more questions?

MS. DEONYAD: Carla Deonyad. So Vince,

I was struck by the fact you were saying the "low diversity was more susceptible and the higher diversity was more resistant".

DR. YOUNG: Mm-hmm.

MS. DEONYAD: But for other body sites, say for example in Human Microbiome UC Project, you saw the opposite. Like in the vagina, less diversity tends to be more resistant and more diversity seems to be more susceptible to bacterial vaginosis. I was wondering what you think different body site.

DR. YOUNG: Right. And that's why -- even though I've been guilty of putting the word diversity in my early papers there -- I realize that diversity is just a marker for how many different kind of organisms that you might have there. And if you have the wrong organism there, or you're missing the right organism, it doesn't matter how diverse you are or not. And it does vary from site to site. As you said, in bacterial vaginosis you actually have much higher diversity. In the healthy vaginal tract which is generally

dominated with lactobacillus -- but not in normal, healthy individuals -- they tend to have that. And that's lower diversity. I published a paper with John LiPuma on the cystic fibrosis lung. And actually, increased diversity was "protective" early on. And when you've just had nothing but pseudomonas, or burkholderia, or staph later on -- and maybe that's because you've had a lot of antibiotics and people say that was bad. Again, we had to be careful what we said, because we're not going to stop treating our cystic fibrosis patients with antibiotics, because that's what's extended their life span by decades. But maybe we do have unintended consequences that over time -- maybe I need to stop here, yeah. We have about till 10:00, right? We have time for one more question?

MR. VOREADES: Noah Voreades from GenBiome Consulting. Going back to the question about stool collection. I think there's still a lot of open questions in regards to what is the appropriate way to collect the stool. Meaning is

a swab from a tissue paper sufficient? Can you take an aliquot with a scoop? Or do you need to essentially take the whole stool, homogenize, and then sample from there? I wasn't sure if you had any best practices or, you know, within the community that you're in if you can provide any insights.

DR. YOUNG: Sure. As far as sampling, you know, those are important studies to do. And boring papers to convince your graduate student to write. But, for example, we looked at that. And we did a study where we compared feces to swab in a number of patients. And we showed that the rectal swab taken matched the fecal specimen pretty closely for all intents and purpose with what we're doing. And I think what happens is then you say, oh, what's the best storage technique? What's the best extraction technique? What's the best way to do your amplification? Which is the right taq polymerase? Which is the -- you know. And I think what we have to come up with is that we're always going to have biases.

And what we need to do -- and I kind of tell my students -- in spite of all the biases we have technically, appreciate that they might be there -- do things the same way and design your experiments very carefully so that you can get an answer despite what the biases might be. And then try to test it in another way. In other words, go to a germ-free mouse. After you've sampled. Go and try to actually do interventional studies, go into bioreactors, go into organoids, to try to figure out that the answer isn't just from sequencing. I hope no one's from N.H. Gary. But the answers not just from sequencing. All right, thank you very much folks (applause).

MS. DEAL: And the next two speakers are from FDA. And the first speaker I'd like to introduce is Bob Durkin. And Bob is the Deputy Director, Office of Dietary Supplement Programs at FDA Center for Food Safety and Applied Nutritional Systems. And his office is the agency lead for the regulation of dietary supplements. Today Bob will speak about how the agency approaches the

regulation of products labeled with (inaudible).

MR. DURKIN: Good morning. I very much appreciate being asked to speak here today. As I was introduced, I am the Deputy Director of the Office of Dietary Supplement Programs at FDA Center for Food Safety and Applied Nutrition. I think an obvious question at the forefront is if we're here at a workshop to talk about the use of microbiome products as drugs. Why do they invite a person from foods to speak? But I think that's a good question and I think it should be addressed at the front of my presentation. Probiotics like live microbials -- you know what I'm trying to say -- are a very quickly growing segment of the products labeled as dietary supplements. They are coming into our market place very rapidly. They're taking a lot of market share. And they have the FDAs attention when they are regulated as a dietary supplement. Products that are rated as dietary supplements or dietary ingredients are really regulated like nothing else at the Food and Drug Administration. There are a lot of

similarities, a lot of cross overs, but even when you think you hear something that sounds the same, I can almost assure you -- I don't want to speak in absolutes -- but it's very likely that there's some nuance or tweak -- a difference between things that sound the same, the way they're handled in a drug or a biological or a conventional food compared to a dietary supplement. That said, I thought the best thing for me -- nope, I didn't do that right. How do I get to the next slide? Okay, there we go. I did it (laughter). So that said, I thought I would start off -- I considered the folks that would be in the room today, the folks that would be interested in this. And on one hand, I think there's some people that are very well versed in the regulation of dietary supplements. I see some familiar faces in the room. On the other hand, I thought there were some folks that might be in the room that are a little uneducated or uninformed about how dietary supplements are regulated. And a basic 101 about that might be a good place for

us to start our conversation. This slide here shows some very different ways that dietary supplements can be presented on the market. We'll get into some of that a little bit later. I'm supposed to find something in particular. So we'll start with the definition of a dietary supplement as found in the The Dietary Supplement Health Education Act. The shade states that a dietary supplement is a product that is simply intended to supplement the diet. That can be translated to mean that it cannot be a conventional food or intended to be the entire substance of an entire meal. A dietary supplement must be intended for ingestion. It cannot be sublingual, topical, injected. Those products fall out of the definition of a dietary supplement. A dietary supplement must also contain a dietary ingredient. There's a list on the 201ff1 of ingredients that can qualify as dietary ingredients -- vitamin, mineral, herb, other botanical, amino acid, dietary substance for use by man to supplement the diet by increasing

the total dietary intake, for concentrate metabolite constituent extract or combination of any of the above. Oh, you're going to have to come here and show me. I'm so happy he was unsuccessful (laughter). You have no idea what a relief that was. One more. Thank you very much. Again, this is the exclusion from the definition of a dietary supplement. It's found in 201ff3d. Essentially it says that an article that was the subject of an approved IND or an ANDA -- for which there were significant clinical investigations that were made public -- is excluded from the definition of a dietary supplement. Basically, you can not do research on an ingredient, and then someone in the dietary supplement industry come in and take it out from underneath you. This was meant to preserve the incentive for development under the Rupert and drugs. Old versus new dietary ingredients. The new dietary ingredient or NDI notification requirement is for those products that contain a new dietary ingredient. A new dietary ingredient is an ingredient that was

not marketed prior to October 5, 1994. This NDIN process is basically the only premarket opportunity that FDA has to look at a dietary ingredient before it comes on the market. Again, it's a notification process, not an approval process. The manufacturer or distributor of the dietary ingredients to be contained in the product labels of dietary supplement has to notify FDA of their intent to go to market 75 days prior to going to market. During the 75-day period, FDA will evaluate the firm's basis for thinking that their ingredient is reasonably expected to be safe to go to market under the labeled conditions of use. FDAs response to a new dietary ingredient notification was essentially two types of responses. A response without objections and a response with objections. A response without objections is known as a good day letter. It means go to market; we don't have a problem with your product. A response with objections can come in a few different flavors. We can disagree with your basis for thinking your product is reasonably

expected to be safe. We can disagree with how you identified your product -- you didn't tell us what it was. We can find some shortcomings in your manufacturing process -- maybe you didn't show us that it was going to not be contaminated or have some other follow-on constituents or components that would be dangerous. We can also send you a letter saying that your ingredient is not a dietary ingredient. Or that you didn't even follow the directions for filing a complete notification. New dietary ingredient proper notifications must include the name and address of the manufacturer or distributor that is introducing the NDI into commerce, identity information on the ingredient -- so we know what we're talking about -- information on the dietary supplement that contains the ingredient, conditions of use, and safety information. The safety information can be based on a history of use or other studies that demonstrate the ingredient will reasonably be expected to be safe, or a combination. In other words, you can show

historically where your ingredient's been on the market and it's been used safely, or you can show us scientific literature -- preclinical, clinical studies -- or you can show us the combination of all the above. New dietary ingredient notifications. While the requirement's been in place for about 20 years, we've received less than 1100 independent NDI notifications representing about 720, 750 individual ingredients. This shows anecdotally maybe that there's an under reporting going on in the industry. And that that might be something you want to address before -- or you can get a visit from FDA. Current good manufacturing process. This is important. I thought it was something that should be mentioned here today in regards to dietary supplements. A large part of FDAs post-market regulation of dietary supplements is based on the good manufacturing process regulations. FDA published the final rule for good manufacturing in June of 2007. This rule is found in 21 CFR Part 111. It's different than drugs. Drugs are found in 211. That's one of our

differences there. Not just where they're at, but also the substance of what the GMPs represent. GMP regulations are an important tool to ensure that dietary supplements are produced consistently in a high quality. Maybe not as high of a quality as someone who's familiar with drug GPAs would think. They're certainly more than conventional foods. Sort of in between. The regulation has an emphasis on production and process controls. Building quality into the product, as well as requirements for the testing of the raw material and finished product stage. This is an extensive regulation -- and may be relative to conventional food, but not so much to drugs or biologics -- an extensive regulation that covers all aspects of manufacturing. From setting up a facility and establishing personnel through product design -- production and testing -- to records and record keeping. A little more on GMPs. They're applicable to all firms to various degrees who are involved in the manufacturing, packaging, labeling, or holding of dietary supplements --

both domestic and foreign. FDA investigators confirm GMP compliance through a series of investigations. We conduct 100s per year -- somewhere between 5 and 700 -- split between domestic and international. Non-compliance with regulations can result in FDA action. Another interesting aspect of products label as dietary supplements are, they're labeling requirements. In addition to the previously mentioned manufacturing requirements, dietary supplements also have labeling requirements. These dietary supplements are a category of food. They must follow the food regulations found in 21 CFR 101. A few requirements that are specific to products labeled as dietary supplements relative to other foods would be that they must be labeled as dietary supplements. They must actually use a statement that describes them as a dietary supplement. It might say dietary supplement. It might say probiotic supplement. It might say calcium supplement. But it has to have something on it to describe it as a supplement -- to show

the intent of the person putting it in commerce. As with foods, dietary supplements must list all ingredients. But the ingredients must be formatted -- instead of a nutrient facts label -- they must be in a supplement facts label. Additional, dietary supplement labels must contain the name and location of the manufacturer or distributor and have contact information -- use phone number or address -- to which consumers or health- care providers can notify the firm of adverse events. A little bit more about supplement labeling. In addition to the required aspects of the label, dietary supplements are afforded three types of claims they can make regarding their products. The first of these claims would be nutrient content claims. An example of this would be a product that is high in calcium or low in sodium. Dietary supplements can make structure function claims regarding the effect of the product on the structure or function of a body. An example might be calcium helps build strong bones. I'll talk more about these in

a minute. Finally, a dietary supplement can make some authorized health claims or qualified health claims. These are actually spelled out in regulations. They can be found on our website. An example might be regarding calcium or vitamin D reducing the risk of something like osteoporosis. A little more on instruction function claims. A structure function claim is intended to describe the role of a nutrient or dietary ingredient on the structure or function of the human body. DSHEA created an exception to the drug definition that authorized dietary supplements to bear these structure function claims without being regulated as a drug. Dietary supplements -- with maybe just one or two other exceptions -- are the only type of food that can make a structure function claim. Any other food that makes a structure function claim has now put itself in the unapproved new drug box. This is a unique exception for dietary supplements that is separate from most foods, cosmetics and such. The exception from the drug definition applies only if the claims were made in

accordance with the information found at Section 403R6 of the Act. Firms tend to get in trouble when they make a claim that is intended to be a disease claim. A slight error about disease claims. Disease claims are hard. They can be in a gray area. Context is critical. You have to take the label and the labeling -- the totality of the circumstances. A good example might be an EKG symbol on a label may in itself not be a drug claim. But if they then make a statement about cardiac health -- the two together may be considered a drug claim. Again, some guidance for industry can be found online -- structure function claims fall into the compliance guide. No claim is ever likely to be absolutely violative or absolutely okay. Again, it's all a matter of the circumstances and in evaluation. A little bit about adverse event reporting. FDA post-markets for balance of diet products labeled as dietary supplements includes adverse event reporting. This is a result of the Serious Adverse Event Law which took place in 2006. Dietary supplements

must submit serious adverse events to FDA for review. The reporting system works through FDAs MedWatch Program. And submissions can be through an electronic portal, e-mail, phone call, letters. While manufacturers are required to submit these reports -- consumers and health-care providers can do it on a voluntary basis. If the manufacturer receives an adverse event and determines it can be serious, they have to report it to the FDA in 15 days and follow up on that specific event for a year. A little more on adverse events. Once the reports are entered in the MedWatch, the dietary supplement-specific adverse event reports are entered into our care system. Which stands for the Signal Adverse Event Reporting System. ODSP has medical doctors that review every single adverse event to make a determination if there's association or causation. They'll look at the trees, they'll look at the forest from all different perspectives to see if we have a problem with a product or maybe even an ingredient. If there's signal of a risk to the public health, we

do what's appropriate. We do other investigations. We'll call up the person that provided us with the adverse event. We'll investigate and if required we'll do what we need to do to protect the public health from enforcement actions. Just a little bit here about what we deal with when we talk about the dietary supplement market. When DSHEA was enacted on October 15, 1994, there were about 600 manufacturers, 4000 products, worth approximately 4 billion dollars. Today there are over 7000 registered facilities, there are over 75,000 independent SKUs for products labeled as dietary supplements. And external sources estimate that the industry is worth upwards of 40 billion dollars. How does FDA approach this large, diverse, fractured industry? We try to regulate it. This is a basic pictogram or organizational chart for the FDA -- as a larger entity. You can see the Office of the Commissioner up top. Down bottom you see what we call product centers. And you can see the Center for Food Safety and Applied

Nutrition is the second from your left. We see the Center for Biologic Evaluations and Research as the fourth one in. We don't work with the folks directly that work for CBER. We're in different product centers. We know each other, we have relations, we have good communications. But we don't actually work together in the same building or even the same office structure. There are some other offices on here that impact the regulation of dietary supplements. You have the Office of Operations. That would basically be ORA --the inspectors, the boots on the ground. CBER -- we work with CBER. We work with CDER. We work with the Office of Chief Counsel. This is the organization at CFSAN. As I mentioned, CFSAN is one of the product centers. You can see there are then offices within CFSAN. The Offices of Dietary Supplement Programs is highlighted. We're one of about 12 product offices within the product center. So you say, what does FDA have to regulate this industry that's worth 40 billion dollars? We have 26 people. Yeah. Now that's

directly in ODSP. That's not including folks that we try to leverage in other offices such as ORA and OCC. ODSP wasn't always an office. We were once a division, part of ONLDS -- Office of Nutrition Labeling and Dietary Supplements. Back in December of '15 -- I think it was -- or '16, we became an office. That elevation brought us up to the table. We are now a product office within a product center. It was meant to give us a higher profile and put us in a better position to ask for resources and to work with regulated industry. ODSP program priorities are first and foremost to protect consumers, ensure product integrity, and help to promote informed-decision making (applause).

MS. DEAL: Now we've heard from the Center for Food Safety and Applied Nutrition. And now it's time to hear from the Center for Biologics Evaluation and Research. And our next speaker is Sheila Dreher-Lesnick. And Sheila is a regulatory coordinator in the Division of Bacterial, Parasitic, and Allergenic Products or

DBPAP -- as they say -- in the Office of Vaccines, Research and Review in the Center for Biologics Evaluation and Research Center. And DBPAP is the product review division which is responsible for reviewing product information for regulatory submission for a wide range of products including bacterial and parasitic vaccines, allergenic products, live biotherapeutic product -- the LBPs -- FMT or Fecal Microbiota for Transplantation, and also the PHAGE therapy products. And she has a presentation today. Will discuss the regulatory oversight and considerations for live microbiome products when they're used as drugs. A little different from the previous talk.

MS. LESNICK: Thank you for that introduction. Let's see if I can maybe just -- so today, the first part of my talk will broadly cover the regulatory oversight for development of live microbiome-based biological products. And I'll be just briefly touching on the IND regulations and definitions, and broadly cover the

stages of review. And in the second part of my talk, what I hope to do is point out some additional considerations for clinical studies using live microbiome-based products. And I'll point out a few chemistry-manufactured and control-information points, some CMC points for live biotherapeutic products. And a few points to consider for fecal microbiota for transplantation -- live microbiome-based products. And I'll point out a few chemistry-manufactured and control-information points, some CMC points for live biotherapeutic products. And a few points to consider for fecal microbiota for transplantation. So what is an IND? An IND is an investigational new drug application -- that when in effect examines an investigational new drug from pre-marketing approval requirements. It also allows an investigational new drug to be lawfully shipped across state lines for the purpose of conducting a clinical study of that investigational new drug. The IND regulations require that human research studies be conducted

under IND if the following conditions exist. The research involves a drug as defined in Section 201 of the Federal Food Drug and Cosmetic Act. The research is a clinical investigation as defined in the IND regulations. And the clinical investigation is not otherwise examined from the IND requirements. And pertinent to our discussion today, I just want to point out that a biological product subject to licensure under Section 351 of the Public Health Service Act fits within the drug definition under the FD&C Act. And a few clarifying points about exemptions. What this means is that clinical investigations of drugs lawfully marketed in the United States are exempt from the IND requirement if certain criteria are met as listed in 21 CFR 312.2(b)(i). And drugs are lawfully marketed if they have been approved under the following pathways. The new drug application, a biologics license application, an abbreviated new drug application, or an over-the-counter monograph. And just to point out here, that conventional foods and dietary

supplements are not lawfully-marketed drugs. And therefore do not qualify for an exemption of the requirement of an IND -- as described above -- when they're studied for a drug use. So if not exempt, when is an IND needed? In general, the FDA regulations require the evaluation of a drug or biologic product in humans be conducted under IND. And a drug is defined in part as articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease -- and articles other than food intended to affect the structure or function of the body. So the intended use then determines whether a product is a drug. And the question becomes, is the product in the study being investigated for a drug-intended use? If the answer is no, then no IND is required. And if the answer is yes, then an IND is required. And this is true whether it's for commercial development or for research-only studies. For additional details, I'll refer to our guidance from 2013 determining whether human-research studies can be conducted without an

IND. Who sponsors INDs? Big companies do, small companies, individual-bench researchers, individual- clinical investigators, and other government agencies. And FDAs primary objectives in reviewing an IND, is to, one, assure the safety and rights of subjects in all phases of investigation. And in phases two and three, to help assure that the quality of the scientific evaluation of drugs is adequate to permit an evaluation of the drug's effectiveness and safety. And this slide is just to remind you, really, of the typical phases of development for biological product under IND. They typically start as small phase one study, and then progress to larger phase two studies. Data generated from these phase two studies are then used to inform the design of the larger phase three efficacy studies. And then data from the phase three efficacy studies are then used to help support a biologics license application. And to obtain licensure, the applicant must demonstrate the following. That a particular product is safe, pure, and potent. And

that the facility in which the biological product is manufactured, processed, packed, or held meets standard designs to assure that the biological product continues to be safe, pure, and potent. And a point I'd like to make here is that potency has long been interpreted to include effectiveness. And only those biologics that have demonstrated to be safe, pure, and potent -- and that can be manufactured in a consistent manner -- will be licensed by FDA. And to date, the FDA has not approved the live microbiome-based product to prevent, treat, or cure disease or condition of disease. So that covers the first part of my talk. And now I'd like to get into some additional considerations for clinical studies using live microbiome- based biological products. And I'll start with live biotherapeutics. In 2012 FDA published a guidance document discussing chemistry, manufacturing, and control information -- or CMC information -- to include in an IND application for early clinical trials with live biotherapeutic products. And an LBP is

defined as a biological product that contains live organisms and is applicable to the prevention, treatment, or cure of a disease or condition of human beings. And a commercially-available probiotic may fit the definition of an LDP, depending on the intended use. And while commercially- available probiotics are generally considered safe in healthy adults, safety issues may be critical and clinical-trial populations compromised by specific health concerns or conditions. And recognizing the difficulty that sponsors had providing the CMC information required under 21 CFR 312.23, FDA revised the LBP guidance in 2016 for proposed trials in generally healthy subjects. And the updated guidance describes how for IND studies using commercially-available LDPs such probiotics -- a waiver of the requirement for CMC may be granted if all of the four following conditions are met. One, the LBP proposed for investigational use is lawfully marketed as a conventional food or dietary supplement. Two, the investigation does

not involve a route of administration dose, patient population, or other factor that significantly increases a risk or decreases the acceptability of risk associated with the use of the food or dietary supplement. Three, the investigation is not intended to support a marketing application of the LBP as a drug for human use or a biological product for human use. And four, the investigation is otherwise conducted in compliance with the requirements for INDs. If the investigation meets all these conditions, we ask the sponsor to submit a waiver by documenting the above, a copy of the label and a commitment to record the lot numbers and date of expiry. So therefore, IND is using commercially available LBPs. If the request for a waiver of the requirement for CMC is granted, then the label on the commercially available LBP will generally be sufficient to satisfy the CMC requirements for the IND application. If the waiver is not applicable or granted, then the sponsor needs to submit CMC information in their IND application. And we do

recognize that specifically for commercially available LBPs, that the IND sponsor may not be the manufacturer. And in this case, the manufacturer and the IND sponsor can use the master-file mechanism to provide confidential manufacturing information directly to FDA. And what we're looking for when reviewing CMC for INDs with live biotherapeutic products, is sufficient information to assure the proper identification, quality, purity and strength of the investigational new drug. And as product development proceeds, we ask that the sponsors submit amendments to the IND to supplement this initial CNC information. What does the CMC information look like? So the guidance then goes on to describe what to include. And that would be, strain information as available -- such as the name, the source, the strain and passage history, relevant genotype and phenotype or full genomic sequence. We also ask to include an antibiotic-resistance profile for clinically relevant antibiotics. Information on cell-banking

system, a description of drug substance and drug product manufacturing process, and stability data. And specifically, to demonstrate that the product is stable for the duration of the treatment phase of the study. CMC information should also include information about manufacturing controls and the least testing -- including potency testing. Which is typically a measure of viable cells expressed in CFU. And for multi-strained products we ask that as product development proceeds -- the sponsor work on enumerating all strains in the final product. Potency testing can also include additional biochemical or physical chemical measurements thought to predict potency. Manufacturer controls or release testing should also include bioburden testing. And there, we want to see that the sponsor can demonstrate the absence of extraneous undesirable bacteria. And we've typically asked sponsors to perform bioburden testing per USP <61> and <62>. But I do want to point out here that additional testing may be required depending on the intended population

and other organisms manipulated in the same facility. And just a slide here on CGMP again. Current good manufacturing practices for drugs and biologics followed 21 CFR 210 and 211. And basically, what it states here is that it is sure that a drug is safe and has the identity and strength and meets the quality and purity characteristics that it reports or is represented to possess. And as described in our guidance here, for CGMP for phase one investigational drugs, FDA recognizes that the extent of manufacturing control differs not only between investigational and commercial manufacturer, but also among the various phases of clinical trial. And now on to a few points about fecal microbiota for transplantation. This slide is a summary of the history of FMT guidance from the FDA. And it starts in May 2013, where FDA and NIH held a joint-public workshop. This was attended by clinicians, bench researchers, members of the public, and government employees. And at that workshop, FDA noted that the use of FMT in

clinical studies to evaluate its safety and effectiveness, are subject to regulation by FDA. Recognizing concerns from health-care providers at the time -- that applying IND requirements would make FMT unavailable for individuals with C. diff infections unresponsive to standard therapies -- FDA published a guidance document for immediate implementation in July 2013. And this guidance explains that FDA intends to exercise enforcement discretion regarding the IND requirements for use of FMT to treat C. difficile infection not responding to standard therapies. The enforcement discretion does not extend to other uses of FMT. Since then, FDA has published two draft- guidance documents. One in March 2014. Where FDA clarified that they expect to exercise enforcement discretion only if the donor is known to the doctor or the patient. We received many comments and they were all considered. And in response, we then published a revised draft-guidance in 2016. And in this draft-guidance, FDA clarified that they intend to exercise enforcement discretion

only if stool for FMT is not obtained from stool banks. And I know some here in the audience are really hoping for an update to this guidance. But I don't have new information to share with you today. But what I can say is that we are considering all the comments that we've received to date. And as we move forward, I'd like to point out a few safety considerations for FMT. We can address safety by adequate-donor screening and establishing appropriate donor-screening protocols for the intended population. We can also test stool. But we have continued questions about the sensitivity of available stool tests. And they're ability to detect pathogens present in low numbers. Questions also arise in terms of longer-term safety. What are the potential longer-term effects of the transferred microbiota on the recipient? With regards to purity and potency, questions remain about appropriate measures of potency for FMT. And our current understanding about whether there are specific organisms or a consortium of organisms that

mediate effectiveness. So as we move towards licensure of live microbiome-based biological products, I just want to reiterate here that only those biologics that are demonstrated to be safe, pure, and potent, and that can be manufactured in a consistent manner will be licensed by the FDA. And what this means is that we need clinical data to demonstrate safety and efficacy -- but we need to remember that this is linked to product quality and consistency in manufacture. And all three are needed for licensure. And I want to end with some final thoughts here. Interest in live microbiome-based biological products has increased greatly in recent years. And CBERs regulatory approach is science-based. And this does allow for the novel approaches to be safely tested in the clinic. And also, we are committed to working with our sponsors to find the path forward. Thank you for your attention (applause).

MS. DEAL: Well I think we have time for a few questions. Bob? Did you want to come up at this time?

MS. SANDERS: My name is Mary Ellen Sanders and I'm with ISAP. And I had a question regarding the intent of research end points -- considering the fact that there's an overlap between the definition of drugs and the definition of foods. And both drugs and foods can affect the structure and function of the human body. And both can reduce the risk of disease as well as provide nutritional support for other disease conditions. And the situation exists in the United States today where human research on probiotics is viewed -- even when there's no intent to develop a drug -- it's being viewed as research that needs to be conducted under an IND. And my question is is there a role that CFSAN can play -- where by oversight of human research on probiotics that fits under legitimate legal intentions of use of foods and dietary supplements to affect the structure and function of the human body or reduce the risk of disease -- can be overseen by CFSAN rather than CBER? Because CBER does a great job overseeing drug research. They

don't really oversee food research yet. These are legal uses of foods.

DR. YOUNG: Thank you very much. So the way I understand your question is, if someone is using a product -- say licensed as a dietary supplement -- and if they use that product licensed as a dietary supplement for an investigation for a structure-function outcome -- that would not be diseased and that would not, I believe, require an IND. But if you were to use that product labels of dietary supplement or a disease outcome -- something more than a structure-function claim -- now you're in essence using it as a drug, and it would require an IND.

MS. SANDERS: Okay, but just to clarify my point is -- impact to the structure-function of the human body legally is both foods and drugs.

DR. YOUNG: Your right.

MS. SANDERS: And so CBER very much could look a structure-function end-point at a study and say, "This is a drug end-point. And they'd be correct if the intent was to market a

drug. If the intent is not to develop a drug or -- and in addition to the structure-function you also have reduction and risk of disease -- which are appropriate for foods and supplements -- if you have research end-points that are focused on that --

DR. YOUNG: Right.

MS. SANDERS: -- is there a way for CFSAN to oversee that research rather than CBER?

DR. YOUNG: We wouldn't oversee the research, but we could certainly partner with our product centers or our product offices within the agency to make sure we're all working off the same definition of what a proper structure- function claim is for product labels of dietary supplement. And I think we do do that, actually. We do communicate. When IND requests come in and someone's making a claim -- and it's sort of that gray area that I discussed -- between a disease claim or structure-function claim -- we do actually communicate and try to flesh out which side of the line it comes down on. And if it

comes down on the structure-function side, and your label is a dietary-supplement product, I don't believe you would require an IND. I mean, the devil's always in the details, but based on the high-level description --

MS. SANDERS: This could also be investigational, so it might not even be marketed as a product as yet. It's a question of developing the research on that product.

DR. YOUNG: Yes, it would have to be a dietary ingredient that's legal in the market, I believe.

MS. SANDERS: The things that default those, is to choose on the side of considering that to be drug research not food research.

MS. DEAL: I actually have to say that this is a complicated area and as Bob has just alluded to -- sometimes what on a high level might appear to be a dietary supplement drug -- it actually sometimes isn't when you get into the protocols. And I'd also like to say, we do have that guidance. And as you probably know, the

dietary-supplement in that guidance, some of the requirements for an IND have actually been stayed for certain studies of dietary supplements.

DR. YOUNG: I think it can be summed up with a statement -- there are no CFSAN regulated INDs.

SPEAKER: A question for Sheila. Is there definition for the term, "live microbiome-based product"?

MS. LESNICK: No.

SPEAKER: Is it different from LBP?

MS. LESNICK: No, no. And what we meant to do with that is just broaden the scope a little bit. The definition for live biotherapeutic products, really, back in 2012 didn't take into consideration that FMTs would be available. And so I think what we had hoped to do with this is to really show that this workshop encompasses more than just live biotherapeutic products and medicines.

SPEAKER: It makes a lot a sense that FMTs are now an LBP.

MS. LESNICK: Yes. So you could say that FMT does fit the definition of live biotherapeutic product. And that is actually discussed a lot internally. We did not have that in mind when we wrote the guidance. It's time to reconsider. We do recognize that there are aspects of that guidance that may be not be effective at this time, so. Such as the requirements to sell banks and things like that. But we're not unreasonable and we do recognize this.

MS. DUFF: I am Catherine Duff. Just would like to say, when you had the slide up about the original workshop in 2013.

MS. LESNICK: Mm-hmm.

MS. DUFF: That should not have read, "members of the public". It was just one of us, and it was me. And (laughter) everywhere I go, I'm the only member of the public talking about fecal transplant. And as the touchstone for literally hundreds of thousands of patients around the world that contact us every day, I think I

have 27,000 unread e- mails right now. Our concern has always been that as engineered microbiome-based products come through the pipeline, that that condition of live microbiome-based products -- put the phrase about being able to prove the potency, purity standardization of manufacturing -- will be used to exclude natural fecal transplant. Which I have to say as the voice of the public -- would be a huge wrong-doing and disservice to millions of people. And there would be a public outcry like you cannot imagine. So we hope that that is not the intent. And that that will not be the outcome. And of course, whenever these draft guidance's are published, we rally the troops and we comment vociferously. And I know that those comments are seen and heard, and I appreciate that. But we are watching closely, and we are very concerned.

MS. LESNICK: Yes. And thank you for that. And we're working hard. And really, it's a complicated and difficult place. I think where we

are now is, we're really trying to think of the best way forward here. And we do listen, and we are taking everything into consideration.

MS. DUFF: As the only member of the public in the NIH-funded microbiome transplantation working group, you know, we noted that there is also no way to ensure the consistency of other products -- biologics. Which is blood or bone grafts or tissue. And we all felt that natural stool-based microbiota transplantation falls more into that category than a traditional drug. So we just hope that you will keep the findings of that working group in mind. Thank you.

MS. LESNICK: We have a question from our overflow room. LB20, you can ask your question.

MS. DENUE: Yes, this is Deborah Denue from Bayer. I have a clarifying question, actually. The FDA has gone on record that the prevention of antibiotic-associated diarrhea is a disease claim. And there's several

dietary-supplement products on the market with substantiating evidence that make the claim to help prevent antibiotic-associated diarrhea. Can you help clarify whether or not FDA still considers that a disease claim or has something changed. Thank you.

DR. YOUNG: In the abstract -- I mean, I can't see the claim in front of me. As I mentioned, it's the totality of the circumstances, the label, and the labeling. It's never just one statement. Although a statement, you know, treats or prevents that antibiotic-associated diarrhea -- that would be a disease claim. There are folks in this room that have had this discussion with us. And I'm looking right at Amy right now, because she comes in a lot. We're open to conversation on this. Right now it is a disease claim. And based on our resources and priorities, we may enforce it as a disease claim. We could send you a letter or we could seize your property if it was drastic enough. I don't know that this rises to that level, because as you said, you've seen those

products on the market. It is a disease claim. It is a violation. Whether they have substantiation or proof for it or not, is right now relevant. But for the here and now, something that direct would be a disease claim.

MR. TERI: Barkoukis Teri, University of Nebraska Medical Center. When I listened individually to you, it was very clear, and I understand 100 percent. But when I put them together, I am totally confused (laughter). And my concern is why can't CFSAN have an IND. And the reason I'm asking this question is, we need more research and more studies, and more work done in this field -- not to regulate and not to reduce it. And you also mentioned that if somebody does something as -- they left it as a drug -- others cannot come back and sell it as a supplement. But how you can you stop them? They will say it may help. Or they will cite something. Or even the physicians can use it off-label, I mean all different things. You don't have to have regulated products to be prescribed different.

And how do you answer the question to CBER is, okay, I understand, I am fully with you. I want to develop probiotics as drugs. At the same time you cannot be always developed as drugs. Because if you do that, and even after it is approved, if you believe anything that Vince Young said, that's not the particular organism which is doing the final change in the physiology -- there are other things happening. How can you say that, okay, that's the one which did it? And that's why it's a drug.

DR. YOUNG: Well I appreciate your question because it gives me a chance to tighten up my language and provide some context. When I said there is no such things as a CFSAN regulated IND that's not to say that a product marketed as a dietary supplement couldn't also be studied as a drug. It could be used with the same exact ingredients and the same exact product, but it's looking for a disease end-point or surrogate end-point -- could be the subject of an IND and be studied as a drug. So I didn't mean to say that

you couldn't take a dietary supplement and never study it. For those purposes you can. It's just then you would be regulated through the IND mechanism. As far as the 201ff3b violation, where someone tries to market an ingredient -- that's a dietary ingredient or dietary supplement -- while there's an IND or NDA with significant clinical studies to support it -- that's something that we do take seriously. We do enforce. But because we're stuck with a largely post-marketing paradigm for enforcement of dietary supplements, we don't get the opportunity to enforce them until we find out for some reason. And that maybe somebody who has a proprietary interest in that ingredient letting us know, we might get involved because there's a signal that it's hurting someone. We don't actively look for those types of violations. But when we become aware of it, we do enforce those.

MS. SIROVSKI: Thank you. Boriana Sirovsky, Johnson and Johnson. What is FDAs stance on prebiotics? And what would be the best

reference where we could find this?

DR. YOUNG: Okay. I'm glad I came (laughter). So prebiotics -- as far as I know -- now, we don't really have a hard and fast definition even for probiotic. But a prebiotic -- not that we have a hard and fast definition for that -- but I believe it's something that supports probiotics. Supports the environment to allow probiotics to develop. If they're a dietary ingredient -- if they're an old dietary ingredient or new dietary ingredient for which a notification is required, and one is made -- they could legally be on the market. It would just have to follow the paradigm -- is it a dietary ingredient? Is it not meant to supplement a meal? Is it not derived from tobacco? Is there not a 201ff3b exclusion from the definition of a dietary supplement? It could legally be on the market as a dietary supplement if it did the right things.

MS. SIROVSKI: Yeah, just simply being incorporated in a product as an ingredient and part of it has a different purpose -- then what

would you expect?

DR. YOUNG: Well it would have to be a legal ingredient. It would have to be an ingredient that's on the market legally as either a conventional food, a food additive, or a dietary ingredient, or a dietary supplement. If it's in a dietary supplement, it would have to be on the proper part of the label. If it's not there for a technical effect it would have to be listed as an ingredient. If it's listed as an ingredient it would have to be a legal ingredient -- which means it would have to fit the definition of 201ff. I don't know if that made any sense. I'm a little sorry (laughter).

MS. SIROVSKI: Where can we find this on the FDA website?

DR. YOUNG: How about if we chat?

MS. SIROVSKI: Is anything -- in public -- okay.

DR. YOUNG: Okay.

MS. SIROVSKI: Thank you.

MS. DEAL: Looking at the guidance --

actually Mary Ellen and -- if a clinical investigation of a dietary supplement is intended only to evaluate the dietary supplement construction and function, an IND is not required. And there is a stay on the studies to support a health claim. And with that, I think we should break for a quick break. And be back by 11:00.

(Recess)

DR. MCCUNE: Hello. If everyone would mind sitting down, and we're a little behind. I think it's just --

SPEAKER: Forward?

DR. MCCUNE: Forward, yup.

SPEAKER: Mm-hmm.

DR. MCCUNE: All right. Thank you very much. I appreciate everybody coming back in, a little bit of a brief break, and I'm not going to hold us up. Just so you all know who I am, I'm Suzy McCune. I'm the Director of the Office of Pediatric Therapeutics, in the Office of the Commissioner at the FTA, and the folks kindly invited me to be part of this conversation today

because I'm a pediatrician and a neonatologist, and particularly interested in this area.

So, just to give you an overview of what we're going to do now, now, we have session two, part one and part two. So, the -- session two is entitled "Safety and Effectiveness of Live Microbiome-Based Products Used to Prevent, Treat, or Cure Diseases in Humans". Part one, which is what I am moderating, will be before lunch; part two, which Paul Carlson will be moderating this afternoon, and then we'll have all of our speakers come together, for both part one and part two, to have a panel discussion, and I will say that, after all of our three speakers this morning, we'll hold questions and then the three speakers will be able to answer questions, clarifying questions, before lunch.

So, with that, I'd like to introduce our first speaker, who is Dr. Josef Neu, who is Professor of Pediatrics and Director of the Neonatology Fellowship Training Program in the Department of Pediatrics at the University of

Florida College of Medicine. Dr. Neu's going to talk to us, today, about the use of commercially available products to prevent Necro.

DR. NEU: Thank you, and good morning. Here's my disclosure slide, and, over the next 15 minutes, I'm going to quickly cover historical perspectives and difficulty defining Necrotizing Enterocolitis. This is a big conundrum that, I think, we're just beginning to recognize, more and more, that we do not even have a good definition for this particular disease. Then, I'll talk a little bit about the path physiology of the most classic form of Necrotizing Enterocolitis, and then get into probiotics in Necrotizing Enterocolitis.

Well, let's begin. This is a typical neonatal patient, cared for in the neonatal intensive care unit, and these babies, now, over the last 50 or so years, we've caring for more and more of these babies. At one point in time, when I first started my residency program, we would take babies who were 26-27 weeks gestation, and

put them at the side of the neonatal intensive care unit and allow them to die.

Now, we are taking 22-23 weekers and being very aggressive in trying to save these babies, and, along with this, we're starting to see, more and more, this particular disease process that we call Necrotizing Enterocolitis, and here's a picture of a baby with this problem. This is not a typical inflammatory bowel disease. This is very different than what we see, typically. This is a disease that, once it affects the baby, within 24 hours, that baby can be dead, and, so, this is a problem that is very difficult to treat, and I think we need to aim at prevention of this particular disease.

So, over the last 50 or so years, since we've been starting to really work on these, saving these very small preterm babies, we really haven't made very much progress in this disease, and there's several reasons for this. One is that we've been lumping several disease processes into or underneath the umbrella of quote "Necrotizing

Enterocolitis", and I'll talk about this very briefly.

We have some animal models. For example, there's this rodent model that you asphyxiate and, as babies, and you put them into a refrigerator, and you treat them with antibiotics, and they develop some necrosis of the bowel, and that is called Necrotizing Enterocolitis. That is not the same disease that we see in preterm babies. There are over 100 published papers using that particular model, and then there's been a narrow focus on individual inflammatory pathways, or oxidative pathways, rather than whole systems approaches for this disease, and, so, I think we need to consider looking at whole systems, rather than just individual pathway components.

In the late 1970s, a surgeon by the name of Martin Bell developed these criteria called the Staging Criteria for Necrotizing Enterocolitis, Stages One, Two, and Three. We are beginning to recognize that, Stage One, if you take some of the babies that we care for, today, that are born less

than 750 grams, about 70-80 percent of those babies would -- could be diagnosed as having Stage One Necrotizing Enterocolitis.

Stage Two relies on radiographic criteria, and sometimes we make mistakes with those radiographic criteria. Stool, in the bowel, actually can look like Pneumatosis Intestinalis, which is one of the major criteria that we use for diagnosing that disease.

Stage Three relies on free air in the peritoneal cavity. Well, we have another disease entity called Spontaneous Intestinal Perforation, which occurs fairly early in very preterm babies, and some of our surgeons don't operate on those babies, and this is not Necrotizing Enterocolitis, but these babies get recorded as having Necrotizing Enterocolitis. So, the criteria that we are using for this disease are not very good. We don't have a very good definition.

So, we have, in the middle, here, this circle, intestinal injury that we are calling Necrotizing Enterocolitis, but we can have some

babies who have cardiac problems; for example, Hypoplastic Left Ventricle, or Interrupted Aortic Arch. Those babies don't get enough blood to their gastrointestinal tract, and they develop Necrosis of the Intestine. They get charted as having Necrotizing Enterocolitis, but that's a misnomer. They have Ischemic Bowel Disease, but not true Necrotizing Enterocolitis. Then, we have these spontaneous intestinal perforations. Then, we also have some diseases that are associated, more, with what we are feeding the babies.

So, really, this is more than one disease, and we are struggling with really trying to define a classic form of this disease process. Now, we do think that microbes are associated with this disease, and our group, at the University of Florida, was among the first to see differences in the microbiota in stool samples of preterm babies, prior to the development of the disease, and what we were able to do, working with Dr. Mohan Pammi at Baylor University, we were able to take sequences from several different neonatal

intensive care units that did the same types of studies.

So, we had stool samples from several different neonatal intensive care units that looked at Necrotizing Enterocolitis, versus control babies, and we were able to find, as we see on this particular slide, here, differences in the microbiota, prior to the development of the disease. So, here, we have control babies. Each one of these colors represents a different phylum of bacteria, and, in the controls, you don't see a lot of differences, but, in the babies who subsequently develop Necrotizing Enterocolitis, over time, we see an increase in these light blue, which are the proteobacteria, and a decrease in the firmicutes, okay, also a major phylum of bacteria. We also saw that there were very few Bacteroidetes in the Necrotizing Enterocolitis babies, but, again, these are phyla. These are studies that were done at the phylum level, but they do suggest a difference, prior to the development of the disease in the microbiota.

I don't have much time to talk about any of the other agents, but I do want to talk about probiotics, okay, and the question, here, is are we there yet, and I think there's a lot of debate, right now, a very heated debate, about the use of probiotics in preterm babies, and, in fact, I've seen several review articles that say, "The only disease entity where we have definitely proven that we can prevent a disease is in Necrotizing Enterocolitis, using probiotics." Okay? This is in review articles, and, so, there's this belief out there that we're there, with the use of probiotics. Let's talk about this a little bit, and where this story came from.

In 2010, a meta-analysis came out, in Pediatrics, looking at 11 different centers where they used 10 different probiotic preparations, and, here, we see that, in terms of prevention of Necrotizing Enterocolitis, favored treatment. In fact, death was lower in those babies who received the probiotics. Okay, that's probiotics. So, in this meta- analysis, 11 studies were evaluated.

Ten different probiotic preparations were used. Ten different preparations were used. That's like saying, "I'm going to prevent ear infections using Chloramphenicol, Amoxicillin, you know, Clindamycin. Which one?" That's a service similar analogy, okay?

They found that risk for Nec and death was significantly lower in the probiotic group. Sepsis did not differ, and, in quote, in that paper, "The overall instant evidence indicate that additional placebo control trials are unnecessary if a suitable probiotic product is available." If so, you don't see this very often after a meta-analysis. You usually see, "More studies are needed." Okay? Here, done. Okay, it's all over with, and there was a commentary along with this. Think, "Is it ethical to not use probiotics in preterm infants?"

So, the Journal of Pediatrics, the editors asked me to look at this very closely, and, so, I came out with this commentary in the Journal of Pediatrics, "Routine Probiotics for

Preterm Infants: Let's Be Careful." and I outlined some of the reasons why we do need to be careful and move slowly in this area, and I'm going to go through some of these rationales as we go on.

First of all, I want to start with systematic reviews and these meta-analyses. If you put garbage in, you'll get garbage out. Okay, this is one of the problems in many meta-analyses, after a few years. About 50 percent are proven to be not very good, untrue, and big mistakes is pooling data across trials as if they belong to a single large trial, okay, and, over the years, just about every single year since 2010, there's been another meta-analysis, or at least one meta-analysis, including a Cochrane Review. This is the Bible for Neonatologists, that, you know, the Cochrane Review says that we should be using a certain agent, that we should go ahead and use it. Well, the Cochrane Review recommended that we are -- should be using probiotics, but which one? I mean, there's hundreds of them out there.

So, here's a study that came from

Europe, and they looked at one particular probiotic, and they did this with a couple of other probiotic preparations, and they found no real difference if they just looked at one probiotic preparation by itself, rather than putting them all together. One of the biggest studies, in that first meta-analysis that I showed you, came out of Taiwan, and one point that was not very well discussed in that paper is seen here. See the red arrow pointing to Sepsis? The study patients were those that received the probiotic. The control patients were those that were in the control group, not receiving probiotics. So, we had 12 babies in that study who developed Sepsis, and one baby, in the control group, that did not develop Sepsis. Okay, so, large association with the development of Sepsis in these really small babies, and if you look closely at the meta-analyses, babies less than 1,000 grams were not benefited by the use of probiotics. They were all babies that were greater than 1,000 grams. So, more than two

pounds, it seemed to have to have some benefit. Less than 1,000, less than two pounds, no real benefit.

There was another fairly big study, in Australia, which was not powered to look at Necrotizing Enterocolitis. It was powered to look at Sepsis, and they've had 1,099 very low birth weight infants, and they've found no difference in Sepsis, if -- or all caused mortality, but on secondary analysis, looking back, they saw that there was a difference in Necrotizing Enterocolitis. Okay, Nec went from 4.4 to 2.0 percent on this secondary analysis, with a P-value of.03. The number it needed to treat was 43, with a 95 percent (inaudible) 23 to 233. There was no effect on -- in babies less than 1,000 grams birth weight.

Another study, and this is the largest study and the only study done, thus far, that I'm aware of, this was done in U.K. by Dr. Costello and colleagues. It was a double blinded, randomized, prospectus study, adequately powered

to look at Necrotizing Enterocolitis, using a (inaudible) probiotic, and it studied babies at 23 to 31 weeks, gestational age, and they found no difference in Nec. They had onset Sepsis, or death.

So, the question that was raised this morning, "Is this a food supplement or drug?" It depends. Well, maybe it doesn't depend, after we -- what we heard this morning, if we have a medical claim, prevention of Necrotizing Enterocolitis, usually, should be considered a drug. Drugs that are sold by prescription are subjected to rigorous testing. Foods can be sold by anyone, and not subjected to rigorous standards, for the most -- here is one study, and this is one of several, in a case report that shows certain bacterial species that caused some Bacteremia in babies receiving this particular probiotic. We see several of these in the literature.

A few years ago, at Yale University, a preterm baby died. It was taken to the autopsy

suite, found to have Mucormycosis. The Mucormycosis was traced back to the product. This was -- was this a product that was tainted? Is this a product that did not -- was not well controlled, in terms of its development? This is what we are trying to avoid, and this is why I'm saying we need to be careful.

In the United States, about 15 percent of neonatal intensive care units are already using probiotics, but the types of probiotics that are being used tend to be those happen to be available in the hospital. The most commonly used is Lactobacillus Rhamnosus. Lactobacillus Rhamnosus, the studies that have been done have not shown -- been shown to decrease Necrotizing Enterocolitis, but, here, we see the states, and we have no real evidence for safety or efficacy in some of the probiotic preparations that are being used, right now, in the United States.

There's also no current standards for quality control of this reconstituted product, and good manufacturing processes or practices for the

use of probiotics, as drugs are not available. The quality of some of the products are questionable. People have looked at the probiotics that are actually out there, and some of them are not really what is being sold, in terms of the -- that the product that they say that -- that this particular strain, this particular genus and species, is in a sample. They are finding different genus's and species, using PCR technologies.

We have to be careful. This is a study that was done by a group at Emory. Ravi Patel is also here, and this is an interesting study. Neonatologists are sick of Necrotizing Enterocolitis. We hate this disease, okay? This is a disease that kills babies very quickly. Five to seven percent of these babies are -- babies from 500 to 1,500 grams are affected by this disease, and, when these babies develop the disease, it's very hard to treat, as I mentioned before. If it goes onto surgery, 20 to 30 percent of those babies die of Necrotizing Enterocolitis.

If they survive, five years of age, and if they have a short gut, it takes what -- it costs five million dollars to care for that baby with that short gut. This is not a trivial disease, and these babies who have Necrotizing Enterocolitis also have neurodevelopmental delays. So, this is a terrible disease, and we are looking for something that will prevent this disease, but the problem is that we are, sometimes, maybe a little bit, too aggressive in moving forward.

This is a interesting study that, if you look at the Necrotizing Enterocolitis, prior to implementation of probiotic, there would be Necrotizing Enterocolitis, and, after implementation, we see an increase in Necrotizing Enterocolitis. A study in Europe, and I'm just going to show you the title, here. This just came out very recently. Increased incidence of Necrotizing Enterocolitis associated with routine administration (inaudible) probiotic in extremely preterm infants. Again, this was not a prospective, randomized trial. This was a

retrospective, observational type of a study, as was the study at Emory, which is limiting, but this is something that should be (inaudible)

So, in summary, Nec pathogenesis is multifactorial. Even if we invoke a classic form of Necrotizing Enterocolitis, we need to have better definitions, going forward, in our future studies. Treatment of Nec, once it's developed, is extremely difficult. We need to prevent. Intestinal microbial environment, along with developmental aspects of the GI tract, are key in understanding the pathogenesis of Nec. We need more studies. We need to have better systems, enteroids, animal models, to evaluate mechanisms that fulfill criteria for causality, derived from strong associations found in humans, and, lastly, once we have a clear understanding of the causes of the different forms of Nec, we will be best able to target preventative strategies. Reminded again, let's be careful.

DR. MCCUNE: Thank you so much, Dr. Neu. Our next speaker is Dr. Daniel Merenstein, who is

a Professor of Family Medicine at Georgetown University, where he also directs the Family Medicine Research. He also is Secondary Appointment in the Undergraduate Department of Human Science in the School of Nursing and Health Studies, and, today, he's going to talk to us about the evidences in -- for probiotics to prevent antibiotic associated diarrhea, what is holding up evidence- based use in the United States, and I just will say that we're shifting to the diarrhea topic for the next two talks. Dr. Merenstein?

DR. MERENSTEIN: Thank you very much. I really appreciate this opportunity. I'm excited that so many people are interested in this. I'm going to be speaking on -- about what I study, antibiotic associated diarrhea, or, as I refer to it as, AAD, but I was also asked to speak about why it hasn't taken off in evidence (inaudible) in the United States, and I'm going to give some opinions about that. In my conflicts, I won't be speaking about any of these today.

I put this up: "In God We Trust, and All Others Must Bring Data." because I am going to give some opinions today, and you might not agree with my opinion, and that's fine. That's reasonable, and we should discuss it later, at the panel, or at lunch, or whenever, but I am going to present the data, and, just because you disagree with my opinions, I hope you don't ignore the data because the data are really robust, and really tell a story.

So, if you remember Dr. Young's graph, it just kept going up, the microbiome research, but this is probiotic research. It has been going down, down, down. I assume this will go up a little bit because the year is not over, but it's not going to get to up, up anywhere near there. I'm going to talk about why I think that's happening, and why it's, obviously, a serious problem.

So, I'm going to discuss the evidence behind AAD for probiotic use, and, just to make it a little more robust, I'm going to show you what

other people say about it, so you don't think it's just my opinion, and then I'm going to give you some opinions of why I think we're having a hard time implementing this in the United States.

So, this is the Cochrane Pediatric AAD, and I agree with Dr. Neu that evidence in is only as -- evidence out's only as good the evidence in, but, really, in medicine, this is considered the highest level of evidence. There's over 23 studies, almost 4,000 patients, 11 of which use a single strain. In AAD group -- in the probiotic group -- excuse me -- it was eight percent AAD, versus 19 percent in the control group. If you work your way down, the relative risk reduction's 58 percent. I don't have time, today, to talk about other interventions, but, next time you read an article, think about 58 percent, and where that falls in. The absolute risk reduction's 11 percent. The number needed to treat is nine, and, again, when you read articles, and you see number needed to treat, think about when you see such a low number needed to treat.

So, the initial thing, and this is what Dr. Neu already said, is, okay, what product do I use? Well, there's multiple products to use, but let's just take one product. This is a meta-analysis of one single strain: 12 RCT, almost 1,500 participants, almost the same exact data, 22 percent versus 12 percent, relative risk, 49, number needed to treat, nine. Adverse events, as we've seen in many people, also have seen and shown, and the RAND studies show this, too, are nearly the same in experimental, in the control groups. In fact, a lot of RCTs show they're lower in the experimental group than the control group.

I know we're going to talk about C.diff a little later, but I think there's no way to talk about AAD without talking about C.diff because C.diff is, really, what we're mainly worried about when we're talking about AAD. This is another Cochrane Review: 8,600 participants, 8,672, 27 studies, and I also want to go back. Not all these studies are perfect. I don't mean to say every study was low risk or biased.

They have some problems with some of the studies. Incidence in probiotic group was 1.5, control group was 4 percent, relative risk, 62 percent, absolute, 2.5, number needed to treat, 40. Interestingly, when they looked at this, it really, mainly, is a benefit when your infection rate is greater than five percent. So, if you know your hospital rate's greater than five percent, the data is even -- is much more impressive.

Physicians used a medical letter. Pharmacists used a pharmacist's letter. This is well-respected, evidence-based review. They conclude treating 12 patients with the probiotic prevents one case of AAD. Treating 29 prevents C.diff. They go on to say probiotics reduce the duration of acute diarrhea in infants and children by about one day, and for those who might say, "It's just one day." that's the exact amount of -- that's the exact treatment we get when we give influenza drugs, when we give antibiotics for Strep, we give antibiotics for Otitis Media. We'd

be happy with one day. Usually, it's actually less than one day.

Two years ago, JAMA had three articles about probiotics. The first one was just a survey. It showed 156 increase. People have already talked about that. I think this editorial, though, was even more powerful. They said, "Not all supplements, of course, lack evidence of efficacy. Many supplements, including vitamins, minerals, and probiotics, are important components of modern healthcare." I don't think we would have seen that 10-15 years ago, but it's well-accepted in the mainstream medical journals, the evidence of probiotic, and they concluded with a third article on that, where they talked, again, about the evidence of the AAD, which I've already shown you, three articles, in JAMA, talking about probiotic usage.

So, how are people using probiotics? This is one survey. It found 87 percent of academic hospital formularies carry a probiotic. If you're in this area now, there's three major

hospital systems. Hopkins, I'm going to talk about Hopkins in a few slides. There's MedStar and Inova. MedStar and Inova -- so, there's 10 million people in this area. Not one, if they're hospitalized, has a chance to have a probiotic that has efficacy. There's -- I'm not going to call out products, but there's products on these, and just like Dr. Neu just showed, that don't have efficacy, that hospitals used, mainly, for cost reasons, and it's embarrassing, and if you get hospitalized now, even though I've shown you the data for AAD, in prevention C.diff, you can -- in the local area, you will not get a probiotic, unless you bring it to yourself, that will prevent AAD, and there's a good chance they're going to put you on antibiotic, if you're hospitalized.

The CDC did a review in 145 hospitals, with about two million discharges. They found 96 percent of hospital used a probiotic. You are nine times more likely to get antimicrobial, and 20 times more likely to be diagnosed with C.diff if you are on a probiotic. They concluded, in a

sample of U.S. hospitals, a sizeable and growing number of inpatients received probiotics as part of their care, despite inadequate evidence to support their use in this population. I would just add an editorial. Just because you don't know the evidence, you shouldn't conclude with inadequate evidence. The evidence was there already. The evidence was clear, from Cochrane Reviews, the highest level evidence we have, that the number needed to treat is nine or 40, for AAD, a nine, and for C.diff, 40.

I'm going to talk a little bit about FMT data. I am a big proponent of FMT. I have a son with Ulcerative Colitis. I think the FMT data is very promising. I think it not just teaches us how we can do it with FMT, but we can do it with drugs, but I'm going to present the data. So, before you attack me with FMT, look at the data.

In 2016, there was a review, about 7,500 original articles, not studies, articles, and mainly reviews. This is well-accepted. This review found about 28 percent. You'll see about

30 percent AEs, mostly mild, but some serious infections, and, again, as someone already pointed out, we have no idea about the long-term implications. These are all I could find, and I wrote every author and asked them if there's other studies. There's probably ones in other languages, but there are five RCTs. This is what the FDA changed their discretion, IND, about, five RCTs, with FMT. Two are done with enema, two with colonoscopy, one with nasal duodenal tube, 187 patients. Of these five, two were blinded, two blinded studies, but one, the best highest level, that was placebo controlled blinded, found efficacy of 61 percent, versus placebo of 45 percent. So, that's the data. Look at the data.

Now, IND is saying, which we can't even say it is, if it's evidence-based review or not, looked at this data, just this year. Nace concluded there's insufficient data, at this time, to recommend administration of probiotics for primary prevention of CDI; 27 clinical trials, 8,600 participants. They said, for Fecal

Microbiota Transplant, it is recommended for patients with multiple recurrence of CDI, who have failed appropriate antibiotic treatments, and, again, one is prevention, one is treatment, but just think about it, if we flip those numbers. If we flip those numbers, I would never have gotten a grant for AAD. I would never have even thought of applying. If you told me you have a product, St. John's wort, that has five clinical trials, two of which are blinded, and I wanted to apply for a grant, my Chair would be like, "Can you find a better product to apply for a grant because you're not going to get funded."

So, part of the reason I think it hasn't taken off is lack of understanding evidence, maybe bias, but there's no question, and it's unfortunate. I appreciate Seiber inviting me, and they asked me to speak about this, that Seiber has part of the blame. That was a horrible death that Dr. Neu talked about, horrible death, and this is what Seiber did with it after that. They said, "The FDA encourages healthcare providers who use

dietary supplements containing live bacteria's yeast, probiotics, to submit an IND for FDA's review.

FDA's primary goal, in reviewing IND, are to ensure the safety and rights of subjects, and help ensure the quality of the scientific study of drugs is adequate to permit an evaluation of the drug's effectiveness and safety. This is what they sent out. This is what happened. A couple -- there's headlines that you can -- tons of headlines, but one on Forbes, "Infant Death Triggers FDA Health Providers Warning of Probiotic Risks," but this is what happened at Hopkins, one of our top institutions in the country. They outlawed all probiotics. This is what they wrote, "Due to the documented risk associated probiotic use in the hospital, probiotics are not available for use at any Johns Hopkins Health Service Hospital, not purchased, stored, administered, or dispensed." I'm going to read that again, "Not purchased, stored, administered, or dispensed. The use-ation of patients' own supply of

probiotics, while in the hospital, could put patients and healthcare workers at risk for possible infection, and is, therefore, prohibited."

I did my fellowship at Hopkins. They let me give Benadryl. No study was ever shown at our -- it was one study ever, in 1976, to infants, six months, or six months to nine months, to see if it helped them sleep through the night, Benadryl. We know, as physicians, there is major side effects of Benadryl. That was fine. The IRB approved it. This is what they wrote about probiotics, "You are not allowed to bring in your own probiotic, into Johns Hopkins Hospital, because of the danger of other people."

So, clearly, we know, because there's bright people at Hopkins, this was written by the lawyers, and the lawyers looked, and they said what FDA wrote in the letter, "FDA encourages healthcare providers use dietary supplements to submit an IND." It's pretty clear, actually. You can't blame them. If you're going to use a

probiotic, you need to submit IND, but doctors can't do that. They're not going to do that for Nec. They're not going to do that for AAD. They're not going to do that for (inaudible) They're not going to submit an IND every time they use a probiotic.

Just a few months ago, we had a horrible transfusion problem, with platelets causing infections with ACB. This is what happened: the Centers for Disease Control mentioned working with two states, investigated the potential ACB complex transmission, through platelets transfusion, has issued a nationwide call for cases. Please report any patients who develop or developed Sepsis, due to ACB Complex within 24 hours of receiving platelets. Imagine if the Seiber did that. This was a horrible death, and it was an infection with a contaminated product that should have been called (inaudible) it was, but instead of saying, "We should figure out the products are safe, or if you see anything in your hospital -" They sent a letter. It says, "You need to give INDs when you

give probiotics." That's greatly impacted probiotic research in the U.S.

The second, and the final, thing that's greatly impacted is the definition, and you heard it today, and I think you heard it really clearly, with the two speakers going back and forth in the confusion. So, I study the yogurt. It is a yogurt, and I brought enough for everyone to taste, to prove it's a yogurt. In fact, this -- I study the same exact strain that's in every one of these products, including infant formula; same exact strain, at the same exact dose, or they have a higher dose than I have. So, I have a lower dose of the same strain. I'm on my fourth IND. The first two, you could argue, were reasonable because it's antibiotic associated diarrhea, and, we already heard today, that's considered disease by the FDA.

Now, I already had 15,000 days because I had done a prevention of preschool absences study with the same yogurt, here, on -- so, they could have said, "You can go to a phase three trial."

because that's what NIH funded me to do, but they didn't. They said, "You do a phase one." So, I did a phase one safety, in adults, then in kids. Now, I'm doing a phase two, but even more surprising is, about five months ago, I got funded to do a mechanism study of AAD. My outcome is short chain fatty acid changes. That's it, short chain fatty acid changes. My secondary outcome is microbiome changes. There's no question, and it was already explained this morning, that's a structure functioning claim. There's no debate. There's no clinical outcomes. Healthy people -- not hiding anything. I'll send you the protocol. Healthy people, 60 people, the FDA required an IND for that, and this is slowing down research in the United States, and I'll show you that, and, just quickly, I think I have time. This doesn't have to be. You can -- well, there's lawyers, here, who can tell you -- explain it, too, but I also applied to do a chamomile tea study, to see if it'd help kids sleep through the night. I do lots of crazy studies like that.

So, I wrote to Seiber because my IRB said, "You know, it's never been studied. You know, you need to ask them if you need an IND." and they said, "What we need is your CV, to make sure you're a legit person, and your protocol." Two days later, they sent back, and they said, "You can go ahead with your procedure." That's what they could do. It took the -- for the structure function claim, it took the FDA about three to four months to -- for me to go ahead with my study, and, because of that, I have to wait till the next budget season because we missed the budget season this year. So, those are all the products that have the same exact -- as the one you can taste, if you want.

Okay, FMT versus probiotics. Most hospitals, not all, because of Hopkins, and I'm afraid some are going to follow because they're going to follow a place like Hopkins, are using it, but let's talk about it, what we always say about why you don't use data. I'm thinking Dr. Neu, actually, said a lot of this. Why don't we

use it? We don't know the strain data yet. We do know the strain data.

There's multiple products that are well-proven for AAD. I showed you one. There's other ones. We don't know how to give. FMT, in the five studies we had, was given three different ways. What's the dose? Well, that -- you know, tell me the dose of FMT. We know the dose of that, probiotics. What are the adverse events? The adverse events are minimal. There are horrible cases of contamination, and there are some evidence of some Sepsis, very infrequently, but it's unbelievably low, unbelievably low; and what's the long- term data? Well, we don't have long-term data, really, for most of the drugs I use in clinical practice. It's not an excuse, but we just don't, and we, clearly, don't have it for FMT. We have, again, better for probiotics than we do for FMT, but Seiber, rather quickly -- I was impressed. I didn't realize it was as quick as Sheila showed; within two months, changed their role, and let people go ahead with that. Why did

they let this happen in two months? It's an interesting question.

These are all studies, throughout the world. So, if you see, in the U.S., and this is what happens in the U.S., about 40 percent of clinical trials are done in the U.S. We can argue about what I used. Again, I thought these were reasonable comparisons. Omega-3 is about 37 percent, vitamin D, about the same. Probiotic trials are about 17. From my anecdotal evidence of people calling me and asking me how to do trials, I think that's on its way down. So, the U.S. is falling behind in probiotic trials. In the age of the microbiome, the U.S. is not doing probiotic trials.

So, we need more AAD studies. I'm a little biased. That's what I do for a living, but I think we do need more studies, okay? We need to know the time, the dose, how long, when you take -- we need to do that, but physicians and patients are using these, I would argue, often, incorrectly. FDA and, specifically, Seiber's

action, via the letter, and lack of waiving INDs has slowed research down.

I think, to conclude, Seiber needs to remember their mission. It's responsible for advancing the public health, by helping to speed innovations, and I think they've done the opposite of probiotics. Thank you for your time.

DR. MCCUNE: Thank you, Dr. Merenstein. We'll do questions for the group after Dr. Freedman's talk. So, Dr. Stephen Freedman is a member of the Sections of Pediatric Emergency Medicine and Gastroenterology at the Alberta Children's Hospital, in Calgary, Alberta. In 2016, he assumed the role of Chair of Pediatric Emergency Research Canada and was appointed the Alberta Children's Hospital Foundation Professor in Child Health and Wellness. Today, Dr. Freedman is going to talk to us about use of probiotics in Acute Pediatric Gastroenteritis, two large North American clinical trials. Dr. Freedman?

DR. FREEDMAN: Thank you very much, and it's a pleasure to be here, today, and I think

this is a nice segue from the two earlier discussions, and I do have several disclosures.

So, I do actually hold an IND, or, actually, I'm not the holder. It's actually -- David Schnadower is the holder of an IND, related to funding from NICHD for the conduct of one of the trials that was conducted in the U.S., and also, similarly, helped Canada. Approval was obtained by NHPD for the CI Chart funded trial, in Canada. The study -- drug and placebo were provided by the manufacturers of the LGG, as well as Lallemand Solutions for bay -- lactobacillus rhamnosus helveticus.

So, I'm going to segue from antibiotic associated diarrhea to acute infectious gastroenteritis, which is one of the most common diseases of childhood. It is the second most common cause of death, globally, in children under five years of age. It is a -- different than Nec, where children in the U.S. don't usually die from this, but it's the global burden of it, in kids, and on the economy, and on healthcare providers,

and on schools; 1.7 million ED visits per year, in the United States, nearly 100,000 hospitalizations, and there are few options to modify the disease course. So, probiotics are being touted and advertised. That's just actually changing the disease course in kids. We do, currently, have other options for symptomatic short-term relief and treatment of dehydration, should it occur.

So, I'm going to -- we've been hearing about Cochrane Reviews and the pros and cons. So, the biggest Cochrane Review of this topic was done, and the latest was in 2010 by Allen et al, and, as you can see, there was a decreased duration of diarrhea. They concluded about 25 hours till the timing to the last diarrheal stool. Several challenges, though, that can be -- come up from this.

Number one, it's mostly inpatients, primarily in an era of rotavirus, which has been dramatically reduced, due to the introduction rotavirus vaccine in North America. Most of these

studies were single center, very small sample sizes, generally. Although, there were many studies, as you can see, but, unfortunately, this led to significant heterogeneity. So, there's 97 percent heterogeneity between studies in this Cochrane Review. They employed variant probiotics in varying doses.

Nonetheless, based on this data, several organizations issued strong recommendations, but they then go on to say, based on low quality evidence, that support the use of probiotics, and the most notable being ESPGHAN, which is a large European group. There was no position statement, really, on this. The last one, from the CBC, was in 2003, and didn't really address this issue very much.

So, this raised one question that two networks decided to try to answer. So, I'm the Chair of Pediatric Emergency Research Canada, on the right, and then we work closely with our sister network, PECARN, Pediatric Emergency Care Applied Research Network, in the U.S, to conduct

one question, across two networks, using two different probiotics. They shared a common hypothesis, however, that probiotic administration would result in a significantly lower proportionate of children with moderate to severe disease, within the subsequent 14 days, compared with placebo, and we didn't just look at -- and I'll come back to one isolated symptom.

We looked at the global burden of disease as our outcome. They were conducted as randomized, double blind, placebo-controlled trials. Eligible children were age three months to 48 months. They both, in both studies, had clinically died -- had been clinically diagnosed with an acute intestinal infectious process, defined as greater than equal to three episodes of diarrhea in a 24-hour period, which is the working definition for gastroenteritis, accepted by all organizations. We used a web-based random number generating software, randomize.net, employed random block sizes. We stratified by sites, and we used a one to one treatment allocation ratio.

Several differences between the studies, which I'll try to highlight as I go through, I decided to present them, kind of, in parallel because they are so similar, as opposed to going back and forth between the two. The U.S. study included 10 emergency departments, all pediatric centers. Kids were able to have symptoms up to seven days, so up to a full seven days of symptoms, and this was based on the only one real prior study in the U.S., which was conducted by Nixon, in -- out of Albert Einstein, which found, actually, that they did not see a difference in the group administered probiotics, but they did, maybe, see a trend amongst those who had a longer duration of symptoms of baseline. So, they focused on that group of kids. They studied LGG, a dose of one times 10 to the 10th CFU BID for five days, compared with a placebo, and then randomization was also stratified by the duration of diarrhea, given the importance of that, as a -- in a priority hypothesis.

In the Canadian study, we included six

emergency departments, focused on children with shorter duration of diarrhea because most of the other studies in the literature had shown greater benefit in shorter duration events, up to 72 hours, and we studied Lacidofil, which the combination of a lactobacillus rhamnosus and helveticus product, in four times 10 to the ninth CFU, twice daily, for five days. Both of these dosage ranges were what was supported by the existing literature. In Canada, they actually held an indication for that dose in the use of the product, and, in the U.S., it's a commonly recommended dose of LGG.

So, we excluded children who were at risk for invasive disease and infection. I didn't go into it, but there are -- actually are numerous case reports in the literature of individuals with central lines who developed Bacteremia, with the probiotic strain, particularly in ICU settings. So, we excluded all children indwelling vascular access lines, congenital heart disease, because of the risk of reports of Endocarditis,

immunodeficiency, immunosuppression, on a GI problem, such as IBD (recording cuts out) particular pancreatitis because of a large European study that showed increased mortality in that group, and then kids who may not have Gastroenteritis, so, Bilious Emesis, or Hematochezia, bloody diarrhea, so, not tradition, at least North American Viral Gastroenteritis.

The studies also had some specific peculiarities, kind of, at the pushing of some of the local Federal agencies. So, premature infants and those less than six months corrected age were excluded, those on supplemental probiotics, or an allergy to LGG, or the antibiotics that would be used to treat a Bacteremic episode. In Canada, we excluded those who had had recent or gastrointestinal surgery, preceding probiotics in the two weeks prior to enrollment, and then soy allergy because the soy-based culture medium was used to grow the probiotic.

We conducted follow-up surveys every 24 hours until symptoms had resolved for at least 24

hours, as well as day five and 14, post randomization, and then, actually, in the U.S., the FDA's urging we conduct a follow-up, for safety, at one, three, six, nine, and 12 months, following conclusion of this very short study. Stool specimens were also collected, and, actually, we used rectal swabs, and we can discuss that if people are interested, and we, actually, collected specimens on all individuals who were -- participated. We analyzed them for infectious agents, including 15 pathogens, in both sites, using a multianalyte pathogen panel, and then we used an in-house viral panel for five viruses in Canada, along with bacterial culture, and then we also did independent testing of the batches of the probiotics, in both studies, to ensure that they were delivering the CFU counts that we had intended to deliver.

A primary outcome was moderate to severe disease, defined by a modified Vesikari scale score greater than equal to nine, and I'll discuss that on the next slide, which ranges in score

severity from zero to 20. We secondarily looked at duration of diarrhea, duration of vomiting, future healthcare provider visits, as well as adverse events.

So, this measure is a composite score, and we chose to use a composite score, as opposed to individual measures, because what if you reduce the duration of diarrhea, but they actually have more diarrhea for two days, but they have it for two days instead of three. What's better? I don't actually know. I don't think caregivers really have an answer to that or is an easy one. So, this is a score that's actually emerged from the rotavirus vaccine files and been adapted for use in the outpatient setting. So, it actually looks at duration of diarrhea, duration of vomiting, maximal frequencies of diarrhea, maximal frequency of vomiting, fever, which is very concerning to caregivers when their child is ill, and then we also looked at interventions, so, healthcare provider interventions, either as an outpatient or in the emergency department, and

need for IV fluids or hospitalization.

Our sample sizes had 90 percent power to evaluate a 25 percent rate in the placebo group, aiming for a number needed to treat of 10, as you were hearing, but number needed us to treat, which would be based on a minimally clinically important difference of 10 percent. We conducted two-sided analyses with five percent significance, and adjusted for follow-up, for drop-ins and drop-outs, and many people who take probiotics over the counter, even though they're not randomized to it, and then we did interim analyses. So, we adjusted for that as well.

In the U.S., the calculated sample size had to be increased because, on one of our analyses of the probiotic product, it was found to have too low of a CFU content, lower than what we had intended to deliver. So, we worked with our DSM-V to increase our sample size, appropriately, to 971 participants in the end. The Canadian trial, there were no concerns in that regard, and ended up enrolling 886 participants.

Our analyses were in by intention to treat principles, multiple mutation, and we employed with logistical reaction stratified by sight, the secondary analyses looking at other covariates, and then we conducted subgroup analyses, looking for interaction.

I'm going to present the Canadian data first, after this slide, which essentially shows that the groups were similar in both studies, around -- just over about 16-17 months of age, the only difference being the duration of diarrhea, slightly higher in the U.S. cohorts. As you can see, over here, 57 hours, based on the eligibility criteria, and, hence, their baseline modified Vesikari scale score was slightly higher, 12, compared to 10 in the Canadian cohort.

So, in the Canadian study, as you can see here, if we look at all participants, the proportion who actually had the outcome of interest, the primary outcome in the probiotic group was 26.1 percent, but at the 24.7 percent in the placebo group, and we look at some of our A

priority identified subgroups, kids less than one year of age, exclusively breastfed, antibiotic usage in the preceding 14 days, or greater than 70 percent compliance. As you can see, there was no difference between groups.

Look at some of these secondary endpoints. These were particularly important because of the meta-analyses that had shown reduced duration of diarrhea. When we look at that, there's no difference in diarrhea duration, no difference in vomiting duration, no difference in follow-up healthcare visits. Traditionally, were no differences in adverse events between groups.

When we looked and dove a little bit deeper into this issue of duration of diarrhea, because that's been the greatest claim for our usage in acute infectious gastro, we looked at daily episodes of diarrhea, from our diaries, and, as you can see, they're actually essentially identical between groups. An incident rate ratio of 0.98. The only difference we did find was in

vomiting. The incident rate ratio was slightly higher, and that's -- was primarily due to a difference on the first day of treatment. However, the magnitude is actually relatively small, and probably the clinical significance of this is minimal, at 0.83, versus 0.55 episodes, on the first day, after randomization.

Now, we're going to move onto the U.S. side of the PECARN study, and the results are actually remarkably similar. The proportion, having a modified Vesikari score greater than equal to nine, which was our primary outcome of moderate to severe disease, 55 percent -- sorry -- 11.8 percent in the LGG group, compared to 12.6 percent in the placebo group. No -- the P value was 0.83. When they looked at mean episodes of diarrhea, per 24-hour period, or the mean episodes of vomiting, per 24-hour period, a very similar graph is displayed. There were no significant differences on any of the days or either -- on either of these parameters.

This is a busy figure. I'm want -- I'm

just going to try to highlight -- is what we looked at, here, to show you how we tried to stratify and look at different things. So, on the top five, the five column headers, are the different A priority stratifications, so age, less than one year, greater than one year, duration, less than 48 hours, greater than 48 hours, antibiotics versus no antibiotics in the preceding 14 days, and then some of the early analyses that we've done related to the etiologic agent. We looked at no pathogen identified, a bacteria pathogen identified, or a virus identified. It gets more complicated than that, and I'm not going to go into it too much right now, and then, on the left, here, are the seven different outcomes of interest, so moderate severe disease, repeat healthcare visits, health, cold, members becoming sick, time to last watery stool, time to last vomit episode, hours of working, this applies to parents because, actually, that's a huge impact on your GDP, and a big reason, and the economic reason for giving probiotics is lost work and

wages, and days of missed daycare. On all of these seven outcomes, across the four different subgroups defined in the columns, there were no significant differences between groups.

So, both of these studies are subject to several limitations. One is based on recall bias. So, there's no hardcore evidence. We don't have biologics that we've analyzed yet. We do have data, that we will be going into later on, but, basically, it's based on symptomatic recall of parents. We did contact them ever 24 hours, and, very robustly, I think, did the best we could to accurately report that.

We used composite outcome measures as our primary, which can be criticized because our composite -- however, I would argue that they're much more meaningful than individual outcome measures, but, when we broke it down by looking at all the individual symptoms in these outcome measure scores, none of them were significant, and we ultimately only studied two products, one dose of each, and that's all that we studied, and

that's really where I restrict my conclusions to, at this point in time, but, based on the data that we have presented and analyzed so far, in children presenting for -- to an emergency department with acute gastroenteritis, probiotic administration does not prevent development of moderate to severe disease within 14 days, and a huge thank you to David, Dr. David Schnadower, who really led the PECARN study, and then to all of our coordinators, site managers, program managers, our laboratory partners, and our funding agencies, so CHR, as well as the NIH, NICHD. Thank you.

DR. MCCUNE: Okay, we're standing between you and lunch, and we're going to go a couple of minutes over, into the lunchbreak, for questions, but I want to ask our three speakers to come up, and I'm going to open up the session for clarifying questions, recognizing that we are going to have a panel opportunity, this afternoon, to hear from all of them again. So, I -- if you want to -- are the microphones working at the table? Just push. They just -- you just need to

push down when you're talking, so.

DR. SANDERS: Mary Ellen Sanders, from ISAPP. Josef, thank you. That was a, I think, very nice talk, and I wanted just some clarification. You mentioned some case studies about Bacteremia and adverse effects from probiotic administration to premature infants. What is the overall number needed to harm, for probiotic administration?

DR. NEU: I could not tell you the answer to that. I don't know.

DR. SANDERS: Is it fair to say that, when you're considering an intervention, that number needed to treat, compared to the number needed to harm, is a relevant comparison, versus just, well, here's flaws in the particular data, and, therefore, because there's flaws, it's not perfect data. We're not going to act --

DR. NEU: Don't --

DR. SANDERS: -- without considering the number needed to harm.

DR. NEU: Yeah. I think that the --

that these harms are very likely, and this is, again, opinion, are probably highly, highly underrepresented because so many of these babies do have problems. They have -- they develop Sepsis. Much of the Sepsis that we see in our preterm babies is gut-related translocation of bacteria. So, I think that a lot of the problems that we see are actually underrepresented with the use of probiotics. Again, that's my opinion.

MR. LILLIS: So, Christian Lillis, from the Peggy Lillis Foundation. My mother died of a community acquired C.diff infection in April of 2010, and, so, listening to Dr. Merenstein, in particular, talk about this mishigas with the FDA and letters and such, I find that really troubling, and I would like to know what patients and caregivers can do, in this space, because I often feel like I -- Catherine Duff, my partner in crime, earlier, said that she was the only person at the last one of these, and I'm, I think, the other person who represents the public and patients, and, so, these events happen. They

happen in the Beltway. It's really -- I didn't even learn about this until, like, two weeks ago, so. How can we become more involved because I think that's the missing ingredient?

You have patients fighting over Cancer treatments. You have patients fighting for heart disease. When it comes to infectious disease, there's just no patients in these rooms, ever, and I don't buy the whole "I'm also a patient." Nonsense. So, leave that at the door, if you represent it, and just, you know, that's just crap. So, I would like to know more about the probiotic stuff. I mean, if we can prevent these diseases, I think it's very important, and it's something that I, personally, find very frustrating because we get asked about it all the time, and we know that there's evidence, but we don't know exactly where to direct somebody. So, if you have any ideas, I welcome them.

DR. FREEDMAN: (recording breaking up) the exact question, but, I mean, I'm a -- you know, as you can see, you had some high quality,

hopefully, evidence presented, and emerging, and I think the patients and the advocates need to advocate for independently funded studies, Federal funding to look at it. I mean, I think Dan was highlighting the lack of, you know, investigation into barriers to conducting probiotic research, and, so, if it's left in the ER, I, truthfully, think it shouldn't be, being led by industry, and setting their own outcomes, the own measures, et cetera. I think these need to be Federally funded studies, big, large, answering questions important to patients, caregivers, healthcare providers. To me, that's really where it needs to move. The problem in the -- I can comment more on the acute gastro world, is almost all the studies were funded by industry.

We know there's a lot of negative studies that never got published, and, so, I'm going to get all the industry people very upset with me right now, but that's okay. I'm running for lunch somewhere, but, I mean, I think, truthfully, we need large studies, several

thousand people, to answer some of these questions, rare outcomes, such as C.diff, and bad outcomes. We need very large studies to answer them. Numbers of 33 patients aren't going to tell you whether everybody with C.diff should get treated. Obviously, very sorry to hear about your loss, but, to me, I think that is where the advocacy needs come in.

DR. MERENSTEIN: I guess I would add -- I also -- sorry for your loss, and I, you know, this is -- in family medicine, there are often talks about how can we get people interested in these non -- we call them sexy diseases, and it's difficult, and I have a question, from your question to Dr. Neu. So, if I have a preemie, is it ethical not to mention that there's these things called probiotics, to them? You know, you talked about these, all these issues, and you talked -- you showed all the harms of the studies, but the Cochrane Review, and, if I'm not mistaken, I think it was started by neonatologists, is pretty high standard, and if you have a preemie,

and you don't offer probiotics, if you're a Hopkins or something, is it ethical not to even mention it to the family?

DR. NEU: So, tell me what probiotic am I going to mention to the family?

DR. MERENSTEIN: I'm not a neonatologist, but -- yeah.

DR. NEU: Which one probiotic has proven -- have we had that has been proven to be safe and effective against Necrotizing Enterocolitis?

DR. MERENSTEIN: So, your answer is it's not -- it's ethical not to mention that, what your answer is?

DR. NEU: Yes.

DR. MERENSTEIN: Okay.

DR. MCCUNE: So, I think we'll get into a little more discussion of some of these issues this afternoon. I did want to thank -- now, I'm missing where you went, but thank you so much for your comment, and I'm sorry for your loss, and I really do want to say that, from an FDA perspective, we are very interested in the patient

perspective. I would say that there are a couple of venues to be able to be involved in these issues.

One of them, I think, we heard this morning, about providing feedback to the guidance documents, I think is always welcome. I think there's another outlet, right now, are the patient-focused drug development meetings that are being arranged through the agency, and, certainly, something that can be talked about, especially externally-derived meetings, where FDA members come to listen about these issues from a patient-focused drug development perspective, and then the third one, that I know folks in the neonatology space are aware of, but consortia efforts, like the International Neonatal Consortium, where stakeholders from all of the various groups, including industry, academia, patient advocacy groups, as well as regulators, all come together to talk about these issues and how to do the best studies, moving forward in a pre-competitive space.

So, I think that there are opportunities out there. We really want to hear the patient voice. I wasn't supposed to inject myself here, but, sorry, I did.

DR. PANIGRAHI: Pinaki Panigrahi, and from University of Nebraska Medical Center. I just have a comment. I had asked -- I asked this question in the previous session. I think I had answered after I am hearing to -- three of you. My concern, now, is how much -- again, it is directed to Dr. Neu. Like, if we want to ask number needed to harm, we don't know that. Yes, we don't -- again, I agree, fully, with you that -- do I have a probiotical use for Nec? Maybe, we don't know because all of them are using different ones. So, how do we get to that -- get to a point where we can really answer these questions, and, probably 20 years ago, there was a meeting, similar meeting, in college park, and it was told that companies are self-regulating themselves. They want to put out good products. If the one that, you cited one case, that was an accident. I

mean, that was a bad stuff, bad -- it was manufactured in a sloppy way, so. I mean, that can happen anywhere, anytime, but to take that as a reason not to do probiotic research will not be fair, and only as long we do more and more research, we will find out more and more adverse events, and if they're out there, and if we don't do it, then we'll never learn about it.

DR. NEU: So, I can't agree with you more, that we have to do the right kind of research, and we have to have, you know, safety. The problem is with these probiotic agents that, if we treat all preterm babies, and this is what we are talking about, all preterm babies with -- between 500 to 1,500 grams, with a certain agent, and it is a tainted agent, we're going to have a major problem on our hands, not just one baby. We're going to have hundreds of babies that die at one time, and, so, I think we need to be very careful about the product that is available.

DR. FREEDMAN: Pinaki, who just asked a question, is being very modest. He, I think, he

had a study, published last year, with 3,000 infants. The number needed to treat, prevent death -- death, that's the best outcome you can get. I think it was 27. So, I think he has a product that he has used.

DR. PANIGRAHI: Well, I didn't want to elaborate on that. I mean, that was a huge, a very large study done overseas, all NIH funded. Enterprise studies are also NIH funded. If NIH had asked me to do it under IND, which is the case now, if I go to them and say that I want to study Sepsis or Nec, they will ask for an IND, NIH will, but, at that time, they didn't, and without -- had it been the case, then I wouldn't have done a study in 4,600 babies, which not only shows efficacy, it also shows, at the least, that side effects are none, literally, that it's a extremely safe probiotic to use. So, that could have never been done.

DR. NEU: So, I think that we need to compare apples to apples, and oranges to oranges, here, and you were evaluating babies, infants. We

are talking about preterm babies, and a preterm baby that is less than 1,000 grams is different, is a different human, than a baby that is 1,000 to 2,000 grams, and, so, we are talking about a very different individual than those babies, or those infants, you studied.

DR. PANIGRAHI: I fully agree with that. I mean, we take it with a vulnerable population, with the kind of disease, of course, yes, but with the same token, if there is a blanket regulation made by the FDA that any time you do, in a probiotic study, in infants, in sort of a preemie, just a one-year-old infant, or in adults, or in AAD, or in any other disease for that matter, if it requires an IND, it's going to stop. I am a proponent of IND because I won't restrict one disease, one bacteria, but a precise outcome, yes, I will go for an IND, myself, but if it is, at some point, but to take that as a standard and demand that every study needs an IND, I think I differ in that.

DR. MCCUNE: So, I think, maybe, we'll

talk about, a little bit, some of these issues this afternoon. We're in the lunch session. So, I'm going to say the last two questions, and if you might be able to make them brief, in the responses, brief. Otherwise, I'll be in trouble for not letting you go to lunch.

MS. TOPHAM: Good morning and thank you for your presentations. My name is Debra Topham, with Knowledge Bank. I'm a Regulatory Consultant, and I also educate graduate students in food science and nutrition, and my question, comment, clarifying point is how come the studies are poorly designed, as far as how they characterize the background dietary intakes and the placebos because, in any good drug study, you have, of course, the phases of work, but, in a phase one, you're, maybe, even controlling the diet, the environment, and you start throwing all of these kinds of organisms at the public, at large, you bring in other intermediary effects, and I often find that, even in the case of the Canadian study and the U.S. study, that was not necessarily a

fair comparison between two treatment groups, let alone the ignorance of using any kind of background characterization of the delivery system, with or without milk, with or without oligosaccharides, so. Comment, question, clarifying point, if you will?

DR. FREEDMAN: I can try to tackle a little bit of that, so, several things. So, in terms of, you know, breastfeeding, we actually did look at exclusive breastfeeding. We actually had specific predefined mechanisms of delivery, that were the ones recommended by the manufacturers of each of them. So, they were in solutions that were deemed to be most compatible for viability of the organisms. I didn't go into all of that.

The other element, for those who -- people who do clinical trials, such as myself, I'm trying to get in touch with caregivers on a daily basis, nearly impossible. I'm trying to get them to even tell you what they've done for the last day, or how many stools, very hard, trying to get them to tell you what they fed their 18-month-old,

impossible. They're at daycare. So, getting that data is nearly impossible. The other is, actually, and, I would argue, on the other hand, these are -- and, actually, I think where research needs to move, pragmatic clinical trials, real-world. So, in the real world, people are eating whatever they're going to eat. They're going to feed their kids. We can try to tell them, if there was a specific thing, but you actually need to look at effects of interventions in the real world, on real patients, and what's going on, and, so, that's really why, you know, yes, it could have been interesting, were there subsets, but, truthfully, trying to get that data is nearly impossible in these types of trials, and, so, it was not the focus of our effort, post randomization. Getting antibiotics was a big one, obviously, getting breastfeeding status, daycare status. We focus on a bunch of those, but dietary history intakes? Good luck.

DR. MCCUNE: All right. Last question, please.

MS. TEROVSKI: Brianna Terovski, with Johnson & Johnson. You actually answered mine, so, kind of, along the lines of my question, and you mostly answered it, 90 percent of it. I was just -- just a little piece, I'm missing. How did you go about selecting the probiotics that you would study, for the indication that you were studying in the organisms? I think you said a lactobacillus. What was your -- the process, if you could share (overtalking)

DR. FREEDMAN: How long do I have?

DR. MCCUNE: I'm holding everyone up (inaudible)

DR. FREEDMAN: So, really? If you go for -- no. So, in short, actually, it was a several step pragmatic process. So, the -- initially started working in the Canadian side, with a product made by Lallemand Heath Solutions, or Lallemend Health Inc., which is a Canadian company, also subsidiary through France, where one of my long time collaborators who'd been working and studying this product in vitro, and working

in, then, in animal models, and demonstrating efficacy in immunomodulatory properties and cytokine mediation, and improved benefits in the animal models. They had some human data, as well, showing evidence of benefit, and, so, we went to that.

We, then, did a dose finding study that we've published in Clinical Pediatric several years back, where we actually looked at the higher dose. So, they had their recommended dose. We, then, said, because I didn't want to have too small a dose, we, then, doubled the dose, and actually did a study looking at safety of that, and we found that the higher dose was safe, no adverse events. So, we used the higher dose because I didn't want to be criticized as having too high a dose in that study, and, so, that's how we moved forward on the Canadian side.

We, then, went to the, and, if you ever want to get into INDs, we, then, submitted a study, in the U.S., funded by NICHD. We, unfortunately, could not get an IND for the

product I studied in Canada, from the U.S. We couldn't get the purity piece. "Oh, well, I don't make it," the manufacturer. They had too much contamination, couldn't get an IND. We, then, decided, okay, can we just tweak this and look at who else, and we looked at the number one player in the market, most commonly used, and, with a fair amount of evidence of benefit, which was LGG, and then they also held an IND on their master file, and we were able to use their master file to obtain an IND to do the study that we, then, conducted, and, so, that's how we ended up using LGG. You know what? Truthfully, we didn't start out that way. I wanted to study the other product, both countries. Actually, it's probably to a certain degree, I'm actually happier, at the end, that we studied two different products, two different countries, and, so, I actually think it was a good resolution of the problem, but there are issues of -- you know, we couldn't get the IND to study it.

DR. MCCUNE: So, I want to thank all

three of our speakers from this morning. It's 12 minutes after. We would love to have you back by 1:00, please, so that we stay on time.

(Recess)

MR. CARLSON: As Susy pointed out earlier, this is the second half of our -- of this this session, looking at live microbiome-based products, for disease indications. This part of the session is really going to focus on C.diff, both from useful commercial products to discussions of FMT, and then CMC considerations for this type of product, in the context of C.diff. So, we'll go ahead and get started. I'll introduce the first speaker as Krishna Rao, from the University of Michigan, who's going to talk to us about commercially available products for prevention of C.diff.

MR. RAO: Thanks, Paul. I get to be the guy to talk about diarrhea, right after lunch, but I'll do my best to keep this civil. So, I only have one disclosure. I'm a COI, on a grant from Merck, not related to this particular topic.

So, I want to talk a little bit, briefly, about C.difficile infection. Vince Young gave a nice overview, this morning. So, I won't touch on that too much. I do want to talk about some of the basic science and mechanisms of the Hind Probiotic Development for C.difficile, specifically, and I think it will be clearer, by the end of the talk, why I wanted to spend some time on that, and then we'll delve into the clinical literature and talk about future directions that, I think, may be helpful.

So, briefly, on C.difficile Infection, so, as we know, it a gram-positive spore-forming bacillus. I won't go through all these numbers, but I will point out that it's striking. These numbers, here, that you see, are very high, and they're for the United states alone. So, clearly, this is a major problem, if we're having nearly half a million people a year, getting this every single year.

What can we do to actually prevent this, and, again, Vince showed a similar slide to this.

I won't go into too much detail. I will point out the important part that we need for our discussion, which is that, you know, C.difficile is a spore-forming organism, and, so, you do need germination of the spores, to a vegetated state, and then production of toxin from those vegetative cells, in order to actually indicate disease. Notably though, this person who is currently symptomatic and red, doesn't go to green once they get treated immediately. It takes some time for that microbiome to recover, and if they get re-exposed, or they have another hit or an insult during the susceptible period, they could end up in this cycle, that sometimes only people transplant, or other measures can fix, and we'll hear more about that in the next talk.

So, how do these even work, and I think, I get this question all the time, from my patients. So, it's only fair that we ask ourselves this question, too, which is: is it even feasible to think that these drugs might work? A lot of them will say, "Doc, you know, I'm taking

antibiotics, right now, at the same time you're giving me this probiotic. Isn't it just going to kill the probiotic you're giving me?" and it does reassure them a little bit, that we've thought about this, and we thought through this, and that, you know, many probiotics can easily make it to the lower gut, lactobacillus, famously, is a lover of acid, tolerates low pH just fine, lactobacillus and bifidobacteria.

I mean, the setting of C.difficile literature can colonize the gut, and we see it persisting, even after it's been -- it's administration has been withdrawn. These are actually, occasionally, a little bit too invasive, and we sometimes see them in extra intestinal sites, and they're often selected to be resistant to certain antibiotics. We give back to lactobacillus, for example, with vancomycin because it's resistant to that, even though it's low-grade antibiotic, and what's important is, also, that these are very strain and person dependent, and half of patients don't colonize at

all. Like Vince, I actually read this study in Cell that just came out, unlike the press reports, apparently, and they don't say that probiotics are useless, but they do make some interesting claims that there are very strain and person dependent findings that need to be accounted for.

Again, this is just kind of a general overview we can look at and think about, these mechanisms, from the more widespread ones, such as, colonization resistance, and production of short-chain fatty acids and secondary bile acid metabolism, and a more rare and very strain specific ones of specific immunologic claims or neurologic claims, but for C.difficile infection, broadly speaking, we have a few areas that we can target.

So, one is this bile acid hypothesis that we've talked a little bit about. Clostridium scindens is one that we'll hear a little bit about, that can target the pathway, by inhibiting -- by promoting the conjugation of primary to secondary bile acid, which are inhibitory to

vegetative C.difficile and sporulation. You can actually have these probiotics producing anti-bacterial compounds, that actually can be sidle or static.

In this case, this is an example of lactobacillus reuteri, which is able to do this, or they can make compounds that inhibit the toxin activity, in particular, protea, so, such as saccharomyces or akhaten, and they can have very non- specific general effects, too. So, some of these will increase mucin production. They'll alter local pH, inflammation, increase production of IGA, and just to pick out a couple of mechanisms to spotlight, so, one is bile acids. There's a lot of data suggesting that bile acids are important in C.difficile infection pathogenesis, been showed one study earlier today.

Here's another one, where we looked at, specifically, fecal transplant patients, who are successfully treated, had their -- a cure of their C.difficile infection, and whether you look at short chain fatty acid or secondary bile acids,

you seen increase in these patient populations. Sometimes, that's a transient bump. In many cases, it's more a persistent bump, but the question remains, you know, what is the direction of causality here?

There are some nice mechanistic studies, looking at probiotics and C.difficile, that are coming out, and have come out, actually, in just the last few years. I'll draw your attention this, to the date, because this is, again, another theme that's important. This is in 2015, just three years ago. Eric Pamer studied inst -- alluded to this briefly, but they looked at mice who were treated with antibiotics, and those who weren't, assessed the microbiome, found that there were a couple ataxa that were highly differentiating, those two populations of mice, and, in particular, clostridium scindens and three other ataxa, seem to confer resistance to C.difficile, in either alone, or if you gave the secundines with other bacteria as well, that they identified.

Looking at both the colonization levels of C.difficile, as well as the survival of the mice, they saw a significant effect, and, in fact, the consortia actually completely abrogated the effect of C.difficile in these mice.

Going on another spotlighted pathway, let's look at bacteriocin, so L.reuteri makes reuterin, and that's one pathway that has also, very recently, again, look at this date, 2017, been shown to be efficacious in C.difficile mouse models. Here, they administered a L.reuteri, along with its substrate glycerin in a bioreactor model, and they found that that actually inhibited C.difficile, almost several logs, and that the populations differed when you assess their microbiome, and again, this is only in the presence of the substrate that lactobacillus reuteri actually needs to make reuterin.

Now, what about the clinical literature, and I would say that, broadly speaking, you can look at individual studies, or you can look at meta-analyses, and when you look at individual

studies, you see a lot of really very poor quality of evidence to a large degree. So, many of these are uncontrolled studies. There's not one large definitive RCT, yet, that's been done for this topic, and there's a lot of heterogeneity. There's heterogeneity at the strain level, at the doses level, at the regimens, and also in the patients, because not all patients come into to C.difficile with the same level of risk, and it's important to define that population, specifically and strategically, when you're designing your studies.

So, what do the individual data show, and, so, there's one study that has been mentioned, this PLACIDE Trial. That was a couple of years back, in 2013, and that was a large negative study for the use of probiotics in C.difficile. Now, it was negative, but it's important to mention that this was a study of antibiotic associated diarrhea, not C.difficile, so C.diff wasn't their primary outcome. Secondly, only a percent of patients, in that study,

actually had this outcome of C.difficile infection. So, even if they had an effect size of 50 percent, it would be really hard to power a study to detect something like that. So, again, and it wasn't focused on a high-risk population, and many of these studies were not. Many of these studies have very ill-defined inclusion, exclusion criteria, in fact many of them allow people to eat yogurt, at the same time that they're on their quote - unquote assigned study probiotic.

There's other confounders. There's one study that actually noticed a pretty decent effect size for C.difficile, but then you read the study, they literally moved to a new hospital, during this study, and, so, of course, their rates went down. So, the preponderance of this evidence, I think, I agree with one of our earlier speakers, that it has cooled the interest in probiotics, specific for this indication. In particular, this Allen Study really did cool the interest in studying this, probiotics for C.difficile, and the current guidelines don't recommend them, as has

been pointed out.

Now, what about meta-analyses, and there have been several conducted over the years, and this is by far not an exhaustive list. I don't think I even include the Cochrane Study on here, the recent one from last year, but these are some of the three that are commonly recommended, and looked at the dates for these, 2012, 2015, 2016. That's actually flipped from the way you would normally think about it. A lot of the mechanistic studies, that I highlighted, were more recent ones, and these studies looking at efficacy in the clinical literature are older ones, and this has been pointed out, about meta-analyses before. So, maybe, I'll point it out a different way, and use a different analogy.

My meta-analysis instructor, in school, liked to use a different analogy to say that a lot of time when you combine meta-analyses, we think, that we're mixing a whole bunch of turds, and out comes a pot of gold, but, really, what sometimes happens is you mix a bunch of turds, and you get

turd soup, and I fully acknowledge that. However, I think there is a signal, here, that we should not ignore, and that needs some elucidating.

So, one of the problems with a lot of these prior meta-analyses is that, again, these individual studies, there's a lot of them, many that are poor quality evidence. So, which ones you include matters. How you include them, and how you extract the data matters a lot, and a lot of these didn't actually follow PRISMA best practice guidelines, which is why there is heterogeneity, even in the meta-analyses themselves. Sometimes, I study different populations. So, broadly speaking, what I've seen is that, if you have meta- analyses that have these broad criteria, that take a lot of studies, they have weaker effect sizes. They don't tend to demonstrate statistical significance.

There's more heterogeneity in those studies, and when you have narrow criteria, not surprisingly, you actually get better results, and when you focus on a high risk population, and only

include, for example, RCTs, but placebo controlled, you actually get a little bit if a signal that you're able to tease apart. This, I think, not to pick out just one meta-analysis, but I think this is one that's really clarified what's going here, for me, a little bit, at least.

This was done last year, in the gastroenterology, and they point out that there is a lot of heterogeneous data in the past. Here, they focused on RCTs, so controlled studies, in hospitalized patients on antibiotics, so among the highest risk population that we have. They rigorously adhered to the PRISMA guidelines. In fact, they even went so far as -- a lot of these studies have attrition basis. Some of the prior meta-analyses didn't assess for attrition basis. This one did. They went so far, in their sensitivity analysis, however, to actually assume that, in the patients that were lost to follow-up in these studies, the rate of C.diff was five times higher than in the other population, and even when they made those very conservative

assumptions, and, actually, they had very low heterogeneity there. Their calculated I squared was zero percent for this meta-analysis, but they did a meta-regression anyway, and use mixed methods anyway, and, even with very conservative methods, with over 6,000 patients, included among 19 different trials, you can just look at this forest plot, and if you would guess that the heterogeneity is low, you'd be right. There is a significant effect here, and it's, you know, it's about 40 percent relative risk, or number needed to treat of about 43, and even with the very conservative five fold increased incidence in the untreated group, and with the missing data, they still see an effect size of a 40 percent reduction, a relative risk of 0.6 and a number needed to re-treat of 63, and, you know, as Dr. Merenstein mentioned, you know, this definitely with in the acceptable range of what we do, clinically, just to give you something to anchor your thoughts a little bit.

We routinely prophylaxis against Venous

Thromboembolic Disease among in-patients who were admitted, who meet certain risk criteria, and the number needed to treat there is in the 250s, and the number needed to harm is quite a bit more than with probiotics, but we're not using probiotics, and the question is why? Before we get to that, real quickly, the other place you can look at probiotics for C.difficile is not in preventing the primary C.diff, but in preventing a secondary C.diff episode, or that recurrent cycle that we talked about, and the punchline is, here, is -- actually this is much less robust, in terms of literature, and I'm not, it's not clear that there is much of a signal here, at least with single agent probiotic. We'll hear about FMT shortly.

So, why aren't we using probiotic for C.diff? I just told you that there does to seem to be a little bit of a signal, even in a really well conducted meta-analysis, with very rigorous adherence to the best practice guidelines. So, maybe they aren't safe. Maybe that's one concern. They're officially, generally regarded as safe.

There are some symptoms people report, IBS-like systems that can occur, that are actually fairly common, and this recent paper, in Cell, that we had talked about, did highlight some of those.

However, there are some major concerning things that we have to be aware of. So, there are patients who are treated with saccharomyces formulations, the lactobacillus formulations, that have had Bacteremia and Endocarditis respectively reported with those formulations, and they -- yes, they went back to actually verify that the strain in the probiotic was the one that isolated clinically. There's this other famous study, on in-patients in the ICU, that were given probiotics for their Pancreatitis, where there was actually increased mortality in the probiotic. I'm not sure what that's about, but that definitely has cooled a little bit of the interest in this, and also notable is that many of these trials that I just talked about, in these meta-analyses, they excluded immunocompromised patients, IBD patients, ICU patients.

Those patients who are among the most highest risk for getting C.diff in the first place, and, so, it's hard to generalize, to some of these other populations, where we would actually want to use these agents. Maybe we're not using them because there's lost of major evidence gaps that need to be filled, and I think that's part of it, and there's some examples here, things like what are the interactions between specific class of antibiotics and probiotics on C.diff risk? To what degree do dietary probiotic use impact the results of prior RCTEs, and ways that we can, maybe, navigate some of those discrepancies and gaps.

Maybe there's too much heterogeneity, and we've talked a little bit about this, but, in the past, it seems like we've almost been moving bedside to bench, and, only now, in the last few years, that we've finally kind of -- developing, I think, the rigorous pre-clinical research, to say, "Hey, these are the strains that are actually showing effect efficacy in these really nice

models." Now, the challenge, of course, of that is translating these models to actual humans, and that can be challenging. Bioreactors are a little bit easier to do, than with mice, because you, actually, can actually use a human microbiome in a bioreactor, but, and there are humanized mouse models, but there's all kinds of issues with that, but those are challenges that I will acknowledge.

However, what's really encouraging is that the strains that we're seeing, in these newer pre-clinical studies, are largely a lot of the same strains that we've seen in these trials before. So, I think it's encouraging that there's a signal here, and that we may be able to kind of make some progress. However, that's going to be challenging for this other reason, another recent study, looking and really showing that there's only strain specificity, but disease specificity. So, whether you're talking about a specific strain, and you look at different disease processes, they're all over the place, in terms of the efficiency, or if you look at a specific

disease process, C.difficile, as we've seen, you're all over the place, in terms of what strain is actually going to make a difference here.

So, what are the future directions? Where do we go from here? What advice could I have, and one thing that I think is happening, and that needs to happen more, is we really need to go back to the bench, and make -- I'm making an argument here, that we need to have a rational mechanistic approach to how we actually design these probiotics, and these are examples of all the mechanisms that are currently being studied and have been studied in the last couple years, and are -- have yet to make it into the clinic literature, and I also think that, on the clinical literature side, we need to have very strict, well-defined inclusion, exclusion criteria. Don't let your subjects eat yogurt. I think that clouds the picture. Actually, have good randomized placebo control trials. We need to power it appropriately for C.diff, and, you know, one of the other recent developments, not to plug my own

research too much, is -- we're actually starting to have electronic health records being able to be used in machine learning algorithms to re-stratify people.

So, this is a study we published last year, where we can actually re-stratify people, and actually predict a episode of C.diff, four to five days before it happens, in the admission setting. Using a risk model like that, and randomizing those patients to an intervention, may be a lot more fruitful than prior approaches.

So, conclusions, I think, no argument here, that C.diff prevention remains a major need. The rest of this is opinion, but I think, the current clinical evidence does support that there is some role for benefits of probiotics in C.difficile. So, the reason I can't recommend this, though, is it's one thing to say that there might be a benefit here, that it looks like there is a benefit. It's another thing to make a very specific falsifiable scientific hypothesis, another thing to make a very specific claim of

about. Here's the strain and dose that a patient should take for benefit, and we aren't at that point yet. With the interest of time, let's end there.

MR. CARLSON: Thank you, Krishna. We're going to -- like we did for the last panel, we're going to do questions for all the speakers, after. Our next speaker is Colleen Kelly, from Brown University, who's going to talk to us about clinical evidence on FMT for C.diff.

MS. KELLY: Thank you very much. Thank you for the invitation to speak today. So, I was asked to summarize the evidence that we have from randomized controlled trials for C.diff infection. I want to just, sort of, start off with my early experience with what we called fecal bacteriotherapy, at the time, and in my first year, I treated two patients just like this, a 61-year-old woman, who had had six intensive care unit admissions, over a twelve-month period, during some of those, almost like lost her colon, almost went to surgery, almost died. Each one was

very dramatic.

The second was a young girl who was 19, who got a dose of clindamycin, getting -- for some dental work, and this was during her first year of college. She, then, developed recurrent C.difficile episodes. She had to quit the soccer team at her college, and take a semester off of school, and both of these patients were treated with repeated courses of Vancomycin, Metronidazole, (inaudible) and probiotics, and with -- to no avail, and both resolved their C.diff infection with a single FMT, and, in fact, in the first two years of fecal bacteriotherapy, at our practice, 24 of the 26 initial patients I treated did not develop a further C.diff occurrence after that first FMT, and, by 2011, we weren't calling it fecal bacteriotherapy anymore, this terminology FMT, and, as Vince Young spoke this morning, we're transferring these entire communities of micro-organisms from one person to another, to increase the diversity, and repopulate some of those beneficial anaerobes, and, at that

time, when I was kind of having my early experience with FMT, others, also, were seeing that this was working, and this is the results of a paper published by Zayn Kassam, around that time, that just demonstrated that we were seeing really high cure rates in open label clinical trials and case reports, close to 90 percent overall, with some evidence that it may be more efficacious when given from below, than from above, but the real game changer was in 2013, when this Dutch group published the first randomized controlled trial in the New England Journal of Medicine.

It was relatively small trial, 42 patients, who had a least one C.difficile recurrence, and they were randomized one of three arms, either a short course of Vancomycin, followed by a bowel lavage, like a bowel prep, and infusion of 500 CCs of donor stool, by a nasoduodenal tube. The other two groups either got a standard course of Vancomycin, 14 days, with or with out that bowel lavage, and that study was

actually terminated at the interim analysis because FMT was so effective. Eighty-one percent resolved their c.diff after a single FMT, and then the couple that needed to get retreated, it was up to 90, close to 94 percent, compared to 20 to 30 percent in the Vancomycin groups, and others have also looked, in a randomized way, at FMT versus this standard of care, which is Vancomycin taper. Camrhoda and colleagues, in 2105, reported on a similarly sized patient population, with recurrent C.diff.

Their intervention was, again, a short course of Vancomycin, followed by FMT delivered by colonoscopy. If they saw pseudo membranes, which are indicative of more severe disease, then they would repeat and do, potentially, more than one FMT, but most patients, in the trial, only got a single FMT. This was compared to a group that just got a standard course of ten days of Vancomycin, and then a pulse taper dosing over the subsequent three weeks.

That study was actually also stopped

early, for superior efficacy of FMT. You can see the numbers there. Conversely, Susy Hota and colleagues, in Canada, more recently, published a study of patients with recurrent C.diff, who were treated with either the standard course of Vancomycin, followed by a taper, or, instead of tapering the Vancomycin, they were given a single FMT by enema, and, in that study, that was also stopped early, but for futility, in that that single enema did not appear effective at resolving the recurrent C.diff cycle in those patients.

So, I was the PI for this clinical trial, which was published in 2016, and we did our best to find a placebo, which in this case was autologous FMT. So, patients would submit their own stools to us, and then they -- I would -- picked a card, and they either got a fresh donor FMT by colonoscopy or they were reinfused with their own stool.

It's important to know that they were all treated with, at least, a standard course of Vancomycin, 10 to 14 days, and symptoms had

resolved prior to getting that FMT. Vancomycin was stopped three days prior to the procedure, and they were infused with either their own or a donor stool. Overall, 91 percent of patients resolved with FMT, versus 63 percent who got the placebo, and at -- you can tell that there were some differences between sites, and, if you'd just let me, I'm gonna leave that till later. I promise you I will address it, but I do want to say there were no SAEs, in the FMT related group, you know, no related SAEs related to FMT, and I do want to point out that we were limited by the IND. This trial did not include patients over age 75, or patients who were immunocompromised.

Others have compared FMT to FMT, comparing different delivery modalities, so, Youngster and Libbey Hoang, in -- published in Clinical Infectious Disease, in 2014, looking at recurrent C.diff patients. They were treated with 41 grams of stool that was frozen, and thawed, and administered, either by nasogastric tube or by colonoscopy, and there really weren't differences

between those groups in efficacy, and, more recently, Dina Pao, in Canada, randomized patients who had had at least three episodes of recurrent C.diff, to frozen capsules, which were then thawed and administered, or at the same amount of stool, 100 grams administered by colonoscopy, and they actually found them to be equally effective, 96 percent for the first dose, either by capsule or colonoscopy, though the capsules were rated as cheaper, and preferable to patients overall.

Other groups have compared dosage formulations. Fresh FMT was compared to frozen, in a large trial, by Christine Lee, also conducted in Canada. This was published in JAMA, a couple of years ago, that over 200 patients who had at least one recurrence -- so, I do want to point out that this study, patients were get -- kind of interrupted on much earlier in the C.diff cycle. It was after a single recurrence, versus three or more, and many in this study, it only had a single recurrence, in fact, 92 percent of those patients.

Their overall efficacy with a single

enema was 62 percent, and didn't appear to differ whether they got stool that had been frozen, or that that was fresh and administered, that way, but you can see to get up to those 90 some percent numbers, you needed three to five enema FMTs. Another group, Baylor, published just this past year, looking at fresh versus frozen, versus lyophilized formulations of FMT. This was about 50 grams of stool. So, it's the similar, similar dose, but the preparation method differed, and really fresh, a 100 percent resolved, 83 percent after getting previously frozen stool, and 78 percent after lyophilized, and the differences were not significant because most of these studies are small and really underpowered to detect meaningful differences there.

So, this, as, you know, we've heard a little bit of versus a not necessarily trust systematic reviews in meta- analyses, but Paul Moayyedi did a good job with this one, published last year, looking at summarizing the five big randomized control trials for FMT, and those

included 284 patients, and I want to point out the number needed to treat was three. That's huge. There were significant heterogeneity across these studies because of different modes of delivery, and doses, but the despite that, and looking through all these with grade type criteria, it was determined with moderate quality of evidence, you know, to be effective, and importantly, in all those patients, there were no FMT related severe adverse events, and this is something that I've also seen in my own practice.

I'm up to nearly 300 FMT's at this point, 10 years in, and I have, to date, not seen a definite FMT-related complication. So, they are certainly, I'm sure they occur, but they are rare, and, since 2013, American Gastro Society Guidelines, and European Guidelines, have promoted Vancomycin, I mean, excuse me, FMT after patients have failed standard treatments with pulse in tapered Vancomycin, and, more recently, the IDSA guidelines, which were published last year, also support using FMT for patients with multiply

recurrent C.diff, used with strong recommendation, despite the moderate quality of evidence.

So, here's something that works. We know it works. Handing the ball to industry, we're all, as clinicians, looking for something easier than putting fresh stool in people, but the results, so far, from the industry funded trials, in this population, have been disappointing. Seres Health, in 2016, reported in their capsule study, and I do want to -- there's a little caveat that Seres' product was not FMT, per se. It was derived from human stool, though it was ethanol treated to kill off vegetative forms, and it was basically clostridial spores, but there was no significant differences in those who received the placebo and those received the Seres capsules.

Rebiotix helped -- or presented an abstract form, and also, more recently, published results of their phase two trial, comparing placebo to a single FMT, or two FMTs, and, interestingly, two doses of FMT was not more effective than placebo, though a single dose of

FMT was. However, their -- a (inaudible) endpoint was resolution with two FMT enemas, and, therefore, their study was also not significant. So, lessons learned from all of this, delivery method certainly matters. Single dose enemas are less effective, and we see this from a couple RCTs now Lee study, Suzie Hota, in Canada, and then the Rebiotix results, very similar in efficacy to that single dose FMT.

Fortunately, freezing doesn't impact efficacy. So, we don't have to worry about keeping fresh stool around, and also, fortunately, capsules and colonoscopic FMT appear equally effective. So, we don't necessarily have to instrument these patients and put them through the procedural risks, and why are we having, you know, why are we having these difficulties, and I think, one of the things that the diagnostic challenge is around C.diff. Though, about ten years ago, everyone went to the PCR because it's more sensitive, and we weren't going to miss any cases. The problem is is we pick up a lot of colonized

people, and colonization rates are high, up to 15 percent of healthy adults. I think that's a little high. I think it is closer to like three percent, but this was just from some Seres Hospital in-patients, up to 29 percent, and residents of long term care facilities, up to 50 percent of these people are gonna test positive for C.diff, the organism, without actually having C.difficile infection, being said.

The other thing that we see, after C.diff, is post- infectious IBS, that occurs in close to 25 percent of people, where they may go on for a period of time, to have loose stools and diarrhea on and off, and some bloating, and discomfort, and that may, in the setting of colonization, be mistaken for a recurrence, and, you know, called that in a clinical trial, and treated as such. So, don't rely on PCR for diagnosis in these studies, and enroll from highly experienced FMT Centers because we're seeing this all the time.

In our center, we published 25 percent

of patients referred to me. It was a -- subsequently, like 100 people, consecutively referred, and a quarter of them actually did not have recurring CDI. I didn't need to give them another treatment for the C.diff, and I found all kinds of things, and that's just the, you know, the list of things that I found. I found undiagnosed Crohn's disease, Celiac disease, lactose intolerance, three cases of fictitious diarrhea, people who just like to come to the hospital and get attention, and one of things that we found, interestingly, that there was an inverse relationship between age and these alternative diagnoses. The younger people in these trials are less likely to actually have real true C.diff, compared to older patients, and I think John's going to talk more next, a little bit about the Seres data, and, I think, that they did see more efficacy in the older groups in their paper, but, importantly, I think people were cured, and I think that that's kind of what, I think, happened with a lot of the patients, at the New York site,

in our study.

Some of them had been on continuous courses of Vancomycin for a very long time, waiting to see Dr. Brandt. He did not, necessarily, stop that Vancomycin, he said, "Okay, I'll enroll you in this study, and then we'll stop the Vanco three days before, and give you and FMT." but I think that a lot of them were probably already cured. One had been on a continuous course of Vancomycin for 148 weeks. That was an outlier, though, so. So, I think, keeping these things in mind with your study design, and how long patients should be treated with Vancomycin, prior to being enrolled in an FMT trial, is important.

So, to summarize, here, I think FMT works for C.diff. We just don't know exactly how well yet, but I'm certain that it works. It also appears to be very, very safe, and we need to really take into consideration these things when we're designing clinical trials. Who are the most appropriate patients to enroll? At what point in

the cycle of recurrence should it be, after the first recurrence, or a second, or a third? Should we be looking at FMT for a patients with severe, or severe complicated C.diff, or essentially lose their colon, or die, or even as a treatment for primary C.diff, and there's been a couple of papers, recently, suggesting that, maybe, instead of an initial course of Vancomycin, or Flagel, giving a dose of FMT, and then what should be the best end points, and for how long after, you know? Are we looking at eight weeks, 12 weeks, diarrhea free, of course, like the PCR, versus the enzyme iminoacetate? So, all of these things, really, should be important to those of you who are in the audience, who are looking to design a pill for us to use. So, thank you, very much.

MR. CARLSON: Thanks, Elaine. So, we'll move on to our final speaker in this session now. John Aunins is going to come talk to us. John's from Seres Therapeutics. He's going to talk about CMC considerations for microbiome-based products. John?

MR. AUNINS: Thanks, very much, Paul. So, now for something completely different, as they say. So, the benefit of going late in the afternoon is that a lot of your intro slides have already been covered by people in various forms, and, so, you can kind of go through them. So, microbiome, as an interesting subject for a pharmaceutical development, is a fairly recent sort of evolution. It's paralleling in my mind. A lot of what went on were for stem cells, about 20-25 years ago, where people first view them as tools to understand disease, next as targets to manipulate, and then only later to become therapies, and you can see that, in, sort of, the applications that people have started to develop. So, microbes as tools, obviously, as Vince Young, pointed out, people want to understand how their drugs are metabolized, but then also try to maybe sus out exactly which compounds bacteria is treating, to create new drugs.

This is an approach that's kind of favored by the larger, more conservative players

in the industry. Microbes, as targets, I think, everybody would like to have surgical strike kind of antibiotics that only get the pathogen of interest, and don't have the collateral damage of the broad spectrum antibiotics that we currently have, and then there is a fair amount of research in prebiotics. If you look for interventional studies and clinicaltrials.gov, you'll find almost 300 studies, on prebiotics, attempting to manipulate levels of microbiome components.

It's not obvious to me, I think, that there is a miracle food that you can eat that's going to cure you of disease, but, you know, there may be certainly concepts, like Xenobiotics, that we talked about in just second ago, that could be valid. What we're here to talk about, of course, microbes as therapies, where we're trying to, not so much, do antibiotic-like maneuvers of loss of function, but really have gained a function, or in some cases modulation of function, for example, for immune system, by replacing or altering the microbiome.

I don't know that I need to really belabor the different types of microbial therapies. Clearly, we've got two different sets of equal here, the traditional probiotics, and then the newer area of gut commensals. I think the traditional probiotics -- these are, basically, dietarily acquired organisms. You get them with dairy products, fermented foods, and such, by and large, or the strains.

I'll disagree with Dan Merenstein, in that, every time I talk, I'll update the clinicaltrials.gov search for interventional studies, and it keeps growing, and growing, and growing. It's over 11,000, 1,100 studies, over a 110 in the past year, that I found. So, I think there's a robust amount research on it, but I think the results, by and large, have been -- seem to be modest, for various reasons, that we've heard this morning and this afternoon.

I find it interesting, Bob Durkin didn't have a kind of an equivalent sort of metric, but since the -- they're a European equivalent, the

European Food Safety Authority, put in place a rule, that said, basically, "You can't make health claims, unless you submit a scientific dossier, and you proved your claim." It reviewed over 300 of these things, and they've only approved one, and that was for a fairly obviously secretion of cobalamin, which is known to occur by bacteria. So, there's not a heck of a lot of evidence.

I also don't need to probably talk about safety so much as to -- because we've talked about that a bit. I don't know how many of you caught Bob's subliminal drawing of the lion, though, where he said there was something like 500 inspections, and 7,000 production facilities. Work that out. It's about one inspection about every 14 years. Would it surprise you if things get a little sloppy in the interim? I think not.

Gut organisms, as we've heard, have gotten a lot of interest since the Human Microbiome Project came along, and the confluence of the C.difficile epidemic, and the advent of FMT as a potential curative for that. Clearly, FMT is

-- it's a good initial staff. It's doing great things for a lot of people. I think the efficacy in safety, as Colleen just described, is still a bit ambiguous, and could be further refined, and, of course, for any of these products that are made on gut commensals, I think, it says, yet, TBD, that they actually, you know, they put the proof in the pudding, too, for safety in efficacy, but I think it goes without saying, that where we would all like to go, is to get to designed microbiome therapeutics, which would be either single strains or a consortia of strains of purified organisms for the GI track. In some instances, such as our colleagues here from Senlogic, they might be genetic engineered for heterologous gene expression.

I don't think I need to go to this slide very much, either, because Vince Young described how, basically, the microbiome works as an ecology, how it has steady states, unless they're disrupted by certain events, such as pathogen infections, or broad-spectrum antibiotic use. I

think the interesting thing that came out of the Human Microbiome Project, is that, whereas, if you look at the strains of microbiological diversity, you see that everybody in this room would have a vastly different microbiome, but if you look at the gene content, as a functional diversity, it's fairly consistent, and so, I gave companies, like Seres, hope that you could actually, potentially, develop drugs that don't have to be, say, tailored to individual microbiomes, that you can simply try to design things that have the proper function, and replace that function.

As I mentioned in the last slide, so, whereas traditional probiotics tend to have very short half-lives, they wash out pretty much as soon as you stop dosing them. On the other hand, the gut commensals tend to stay persistent, and that's been seen in the trials of fecal transplantation, and other trials as well, and, here, basically, the idea is you take a disrupted disease ecology, and you're going to replace it and stabilize to some normal ecology. Per this

cartoon here, where you'll have microbes from your product that will engraft, and then they'll be augmented by other microbes that come along, and you get rid of your disease microbe, such as C.difficile.

This upper right panel, here, is data of engraftment, from the trial that Seres did, in ulcerative colitis, with a product called SERE-287, which is a spore composition, and, basically, what you can see is that, over the dosing period, depending on the regime that we gave, whether it was a weekly or daily dose, you get engraftment that starts to plateau out about day ten, and through the end of dosing, and so you can create a persistent change.

The interesting thing is that, after you stop dosing, a month later you still have the persistence of the microbes. So, they seem to have engrafted longer term, and that engraftment appears to change the structure of the microbiome. This is a principle components analysis plot that simply shows subjects who went into remission for

Ulcerative Colitis, versus those who didn't. You can see that you got a distinct difference in the structure of the microbiome. So, these are the kinds of things we're trying to do at Seres, is to develop drugs, in that vein, that are going to be commensal microbes, consortia of them, to alter disease, and our paradigm really is to use proof of concept, consortia, probe consortia, like FMT or other natural consortia.

Basically, take the results from studies of those interventions, which are really the gold standard, rather than using observational studies. Try to find organisms that seem to have impact, that are present in your drug, and are associated with success of your trial. Identify the metabolites that are associated with those organisms or those changes, and then try to map those pathways that are expressed by the organisms, and then devise novel consortia that you can use to develop into drugs, right? That's probably the novel part. More conventional is doing the screening for your drug candidates, in

vitro and in vivo, and really pulling from large stream libraries to construct those candidates, and then the next novel bit is the manufacturing, which what I'll talk about from here on.

So, there's several unique features to manufacturing consortia of gut commensal microbes, right? These are not your grandfathers' industrial microorganisms. They're not Chinese hamster ovary cells. They're not E.coli recombinants. They're not saccharomyces. Most of these have never been in any kind of an GNP production. They're generally strict anaerobes, quite often not aerotolerant. So, you have to keep them isolated from oxygen, and many of them are spore formers, which is a unique feature.

So, when we're making consortia bugs, we have to deal with a multiplicity of organisms in the product, making all of them, as you heard from Sheila, you need to, basically, be able to count them all. You need to make sure that you've got their culture behaviors down. You need to preserve them all. Make sure that they all

survive your formulation, and they get to the side of the gut, where you want to deliver them, and then you have to be able to count them, and then, last but not least, you need too be able to manufacture them in a GNP fashion. So, just going through those, the first bid is to actually be able to grow microbes, and just like the, you know, slides that you'll see with throwaways of ten times as many microbes, as human cells and so forth. You'll also hear throw away statements like, "99 percent of the human gut microbes are uncultivatable or haven't been cultivated."

Well, it's -- there it is, for lack of trying, basically. Coming out of the Human Microbiome Project, there was a list of most wanted organisms. Seres has about 75 percent of those most wanted organisms in our strain libraries. The problem, from the CMC production perspective, is that, quite often, they are isolated in things that you wouldn't normally take into production, things like brain heart infusion augers, rumen fluid media, blood augers, and so

forth. So, the trick for the CNC guys and gals is to, really, to take that strain of interest, and be able to grow it in GNP acceptable media and do that in an efficient fashion.

So, we have a multi-stage screen that we use to, basically, get away from complex, and ill-defined and, perhaps, undesirable components, and get to something that's much better defined in an optimized process, and the trick is, there, is to have set up screening paradigms that make use of high throughput robotics, that make use of bioinformatics and Omex Technologies, in order to be able to do this with no -- a modicum of manpower applications, so you don't burn yourself out to death. I'll also note that, you know, you not only need to be able to adapt things and grow them in culture, but you also probably need to be able to optimize phonotypes. So, in Seres' case, we're interested in a lot of firmicutes, to date, and, so, we're interested, specifically, in sporulation, and optimizing that sporulation, especially in the GMP media, can be a complex

endeavor, and nevertheless, you know, we've managed to have pretty good success at doing this, and getting to productivities in our fermentations that are acceptable for future use.

The next thing I mentioned is formulation and delivery; similar problems, here, as you've got for the fermentation. You need to be able to preserve a range of phonotypes, right, and, here, you know, basically, your formulations, and chemistry, and processing has to be acceptable for grand negatives, for grand positives, a range of different types of organisms, cocci, and bacillus, and so forth, and, so, you need similar sorts of platforms, screening methodologies, which I'm not going to go through here, but sufficed to say, we can take some very sensitive clostridial strains, and do a lot better than what you can find for, say, commercial buffers.

So, this upper right panel simply shows losses are tighter through freezing, drying, one week and four weeks, at accelerated temperatures. We can substantially knock that down with pretty

straight forward optimization, and then lastly, well, before that, I'll just note that, I think, it's axiomatic that, once you get away from spore phenotype organisms, you're going to have to go to dried state.

It will, you know, other than perhaps some products that could be frozen as liquids, such as FMT, your ideal product is going to be an oral capsule. You want to be able to put that on a shelf, right, and so, you're going to be dealing with dried powders, and those can be challenging to handle because now you have to prevent, basically, aerosolization of the powders. You want to prevent exposure of powders to moister, to oxygen, and so forth. So, that can be tricky to handle, and I'll just opine that it'll be a miracle if people get actually room temperature stable microbiome therapeutics, in general.

My guess is that most of them will be refrigerated, cold chain products, accepting the spore products. Lastly, delivery, of course, I think it would not be lost on anybody, here, that,

yes, as Colleen mentioned, people do prefer to take capsules, rather than have enemas, well, maybe rare exceptions, but you have to address bioavailability, and get your bugs past gastric acid, and bile acids, and, again, you know, basically there are multiple technologies, capsule types and coatings for capsules, or tablets that allow you to preserve the bacteria in the face of acid exposure, to the extremes. So, it can be done.

Perhaps one of the more interesting aspects of CMC, or microbial therapies, is the quality control aspects, and, here, the challenge is to devise, basically, all of the elements of SesPQ to, really, thoroughly control your product. For safety, you can read in the live biologic's products guidance, you know, there's some motherhood in apple pie, there. Yes, you should know your bug sequence. You should have it characterized for antibiotics resistance, and so forth. You want to understand whether it's got prophage. An interesting feature is toxins. For

a lot of these gut microbes, you may not have a reference genome, or you may have a poorly matched toxin gene, and so, really, you may need to screen functionally phenotypically, rather than by genetics, to understand toxin expression.

Identity, that's pretty straightforward. Strength, initially, of course, you can use colony forming unit assays for species detection. Potency for activity, though, is an interesting concept, right? Even if you have a single microbe drug, it doesn't take very much thought to realize that, basically, even a single microbe has a secretum of hundreds, if not thousands, of compounds, right? So, unlike a, perhaps, more precise single molecule type biologic, where you're trying to hit one pathway and activate it, you're going to be doing polypharmacy, and, in some diseases, you may actually need polypharmacy to have an effect, and, so, devising these potency assays will be interesting.

The other thing that's really unique is, for gut commensals, is that USP6162 are not

generally useful. You will get product breakthrough on these, and, so, you have to devise ways of suppressing that product breakthrough, or enumerating it as being product among product.

Lastly, I'll just finish up by saying GMP Manufacturer of commensal organisms is also a specialized endeavor and complex. I particularly like this phrase that's taken from the FDA's 2006 Guidance on Manufacturing of Spore Formers, is it -- basically, manufacturers are encouraged to identify alternatives if they can, right? If I'm not putting spores in my plant, I've got a problem, unfortunately. So, I have to deal with that, as would probably most people who are going to make products from gut commensals, and, so, you really have to make sure that you've got unique facility designs that have appropriate classifications, that have appropriate pressure gradients, so that you can both keep bugs you don't want out, keep your bugs in.

You need to supplement that with contained product operations. Try to minimize the

use of reusable equipment, so you have a minimal chance of cross contamination, and then use extensive decontamination procedures to make sure that you have address concerns of cross contamination, and, then, last, but not least, you also want to, basically, make sure that your environmental testing, that is, more or less, you know, well established for traditional biologics, will actually address the microbes that you're producing, so that you can detect if there was something left from a prior campaign, right, and then, you know, for consortia, basically, we have to deal with multi-strain product considerations, and being able to operate in a rapid fashion, right?

If we had to produce things serially, making a master bank, a working bank, and drug substance, and repeat that every time for, say 15, 20 strings, you've got a campaign that's well over a half a year or more, right? So, giving -- getting your procedural and temporal segregations down, and having appropriate decontamination, to

deal with that, is key to having elegant manufacturing. So, thank you, for your attention, and thanks to patients, to collaborators, and to all the internal team (inaudible).

MR. CARLSON: So, now we will have the three speakers from this session come up. We'll do about ten minutes of questions for them, and then, after that, we'll go on to invite the three speakers from earlier up, and we'll do the panel discussion. Anyone have any questions? All very clear?

MR. FORRY: Sam Forry, NIST. I wanted ask a clarifying question for the manufacturing controls, about what kinds of evidence you were able to present to the FDA, to regulators, to demonstrate the validity of your -- the analytical methods that you used to demonstrate that your control processes -- you have to provide those measurements in supporting validation measurements. What kind of measurements are you able to show to validate the protocols?

MR. RAO: Oh, there we go, yeah. It's

-- I don't think it's any different then any other biologic. There's a, perhaps, a slightly different spectrum, in the sense of you got a lot more microbiological assays, obviously, right, and so, you know, for example, on, say, bioburden testing, you're going to need to do a lot more extensive work to show that you're detecting your product, that you can pick out contaminants, right?

There are a fair number of sequence-based assays, too, which is probably the more novel thing, I think, for biologics production, and, you know, having validated sequencing, and, for that matter, data bases to go along with that sequencing. You can produce sequence, but then how do you interpret it? How do you know what it is, is a whole another kettle of fish, right, and how you validate that's a different story.

SPEAKER: John?

MR. AUNINS: Sort of a follow up to that, based on sequencing, so, you have an

organism, or a consortium of organisms, and we're very good at doing genome sequencing, and one of the things is purity, right? We talk about purity, and also derivatives, you know, a common thing in pharmacology is, "Oh, let's just throw a different methyl group, and we'll change these." At what point do we decide that, "Oh, how many single nucleotide variants do we have before we actually have to revalidate this as a brand new, or derivative drug?" quote, unquote, and the reason I'm sort of asking it is, can we really work this under the existing rules that we have for drugs, right now, in your opinion?

MR. RAO: Well, so, I mean, historically, or currently, I guess, you know, when you go to license a biologic same monoclonal antibody, you're expected to sequence the cassette, which is, you know, the 3,000 base pairs or something like that, so that you don't have mutations, or characterize them, whatever, understand that, a loci of insertion. That's pretty tractable and understood. I would agree

that it's, you know, once you're dealing with the five mega-base bacterial genome, how do you look at snips? How do you look at indels, and so forth?

I think, you know, the key thing, for all of this, is you need to show stability from your initial materials, and in your master bank to your final product, and then clinically demonstrate that the stuff works, right?

MR. FORRY: Yeah, I was just wondering, just a point of clarification. So, now, in your practice, are you using standardize preparations, or are you using related, or household contacts for donors? I mean, because it's changed a little bit. So, what's your current practice right now?

MS. KELLY: There we go. At this point, I'm using, almost exclusively, stool from open biome, and it's just a matter of -- it's the easiest thing to do --

MR. FORRY: Yeah.

MS. KELLY: -- and it really -- these patients are really eager to just get everything

done with, but if a patient requests that I use a related donor, I give them that option, and we go looking for one, and I do explain it kind of. It might take a little longer. There's no guarantee that the donor's insurance is going to pay for all of that laboratory stuff, and I don't cut any corners, even if they've been married for 50 years. They go through all of the HIV testing, and everything else, so. Most of them opt for the open bile.

MS. WALLS: Thank you. My name is Isabel Walls. I work for USDA. In your talk, you mentioned, I think it was the lactobacillus reuteri, and it needs glycerol as a substrate to making reuterin, and, so, I'm wondering, when you do the clinical trials on, I guess, anybody, do you consider the substrate? Do you consider -- is it what the people are eating, and if so, do you control what people are eating, assuming they're in hospital, they're already sick. You should know what they're eating. Is that the substrate, and do you control for that when you do clinical

trials?

MR. AUNINS: Yeah, I think you should control for that, and so you either do that in a, you know, as you mentioned, a controlled population, like an inpatient setting, where you know exactly what they're eating, or what they're being given, at least, or you coformulate it, and the term symbiotic has been used a couple of times by some other speakers, and questioning, and audience members, and I think we'll hear a little bit more about a talk where a symbiotic was very successful in -- after a coformulation in preventing neonatal Sepsis, later today, but, yeah, I think, either -- you either -- it's so universal that you expect it be in anyone who's got a normal diet, or you co-formulate it, as it would be the way to go.

MR. RAO: I think we would fall more in the Stephen Freedman camp, that it's kind of futile to control what people eat, through the course of their disease.

MS. WALLS: Even when they're in

hospital?

MR. RAO: That I would have to defer to my clinical colleague, Shirley Trexess. That's who I would refer you to, her, over there.

MR. AUNINS: Yeah, I would just say, the clinician in the hospital, you can control what you order for the patient to eat, but what they actually eat is completely different, but --

MR. CARLSON: If no one else is going to ask a question, I can ask one. So, we had a talk on the use of probiotics for prevention of C.diff, and on use of FMT for C.diff. So, as clinicians, we have those options, what we do, I think. It seems like you are almost exclusively using FMT, or maybe it depends on the state where you're at, but, in practice, are using probiotics versus FMT?

MS. KELLY: So, this actually comes up quite a bit because all of these patients, once they've gotten over C.diff, if -- there's kind of like a PTSD. So, any time they're ever going to need another antibiotic again, or going to have a surgery, or anything, they're calling me and

asking me what they should do, and I -- actually, if they're not immunocompromised, I do tell them to take a probiotic, along with, and then for a period of time, like about a month afterwards, and does it work? Maybe. If it's going to really break the bank, and they can't afford it, I tell them, you know, there's not great evidence that it's going to do anything, but I think it really empowers them. They feel like they are doing something, and I think that that's meaningful, in some way. There are people who recommend giving antibiotics, along with, like, an anti-C.diff antibiotic, like Vancomycin, or Metronidazole, along with whatever antibiotic they're taking for their UTI, or their Pneumonia. I don't do that. Just knowing what I know about C.diff, it's caused by Dysbiosis. Just throwing another antibiotic into the mix never seemed like such a good idea, but that's, you know, that's definitely recommended by some other people.

MR. AUNINS: Yeah, I would echo those, those same responses that you -- and just add

that, most of the time, I don't have to make a recommendation about these things. Patients are telling me what probiotics they're already taking for their C.diff. Part of this is, you know, my filter, as an infectious diseases physician, I'm not seeing these patients, until they're on their third, fourth, sometimes, fifth episode, or more, of C.difficile, anyway, and, so, by that time, they've already gone on the internet.

One of the first things they found is probiotics for C.difficile, and they're just picking things, and, right now, my practice is I don't stop them, and I don't say -- and I say, "You know, I don't have much evidence, either way, to tell you what to do. I can tell you the evidence does show that there is some signal, that there might be some benefit here, but, specifically, the probiotic that you're choosing to take, I have nothing, I have no guidance to give you on that, specifically." I have been using Kefir a lot more, so, you know, not a probiotic, according to the strictest definition

in undefined consortia. It's just a yogurt drink. It doesn't taste particularly good, in my opinion, but my patients like it, and they drink it when they have C.diff, and we have some uncontrolled data. Again, the (inaudible) case series, suggesting that there maybe some efficacy there. So, I certainly don't stop them, but I take kind of a more balanced approach of -- I don't even have to bring it up, and part of this, also, is I practice at the University of Michigan, in Ann Arbor. We draw from wide catchment, but a lot of our patients are, you know, educated.

Some of them are coming in with notes and printouts from web pages that they've researched. So, it's a different crowd, but, usually, I don't have to bring it up in clinic.

MR. CARLSON: Any other questions? If not, I'll invite the eight -- you have one more.

MR. AUNINS: Well, I will say that there have been studies looking at fresh and frozen, as we've heard, and the frozen preparations. There will still be spores, spore fraction in that

stool. So, they're -- it's not killed, per se, but it's certainly reduced in terms of the vegetative contact there, but then, also, people have -- recently, there was a case series. I don't remember which group it was, but it was about five patients, I want to say, that were successfully treated with FMT. These were fecal filtrates, and they were submicron filters, where they actually tried to do cultures afterwards. They weren't able to culture any bacteria. So, certainly, there could have been viral particles, and other microbes in there, but not bacteria, and those patients were all cured. Now, that's just a case series, again, uncontrolled data, a series of five, but I don't know that we've established, completely, that microbes, themselves, are the necessary component of stool, when it comes therapeutic effect.

MR. RAO: Just to add to that, you know, and Seres was trying to develop our C.diff drugs. We wanted to understand whether it was the bacteria or not, and, so, we did do animal

studies, where we took the material, the spore fraction, 0.2-micron filtrates, 300 kilodalton ultra filtrates, two kilodalton with revolt rates, and, basically, you saw elimination of the activity, once you take the bacteria out.

MR. CARLSON: Okay, so, with that, I'll invite the three speakers from earlier up on to the stage, too. We only have two. Oh, we had one that had to leave. Okay, two speakers from earlier, and my co-moderator, Suzy's going to come back, and we'll have a discussion of all of these topics.

DR. MCCUNE: I just will say that, unfortunately, Dr. Neu had to leave us. He had a plane to catch, and, with all of the plane issues going on, didn't want him to, potentially, miss his flight. So, unfortunately, he won't be joining our panel, but we have five panel members. Do you want me to start?

MR. CARLSON: Yeah, go ahead.

DR. MCCUNE: All right, so, we had talked about having a number of questions, for our

panel members, but we would like to encourage folks from the audience. Earlier, there was a good discussion that was going on this morning already. We have three slides worth of questions. This one is the most packed; first one, talking pretty much about the microbiome; the second one, really talking about organisms in general, and then the third, really, about logistics, which is kind of the areas where I think we've been headed this morning.

So, while you're coming up with your questions to ask the group, and, actually, I would encourage if the group has questions for each other, to think about that, but what we had thought up front, in terms of just talking about the microbiome, under all of the different circumstances that we've heard this morning, is how do we characterize the path of physiology of all these different illnesses, with respect to the microbiome? Do we need to personalize therapy, based on an individual's microbiome? How important is the strain selection, and the

treatment of a given indication, and for products with bacterial consortia? How important is strained synergy, and we'll get a little bit into the strains, in the second slide, as well, and then how do we differentiate treatment from prevention, and then how can current associative data be used to support clinical decision, and or to advance a development of new products? So, we'll throw all of those out there. You know, it's kind of -- you can pick a question you would like to start with. You can ask one of your co-panelists a question, and I would like to encourage the audience to come up and have conversation. We are probably going to stick with this for about 15 minutes or so, and then kind of go on to next group, but --

PANEL MEMBER: I'm happy to tackle a little bit. Less on the microbiome, as it relates to gastroenteritis, but more on the pathogens, which I think is really important. So, the advances, now, in diagnostic technology has been great, and, so, there are multianalyte syndromic

panels that are available on the market. They have their own challenges on the clinical side, but from a research side, they do enable good characterization of the infectious agent because not all diarrhea is the same.

You don't always even find the pathogen in -- I think the comment was a lot of C.difficile infection referred for fecal transplants aren't even C.diff, and they've got other diseases. So, I think that really helps us talk -- know what we're talking about, and that we are able to separate apples from oranges, and be able to figure out probiotic or agent disease, meaning which pathogen is actually causing the symptoms, is really, really important, I think, in terms of where our research should be at, at this point, in terms of gastroenteritis, and just kind of broad treating. Broadly treating all of them the same is probably not the way to go, and some of the studies that we did, we're actually now looking at that. We have that data. We're just getting into deeper analytics on that, and then the other piece

is, also, to characterize the disruption in the microbiome, and then the healing from the acute episodes, as well.

So, we've been collecting stool down, five days down the road, and 28 days down the road, in these kids, after the randomization of probiotics, or not probiotics. So, we can start looking at the impact on that, as well.

PANEL MEMBER: (inaudible) a little bit, just again, that, to talk a little bit about the IND issues, and I was really happy to hear the discussion that was earlier, that talked about how, with dietary supplements and foods, that we really don't need to have structure -- INDs for structured function, endpoints and studies, which I think is a huge declaration to come out of this, and I know it's part of the guidance documented. It was just good to have that reaffirmed, and that, you know, use of endpoints that are focused on reduction of risk of disease, also, probably don't need an IND, necessarily, and that's great.

I guess the one other component that I

wanted to talk about, in terms of the insistence that human studies and probiotics be conducted under INDs, has to do with is it the guidances and the FDA stances that have occurred in the past, that I think are, you know, cast the net quite broadly, in terms of how they view drug endpoints, and now I -- granted, there is a definition, treatment, cure, prevention of disease, but I do think there have been some judgements that FDA has made. For example, the example of antibiotic associated diarrhea was brought up.

So, right now, in FDA's mind, or interpretation, any substance that's used to prevent side effects of antibiotics would, in itself, be considered a drug application, and my point is, is that, that's actually a judgement call on FDA's part, and I think to the extent that studies can be conducted in reasonably healthy people, that are safe studies, on endpoints that you may be able to consider structure function, you may be able consider disease, that, to the extent that they're safe and it can pave the way

for innovation in the food and dietary supplement category. My appeal is just to see if the FDA would be willing to just consider those things a little bit more broadly, so that we don't have such a narrow view of what a structure function claim is and what (inaudible), versus a very broad view of what is encompassed on the (recording fading out)

PANEL MEMBER: I'm not a clinician treating patients, but, I guess, you know, I'm a bit mystified, I have to say, coming from a, you know, person who's worked in the pharmaceutical industry for around 28 some years, about the, you know, the seeming confusion, and, I guess, my question back to you would be what do you want to do with the information, and it seems like what you want to do is you wanted to make a claim about a treatment or cure of a disease, and if that's you do, you know, it's the old drag racers' run what you brung, put up or shut up. You know, do the rigorous trials, under IND, prove efficacy, prove safety, and show that your product's under

control, and get a license.

PANEL MEMBER: No, and I completely agree with you, that there is -- conducting a trial, not under the IND rubric, does not compromise safety or appropriate design of clinical product or product definition. All of those things are assumed.

PANEL MEMBER: You know, doing studies in healthy people who are seeking to, you know, have supported organs or better, this is different than doing it in somebody who's diseased. I think that's --

PANEL MEMBER: No, and that's a fair decision in itself.

PANEL MEMBER: -- and there you need that -- you need to take care, and you should have a lot of -- a lot more controls, that really call for IND filing.

PANEL MEMBER: And I'm not objecting to that. What I'm -- when you said, what's the goal? The goal is to provide dietary support for people who need it, either healthy people who are at risk

of developing something, or someone who may be considered generally healthy, a child going in for an antibiotic for and ear infection, that may be able to use dietary support to be able to prevent the development of some kind of side effect, or worse, where you might get -- you get, you know, some kind of pathogen emergence because of the antibiotic treatment, and if a dietary approach that doesn't require getting a prescription, and something that's generally available, as long as it is safe, and you want to look at it from the research point view, and you control the study properly, you make sure the safety is there, and the manufacturing is appropriate, I don't think that it serves anybody the course that, into the drug rubric, when the intent is never to market a drug. The intent is to market dietary supplement.

PANEL MEMBER: But, we can probably continue this over a lot of beers, but you still seem to be going back to -- you're talking about at risk populations, and so --

PANEL MEMBER: Well, I mean it's --

PANEL MEMBER: -- if there's an at risk, then --

PANEL MEMBER: -- I'm saying either, prevention in healthy, or there are at risk, but we currently allow foods to address at risk people. I mean, an at-risk person who -- with lactose intolerance has to eat the lactose reduced foods. They are at risk for developing symptoms from consuming too much lactose. A person with high cholesterol is considered to be at risk, but that's a general population targeted group, where you can use foods to address that, and my point is, is a child taking an antibiotic for an ear infection, is at risk of developing some kind of intestinal potential problems, and I'm not saying there isn't room for drugs. There obviously are. I'm just asking for there to be more room for dietary support for conditions like that, but you are absolutely right. You have to have the same, you know, you have to have good control of the study. It has to be properly designed, properly powered, all of those things.

I'm not talking about study quality here. I'm talking forcing it under the drug rubric, where it -- there really isn't intent.

PANEL MEMBER: So, can I just add into this mix? I would love to hear a further discussion of -- one of the questions that's up there is about the strain selection, and what are you using to be able to do the studies that you wanted? Now, I --

(inaudible)

PANEL MEMBER: -- no, because that -- you want to do studies, but I'm just curious as to how you do the strain selection associated with that?

PANEL MEMBER: There's many different ways to go about doing strain selection. I think we heard some preclinical type studies that have been conducted, already, today. To me, that's the science behind it. You develop your hypothesis base whenever -- whatever preclinical data you have, but, to me, that's not really germane to the regulatory conversation because you have to

determine safety of whatever intervention you're going to define, or dietary intervention you're defining, and I'm not sure why it's important to tease apart the exact rational for a particular strain to be chosen.

PANEL MEMBER: Safety is part of your strain.

PANEL MEMBER: Sure.

PANEL MEMBER: Right?

PANEL MEMBER: Oh, no, of course, yeah. I mean, that's -- I'm sorry if I didn't make that clear. Obviously, you have to choose strains that are safe, yes.

QUESTION: May I ask you a question because I really -- what are you proposing to develop? Is it a food with probiotics in it, like accepted probiotics that we know is -- I mean, lactobacillus GG oswedus, or is going to be a food with some other strain, but don't have that long history that we have with probiotics, like strains, from, like, someone's gut, or something? That's what -- what are you exactly talking about

doing?

PANEL MEMBER: Oh, I think what I was talking about is more kind of the old school stuff, in terms of set strains that we know quite a bit about the safety. We've got good history of safe use. They're on the European QPS list, okay, were you know what's going on with them, for the most part, in terms of a safety assessment, but I think the broader question could be relevant to next generation probiotics, where you say, "Well, then, if we do find some halobacterium, or something that looks interesting, would that be an appropriate addition to a food?" but, that, I don't think is such a difficult question. You just have to go through the proper safety evaluations, and you have to submit that (audio faded) class act, you know, affirmation or a notice, and get a ruling on it.

PANEL MEMBER: Alright, and you wanted to address one of the questions.

QUESTION: We have a question from our overflow room.

PANEL MEMBER: One minute.

PANEL MEMBER: I want to address the question. Do we need to personalize a therapy based on individuals' microbiome? I get that question a lot. It's been in the news a lot after the two Cell studies, and I think it's a sort of a red herring question. I think the answer is clearly, yes, we would love to do that, but to pretend we're anywhere close to that, in medicine, is really to -- not to understand what we're doing in medicine. We do that, and people can just -- treated me for a few Cancers, and that's almost it.

You know, people have been talking about it for 20 years. You're going to get a genetic test, and then I'm going to tell you if you take a betablocker, or an ace inhibitor, but we're not close to that yet. So, for a few Cancers, we do it, maybe sometimes for IBD, and I know we looked, maybe not, even, but it's very few things we personalize treatment for, and to pretend that you need to do that for probiotics, I think, is

inaccurate.

Now, I think it would be great, if we could do it, but, we're, you know, we're probably 50-60 years away from even being close to that. So, I think, it's a, and again, I think it's more than addressed because I get that question all the time, and often as people, like the self-authors, who are selling sometime in personalized medicine, and then publish something that says you need personalized medicine, which is a little suspicious.

QUESTION: LD-30, go ahead.

QUESTION: Hi. This is Joella Woolston. I'm from a company called Intralytix, in Baltimore, Maryland, and I was hoping the panel could address the question: how do we differentiate treatment from prevention? The question is specific to --

PANEL MEMBER: I have the same --

QUESTION: -- okay.

PANEL MEMBER: -- same question that Mary Ellen had earlier. Maybe I'm unable to ask

it eloquently. We are not against saying that it has to be a characterized strain of -- the CMT has to be wonderful. It should be 100 percent safe, but, then, if it is, I can give an example of vitamin D. So, if you give it in really high dose, of course, it has to be on a prescription, but you're still selling it over the counter. People can take it in small amount. Imagine something like that, that it hasn't reached that stage of development. Are you going to stop vitamin D trials and research, and demand that all vitamin D research should be done under an IND, or would you still let, and vitamin Bs, all other vitamins be sold? What do you see, and at the same time, concurrently, double up high dose vitamin D as a drug for a particular medical ailment?

PANEL MEMBER: I think -- very different situation. I mean, you're using a vitamin as a comparison, here, and I just want to -- this is also off topic, but the panel is meant for discussion with our panelists and our speakers,

not for direct questions to the moderators and to the FDA. So, let's go ahead and address the questions.

QUESTION: I have a question for the actual panel. Are you guys ready? So, I represent an organization that works for advocacy and education for a peaceable -- and caregivers for people with C.diff. So, I feel like the FDA has drug its feet on determining what a fecal transplant is, or what that is, as a product. Is it like blood? Is its own thing? Is it a drug? Is it -- and, maybe, it's potentially all of those things, but what I would be interested to hear, from each of you, as possible, and I think probiotics would fall under this, too, is like, would determining a designation of these things, what would be the pros and cons of that? Like, would there be the pro of, like, this is its category, we understand it, we can move ahead with it, and would the con be this will limit us in the way that has kind of been addressed, in talking about some of the probiotics, with like, "Well,

this isn't a drug. It's a probiotic, but if you want to use it this way, it is drug." Because, as a lay person, this all sounds really arbitrary. Like, it doesn't come across as driven so much by science, as by arbitrary rules that -- we could get into how those rules get established, but it doesn't feel very science-y, it feels very lobbying indeed. So, just curious what you think the pros and cons are?

PANEL MEMBER: I'll just start with that. When I obtained the IND to do my clinical trial, and I do it through, at that point still, the pre-IND process, and I didn't really understand what I was doing. I was kind of going for -- I really looked at fecal transplant as a transfusion, or as a -- almost like you would like at an organ transplant, and, you know, you would screen a donor, make sure they don't have any underlying diseases, or any communicable problems, but I was told, that, no, it's drug, and it's a biologic.

For this reason, and my understanding

was that because it is excreted, and things that are excreted can't be in the transfusion paradigm, and, you know, correct me if I'm wrong, but I think a lot of us have been thinking about this, and thinking that you're right. We're kind of trying to kind of jam, sometimes, like a square peg into a round hole, making some of these whole stool very complex FMT's, you know, what we're talking about, like multiple microorganisms, and how they might be interacting with each other, you know, coming from these, you know, fresh stool from donors, and what we can learn from that, and trying to say that that's a drug because it's very difficult to have it, the identical batch per batch, and all the things that you have to do to have a drug.

I know, in the audience, here, and whether -- you know, I was part of a working group at University of Maryland, that Diane Hoffman put together, to kind of talk about going forward, and had regulatory aspects of gut microbiome therapeutics, and, really, the outcome of that was

published, last year, in Nature. I think it was very interesting to look at, maybe, whole stool FMT, done from -- just like a patient of mine, who wants to use his wife's stool to treat his C.diff. That's the practice of medicine.

The FDA doesn't need to be involved. It's me. It's my hospital. That gives me privileges. It's my state medical board that makes sure that I'm doing things appropriately, and then as you kind of move up the ladder, and things become either more characterized, or, you know, you go to the level of, like, open biome that has a stool bank. Obviously, they're doing a great job, but not -- you can't trust that anyone couldn't open up a stool bank and just start shipping stool all over the place.

There needs to be some oversight, and that might make more sense in the kind of tissue transplant, almost transfusion paradigm, and then, as you get into things that are more and more characterized, that you're looking to encapsulate, and sell as a drug, then, maybe, that would make

more sense for, like, the IND, and the typical drug pathway.

So, again, so not my idea, it came out of a huge working group, but that was really, you know, kind of put together as, maybe, one solution to all of this.

PANEL MEMBER: Hi, sorry, if I can just also talk about probiotics a little it, and maybe, I think, the reason -- even though we're talking about probiotics, actually, in a way we never talk about antibiotics. We don't talk about antibiotics are good for, we talk un-antibiotic, a dose, a regimen, a duration, and an indication, and we really talk about it that way, and, actually, I think -- once again, I'm willing to get things thrown at me.

I think the probiotic industry, the way it's marketed of, it doesn't make sense to most clinicians that all 700 probiotics available at Wholefoods, today, are all good for everything, and, if we are not -- I mean, I'll say, I think they should be studied, well, rigorously and

regulated, and they should be regulated like a drug, and then, you might be able to get at some of Dan's comments of why aren't we using them because I think the way that the industry has set itself up, it doesn't conform to the way most clinicians like to think of. A probiotic is good for this, in this patient, for this long, at this dose, and I think, as long as we talk about probiotics, like, for one thing, I mean, there's how many, 10 million in our, trillion in our body, sorry, and number of brands out there. If we talk about it like that, as -- to clinicians, I think we glaze over because it just doesn't register with us, the way talking to me about his drug for this treatment, in this patient does, and I think that I actually would encourage regulating it more.

I hate to say this, but the more we regulate it, the more people will use evidence-based therapies, and feel comfortable in them.

PANEL MEMBER: So, no, no throwing

stones. I was just going to elaborate on one other reason why. I don't envy the role of regulators, when it comes to fecal transplant, because, you know, on the one hand, don't do this, but if you were to Google fecal transplant, and go to YouTube videos, you will see that people are doing this stuff, and sometimes it's very sophisticated, sometimes turkey basters, and things are involved, and the other thing that I didn't mention is, in addition to taking probiotics, a lot of the patients that I see, have tried fecal transplant on their own.

I don't know if you've encountered this, but it's not super common, but it's getting more common now than it used to be. I think, a large -- to a large degree, because this is kind of a limbo zone, and it's not clear, and the patients are desperate. They do get desperate that, you know, there is enough regulation of this, that it's not, you know, wide-spreadly, you know, it's not available at your local community physician for a lot of patients. So, that's why they're

coming to places, like universities, to receive this treatment.

So, on the one hand, you know, there is unregulated use happening. On the other hand, although, I think, it is safe in the short run, is, you know, from the data that Colleen showed us, that in the long run we don't know, and I tell my patients this, that I'm treating your C.difficile, right now, but I don't even know how to answer their question of whether 10 years down the line, did I give you Diabetes, did I give you higher risk of obesity, or cancer, and those are, I think, that's one of the roles of really trying to characterize this, and establish, you know, better precautions, and actually be able to answer some of these questions that regulation can have.

PANEL MEMBER: Two things. One, I agree with most of that. Although, I'd say that, you know, we don't know that for lots of things. We're just learning that statins now, one in a hundred people get Diabetes from a statin. You know, we didn't know that for years. We have

everyone on a statin, right? So, you need to follow these long-term, as you called it. I would say, when you get back to over-regulating probiotics, that the way it's set up now, and the way they're defining on an IND, is I can't actually go to the supermarket -- I can't go to Wholefoods, and pull a product and try to study it.

I've talked to the FDA about that. Unless the company wants to work with me, and help me get the IND, so regulating it more, I think, would cause more problems, actually.

PANEL MEMBER: And I would concur completely, as I couldn't get an IND for one of the drugs we wanted to study. So, how you get an IND, is a different process. Let's not go there, but, conceptually, I think, doing studies properly, and doing them, you know, in controlled manners, and under proper regulations is the way to go. How that's regulated, I'm not even going to touch on that, I didn't go there.

PANEL MEMBER: And this really gets into

some of the questions we have. I've switched to the second slide, here. On this slide, talking about how do we ensure that the product is what the product supposed to be, the high quality and reliability, in terms of, consistent manufacture and purity, and then we can get into the some questions about symptoms verses inpatient, but, again, I think, regarding, it's best to (recording fades out) I would like to hear, at some point from those who are conducting clinical trials for FMT products, that -- to weigh in on some of these questions (recording fades out)

PANEL MEMBER: So, I think one of things that I've heard, in all of your talks this morning, whether it's NEC, C.diff, you know, antibiotic associated diarrhea, and I think also the paper that was published at the Watson Institute, last week, started to, I think, open up the question, as far as why are we seeing efficacy in one sub-population, you know, and potentially damage in another, and I feel like we've amassed a substantial amount of data, but we keep just

trying to do studies to understand efficacy without looking at some sort of diagnostic tool, or resp -- like really digging into responder, non-responder dynamics. So, I guess, my question is would a tool like this be helpful, and, obviously, it would have to be condition specific to some degree, and maybe gender or population specific, but do we have, I guess, the questions are: do we have enough information in the field, right now, to start creating some of these tools, like they did in the Cell paper, where they created an algorithm, based off the experiment they ran, and then, you know, validated the algorithm. So, can we start moving in that direction, as a way to, kind of, overcome some of the challenges that we're seeing from a personalization perspective, and help prove efficacy in a better way, maybe help you guys out a little more?

PANEL MEMBER: Well, yeah. I mean, I'm not the microbial ecologist on the panel, but I, you know, I will say that, I think, we're not at

that stage, yet, and I think we might have the tools that it takes to get there. I mean, you know, as has been brought up, there's the whole idea of anatomy versus physiology. It was on Dr. Young's slide, and, I think, and, John, you talked about it, about how the structure of the community is something -- is one thing, but you can have lots of different structures that functionally perform the same functions, and look the same, and, so, we're very good at -- we've gotten very good, I think, with next generation sequencing, at looking at structure, at looking at 16S, but we're not quite there, yet, when it comes to bridging those various disporous structures, and figuring out what the functional phenotype is from a different set of structures, just yet. Maybe, once we get there, I think we'll be able to make better progress at individualizing therapeutics, but not, not quite yet.

PANEL MEMBER: Yeah, I guess I would just add to that, by, you know, again, going back to what I was trying to say about the sort of the

bedside to bench to bedside paradigm. You need to do the interventional controlled trials. You need to be taking specimens, whether it's stool specimens, tissue specimens, and then you have to use all of your powers of analytics to try to figure out what's going on, and why things work, in order to really build a true understanding, and yet, every -- you know, that's what we are trying to do at Seres, and I would assume, a lot of other people are as well.

PANEL MEMBER: As I was mentioning, I mean, we want to diver deeper into our negative studies because there may be populations in there, whether it be pathogen or response, that will actually tease out. Maybe there are certain responders in here, and who those are. Then, down the road, you can decide whether you use that therapeutically or not, but I think 100 percent. Actually, negative trials are almost more important to find out why it didn't work.

PANEL MEMBER: All respect to our hosts, I would like us, for just a moment, not to talk

about regulation, and remove that, and have a philosophical argument because we have this tension here. I hope it doesn't take 56 years before we can do precision medicine, okay, but there's a tension between doing science, someone already said science, as a bad word, you know, has become, has this other meaning about being science-y, et cetera, but how do we balance the time it takes to do studies, both preclinical and clinical, and amongst the clinicians, the desire to do something for our patients, in the here and now, and what have you guys used to try to kind of -- there are two opposing things.

One takes longer time. One, you have a patient sick in front of you. What have you been doing to try to kind of balance that sort of tension, outside of regulation? Okay, forget about whether or not you get in trouble doing it. How do you balance it?

PANEL MEMBER: So, to me, that gets to where you do the -- the question was asked, number needed to treat, versus number needed to harm, and

I wish Dr. Neu was here because that's, to me, is the question when it comes to the -- to Nec issue because, as long as you can get a safe product, which I -- there's no one going to disagree with that. You need a safe product that's not contaminated, that's been studied. It seems like that's the kind of thing where you look at it, and you say, the number needed to treat, versus the number needed to harm, and you need to try experimental, before the evidence is there, and I would agree the evidence is not there for all the things, but that to me is a clear-cut thing, and, I think, it's same with FMT, but, you know, and lots of other indications.

PANEL MEMBER: The other thing that going on a bit more in Europe, than it is in North America, I think, are registry-based trials. So, I think, there are certain ways of capturing a lot of data, and actually I'm starting to plan a clinical trial of a diagnostic device, but I'm going to embed it into care.

So, it's going to be embedded into care,

it's not going to be a therapeutic option, and then collecting the right data to answer the questions at large scales. So, I think about -- registry based trials are probably the wave of -- thing to look forward to of being able to capture all the patients who are getting FMT's or C.diff at an institution, or health care networks that, I think, capture a lot more data.

PANEL MEMBER: We actually have an FMT Registry. I'm glad you brought it up. That was a good plug. No, so there is a National FMT Registry, that's NIH funded, and that's kind of a joint collaboration between the GI and ID Societies, and we're hoping to get --

PANEL MEMBER: Brilliant.

PANEL MEMBER: -- 4,000 patients. The problem is, a lot with -- I mean, one of the difficult things is, you know, it's very expensive to follow patients for up to 10 years. I'm like, how are we going to retain them? How logistically is this going to work, but we're working really hard on, kind of reaching out to the patients

through apps and emails, and things like this, but our hope is that having, you know, being able to follow and get that real world efficacy data will help answer some of these questions, and have a bio- bank even tied to that, so that if something does come up, we can, like, reach to that stool, and say, "Okay, this person developed this unusual condition. Was it something form the donor?"

PANEL MEMBER: And there were some patient advocacy groups here, and actually they were asking how you can help. I actually think getting patients to be willing to participate in trials, and to actually be willing to have their data collected, integrated into registries, is crucial, and that's one of the biggest barriers to research, period, is declining to participate in clinical trials, and then even declining to have data used, in anonymous DI identified registries, and, so, patients should advocate for this, if that's what -- I meant -- well, I can't you what to advocate for. I think it would be an important thing to consider advocating for.

DR. MCCUNE: So, let me just chime in one second. I'm going to move the -- to the last slide, for us, just so we -- I have, oops, sorry, just so that we have all of the slides up there, and this one gets to some of the logistics that we've been talking about, in terms of how do we address the lack of equipoise from -- by some health care providers, and the ability to conduct clinical trials, some questions about funding needs, and, then, how to take advantage of some of the networks, and some of the registries that are out there.

So, I just wanted to throw, so that's the end of our kind of answered questions, so to speak, but we just wanted that to be out there as you're continuing the discussion.

QUESTION: Hi. I'm Lee Jones. I'm with Rebiotix, and I have been in involved in conducting clinical trials with human stool derived drug products, but I just want to remind everybody, kind of, how this got started in the drug world. So, early on, we connected with the

FDA, and asked about, you know, Colleen was talking about what, you know, how it was categorized. The FDA said that because they're organisms, and they're not human tissue and cells, that they don't fall under the human tissue and cell regulations, and, therefore, it was a drug product in involved in Seiber.

We've conducted multiple phase two trials, and I just wanted to remind everybody that there hasn't been kind of any finalized, formalized, you know, products that have been regulatory approved, at this point and time, I think, anywhere, and, so, we're all in early, early stages, looking at it, and I want to echo the fact that we do need these clinical trials, and I think it's a little bit disingenuous to put things up there without the context, when you're trying to compare non-regulated to regulated studies. I think, we're early, early, early, early, early, and that's my main message, is that it's hard to draw conclusions on anything, when there's -- it's so early in the thing.

So, I just want to remind people that there is a process. People are going through that process, and I think, at some point and time, more data will be available. It's not going to be be all, end all. I think there's still a lot more to discover.

PANEL MEMBER: Oh, I just wanted to comment on a previous comment about -- there has been a lot of discussion about number needed to treat, number needed to harm, and the idea of harm, and I just wanted to remind, or comment that, I think a portion of harm that's underappreciated is when patients are doing something that they think is efficacious, but isn't actually efficacious because then that delays treatment of something that actually could have helped them. So, I think that's important to keep in mind, and, so, we do want patients to be using things that we -- when we -- as physicians, we make recommendations. We want to have some sense of trust behind those recommendations and what we say, and I think it erodes trust if we

recommend something that isn't necessarily a specific product, but it's more of a category of product, with variable efficacy, some of which may help, and which don't help, and especially if it's a very serious condition, and the patient is delaying or avoiding some other potentially efficacious, no improvement efficacious therapy, in leu of something else, but that would be my opinion.

PANEL MEMBER: I would agree. Although, I would say the data, from choosing wisely and stuff, is that we overtreat people. So, maybe staying away for the doctor for lots of things is pretty beneficial.

PANEL MEMBER: And then, just about when patients come to me, and most of them come to me after they've suffered, you know, two, three, or more reassurances, and I do have availability and accessibility to some clinical trials. Some are open label, the others, the finished study, that's starting to, you know, looking -- another capsule study, and I give them the choice.

If they are a candidate for a trial, I give them the choice to be in it or not. I don't feel like I should, you know, force someone to take a placebo for something. In my -- I'm bound by my relationship with that patient, you know, and what they want to do and what's best for them, and not, you know, this greater good, you know, of helping this company develop their drug, or, and there are definitely patients who aren't appropriate for FMT.

I see them. There may be limited life expectancy, very frail, and I maintain those patients on just Vancomycin. I said -- maybe, for the rest of their life. That's, you know, six months to a year, but I think that the position I'm in, you know, I conducted that placebo controlled trial, double blind, as best as I could, did not just convince, I guess, the world, didn't convince myself that I wasn't imagining that this worked as well as it did, and I can say, there has never been anything that has surprised me more than FMT, in some cases, where there were,

you know, people, severe, complicated, septic, in the intensive care unit, and, after two or three doses of stool enemas, were turning things around, and, you know, I think it's very hard to go backwards from that, and to say, "Okay, well, now, we need to kind of pull back. Let's get some animal data. Let's figure out what strain." and I'm not saying that's not important, but I don't think we should lose the momentum that we have in combating this epidemic.

QUESTION: I have a question in 1DR6. You can ask your question.

QUESTION: Hi, there. This is Richard Ethier, from Lallemand Heath Solutions. Just a general point on the IND restriction for dietary supplements. Industries sometime --

QUESTION: My name is Jerry (over talking) Crones and Colitis Foundation. We're a patient advocacy group, and research funder. So, I wanted to address the question, how to address funding needs and also return to the lap --

QUESTION: Yes, well you didn't --

(audio is breaking up)

QUESTION: -- so all of you are focused on advancing a certain type of microbiome based product, through -- and the use of your resources for that differ in different ways in -- and, so, we've discussed the need for a variety of things, like really good CMC, like long, long term registry studies with really good data collection, and RCTs, and all these things have a role, and we've also just learned that there can be a variety of different regulatory pathways that a microbiome-based product can take, even, in the direction (audio fading in and out)

PANEL MEMBER: So, given that, we've seen one beautiful presentation about how a CMC process can be developed, and then it seems like something is that's very resource intensive, and that's presumably incentivized by the way in which the eventual product would be paid for and regulated. For those of you that are seeking resources for products that don't fall into that drug category, do you -- is there a way to address

the funding needs? I mean, it seems woefully inadequate to address these challenges that you're talking about with the current mechanisms that we have for funding. So, I just wanted --

PANEL MEMBER: Here, the only thing -- I mean, I go to -- for Federal funding, generally, both in Canada and the U.S., and the one thing that I did notice, and I don't know if anymore knows more about it, but ENCAM has stopped funding RCTs of probiotic research, and I don't know how that's impacted people down here, but that was quite surprising to me, given that they would seem to be the natural institute to do it, and I don't know if that plays into Dan, some of your noticing a reduction in clinical trials, but they have made it very clear that there are (inaudible) generally are no longer eligible for FCTs, or for probiotics.

PANEL MEMBER: Yeah, they did fund my first two, and now they're funding the mechanism trial, but they don't fund RCTs for it, but I think that's for lots of supplements too, not just

for, but it could be, yeah.

PANEL MEMBER: I'm going to make a comment about the regularly environment because I think there's confusion here, at least among some of us, in the way we talk about this, but we need regulation, and the more reg -- that's a confusion because probiotics, food in general. It's not only drugs that are highly regulated. There are processes. The difficulty is that we have a different interpretation of what that process entails and what the conclusions are, and I think it's important, especially for FDA Industry Clinicians, to have, and this is a knife (inaudible) -- but, frankly, those issues are the regulatory issues, FMT, dietary supplements, et cetera, are not going to be addressed in this kid. I'm suggesting that, maybe, we should get together in a true working group to talk about how to interpret structure functions, et cetera.

QUESTION: I wanted to deviate from the questions on the slide. There's already momentum in that direction, and I hear a couple keywords,

momentum. We've talked about confusion, regulation, industry, and I'm standing here from the (inaudible) perspective, probiotic companies. What is the laundry list, from a clinician's perspective, industry needs to meet because we can talk about FMT verses probiotics? We can talk about efficacy safety. We can talk about a whole bunch of things, but I mean, I'm a scientist who works for probiotic companies. We have evidence-based products. We have, at least, multiple clinical trials behind products. I don't work for the company anymore, but what is it that industry needs to do to support the process to keep the momentum going, to allow you to keep working with your patients, and, I mean, that can be anything from safety and better studies, but can you give some specific things that we can work on, not just better products, more evidence?

PANEL MEMBER: You can say that -- when I'm recommending, because patients always ask about probiotics. I see a lot about IBS, and, so, I try to -- if I do recommend one, I will,

usually, for instance, align orbifidobacteria infantis, but they did, they took some initiative and conducted a clinical trial, and showed that it worked. So, I think, if the -- if industry then sells particularly probiotics, puts a little into these, you know clinical trials, I think that's always helpful.

DR. FREEDMAN: I mean, I really think, you know, for some disease process, maybe there isn't a need for more evidence, you know, and Dan talked, obviously, about antibiotic associated diarrhea. I think, for others, you -- we do -- we are looking at phase three clinical trials because, generally, they haven't funded very large. They haven't done them investigator initiated. So, they've been very directed by industry conducted in that manner, and, so, I think, really, allowing for large phase three trials that will definitively answer questions -- not going into the metanalyses of small little subcenter, single center studies where they control the data, but really total independence of

data and access and allowing investigators to choose the outcomes with patients that are important to them directed and dedicated out of the disease process. That to me is really -- I mean, that's what distinguishes -- and then you can start talking about oh, the really, really large clinical trial of probiotic X for drug X as opposed to the generic, you know, hodgepodge of the metanalysis.

SPEAKER: I'm a microbiologist by training and my basic understanding of the (inaudible) evidence in medicine (inaudible) meta-analysis and systematic reviews, but if it's a strong enough trial as adequately powered do we need all the Phase 2 trials?

DR. FREEMAN: Well, there's pretty good evidence from most pharmacologic drug studies that after early days of excitement from early, kind of, smaller clinical trials that might be the in Phase 2, Phase 3, when you get into the large robust Phase 3, 4 trials, the excitement kind of dwindles and so I think that, you know, in order

to be able to even get to the large Phase 3 to make -- people don't adopt so adoption in medicine takes 18 to 20 years from the time a clinical trial is done and sometimes during that 18 year window there's contradictory evidence that emerges that diminishes the excitement of the original ones. And so, there's a lot of smaller earlier ones that might be powered as a single center even a multi-center trial that's powered. How good is your power? Don't get 5 percent of studies will still be positive, but they're actually truly false positives. And so, really I think the rigor of those studies needs to increase to able to support it and so, yeah, I think kind of some of the small -- just because a study is positive.

Let's put it this way, too. How many probiotics are competing in the same field? So, if we do 50 probiotic studies for prevention of asthma, okay, how many of them would be positive by chance alone and then does that company then get to say, "Well our was positive, let's go for asthma." And then another is done prevention of

atopic dermatitis and there's also 50 companies doing studies of that right now.

And the list goes on whether it be autism or other concerns, IBS, IBD prevention of and so the number of studies are massive. So, the number of false positive studies, I haven't done the math, but we need a statistician who can run the data of, like, I can't remember how many studies? There's a thousand some odd --

SPEAKER: 1,142.

DR. FREEMAN: -- this year so 50 some odd positive studies alone this year. Should we adopt them?

SPEAKER: Good point.

DR. FREEDMAN: So, I think we need to be careful when we say a positive study was done and we should adopt IT.

MS. MCKEON: Can we go from the premise that -- I appreciate the word you said robust. You said if we have a well-designed study with a sort of consortium of knowledge surrounding it whether that's pre-clinical, you know, (inaudible)

animal in vitro supporting with a couple of good studies that might not be Phase 3 powered, you know, in the numbers, but that make a logical clinical argument and that show efficacy, would that be enough? Because we also now start to intersect at the level of efficacy regulations, the legal aspects, the manufacturing, the upscaling. There's all sorts of issues that play, but all of that aside, what is enough? And if we're talking about a treatment, for example, we take neck, we take any of the conditions we talked about today. I mean, these are -- patients are suffering here and that's --

DR. FREEMAN: I guess the question is what do you mean by enough? Enough for clinicians to adopt? Actually, generally, clinicians will adopt. I find when the pharmaceutical reps drop the samples off at their office, the patients adopt because they're given free probiotics and they come into the emergency on probiotics because their family doctor gave them to them. So, I don't know what you really mean by adopt. I mean,

this is a longer topic of what drives medical adoption and maybe deeper than we want to go today. I'm happy to chat about it some more afterwards from my personal perspective.

MS. MCKEON: So, I will say that we are technically over our time, but there have been two people patiently waiting in line so if you can ask quickly that would be lovely.

QUESTIONER: (inaudible) Health Solutions and I wanted to ask Dr. Freedman a little bit about -- he had mentioned that one product not allowed to get an IND in the United States and just maybe talk about what exactly were the issues there because as far as I know there weren't any action problems and maybe a little bit about the differences in Canada and the United States with natural health products.

DR. FREEDMAN: Did Dr. Thompkins suggest you ask the question? So, there were the issues where I worked very closely with Lama and Dr. Thompkins on the submission of the IND and they were fully supportive of us getting the IND. The

issue was we couldn't meet the purity requirements by the, I believe it was the FDA at the time. This is going back what six years. And the (inaudible) file couldn't meet that purity requirements and we decided based on the decision made by Lama was the production costs were going to be exorbitantly high to achieve those requirements and the decision was made not to pursue the IND anymore. I don't know if there's something else you want to add, Richard.

SPEAKER: Because it says up there how to take advantage of the pediatric (inaudible) that works. With NHP's in Canada, you're allowed to make, you know, disease claims and run clinical trials. Probiotics are one of the classes in NHP's. I think this could be a very positive network for the FDA to use.

DR. FREEDMAN: Well, a good plug for Canadian research. So, yes, we were able to get NHPD approval and Health Canada approval for the conduct used in the product with the purity data that we had at the time from Health Canada. So,

perhaps it is a little more liberal on the requirements, but I'm not going to go too deep into the politics. Let's stay out of there for now.

QUESTIONER: Hi, I'm Caroline Edeltine. I'm Executive Direct of OpenBiome. We're a non-profit stool bank in Boston. I wanted to begin by saying that were absolutely echoing the panel in that, you know, in the long run the right option for C. difficile patients is to have, you know, a rigorously tested product that has been evaluated and approved by FDA and that we share the aim of seeing enrollment to the trials that will get us to that point.

You know, and on other side, I think we've -- so in the five years since we've started the service of providing fecal microbiotic products under the current policy enforcement discretion we went sent out about 38,000 treatments to a network of 1,100 sites and that's, you know, we're very proud of that work. We're also pretty surprised by it. I think by now we

would have expected maybe faster progress in the field. And so, I think, you know, my question is really, you know, is there more that we can be doing? Doctor, I think you were the speaker who made the point that what's so unusual about FMT is that patients can do this themselves at home. And that part of the tension of balancing access to material and wanting to make sure that there's access to something that's been rigorously prepared and is available through the medical system is running up against this challenge of enrolling these trials and I think there's probably more that OpenBiome can be doing. There's probably more that we can all be doing to navigate that tension and I would be curious to hear the panelist's thoughts.

SPEAKER: I would just like to say I'm impressed that you sent out 38,000 and I'm nine years in and I'm still studying yogurt and I still need an IND to study yogurt. So, I think that says a lot about where we stand in the U.S.

SPEAKER: So, like, (inaudible) at my

side of the University of Michigan we also used your product for our patients and we provided the option so, you know, we have the OpenBiome product available and we have directed donor stool available as well and basically, shortly after we introduced your product, essentially nobody wants to do this through directed donors. And before that, that was the only option was we had to have the patients go and find their own donor and find their own stool and they were forced to ask their neighbor or their pastor if they didn't have a spouse that would qualify and things like that. And so, the patients didn't want this either. It's not like we were saying, "Oh, we have this OpenBiome product and this is the only way to go now." Nobody wants to do this stuff on their own and nobody wants to go use their spouse's stool or their relative's stool or their neighbor's stool and I think physicians appreciate the service you're providing because it helps our patients a lot and it also helps us in that, you know, it's not on us now to ensure the quality of the product

and do all the screening. Even when we were doing directed donor screening, you know, the American College of Gastroenterology there's has been some position papers about what do you screen for as individual clinician and it's an order of magnitude less than what you guys are able to do and what you're able to offer as a centralized repository.

So, I think keep doing what you're doing, look for regulatory guidance, operate within regulatory guidance, but, ultimately, we want a good, safe, effective product.

SPEAKER: All right. Thanks to all of the speakers. We're going to have to call it there. We are behind schedule so let's do a ten minute break and we'll start back up and 3:05.

SPEAKER: Strains based on their under therapeutic potential. So, the idea behind this last session was to look at a rationale selection and have speakers address rationale selection of strains and we'll hear a number of reasons for that based on, you know, modulatory properties,

resolutions, CDI, resistance of colitis, and even behavior modification. So, there is going to be some preclinical work and some early development work. So, we've organized this and thanks to Neil, one of our speakers, had a great suggestion. We've organized it to go from talking about bacterial consortia to a pivot on how to isolate individual strains and then some examples and we've already heard a little preview to our final speaker on a LBP to prevent sepsis.

So, with that, I'm going to introduce my first speaker. His name is Burnette Oli. He's from the Dante of Bioscience. He's the Chief Executive Officer. I met Burnette back in 2013 actually whenever we had a similar meeting and it was just after the release of the 2012 guidance on LBP so, with that, I'll introduce Burnette who's going to talk to us about drugs based on rationally defined bacterial consortia. Thanks, Burnette.

SPEARKER: Thanks, Ryan. So, the audience is very sophisticated for the average

microbiome discussion. I'll skip all the background other than to say that when I use the term defined bacterial consortium to be precise what I mean is that we make a product based on multiple bacteria that are made starting from pure chromosome banks, not from the source material from the fecal donor. The way our drugs work I think should also be very easy to understand to everybody in the audience. We give them in capsule form as a lyophilized powder. It's been freeze dried. The capsule releases the bacteria out through the stomach and the bacteria can colonize the intestine. In our hands, the colonization is important and if the bacteria are dead and if they've given dead, they no longer work across the range of problems that we study. And we also see that depending on the bacteria that we peak, we can see them at least in the range of immune responses in the mucosal surface of the intestine including both the immunoregulatory and immunostimulatory responses.

With the cofounders of the company,

we've done, over the last few years, a range of work to try to systematically understand and explore which groups of bacteria in the intestine stimulated which types of immune responses. So, for example, starting on the right of this slide this is work that we've done with one of our cofounders, Dr. Kenya Honda, now at the University of Kenya where we have defined groups of organisms that are protein inducers of regulatory t-cells and we are exploring this biology in the context of IBD in partnership with J&J and also theologies and all the way to the left of this slide you can see counter examples where we found bacteria also from healthy individuals that have opposite properties. They have the ability to inducing the Th1, Th17, or cytotoxic cd8 t-cell responses.

In the continuum of the approaches that are being pursued in the field, and that slide is not meant to be comprehensive, my view is there is a fundamental tradeoff when ecosystem effects in specificity. What I think ecosystem approaches, like, for example, fecal transplantation, bring to

the table that's really unique is the ability to do something that would be very difficult to do without (inaudible) modalities, which is change the composition of the (inaudible) microbiota in a somewhat controllable manner. I'll say controllable in quotation marks.

And, of course, at the other of the spectrum, if you go full reductionistic, you can gain in specificity that you don't have with a fecal approach, but in our hands we've seen that come at the cost of losing the poly pharmaceutic effects of microbial communities and also the ability to robustly change the composition of the microbiota.

You know, we ask is can find some intermediate stage where we can still retain the ability of a community of bacteria to change the composition of the gut, but do it in a controllable manner with more specificity. And here's an example of some more work done. For example, looking at bacteria that can (inaudible) regulatory responses. In short, finding that you

can identify a number of bacteria in the human flora of subjects across the world that have the ability to use regulatory t-cells. It's only a certain assemblies in consortia that can really saturate the phenotype in animal models.

So, I think this brings me to a question that I think this is a good forum to bring up, which is how do we think about the contribution of different components of a drug after the final activity and a lot of work that we've done in the field has called my opinion on that and I'll emphasize its opinion. We've done often top down work where we start with the full fecal community. We say that that community has the ability to change the phenotype. For example, Th1 reduction or Th17 reduction and then we'll scale back and find when do we lose that activity. We usually like, as you can see for example in the middle panel, we cannot identify fractions that are equally active or sometimes are more active than a full fecal transplant exaggerating a given phenotype.

And then there's a tricky bridge to cross when you try to really bring down that activity to the absolute minimum number of bacteria, but we often found in our hands that we've seen that when we've looked at different phenotypes for (inaudible) induction it that we are really low in membership of diversities of species in a composition we'll see often the effects wash away. And so, the thing that suggests is there an important role for ecological redundancy within a construction unit to help give a product or physical composition the best chance of success.

And the reason I thought I'd bring this up is, you know, in other contexts of, you know, we had the discussion of is this a combination product? Can you draw a parallel with say a multi-component vaccine and I see a fundamental difference in that if you pick the example for example of a multi-component vaccine every immunogen is there for a reason. You know, they are targeting a certain pathogen so justification

is straight forward.

But, when you try to change an ecological community, the issue of redundancy or the aspect of redundancy comes and also, the specific contribution of a strain is actually going to change from patient-to-patient depending on the study of microbiome. It is not really an inherent property of the component of the product.

This is in a snapshot the process that we follow to debate to try to identify new compositions of bacteria that we define as consortia. Basically, we try triangulate within human (inaudible) in vitro data so arrive at clinical packages that give us confidence that we're not just chasing a correlation but there's actually some evidence of causation, but, at the same time, we're not over relying on anymore models and chasing a causation pattern that has not validness to humans. We interrogate human data sets from studies that we sponsor or collaborate with clinical academics across the world where we try to identify if often in the

context of using fecal transplantation for a range of conditions there is a pattern or any pattern of (inaudible) of strains from a healthy donor and we correlate with the clinical response and I'll emphasize correlate because that data by itself doesn't tell of anything about whether those given bacteria may be actually causing a phenotype. If that's the case, then we will often go and find more models and do the systematic experiments to remove and reintroduce a full micro biotic phenotype is obligated and then reconstituted.

And, if we then have confidence that that's the case and that, therefore, we're not just causing chasing association, then we'll ask, "Okay. Which are the bacteria that have the properties that we need to be interested in?"

For that, strain number 3 we've created a very large library of bacteria from humans across the world somewhere between 60 and 80,000 isolates now sequence the genomes of a few thousands of them and also, generated hypothesis to understand and characterize their properties

and I'll show a little bit more later how we do that.

That's gives us hits or in other words bacteria that have specific property that may be useful. Then we still have to figure out how to assemble any consortia that are more important than the individual strain and for that we use a combination of algorithms that we've publishing with collaborators at GMS and also go back to the human data and ask ourselves from all the potential combinations which ones are actually occurring as (inaudible) humans that have a clinical response.

We then have in house our own manufacturing facilities through the GNP production of bacterial consortia. As we have noted before, these are complex procedures. They have multiple ingredients. If you get them to grow then they need some customization. So, we found it best to basically do all this trial and error work inhouse and there's a lot of it.

And then we've moved in one of those

consortia into human testing now and we're just about to announce the results. This should give you a sense of some of the actual activities from culture collection, strain screening, drug substance production, and drug product production. So, starting up on the left, we did a lot of (inaudible) of picking of colonies from donor material, which let's us go from fecal material to actual pure strains from which point on we never again have to go back to fecal material as our source.

On the upper right, you can see some of the screening that we do to test multiple different types of bacteria, all combinations of bacteria, against activity assays or other forms of (inaudible) to understand what the bacteria do.

On the lower left, it's a (inaudible) in the drug substance production so that's in the (inaudible) where we do the (inaudible), separation, and (inaudible). And then on the right you can see some of the activities, which we have in a separate facility with the actual drug

manufacturer, which involves (inaudible) and as John mentioned before there's some challenges associated with that so we have it in a different facility and that's where we produce the actual final product that's going to be bottled and sent to the clinical sites.

We have a range of different projects from infectious diseases, immune diseases including C. difficile, IDD, (inaudible), and immunotherapy at different stages. I'll use the first as an example to walk you as a case study through the steps that we've used. The target for VE303, which is the fine consortia that we are developing for C. Difficile. This is an LDP that is administered as an entire capsule. It has eight pure colonic strains of bacteria as its components that those regimen is repeated oral once day following (inaudible) antibiotic and the number of days we'll treat is one of forms of a Phase 1 study that we are running now. In terms of PK, we believe there is going to be better restricting and absorbed and also, we expect

abundant strain colonization lasting for a window of time longer than the time it takes for most occurrences in C. difficile to occur. We think that one of the key differentiators from antibiotic approaches would be of an ideal target profile the ability to reconstitute (inaudible) resistance after an antibiotic, but also potentially to start helping address the transfer of antibiotic resistance.

We started this work to follow as a case study the framework I laid out before, had an ongoing collaboration with the University of Leiden. We followed a group of subjects that are being treated with FMT for recurrent C. difficile at any number of occurrences and look at pre and post FMT samples to understand if there is patterns of denying (inaudible) clinical response and to make a long story short, we do see that there is a range -- basically, we are just seeing this heating up on the (inaudible) sample from individuals on the right to be subject and the white as you see the different (inaudible) of

bacteria presented. And, again, to make a long story short, you see the C. difficile subjects have a group of bacteria up in the top left largely absent from healthy donors and then largely gone after a successful response to FMD and also, that healthy donors have a groups of bacteria that are relatively abundant and largely missing from C. difficile active infection subjects, which you see (inaudible) this chart and then get (inaudible) after successful clinical response.

Basically, we've made sure that the species that we select for VE303 are represented. There is I think plenty of evidence in the field some of it actually by Vince Young showing that using certain antibiotics that are associated with C. difficile infection can result in very extensive elimination of (inaudible) which are two groups of abundant bacteria within the (inaudible) which we had found to be associated with (inaudible) clinical responses. Our hypothesis is that by reintroducing those groups we can restart

colonization resistance and then render the (inaudible) infection. Some of the basic (inaudible) that we do with the strains starting with safety layout here, we conducted tests as to determine the extent of which antibiotic resistance and (inaudible) is transferable from a product strength surrounding microbiota and that included (inaudible) presence of antibiotic resistance genes, (inaudible)

Starting on the right of this slide this is work that we've done with one of our co-founders, Dr. Kenya Hundra now at the University of Kenya where decide groups of organisms that are put in ducers of regulatory T cells and we're exploring this biology in the context of IBD in partnership with J and J and also food allergies. And all the way to the left of this slide, you can see counter examples where we found bacteria also from healthy individuals that have opposite properties. They have the ability to introduce TH1, TH17 or cytotoxic CD8 T-cell responses.

In the continuum of approaches that are being pursued in the field, and that slide is not meant to be comprehensive, my view is there is a fundamental tradeoff between ecosystem effects and specificity. What I think ecosystem approach is like, for example, fecal transplantation bring to the table, that's really unique is the ability to do something that would be very difficult to do without drug modalities which is change the composition of the gut microbiota in a somewhat controlled environment, I'll say "controlled".

And, of course, the other end of the spectrum if you go full reductionistic, you can gain in specificity, specificities you don't have with a fecal approach. But in our hands, we've seen that come at a cost of losing the polypharmaceutic effects of microbial communities and also the ability to robustly change the composition of the microbiota. And what we asked is, can we find some intermediate stage where we can still retain the ability of a community of bacteria to change the composition of the drug to

do it in a controllable manner than with more specificity.

And here's an example of some work we've done, for example, looking at bacteria that gives microbiota responses. In short, finding that you can identify a number of bacteria in the human flora of subjects across the world that have the ability to use regulatory T-cells. It's only certain assemblies in consortia that can really saturate the phenotype in animal models.

So, I think this brings me to a question that I think this is a good forum to bring up which is how do we think about the contribution of different components of a drug to the final activity. And a lot of the work that we've done in the field has called my opinion on that and I'll emphasize it is opinion. We've done often top down work where we start with the full fecal community. We see that that community has the ability to change the phenotype, for example, T reduction or T17 reduction. And then we'll scale back and find when do we lose that activity. And

usually, like you see for example in the middle pile, you can identify fractions that are equally active or sometimes they're more active than a full fecal transplant at saturating a given phenotype.

And then there's a tricky bridge to cross when you try to really bring down that activity to the absolute minimum number of bacteria. But we often count in our hands that we've seen that when we've looked at different phenotypes including T1 induction, T17 induction and CD8 induction is that as we were really low in membership where adverse species in a composition will see often the effects wash away.

And so, we think that that suggests that there's an important role for ecological redundancy within a consortium unit, to help give a product or a specific composition the best chance of success. And the reason I thought I'd bring this up is, you know, in other contexts, we've had the discussion of, is this a combination product. Can you draw a parallel with say a

multicomponent vaccine and I see a fundamental difference in that. If you take the example, for example, of a multicomponent vaccine, every immunogen is there for reason. You know, they start certain pathogens so it's justification is straightforward.

But when you try to change an ecological community, the issue or redundancy or the aspect of redundancy comes in. And also, the specific contribution of a strain is actually going to change from patient to patient depending the next time you make microbiome. She's not really an inherent property of the component of the product.

This is in a snapshot, the process that we follow to debate to try to identify new compositions of bacteria that we define as consortia. Basically, we tried triangulate between human and in vitro data. So, right clinical packages that give us confidence that we're not just chasing a correlation but there's actually some evidence of causation. But at the same time, we're not over relying on animal models

and chasing a causation pattern that has no relevance to humans. We interrogate human data sets from studies that we sponsor or collaborate with with clinical academics across the world. We try to identify if often in the context of using fecal transplantation a range of conditions, there's a pattern or any pattern of engraphment of strains from a healthy donor and they correlate with a clinical response. And I'll emphasize correlate because that data by itself doesn't tell us anything about whether those given bacteria maybe actually are causing a phenotype.

If that's the case then we'll often go and find animal models and do systematic experiments to remove and reintroduce a full microbiota and see if a phenotype is aggregated and then reconstituted. And if we then have confidence that that's the case and that therefore we're not just causing changing association, then we'll ask okay, which are the bacteria that have the properties that we may be interested in.

For that stem number three, we've

created a very large library of bacteria from humans across the world, somewhere between 60 and 80,000 isolates now. Secret is the genomes of a few thousand of them and also generated (inaudible) to understand and characterize their properties and I'll share a little bit more later how we do that. That gives us hits or in other words, bacteria that have a specific property that may be useful. And then we still have to figure out how to assemble them in consortia that are more potent than the individual strain. And for that, we use a combination of bioformatic algorithms that we've been publishing with collaborators at UMass and also go back to the human data and ask ourselves, from all the potential combinations, which ones are actually occurring as co-networks and premiums that have a clinical response.

We then have in house our own manufacturing facilities through the GMP production of bacterial consortia. As our product from (inaudible) has noted before, these are

complex products. They have multiple ingredients, they're anaerobes. They're difficult to grow, they may need some (inaudible). So, we found it best to basically do all this trial and error work in house and there's a lot of it. And then we moved one of those consortia into human testing now and we're just about to announce the results in the next (inaudible). These are some of the actual activities from culture collection, strain screening, drug (inaudible) production and drug product production. So, starting up on the left, we do a lot of high (inaudible) colonies from (inaudible) material which let's us go from fecal material to actual cured strains from at which point on never again have to go back to fecal material as their source. On the upper right, you can see some of the high (inaudible) screen that we do the test multiple different types of bacteria or combinations of bacteria against activity assays or other forms of characterization to understand what the bacteria do. On the lower left, you can see some of the operations in the

drug production (inaudible) where we do the permenatation, separation, (inaudible).

And then on the right, you can see some of the activities which we have in a separate facility of the actual drug product manufacturer which involves solid handling. And as John mentioned before, there are some challenges associated with that so we have it in a different facility. And that's where we produce the actual final product that's going to be bottled and sent to the clinical sites.

We have a range of different projects from infectious diseases in wound diseases including C diff, IBD, food allergy and trans immunotherapy at different stages. I'll use the first as an example to walk you as a case study through the steps that we've used in our population, the target for file VE303. She's a defined consortium that we're developing for C diff. This is an LDP that is administered as an enteric capsule. It has eight pure clone strains of bacteria as its components. The dosing

regiment is repeated oral once daily following center of care antibiotic. And the number of days we'll treat for is one of the outcomes of a case study that we're running now.

In terms of PK, we believe there's going to be better restricted not absorbed and also, we expect abundant administering colonization lasting for a window of time longer than the time it takes for most recurrences in C diff to occur. We think that one of the key differentiators from an antibiotics approach is that the antibiotic approaches would be open ideal target profile the ability to reconstitute colonization resistance after an antibiotic. But also, potentially to start helping address the transfer of antibiotic resistance.

We started this work and studied the framework I laid out before had an ongoing collaboration with the University of Leiden where we followed a group of subjects that are being treated with FMT for recurrent c difficile at any number of occurrences. And look at pre and post

samples to understand if there are patterns of (inaudible) with clinical response.

And to make a long story short, we do see that there's a range, basically what you're see on this heat map is on the X axis samples from individual either healthy models on the right (inaudible) and the Y axis you see a different general bacterium presented. And again, to make a long story short, you see the C diff subjects have a group of bacteria up in the top left that are largely absent from healthy donors and then largely gone after a successful clinical response to FMT. And also, the healthy donors have groups of bacteria that are relatively abundant and largely missing from C diff active infected subjects which you see on the bottom of this chart but then get reingrafted after a successful clinical response.

Basically, we've made sure that the species that we select with VE303, are representatives of these (inaudible) associated with clinical response. There's, I think, plenty

of evidence in the field, some of it actually generated by Vince Young showing that use of certain antibiotics that are associated with C diff infection and result in very extensive elimination of post reading clusters 14 and 14a which are two groups of abundant material within the firm (inaudible) which we have found to be associated with better clinical responses. Our hypothesis that by reintroducing those groups we can restart colonization resistance and then render the host less susceptible to the infection.

Some of the basic characterization that we do with the strains (inaudible) we've laid out here. We've conducted tests to determine the extent which antibiotic resistance and viral is transferrable from a product strain surrounding microbiota and that included cecical presence of antibiotic resistant genes, virulence factors and phages near (inaudible). And we mapped out their location with respect to predict that (inaudible) and basically found that there were none of the strains. ARG's or phages, (inaudible) ARG's near

(inaudible) or ARG phages associations. And also, we've tested the clinical sensitivities of each of the bacterial strains to antibiotics and found that each of the strain products, each of the strain substances are susceptible to multiple clinically relevant antibiotics.

I think a relevant point here is this is one of the advantages of working with a fine material. You can design and control and make sure that your product actually doesn't harbor patterns of resistance or villains that could be problematic but there's also a limit to that. In this case, we've been able to find multiple clinical relevant antibiotics that can knock out the whole consortium at once. But just to make an obvious point, the larger the consortium and the more diverse genetically, the more difficult it's going to be to find a group of clinically relevant antibiotics that work for all the consortia at the same time as opposed to individual strains individually. So, that's maybe like a little detailed but I think it's important from a

regulatory standpoint.

We've done a range of models both in vitro and (inaudible) to characterize the potency of each of the individual strains, can they directly kill C difficile of not. And also tried them in animal models, actually the model that's been developed is the one I'm showing here is showing that we can match the activity of the fecal transplant in animals by using the consortium.

And now to wrap it up, we're in the process of wrapping up phase 1a. We study where we've studied healthy volunteers that were treated with Vancomycin in a course that tends to emulate the typical course of C diff subjects. And looked at safe TPK and PD in normal healthy volunteers. And here we lay out the objectives of the studies. We're looking for safety, tolerability and what we would like to see is that this consortium of organisms can rapidly and durably colonize the intestine. We'd like to see them stay behind after you've given the last dose. We want to see

abundant colonization we also want to see robust colonization. And by that, we mean that all eight bacteria colonize all the people, not some bacteria colonize some people and not others.

And this is my last slide. Just to make a point of some of the techniques that we've developed to be able to measure pharmacokinetics. In the clinical studies, we have the benefit in contrast with the fecal transplantation approach of actually knowing exactly what strains we're putting in. We have all mitogenome sequences for each of them. So, we've been able to create a panel of markers for each of the genomes. And then when we look at stool, mitogenome sequences from fecal samples from the actual study, you can look for both the depth as well as the proportion of markers that we detect and basically feed that to statistical distribution. To have confidence that what we are detecting is exactly the strain that we gave, not a close relative that happened to be in the person before we dosed them or acquired by the person after we dosed them.

We think that some of these tools are going to be a basic starting point to start understanding PK in the field. And to be clear, when I use the word PK, I'm not talking about administration distribution, I'm talking about organization. How quickly, abundantly and durably are the microbes trying to find this. It has to start with having a reliable technique to measure the microbes you gave not something that was already there to begin with. So, I'll wrap it up here. Thanks a lot.

PANEL MEMBER: Okay, so we're on time. We're going to roll along. We're going to hold questions until the end. The next speaker is Elaine Petrof who is an Associate Professor and Clinician Scientist ID physician at Queens University in Canada. And Elaine is going to talk to us about the development of a defined consortia for recurrent C difficile.

DR. PETROF: I'd like to thank the organizers for inviting me here to speak today. And I'm going to talk to you about the development

of defined consortia treatment of recurrent C Diff. And I'm just going to start with the slide. So, thanks to Vince I can skip a lot of my early slides and zoom along to the end. But I just wanted to throw this up here. I often use this slide when I give these talks and every time I use this slide, I have to go in and update it and add another disease on here. So, pretty soon I'm going to run out of room at this rate.

But having said that, even though there's been an explosion in this field, I think everybody would agree that really the strongest clinical evidence is probably for recurrent C diff when it comes to microbiome. And what we see with this is basically ecosystem collapse. And on this slide, actually is one of Vince's earlier studies back in 2008 actually I believe it's been ten years. But basically, he was one of the -- his group showed that recurrent C diff patients have lower microbial diversity and, in fact, he showed the graph from this study compared to controls but also compared to Rick Spine Hummers who developed

C diff and then recover.

And so, this really illustrated how this subpopulation of patients that get C diff is different. And these are the patients that do not respond as well to Vancomycin. And there's several studies now that have corroborated what was shown in the New England Journal paper when they showed that about 30 percent of the patients respond to Vanco and the other two-thirds or 70 percent don't. And that's been since corroborated with (inaudible) subsequent studies.

And so, what do we do with these patients. This is sort of how the whole transplant programs took off, at least at our hospital. And really what we're trying to do here is ecosystem repair. So, I won't go through this in a lot of detail, it's already been covered by several (inaudible).

So basically, we're trying to take a healthy ecosystem and put it in to replace or replenish what is essentially a sick ecosystem. And so, by healthy, we mean diversity of species

that provide functional redundancy amongst the organisms. So, there's some overlap and some function (inaudible) organisms and it provides resistance to disease. As opposed to a sick ecosystem where we're dealing with low species diversity and an imbalance or dysbiosis is the other term that sometimes we hear which leads to an impaired function and a susceptibility to disease. And this is made worse by giving patients Vancomycin because yes, it does clear out the C diff that's in there but unfortunately it also kind of parches the forest, so to speak, and it kills the innocent bystanders which are kind of exacerbating the problem when we can't recover those organisms. And so, what we're left with is a ravaged ecosystem that really can't get back up on its feet.

So, what are some of the options. We've kind of gone through all of these today so I will probably go through some of these more quickly. But I wanted to sort of briefly touch on all three. There are options for ecosystem repair

being probiotics, FMT or defined consortia which is the approach that we're taking. And so, the probiotics, at least for the case of recurrent C diff treatment, I know we've talked a lot about C diff (inaudible) antibiotics. But as far as it goes for treatment, really there is no evidence that this is going to work.

And if you think about it, it kind of isn't that surprising. Because a single organism or a few species of lactobacillus indifido are really not enough to improve (inaudible). And, in fact, if you put into a system that has very little an overload of a particular organism, you can also exacerbate the dysbiosis and cause even more of an imbalance. And this has been touched on a little bit with previous speakers. And also, I did want to point this out I didn't hear anyone mention this. But this Annals of Internal Medicine paper, I don't know if anyone saw this. But it was a paper that looked at prebiotics, probiotics, symbiotics and adverse event reporting. And, in fact, they are grossly

underreported when you look at all these clinical trials. And so, this situation of dysbiosis and imbalance and the adverse events that you get with probiotics I think probably is happening a lot more than we realize.

And then finally, I was hesitating to put this trial in but I think I'm going to throw it in, the elephant in the room. So, there was this paper that came out which we've all alluded to that came out in Cell. And basically, what they showed was that the impact of the microbiome by probiotics is probably not really what we think it is and there may be interference as opposed to enhancement of recovery of the microbiome.

And so, I'm just going to very quickly show you a few figures from this paper. And I would strongly recommend that some of you pull it because it is actually a beautiful study. I'm just going to show you the human data. Actually, they did this in mice, they did it in two separate spans of mice and they showed basically the same thing. That you can see from the design here that

they split them into three groups and these are healthy volunteers and they took them at baseline.

So, the gray baseline that's the microbiome (inaudible) antibiotics. They give them antibiotics and then they either got fecal transplant, probiotics or nothing. So, the nothing group is the spontaneous recovery. Now if you think about it, that's kind of what we always do with patients that come in with a UTI. You give them antibiotics you send them on their way. That's generally how we've done it in the past.

So, they then looked in the follow up period out here past three weeks and actually they followed them out to like five months. And what they found is that the probiotics group actually had fewer species then the spontaneous recovery group which is kind of interesting. And the same was true for the bacterial load and then fecal transplant is in brown, you can see here.

And then if they looked at the communities, so this is kind of a busy slide. But if you just focus on the UniFrac distance on way

to baseline. So, what that means is the further away from baseline is basically shows a disruption of the community. And so, if you look at there you can see that the probiotic group is further away from the baseline of the naïve gut microbiota of these patients then either fecal transplant or spontaneous recovery. Another way to look at that is in the PCA plots. You can see that the probiotics and the antibiotics groups cluster together. And over here, you have the spontaneous groups with the fecal transplant and the gray is the naïve so they cluster together.

So, what this indicates and then oh yeah, this is another really cool thing that they did. So, they had all these -- this is the probiotic species that they used in the study and they took them out of the analysis and this is the supplemental figure and basically, they saw the same thing. I suspect a reviewer probably asked them to remove those just to see if it was an artifact and see if the data still held true when they took them out and actually they saw

something.

And so, what this indicates is that the volunteers that got the probiotics did not recover their microbiota to the same degree as the patients that got nothing or the ones that got fecal transplant. Indicating that maybe we're doing harm without even realizing. And again, Mary Ellen is going to get mad at me for saying that but I think it's worth discussing. It just is a good illustration of how we think that we understand what's going on but maybe we don't actually fully understand and recognize what we're doing to the microbiota.

And so, what I came out of this or concluded is that microbes work better in teams. So, if you have a few probiotic species that are acting alone, they may not be as affective as an ecosystem which is what FMT is like. It's more like an ecosystem and so there we've got synergy and they all work together as a team. And so, really FMT is sort of the ultimate probiotic ecosystem. I won't go through this. We all know

that it's affective. This is just one of the studies that we did with Christine Lee back a couple of years ago.

I think FMT is great. We've been doing them since 2009 at our hospital but they do come with their own set of challenges. And some of these may be Canada specific but I'm going to mention them anyway. So, the first one which has always made me nervous as an IV doc is the risk of transmitting something. I know this has not happened yet, knock on wood, thank goodness, and I'm not saying on this slide that Zika is being transmitted by stool. I'm not saying that someone has gotten it from a stool transplant. The reason I put this up here is a patient actually asked me this question and I didn't actually know what to tell her because she came to me with this. Which is this Zika don't give blood, you might have Zika and then she asked about stool. Can I get Zika from stool, she was pregnant. And I wasn't comfortable with her getting a stool transplant once she pointed this out to me.

You know and next week it will be some other virus. Like it just seems like there's always something that pops up. And so, even though nothing has happened, I can't actually advise my patients that nothing ever will. This is sort of like with HIV situation with blood back when HIV was new. So, that is a risk that still makes me a little uncomfortable.

The other thing is that our public health labs have become increasingly resistant to do the screening test which has not been very helpful. And part of this, I know, is probably because the screening compared to ten years ago has actually become a lot more comprehensive. So, if you look at the recommendations from the AGA, for example, you know, several years ago compared to what has come out more recently with the ISA, there's a difference there in terms of what they're now recommending that we screen for. And our labs say that these tests are not validated to be run on healthy foreign stool. That's the excuse that they give us and they kick them back.

But it puts us in an awkward situation because then we don't know what to do with this donor and we have to call them back.

And then that leads me to my next point that donors, like maintaining a stable donor supply has been a major challenge and, in fact, at our hospital we don't have any donors right now. I'm having to send people elsewhere because we can't get enough donors. And this came out, this is a joke. It's a program in Canada called this hour has 22 minutes. But it's actually kind of true. We almost have to do stool donor drives the same way that we do blood drives to try to get people to come out and donate. And then quality control, that's a whole other interesting, like I don't have any answers for this. And this was really driven home when we did this study.

So, we looked at a stool transplant donor that we had who has been very good at donating. And all of his stool that he's donated have cured the patients that we've treated with his stool. But we sampled his stool a little over

a year apart. I think it was 12 months or maybe it was even 18 months apart and the composition you can see even though you don't necessarily see all the different species and strains and everything listed on the side there. You can tell just by looking that this is not the same mixture. But having said that, it was effective in both of those. And so, coming up with a generic stool is not going to be an obvious solution.

So, we came up with this microbial ecosystem therapeutic psyche which is basically a cleaned up stool transplant. And so, we're hoping that it will be more reproducively like more like an FMT but just more reproducible and better characterized and we're emphasizing diversity, ecological resilience which I'll talk a little bit about in a sec and safety.

And so, we're looking at human gut thrive commensal so a little different from probiotics. And this is not really rocket science. Actually, I just pulled this up off the British Colombia website. This is some forest

ecology thing. The same principles would apply for a jungle in Costa Rica and essentially, we've just adapted these same ecological principles to the work on the ecosystems new gut.

And so, this is our approach. We take fresh fecal samples. We do a detailed anaerobic culture and then remove pathogens. We characterize old bacteria in there and then we take what we have after we've done all of that, put it back into the bioreactor and test it. And if the community holds together then we would administer that to a patient. And the goal is to come up basically with a cleaned up stool transplant is what we're trying to do here.

And so, what's unique about this is that it's one ecosystem, one donor. So, we don't mix and match strains from different people and mush them up all together and put them in together. These have all co-evolved in the same person so we keep that ecological principle intact. And we take out what we think would be undesirable to have in there such as viruses and if all of that

comes out, the bacteria that we have are identified. We check them for antibiotic resistance, those also come out. And then once we have what's left, that's what we then test and put it into a bioreactor and see if it holds together. And this is just an example of one of these bioreactors. You may have heard the term robo gut, that's also been used to describe this.

So basically, it's an in vitro system that simulates the environment of the distal human gut with an artificial pole and that's another way to look at it. And so, you have food that goes in and then waste that comes out. There's a stirrer here to make a parastoltice. You can adjust the rate that it flows through the same way you can sort of mimic the GI transit time and it's all controlled temperature, anerobic conditions and PH. And this is sort of what it looks like as our protograph and if we pull away all the wiring, you can see in the back those large volume vessels back there.

So, we inoculate identically at the same

time. And then one serves as a test vessel and the other serves as a control. And we can run these for weeks at a time. And the other advantage that this one has over the smaller bioreactors is we can control PH and some of these other parameters that are a little more tricky to control with the small volume ones.

And so, this is just an example of optimization, something that we would do with this. So, this is actually a fail. So, this is showing you that we, as we all know, learn more from our failures than from our successes. And so, here I'll just run you through this briefly. So, here we have fecal transplant material. So, donor stool that gets inoculated into the bioreactor and we run that out and then we can hit it with drugs or we can change nutrients. We can manipulate the conditions here. You can see that we've administered Clindamycin. And so, as long as this percent similarity index is above 90 percent, we consider that the ecosystem is holding together pretty well.

If, on the other hand, there we have the mixture after we've taken things out. And so, then we inoculate that into the bioreactor and in the case of this particular ecosystem, you can see that it collapses. So, after we give Clinda, it does not recover. So, this would be an example of how we can fine tune these ecosystems and test them for resilience and robustness and we can use different drugs to do this as well as different nutrients.

And then the other thing that we can to is compare in vitro and in vivo. So, we did this study as well where we took our mixture and then on day zero, we inoculated it into a king staph or bioreactor and we also inoculated into a patient. And you can see here, day 14 sample from the patient and day 12, they don't look exactly the same but they're starting to look similar to each other. So, we think that this bioreactor represents a good surrogate for in vitro in vivo work. These are some of the animal studies we've done. I'm not going to go through those but those

are just the references. So, we've done C diff, salmonella and DSS colitis.

So, this is the study that we did with the humans and you can see here lactobacillus indifido are in here but they're not the main ones, they're part of the team. And then this is just data showing that the at six months period of time right here and here for these two patients. We have a composite mixture of the bacterial composition of the pretreatment, native microbiota from the patient and the repoopulate mixture of the micro ecosystem therapeutic that we put in showing that these do colonize.

So, next steps we have a new ecosystem, new donor. We've actually expanded to more species and this thing is a monster. It's got a lot of different very interesting bacteria in it and it's a clinical pilot study that's currently under way. So, just as a summary, what we're doing with this stuff that we think makes it a little bit unique is the ecological principles that we're using to develop these mixtures. Known

composition, diversity, patient safety and this came up earlier. Outcomes can be tracked and now we can link them back to a specific bacterial composition because we know exactly what's in there unlike what we were talking about with stool and having the stool registry. My acknowledgments. Both Canada, U.S., I just wanted to acknowledge all my collaborators.

PANEL MEMBER: Thanks Elaine, I appreciate it. Okay so moving from defined consortia to finding a needle in a haystack, our next speaker is Neil Surana. A freshly minted Assistant Professor of Pediatrics in Molecular Genetics and Microbiology at Duke University. He braved the hurricane to come to us and we were on call to give it a webinar presentation but we're really happy to have Neil, thanks.

MR. SURANA: Thanks very much, Ryan, for the invitation to come as well and to get me out of the rather wet Chapel Hill right now. So, there's one thing I want to talk about, how do we move forward in the field. And, I think, as has

been mentioned by many so far --

MR. BATES: There's an issue in the field of moving from associations and correlations to causation. This is a study that Dirk Evers and Randy Xavier a number of years ago where they looked at pediatric patients with new onset Crohn's Disease and identified a large number of different genera in some bacterial families that were either more or less abundant in patients versus (inaudible). The question with these and it's always where do you go from here? And you see all these associations, but how do you either define a consortia or how do you define organisms that actually matter? So, this question on how do you go to causation is challenging.

If you think about this in a different way, you can picture the microbiome as a haystack where each individual piece of hay is a different micro that's there and I think all of the work that's, you know, been described so far today has highlighted the fact there's something there and the post trial for this is really FMT particularly

for prostate and difficile infections. We know there is a needle in there and there may be multiple needles, but how to you actually find that needle and is there a better way than FMT to go without it.

And so, what many in the field have been doing are these microbiome wide association studies to basically subset the haystack and you go from a large haystack down to a smaller haystack and we know that there is a needle in there too, and again, there may be multiple needles. I should say I wanted to update this picture of -- as Ryan mentioned, I just moved from Boston to North Carolina and I want to update this with pictures of my own haystacks, but the weather the last few days didn't really allow for that.

So, instead of going to these smaller haystacks essentially, can we just find the needle itself? And along with this, though, sort of presupposes the idea that a needle is better than the haystack. Just to think about this, you know, if you think about FMT at least for (inaudible)

and difficile, is being tested for a large number of other indications that has biological activity as many have talked about there's questions about whether it's reproducible or not and I think a lot of the conversations in the Q&A sessions have highlighted some questions where in the regulatory apsects of it, batch-to-batch variation if you will. When you think about bacterial cocktails, many of these issues are resolved and also from a company standpoint also improves patent position, but when you think about single isolates, you get all of that and potentially more and I don't mean these checkmarks to be completely black or white as they appear here, but sort of at least one man's opinion as to which one offers a little bit more benefit or not.

And one of them being that with a single isolate it may be a little bit easier to define a mechanism underlying how this organism impacts disease overall. And if one can identify mechanism, then that allows you, as people brought up into concession, to perhaps do precision

medicine with microbiome oriented therapeutics. So, if you know the organism of interest that acts through a certain mechanism, you can identify patients that have a defect in that pathway and then target that patient population specifically. If there is only organism being given, it in theory at least has lower potential for side effects than giving 8 or 10 or 20 different organisms at a time. And, also, I think it allows the possibility of defining specific molecules from that bacterium that can then be used in sort of a classic drug development process. And so, if FMT is essentially the IPhone, if you will, ultimately it will get to the iPhone 10X or 10S which is the actual molecules themselves.

But how do you choose these strains really is I think the issue that has come up sort of repeatedly over the sessions so far today in a work in Dennis Castro's published a year ago, they approach this question from a fairly reductionist point of view. So, they each gave a biogenetically diverse set of organs and it's 53

highlighted by the stars around this plotogram and then generated mice that were mono colonized for each of these and then really did an absurd number of immune phenotypes for each of these mono colonized mice, performed correlations among all of these different immune phenotypes, and created a dendogram based off of those correlations.

But what you get in the end, though, is you look at this -- these are color coded now by fileum in the squares and by genera in the circles and even if I don't tell you what these genera because there are too many to really make it a meaningful key, but what becomes apparent is that the taxonomy doesn't really correlate with the immune team either at the biome level or the genus level and for many of these species, multiple isolates of the same species were used in these experiments and they gave different results.

And so, I think this highlights that not only -- one can't just infer because lactobacillus is a commonly used probiotic that will have the same activity as a different lactobacillus species

and if you say lactobacillus reuteri, a different strain of the same species, we have very different functionalities as well.

So, then it gets back to this question, how do you choose? How do you find that needle overall? And I think what we realize is that all of these microbiome wide association studies share a lot in common with genome wide association studies. They have a lot of the same strengths and some of the same weaknesses, but GWA studies are really an outgrowth or an adjunct to what geneticists have been doing for decades, which are family pedigree analyses and there geneticists will identify a patient that they think has a hereditary disease, look through the family pedigree, identify other family members that has the same disease, look through their G nodes, and identify regions that are shared in those disease, absent in those without, and if you use over enough family pedigrees, you can really hone on at the gene level.

So, we reason can we do something

analogous to this for the microbiome. So, you know, as proof of concept, we used mice, which as Vince pointed out, it makes it a little easier. Now, the colors represent different microbiomes and we can take mice with different microbiota does, put them in the same page, take advantage of the fact they are (inaudible), they eat each other's poop, and now we generate mice, they hybrid microbiota that is reflective of its parent microbiota. It's much like a child has a G node reflective of both of its.

So, with this, if the microbule effect on disease is dominant, we should be able to triangulate microbes that are associated with the phenotype (inaudible). So, as proof of concept, we had multiple genetically identical, or at least related, strains of mice with different microbiotas in red, germ free mice, in blue, a strain of mice that they (inaudible) microbiota that we've been breeding inside of (inaudible) isolators for about a decade, ones with a human microbiota, again, bred in isolators for about a

decade, and then just wild type of mice, which was bought from the vendor. And which you can see these are experiment done with DSS colitis and showing basically just survival. You know, in two cases, they all died and in a couple of cases they virtually all lived.

So, we can take this very stark phenotypic difference and now do microbiome wide association studies. And if we focus just on these parental strains of comparing either the wild type mice that we buy from vendors versus the mouse microbiota or the one for the human microbiota versus mouse microbiota, there's still 100 to 160 different taxa that are differentially abundant between these groups, which, again, leaves us with the question what do we next? How do we choose which organism to focus on?

So, we used this idea of microbial pedigrees. I'm not going to go through all the data, but we found that if you cohouse these mice just for a day, that in both sets you get intermediate phenotypes. The mice that used to

die now live a little longer and the mice that used to survive now die quite a bit more.

But, again, this only gets us down to the 60 to 90 different taxa that are different to the abundant. We applied an additional criteria that geneticsosts would do with a given pedigree, which is look for things that are shared among all four comparisons. And, when we applied that additional criteria, only one thing came out, which is the bacterial family lachnospiraceae, which was associated with survival from DSS colitis. And, importantly, even though all of this was done in mice, our results mimicked what would have been shown in humans. Again, this just keeps going back to that same study by Dirk Evers and Art Xavier that found that lachnospiraceae were decreased in patients with (inaudible). So, our mouse data at least has some relevance to the human cohorts as well.

We went through and much like using a scenario similar to what (inaudible) described or what Kenya Honda had done, several different

examples, we defined a bacterial cult community, a bacterial cocktail, that enriched for lachnospiraceae, gave it back to our colitis prone mice, demonstrated that would protect mice from the disease, but then we went ahead and tried to pick single colonies and identified one species that fell within the family of lachnospiraceae. It happens to be a new bacterial species that we're calling clostridium immunis. As a control, we chose a different bacteria, clostridium innocuum, gave both of them to our widest prone mice. Those that got the control organism still all died with the same kinetics. Those that got the lachnospiraceae isolate are now protected from disease.

I should note that this is done with a single gavage of these organisms one week prior to challenge with DSS though I'm not a company. I have not done all of the dosing regimens that one might be able to do to sort of see if we can improve this from 60% survival to 100. But proof of concept is that we can identify organisms using

this approach that down to a single species that is protected from these (inaudible) in a causally related manner.

And so, what we were able to do is use this concept microbial pedigrees or micro unified triangulation to bioemphamatically pinpointing limited number of taxa that are associated with our phenotype and by doing this, we increase the specificity of our results at a cost of sensitivity. So, we may not be identifying everything, but the ones that we do identify through this approach, are more specific to the phenotype of interest. Using a directed microbial culture techniques are able to isolate the organism of interest and in back to our mice to demonstrate causality.

And Vince earlier this morning had mentioned Koch's postulates and we have now sort of demonstrated Koch's postulates with a commensal organism even though the even though the bulk head intended needs to be where the identification of pathogens specifically, I think that these really

need to be applied to a study of commensal organisms as well to really add to the scientific rigor within this field as well.

We've used this same approach to identify other organisms that are able to induce post expression of antimicrobial peptides, again, in a causally related manner. So this is a (inaudible) result of what we have been able to this least two different phenotypes and now applying to several others.

The big picture though, you know, even though we did this with mice, the approach itself can be applied to human cohorts as well so we can look to our patients, identify pedigrees that matter, to then identify taxa that are related in a causal manner to the phenotype of interest, use concept of microbial pathogenesis that has been owned over the last century to identify the bacterial factor from these organisms that mediate the protection, and then go through a standard drug development process to develop those organisms.

I just want to end with the idea that I think really we've just scratched the surface overall of the roughly truly (inaudible) bacterial species that live in the world or that 10,000 neglected human microbiome. There's a very small (inaudible) about this number, but clearly less 100 or so different immuno modulatory bacterial species in the consortia have been identified with only a couple, you know, very limited number of bacterial moducules have been identified today so there's clearly work that needs to be done at all of these levels as well as trying to understand how to translate this (inaudible). With that, I will stop.

SPEAKER: Okay. So, we're going to transition to the next talk and actually hear about one of those molecules. Greg Bates is a Senior Vice President of Regulatory Affairs at Axial Biotherapeutics, officially my favorite biotech company name, Axial. And the title of his talk is Bacteroides Fragilis used in a mouse model of autism.

MR. BATES: And thank you for inviting me. It's been a very interesting day today and I'm looking forward to more discussions as we move on. So, I'm going to follow what Neil said by talking about maybe trying to identify that needle in the haystack and I think we potentially may have identified one of the needles, but I think there's probably multiple haystacks, which with different needles being important in different diseases. But Axial Biotherapeutics, the company that I work for, is a reasonably new company and we're looking at the gut brain axis. So, we're trying to determine the connection between microbiome and neurologic disease. We're specifically focusing on neurologic diseases have a gut component to them.

So, that's what that slide says. We're really trying to focus on that gut brain connection to figure out how we can manipulate the microbiome to help treat neurological disorders that may have a causality.

The work that we're doing at Axial is

based on some of the groundbreaking work that was published by Sarkis Mazmanian that helped (inaudible). He has published quite a bit on the connection between gut and the brain and the connection between microbiome and neurologic disease and understanding what those connections are. He has published data in ASD, Autism Spectrum Disorder. We also have a program on Parkinson's Disease that's also (inaudible). We have three programs that are expected to be clinical (inaudible) today. We don't have clinical so I'm going to be talking to you about the treatment (inaudible). When we do get to the clinic, our initial clinical focus would be try to look at objective biomarkers as well as GI function because autism (inaudible).

SPEAKER: Can you please speak into the microphone.

MR. BATES: I'm sorry. Which is linked in severity to the neurologic symptoms that you see as well. So, our target, again, is to look and its effect on the neurological disease and

we're trying to target therapies that are focused at the gut rather than the traditional way of treating neurologic disease by getting systemic therapies for obvious reasons. Hopefully, improve safety, decrease systemic exposure, getting around (inaudible) with systemic therapies as well.

Our therapies are both live biotherapeutic products as well as small molecules that are based on some of the activities that the microbial organisms that we're targeting may have (inaudible).

Generally, our approach, and this is our approach for Parkinson's not so much our approach for autism which we are going to talk about in a bit, is to transplant a diseased microbiome from a person with neurologic disease into a germ free mouse to see if we can create disease. So, for instance, in Parkinson's Disease, if you take the feces from a patient with Parkinson's and transplant it into an (inaudible) expressing mouse model, you can actually create the symptoms of Parkinson's in a mouse so that allows us then to

have a handle that we grab on to to try to figure out what is it in that microbiome (inaudible) to cause these symptoms.

With autisms, I'll talk about the work that Sarkis did and that we've continued (inaudible) in the autism area. Well, let me first talk a little bit about autism itself. Though autism, as many may know, is increasing in (inaudible) quite a bit. It's currently estimated that it affects about 1 in 59 children. This has increased substantially over the last 10 to 15 years. The CDC when they come out with their reports every couple of years it goes up every time. And this is more than just an increased diagnosis. It seems to be increasing in the population in general. Poor behavioral deficits really Autism Spectrum Disorder is a spectrum so it's a heterogenous disease that have these cognitive deficits in children have certain things in common and poor behaviors are impaired social interaction, impaired communication, and they have repetitive stereotype behaviors.

There's a number comorbidities that go along with that, irritability, anxiety, and GI symptoms as well. There's currently no currently drugs for approved for treating the -- no drugs or biologics approved for treating the core behaviors of autism. There's only two approved drugs right now. That's Risperidone and Aripripazole and they're approved for treating the irritability associated with ASD. ASD is a wide open field. People have done studies using all sort of interventions including FMT's, probiotics, you name it, with varying degrees of success. (inaudible) As a matter of fact, there have been some FMT studies where B. Fragilis, which is the organism that we're using, has been in the FMT's, but very inconsistent results.

Again, going back to autism, autism is also a disease that occurs much more prominently in boys than in girls, about 4 to 4.5 times more likely to be in a boy than in a girl. There's certainly a reason to that. There's probably a genetic component underlying autism. There's

environmental factors to kicking off the disease and there may be in Parkinson's as well.

It can be diagnosed as early as age 2 and kids start showing symptoms very, very early in life. Importantly, there is a subgroup of subjects with autism who have abnormal GI function. Some have diarrhea, some have constipation, bloating, abdominal pain, it varies from child-to-child, but there is definitely a GI component to the disease.

So, when you look at kids that have ASD and you try to look at information that correlates the gut microbiome to ASD. First of all, you see that, again, there are a number of kids with ASD that do have GI components of their disease. If you look at the microbiome of these kids versus a neurotypical child, there are differences. There's lots of publications on what the differences might be and many of them are different from one another so there is no fingerprint microbiome of an ASD child. There has a tendency to be less diversity in kids with ASD

particularly in the bateroides and the (inaudible) components, but there is no current fingerprint as to what in microbiome is causing ASD.

There are many risk factors that occur in kids with ASD. Mother's that have infections when they're pregnant have a higher risk of having ASD kids. Antibiotic use has been associated with ASD. Birth by c-section. A lot of the things that we hear associated with microbiome type diseases. The kids that do have the GI symptoms also show alterations in their intestinal permeability. So, if you do a Lama test on a kid with intestinal permeability with intestinal problems with autism, they frequently will have impaired intestinal permeability.

So, we started trying to think, okay well, how can these things be connected? What is the connection between the gut and identify a specific organism and create a specific microbial fingerprint that's associated with ASD. What could it from the gut be affecting the central nervous system. So, people have looked at the

(inaudible) around ASD as well and there have been a number of papers written about uremic toxins. Urinary (inaudible) has been published on and was found to be elevated. And there's also literature out there on 4-Ethylphenal Sulfate or 4-EPS, which is a close analog for (inaudible) that is also increased as well. They are very closely related molecules and we're measuring both (inaudible) and 4-Ethylpenal Sulfate.

So, we looked ourselves at a cohort of ASD children who were part of the charged database at UC Davis and looked specifically at the metabolism of these kids and identified that in a subset of about 33% about third of the kids had a significantly increased level of 4-EPS circulating (inaudible) microbiome sourced uremic toxin. So, we've looked at a number of different cohorts now and have been able to reproduce this and replicate this in other cohorts of kids and it does provide a potential stratification opportunity in doing clinical trials to look at high 4-EPS children versus low 4- EPS children. The issue in autism

is that there is a very limited amount of cross-sectional data in the autism population identifying how much one autistic child (inaudible) what sets these subgroups of kids with gut symptoms apart from (inaudible). So, the treatment hypothesis that we're looking at Axial is the effect of the metabolites getting into the circulation and affecting the neurologic (inaudible) and the fact that these kids with gut problems have impaired intestinal permeability and an increased (inaudible). So, Sarkis, in his lab, had been doing a lot of work with B. Fragilis (inaudible) and had shown that actuary Fragilis as well as a number of other actuaries in this group (inaudible) had been improving the intestinal barrier and decreasing intestinal (inaudible).

So, we started to investigate B. Fragilis in mouse models of (inaudible). So, a little bit about Bacteroides Fragilis. B. Fragilis, again, is a compound that Sarkis had worked with before. There are other Bacteroides that also have an effect on the intestinal

barrier. Beta omicron also has an effect. B. Fragilis was chosen because they had a specific strain of B. Fragilis they had been working on for quite a while that was non-toxigenic. Again, B. Fragilis is not (inaudible). There are enterotoxic of B. Fragilis that can actually cause disease. The specific strain that we're on here is specifically non-toxigenic and not capable of producing enterotoxin. It's a non-spore forming brand negative (inaudible). It's very prevalent in the adult population, 50% or more actually get it. It's been shown in some studies to be as high at 90 plus percent in children, which decreases a bit as kids get older down to the 50 to 70% in adults and we believe that the organism functions in part by a direct interaction with (inaudible) epithelial cells.

So, hopefully, this organism will help to improve the intestinal barrier and decrease the exposure of the systemic organism from these toxins that may be (inaudible).

So, B. Fragilis, first of all, when you

look invitro, it does have the ability to repair epithelial cell barrier integrity. So, I think I'm a little bit taller than the microphone. So, if you take a Caca 2 monolayer and you disrupt it by exposure to cyanophytes and you increase its exposure to B. Fragilis, in a dose responsive manner and you see a repair of the integrity of that barrier. So, this is an interesting finding invitro. So, in vivo we see the same thing. So, the rest of the data I'm going to show is from a model that's called the MIA model, Maternal Immune Activation Model and this goes back to the clinical notice that pregnant women who get maternal infections have a higher risk of developing or having ASD children. So, this model is basically taking a pregnant (inaudible) and injecting the mouse with Poly IC, which is a viral mimic, a double stranded ANA but viral mimic and causes an immune activation in the mother. When the offspring are born, by three weeks of age you start seeing leaky gut and you also start seeing symptoms of autism.

So, when you take these mice and expose them to B. Fragilis, you see an improvement. So, this is measured using FITC-Dextran, which is a radio labeled Dextran which (inaudible) across the intestinal wall, if you use DSS you see a great increase in the permeability of the gut barrier. The S here that is a wild type mouse, the P is the Poly IC so that's the MIA offspring that have a naturally leaky gut compared to a non-treated wild type mouse. If you add B. Fragilis to it, you see a substantial decrease in the intestinal permeability that you get with that model.

And (inaudible) these are tight junction protein staining and you can see again if you add B. Fragilis, you get a repair of the tight junctions.

Then when you look at the symptoms of autism that show up in these mice and you can really examine in these mice what or correlates to the core behaviors that you see in children with ASD. So, again, one is repetitive behaviors. So, mice bury marbles if you put marbles in their cage

and mice that have been offspring in this MIA model. So, again, if you get here onto the left, these are the wild type mice. On the right, these are the mice that are MIA mice. So, if you look at standard wild type mice, they vary about 30% or so of the marbles in their cage. You give them some B. Fragilis it's not really different. The MIA mice, their marble bearing behavior goes up to approximately 45% or so of the marbles. They have an increase in this repetitive behavior. You give them B. Fragilis, it brings them back down again to what the normal level was (inaudible).

This is a measure of anxiety and locomotion both so this is an open field exploration test where you put a mouse in a little box and you have a camera on him and you measure what he does. A mouse that has greater anxiety will hang around the edges of the box whereas a mouse that has less anxiety will spend more time in the center or the open area less protected from the mouse in the cage. So, MIA mice spend much less time in the center of the cage. So, if you

look over here, these are the numbers of times that the mice enter, the center of the cage, and the amount of time that they spend in the center of the cage and you can see, again, in the wild type mice they are here. The MIA mice have a significant decrease in the number of entries to the center and a significant decrease in the duration of time that they spend in the center and if you given them B. Fragilis, it puts them back to where they were before. They get a more normal phenotype and it's not because of an effect on locomotion because if you measure the distance traveled for these mice it's the same for all of them so this is really mice having less anxiety and going back into the middle of the cage again.

A communicative behavior is another of the issues that one sees in kids with ASD and again, this is also replicated in the mouse model. You get mice when they are together and make ultrasonic vocalizations towards each other to communicate. In the MIA model, which is here on the right, you look at untreated. They have a

substantially lower number of ultrasonic calls, vocalization, and the duration of those vocalizations goes down substantially. When you treat them with Bacillus Fragilis, their number of calls goes back up to normal and interestingly, the duration per call actually goes above the (inaudible) so there is something that B. Fragilis is doing here to increase and improve the communication that's emerged through ultrasonic vocalizations.

And, if you look at 4-EPS dated here on the left is from the MIA, you will see that in wild type mice they have virtually non-measurable levels of 4-EPS than the Poly IC treated offspring you see a substantial increase. It's about a 46 fold increase and this is the most disregulated metabolic product at the gut that you see in the animals. When you treat them with Bacillus Fragilis, the level of 4-EPS goes back down again.

So, the hypothesis was that perhaps 4-EPS could be one of the factors that's traveling from the gut to the center of the system causing

these (inaudible). So, if you actually expose animals just to 4-EPS, you see impairment in communication, increased repetitive behaviors, and increased anxiety as well and this happens whether you give them 4-EPS orally, gavage them with 4-EPS (inaudible) and this data here is from decolonized animals that were decolonized to specifically produce 4-EP in their gut. So, 4-EP appears to have an effect. B. Fragilis appears to have an effect on the intestine and we will test this in the clinic next. We've had a pre-IND and within the nexthopefully in 12 months we'll be in the clinic and see if we can test this hypothesis.

PANEL MEMBER: I think I need to be fragile. We're about 20 minutes over here so we're going to try and get back on time. So, with that, our next talk if from Pinaki Panigrahi who is going to talk about -- who is a pediatric infectious disease physician and professor and founding director for the Center of Global Health and Development. And he's going to talk to us about a very large study done to look at

preventing sepsis using a strain of L Plantarum and the specific focus on the timing and why he particularly chose this strain.

DR. PANIGRAHI: Thank you for bringing me here. I don't know if I will ever see NIH and FDA under one roof and so many elite group of people listening to me. I don't know if there is anything left because after hearing so many wonderful thoughts that span from bioinformatics, machine learning to (inaudible), I don't think there is a whole lot left for me to add. And I'm not going to talk to you about prenatal sepsis in a developing world setting, how bad it is. I get carried away. I spend half an hour telling you how bad it is. One million deaths and the morbidity is different in this country if you look at the NICU sepsis continues to be a big problem. And it adds to, if you give them antibiotics, it adds to increased incidents of death so it's a bad disease and there is every reason to study and do something about it.

As I go through, I will have to speak

fast and I will show you many pictures so that you can visualize what was done and try to summarize my work in 15 minutes that took me about 25 years give or take. And you can think if you are thinking about other biologic supplement, how do you develop a new one. Do you just pick on and somebody tells you that it will work or you know about the pathogenesis, that's why you think about it or I just do it for the fun of it, I don't know?

Most of us, I think, we know about the disease a little bit, we know about the pathogenesis and we try to address it when we develop a new drug. Quickly, I want you to think about the history of because we are talking about micro and probiotics and the history. And then I have to talk about necrotizing enterocolitis although my topic is sepsis because they're quite related to each other and that is how the whole development took place. And then I will describe you the randomized clinical trial when we used the lactobacillus plantarum strain along with the

fructo saccharide and finally hopefully because I'm the last speaker we'll be able to tell the (inaudible) same sample wins.

This picture, many of you who are familiar with the probiotics still probably know but I think about in 1982 he drank cholera during an epidemic and he showed that he could survive. But I'm trying to make a point that it's about 100 years and another gentleman were less give and take 100 years did something good but which we all remember him. And this is only 30 years ago, I think, 30, 35 years ago.

Here I am giving you necrotizing enterocolitis and sepsis because all the studies that have been done. Ultimately, they look at neck and sepsis together although the primary outcome could be one of the two. And the purpose of putting it together is in 1999 the first study came out which was kind of soft study but it told us that probiotics may work in preventing NEC. But half and half some of them show some efficacy and half of them don't show anything.

If you look at the more recent ones, it hasn't changed a whole lot. Even the last very, very recent paper reviews that have been written, you can see only some work and others don't and sometimes that is given even negative effects. This is in Omaha. There are four NICUs and three of them have been using probiotics for 5, 6, 7 years and each one of them is using a different type of probiotics. If you ask, have they done any three post numbers they don't try to address those.

NEC is a multifactorial disease. Everybody was thinking even now they think is triad of ischemia bacteria and inflammation and no specific agent has been implemented in this disease. It happens very quickly and if you, as Dr. Neil was telling you this morning it's a bad disease. But this was in the early nineties when I started looking at the disease trying to find out what else may be present looking at just really tightly matched controls and NEC cases. And we didn't find anything and we published that

okay, there is no specification, we are looking at bacteria and by culture techniques it was what we could find.

But we did show that the colonization pattern may be important in causing or preventing necrotizing enterocolitis using simple caco-2 cell culture model and (inaudible) models. Here on the left in the panel, you see gram negative E. coli. You throw them, some of them, the ones that come from NEC babies that actually have massive numbers. In the panel B, you have gram positives like enterococcal (inaudible) in this particular case. If you put them together, you don't find too many of E. coli that is there but you find some gram positive still there.

Then we did some translocation model. Again, the same thing we have small transcytosis cells you put gram negative to transfer it. If you put gram positive cells along with it, they don't. And the transcytosis evidence goes down and the productive phenomenon was quite visible that the gram positives do something good there.

So, we wanted to do some animal modeling and I don't know how many years I spent. I was a junior faculty and we had some fellows looking at mice and rats and newborn mice, newborn rats and none of them worked with this model then we went to have a look in this report. And we brought some pregnant rabbits to the facility and looked at weanling rabbits. One thing I made sure that we don't compromise the vascular supply and we made loops in the weanling rabbits and then we injected E. coli into the loops or we injected E. coli and enterococcus faecium or staph epi in combination in the same amount. And they recovered overnight and then we sacrificed them and looked at the pathology.

Saline injected loops normal histopathology. If you put E. coli this is how it looks like. You don't have to be a pathologist and if you put the exact amount of E. coli along with some gram positives and you have some (inaudible) causality now but no (inaudible) adults. So, that told us that gram positives are

really doing something good. So, we could conclude that normal flora gram negatives E. coli (inaudible) do not belong to any pathogenic groups but produce disease and bacterial attachment and (inaudible). And if we put some grand positives, mostly gram negative staph and enterococci, we can prevent disease. And in these models and in NEC patients we always sepsis and the same thing happened in the rabbits. Whenever we had the NEC, we will be able to culture the same organisms from the blood of the rabbits.

So, can we give enterococci and staph epi to our babies. Obviously, no way because those are pathogens for preemies in the NICU. And we thought how about probiotics. Maybe we can, we just heard the term and which probiotics to use and so many are there and some of you who have gray hair might have heard about the story of Lactinex which was an FDA approved drug then it was taken off the drug route and it was put back on the OTC. What to use? We have no clue which one to use. And nothing against any of the

manufacturers and I love them because of them, we are here today but the field has progressed to that extent. But do you see what the line says, comes to (inaudible) and cultural and all of them have different types of mixtures. We had no clue as to which probiotic to use and no wonder people called it snake oil sometimes.

And I was interested that whatever we use it should go and colonize and do its job. And nobody, none of the studies would ever talk about colonization what happens, why did you take that particular strain, because it was available, we took it. So, without colonization, I was not comfortable doing any real studies. So, these are all, by the way, funded by NIH either (inaudible). And with small funding from poverty, we did a study where as usual, we took LGG because of we thought it will colonize and it didn't colonize. Well then, we took some sporogenesis which was called bacillus (inaudible) that also didn't colonize. So, in the mid- nineties, if you think about Forest Gump, that is exactly what was

happening, we didn't really know. Were we really picking them up from the box of chocolates, I think that was happening.

So, we wanted -- I said okay, go to hell, I don't care. We will screen the -- we have the model, we have one invitro model, we will screen the strains before we think -- go to the clinic. So, we screened about 280 plus strains from the model, from here, from some from former Soviet Union, healthy stool and all different sources. First focused on bifidobacterial, none of them did anything in our model, specifically in our model.

Then we went to acidophilus because that was known, I knew about acidophilus and that didn't do much. Then came lactobacillus plantarum which saw something and we had quite a few. I didn't even hear that on plantarum at the time. I said planned and many of these are from babies' stool. I said, why should the baby stool have plantarum?

And finally, we found that there were --

and this is just the evidence of a picture I'm showing you which was of us screening in the same way they were taking through the animal modeling. And we found that there was one strain of plantarum and one strain of salivarius that did the job that we wanted to them do, i.e. stopping bacterial attachment and translocation and injury in the (inaudible). Because salivarius also transfer quickly in our system, we didn't want to give it to babies so we worked with lactobacillus plantarum and we had to do the typical safe toxicity studies and instead of doing it in rodents we went back to the rabbit, the newborn rabbits. These are newborn, they (inaudible) and fed them for a month and then took it to the clinic where we did the first phase one type study. Particularly in this 2 2 1 allocation where we give lactobacillus plantarum plus fructo-oligo saccharide and it colonized really well. After giving one week of therapy they got colonized for about four months.

Then we did a slightly larger study

which can be called as a phase two gives the results of 2 2 1 allocation in about 180 which showed some impact. But sepsis, as you know, the incidents is pretty low if you look at the regular published. Then finally in the phase three trial after the success of phase one and phase two, where we did in a launched the free trial, it was a one to one allocation in the largest trial that we published last year.

And it was an individually randomized trial in the community setting in India and we wanted to reduce sepsis by about 20 percent. Because gram negative sepsis was half and half gram negative half was gram positive. So, we thought at least we will be able to reduce gram negative sepsis. So, for 20 percent power and 20 percent relative death reduction, we wanted to enroll about 8000 babies. And we simply stopped the study in about the middle of when we had enrolled 4600 babies. I have been (inaudible) and only time we have stopped studies is when there is something wrong and we want to open the study up

big and do such things.

And then we found and we were happy to know that it was obviously due to efficacy and this is in the eastern part of India, one state, where we did the study in two different districts that (inaudible) support. And this will take me three hours, I can make a movie how it was run even one grant five years, $5 million to set up the labs to all the infrastructure to train people. And then finally, we did the study within our one because we already had done the preparatory work after that.

So, it was individually randomized blocks of four and we gave same antibiotics starting day two of life, day two, three or four. First day, we didn't give because many babies die to birth asphyxia and they may have early onset sepsis which we won't be able to really do much about. And then they were watched at home for 60 days. And all adverse events and serious adverse events were reported and then we did (inaudible) blood culture and microbiology identification and

stored the samples. And we had to set up NICUs and those had to come (inaudible) and NICUs now use cellphones. The bottom you see 300 workers who each village has one lady who was trained and then a three tiered system to daily monitor the study and bring the patients the moment they become sick.

A lot of focus group meetings, movies were made and they had to be told that okay unless you bring the baby right away the baby is going to die. And at the end, this is what we got. We screened 7000 babies and enrolled 4500 and there quite a few ineligible, I will tell you that. And then we had very few (inaudible) but we had some that were -- by the way, our inclusion criteria was 35 weeks or 2000 grams. We didn't want to enroll really tiny babies because this was the first time and we didn't know they will be dying due to all different things including asphyxia.

And some of them we could not enroll because they were born at hospital, they didn't come home. But it is a flight and some of them

had early onset sepsis. And after excluding those 2500 and it was very tightly monitored by all different groups apart from the investigators who came and said this is how (inaudible) mix and prepare the antibiotic and squirt it into the baby's mouth.

And the results coming to when we look at death and sepsis, there was a drastic reduction in India of 27. I was always bragging about it, now I won't have to. I know it definitely has (inaudible). And when you look at culture positive sepsis, it was also reduced in massive numbers, 27 versus 6. What happened which we had no clue about, the reason this study was stopped in the middle is a respiratory tract infection. In this country, respiratory tract infection or pneumonia, those are different diseases, we know what they are. WHO on the other hand, classifies neonatal sepsis only for the developing world (inaudible) all of these conditions including respiratory tract infection because they can diagnose in the field, they will give them

antibiotics. So, that component, we had quite a few respiratory tract infections that also got reduced significantly which really tipped the balance and that's why the study was stopped in the middle.

Some other infections were also reduced including colitis and local skin infection and diarrhea. But then if you combine all infections, not just sepsis, then the (inaudible) was 18. And if you include diarrhea it was about 15. Other morbidities we collected because it was a provided trial but we didn't expect that they will have less (inaudible) disease in the first two months of life. And we could conclude that this (inaudible) significantly reduce sepsis but it also had some effect where it reduced (inaudible) staff infections. So, we know now that apart from blocking just the bacterial transmission, there are other even mortality effects that are going on. And there is a lot more work to be done which we have started now looking back at the timing when we give the preparation is day 2, 3 or 4,

does it matter. Those of you who are familiar with the (inaudible) on BCG and the non-specifics stimulus of the human system, one could say that it has nothing to do with lactobacillus. You gave an antigen and you give it at the right time. So, now we are looking at does it make a difference because we have 2000 babies we are looking at (inaudible) and see which ones did better if at all. And this was from the very first study where we looked at it has been published from the microbiome. Again, there are tons of changes and just so that you see how many are so diverse if you look at them and now, we are looking at bacterial host cell interaction which all of us are very fond of. And if you look at this just simple attachment, the recent electromicrobial structural analysis that we are trying to do, they are not as simple as we think. They are not just coming and blocking it and basically different actors at different time after half an hour versus one hour, three hours and six hours. These are the lactobacillus and when they come very close to

the cell surface on the left hand side you see the E. coli, on the right hand side lactobacillus.

And if you think about team expression, we are talking about consortia, we are talking about thousands of species. This was a study we published some time ago taking the same E. coli that I showed you. You put it on cultured cells (inaudible) 332. Lactobacillus plantarum alone combine them it's only 86. Something is going on the on transpectal here. So, I will coming back again products have been sold with different -- you can change the color and still (inaudible) for this pink for babies and if it is a chewable it's a junior. (Inaudible) has everything. They add a little bit of -- again, nothing against (inaudible) one of the most studied (inaudible). But you add a little bit of vitamin D (inaudible) and I'm not exaggerating that okay we'll cool you're baby down.

So, that is one aspect that you can't -- this is something that Joe Neil told about this morning, LDG, that the three fourth study has now

shown that it increases necrotizing enterocolitis and sepsis. So, you can't take it for granted. It may be a wonderful probiotic but I wonder if this is a (inaudible). And I will end by saying that many many years ago, all of you have heard about this how ampicillin was developed. Think about the bark, they were eating it and then 1820 and then chloridoid was there for 100 plus years. And then even now, if you go back to those areas, Levaquin is used not just as an anti-(inaudible) it is used for everything. A little bit (inaudible) you want to feel good take a pill. Also, as a food supplement. So, this is -- I was telling you it was only 5 years ago when it was licensed and Sanopy took it as a drug. So, if you're wanting to use it, give it intravenously, give it (inaudible) of course it has to be developed as a drug. But can we stop the people taking the (inaudible) or somebody who is wanting to sell it in a capsule so that they will have less of the pain or it will help the fever, answer is well, you know, they will do it no matter what.

And this was (inaudible) one of my heroes from 100 years ago, Alexander Fleming, also about 100 years who came up with Penicillin. Now what we are trying to tell, think about antibiotic, it took 100 years to do this. And probiotics, it will take another 100 years. Now we are kind of waking up. We can't expect that okay, we have a prospect on probiotic that's going to cure all elements about this exist. So, a lot more work to be done. Thank you.

PANEL MEMBER: Okay so we're going to move right along. I'm going to invite all of our speakers for session three up to the table and we're going to combine clarifying questions in the panel discussion but we'll take questions first, certainly. So, please step up and ask questions.

SPEAKER: Howard (inaudible). So, the amounts that you showed, you get them (inaudible) groups where you added (inaudible). Do you have a control group where you added a different bacillus or someone just to show at least specific (inaudible)?

PANEL MEMBER: No. In that particular experiment, that experiment was focused on B. frag. However, the laboratory has done that with some other organisms that haven't shown these effects. B frag was specifically chosen for these series of experiments though because of its known effect on leaky guy. And we're looking really at 4-EPS and it's 4-EPS potentially what's causing the behavioral abnormalities.

SPEAKER: End of the day we'll wake each other up.

PANEL MEMBER: So, here's the deal. Thousands of bacteria in the gut, thousands of things we could potentially grow, thousands of different diseases we could potentially go through. How do we sort through that huge matrix so that we don't have to do the thousand by thousand experimental design? What have you guys used to try to sort things out? Two minutes.

PANEL MEMBER: So, I think a couple of things that we (inaudible) a little more. I think that leaning on some human data from

interventional studies is useful because of all the potential combinations. There will be many that are just relevant to mice. So, that can make the experimental space more. Something else that we've starting experimenting with that I think will be useful at some point and I don't know if the prime time is there yet but we're working on it. Is try to start using just good old mathematical modeling to predict how communities will behave and put together. And when I say mathematical modeling, today I mean just pure empirical modeling, fitting adjustable parameters to experimental data to then be able to predict how communities of a few will grow together.

Hopefully, at some point, this goes into actual mechanistic modeling, being able to say, you know, from that genome, I expect this needs to be expressed and this is interactions. I don't think the field is anywhere close to that but we'll get there. Personally, I think we're at very exciting time now where we're starting to transition from just enemonology, microbiology,

which is great to actual having a feel, a few assemblies of rules to work from. And I think that when the field gets to the point where engineers and mathematicians can start coming in because there is enough information that you can actually model things. That's when we'll be able to really dramatically reduce the size of the experimental spaces that we can explore.

DR. PANIGRAHI: Yeah, I would have responded the same way. If we want to think about how the consortia is going to change modeling, mathematical modeling is the only way to do it. But even at the same time, we have to think, okay fine, we know this is how the consortium is going to look like. But what will the physiologic scientific change for that to me it may sound like we have to go back to humans. And if it is provided, that is why I was asking those questions. We can probably, because it is not a "drug molecule" and if it is safe, we can do larger studies.

So, whether it is diet, whether it is

exposure, whatever happens we don't really care because these are ammonized. And as long as they are large, all those variables will be taken care of. They will be distributed half and half. So, we call it mod efficacy effectiveness at that point. So, I think there are not thousands of diseases, if you really look at the textbook, there are not that many. So, we have to think about the pathogenesis and see if this has a microbium has a role and then go from there and use the best single one or (inaudible) against consortia. Because once you give it, they are inter consortia even in the newborns that are within a couple of days to health, dozens of bugs.

So, at least I know that I am giving one that colonizes that stays in there. But whatever happens, the argument outcome is what we are interested in and that's what we will check all the changes physiologic and scientific changes.

PANEL MEMBER: I think the other approach is also starting from first principles, on both ends, understanding mechanistically what's

going on in the disease state and better classification of diseases. But then also on an organism by organism basis figuring out what their punitive effects in the host are and basically create this microbial toolbox where, you know, organism A has this affect on these different parameters. And then you can start to pick and choose for this disease that has these defects we will need organisms A, C and E. But for this other organism, for the same disease in a different patient, you may mix and match from that toolbox that's already falls upon it.

PANEL MEMBER: So, I think all these points are important but I still believe in physiology. Going back to Vince Young's comments earlier about understanding the physiology and how these bacteria are interacting and what they're doing. I think mathematical modeling is useful but we still need to keep our eye on what happens physiologically when we put these organisms together.

I guess you had mentioned going after

all these diseases. My feeling is do no harm, first and foremost, and baby steps. To me, recurrent C-diff is one of the easiest ones to go after first and using that as sort of a learning experience and branching up from there is sort of what I personally would think would be the way to go. And always keeping in mind that organisms don't always do what you think they're going to do the same way teenagers don't always behave the way you think they're going to do. No matter what kind of mathematical modeling you do, they'll always come out and surprise you. And I've seen surprising things come out of these bioreactors when we put things together that we were not expecting at all. And then we could go and look in animal models to kind of dig into that a little more deeply. But yeah, I guess I would err on the side of go slow and do no harm.

I mean, the other thing, I have a colleague, Erica Claude who is a neonatologist. And we've had a lot of interesting conversations about NEC. And she pointed out to me something

that I had never even thought about before and that was that for prevention for NEC, is it's 1 in 10, that means there's 9 out of 10 babies that would be getting probiotics that didn't actually need them. And for a preemie, the different for neonates that are healthy born babies, for preemies, she was questioning what this will do long term if we now set their set points with these new bacteria that we put in. Nobody knows what's going to happen. And so, she's very hesitant to use probiotics and, you know, I never even thought of that before. I think we just have to be careful.

Well, actually so what she's promoting is decreased use of antibiotics and push breastfeeding because breast milk has been one of the most protective elements. And really low birth weight seems to be the risk factor. So, if she can get them to grow, gain weight, they don't have this same risk of NEC.

PANEL MEMBER: I would agree with what's been said. I mean, we're dealing with incredibly

complex interactions and incredibly complex systems here. So, we just need some levers really to get in there and really start understanding NEC (inaudible) from this point on. And one of the reasons, obviously, why B fragilis was attractive was because we knew it was likely going to be safe. But it also has some of the features that we want to see in some of the effects in animal models that we think might be able to make a difference. B fragilis is probably having an effect on other organisms and it's probably well, we know, it's changing micro bio makeup to a certain degree. It is turning it a little bit back to what it is in wild type animals.

So, it's not B frag alone and it's probably not 4- EPS alone. It's probably a whole slew of things that are stewing around in the soup that might, some might be getting in anyway because they penetrate through diffusion. Some maybe can only get through a leaky gut. I think we just have to start chipping away and figuring out what these things do and that will lead us

into other directions, maybe into other diseases.

DR. PANIGRAHI: I'll just add one point to this in terms of changing the micro bio permanently or doing some damage to it. There have been, in our studies, we looked at the microbiome for six months and after the fourth month, it goes down to zero. By six months you don't see that particular strain in there. But others have done, not in neonates but in infants, Isa Lorri and others in Europe, for the asthma allergy studies that it doesn't stick permanently, it goes away, so you're not changing it for good.

And, in fact, we were asked by the India IRB. One crazy person came and said, you have to follow them for 18 months, 2 years and finally not 2 months but 2 years. I said what, 2 years, our protocol has been approved for 2 months, we can't follow them for 2 years. Why, how do you know you have sepsis, you have millions of babies. How can you prove to me that they're not going to grow horns in 2 years?

And that is the real critical period and

what happens is you have all stunting and everything and the GI dysfunction takes place. How can you, I said, how will they be able to better fight against others. But we had to follow for 2 years. We didn't do microbiome but we had to follow and show that there was nothing drastic.

So, long term follow up, longitudinal assessments and with all the tools we have now, I think we should be fairly comfortable telling how we are changing and if the change is good or bad. And if something wrong happens, that happens when you're trying to discover something.

SPEAKER: Debra Topam with Knowledge Bank. Dr. Panigrahi, could you talk a little bit more about the dosing that you used for lactobacillus along with what dosing you used for the FOS and what kind of type of FOS that you used?

DR. PANIGRAHI: Yeah it was fructo-oligo saccharide 150 mg in each dose and ten to the part one billion organism's lactobacillus plantarum. So, it was available as a levelized power which

was mixed with 5 percent extra saline on site and then it was put into the baby's mouth for seven days depending on whether we started on day two, we gave it for all of them received about seven doses.

SPEAKER: Of the 150 mgs of FOS?

DR. PANIGRAHI: Yeah.

SPEAKER: And I guess at that point, are all of the babies in your group primarily breastfed? So, they might have gotten the MI million oligo saccharides along with the fructo saccharides?

DR. PANIGRAHI: Yes.

SPEAKER: That would be the sugar (inaudible).

DR. PANIGRHAI: Yes.

SPEAKER: (Inaudible) at that point?

DR. PANIGRHAI: And, in fact, we had some very angry people writing to NHO that you have been unethical. Breast feeding is the only thing that really helps that has reduced this and that. We said no, you are unethical because you

have been chanting about breastfeeding for hundred years. Nothing has happened, infection has gone up. Although breastfeeding rate has gone up in developing countries, infection rates haven't gone down at all, it has gone up. So, all the ones that we are showing that are exclusively breastfed, unless breastfeeding was established, they were excluded, there were quite a few. So, in spite of having breast milk, in spite of having oligo saccharides, maybe whatever there is not enough good bacteria that could protect them during that window.

SPEAKER: Christian Riel here with University of Michigan. So, my question is, I guess, related to that and I maybe the other panelists can chime in too. How did you decide to co-formulate with FOS to begin with? Was it based on preclinical data, was it based on some idea of what substrate this would grow best on and in general, how do you make those decisions? When do you arrive at the decision where you say hey, do you know this probiotic is not enough we need to

co-formulate this?

DR. PANIGRAHI: Well, I can answer. That was the only thing I have done in my life without solid scientific evidence. If you ask me, give the same plantarum without fructo saccharide. With it colonize, will it downsize, we didn't do that. I think we were impatient because we had already spent four or five years doing two or three other clinical trials. That we expected that they are going to colonize and have something. When that didn't work, we had one organism that was expected to colonize. We wanted to do it better. And we have enough evidence that if it doesn't get probiotics, that's not enough. You have to get something from outside so it was just we wanted to increase our chances of success so we added antidote for (inaudible) be better, all that we don't know we have to work on it.

PANEL MEMBER: So, just to keep things going on this, I put up a slide, we have a couple of slides, one focusing on models. Everybody talked about a model in one way or another. And,

you know, so in essence, you know, we've talked about it, we've heard about the complex relationship between host and microbe and that complicates these models. We have everything from a hostless fermentation model to humanized mouse models to, you know, the human model itself.

And the focus of this session is on strains. And so, I think I've heard on multiple occasions that strains matter and that just a simple L. plantarum, out of the 20 is not the same. So, how do you leverage these models and if I could go one by one how have you leveraged these models? Is it one particular strain of B. frag, are the strains falling part? In your robo gut are you checking multiple strains of the same gene species? So, maybe just kind of briefly go through and talk about why strains matter and how leveraged models.

PANEL MEMBER: Yeah, well with regard to B. frag, definitely strain matters, there's no question about that. There are enterotoxic strains, we wanted to avoid that. The strain that

we ended up using happened to be the strain that was Sarcuses lab but we have used other sources of B. frag that have not known the same level of efficacy. So, there seems to be some magic sauce in the particular strain that we're dealing with. We don't really know what that is. You know, again we know what some of the metabolites are but we don't know really whether -- well, it's certainly not the only metabolites and are those the only metabolites that are having an effect distantly in (inaudible), we don't really know.

PANEL MEMBER: Yeah, so to probe a little bit more on the basis of this question is that B. frag paired with mouse model or is that B. frag going to be something relevant to humans.

PANEL MEMBER: Yeah. There are other mouse models, there are other animal models of AST. There's a BTBR model, you know, which is an inbred spontaneous model. There's the cat nap two model which is a genetic model and we do see efficacy on the behaviors in all of them. But all the models are different. They all cause

different aspects of the disease and they may or may not replicate what's happening in humans. Not all of them have a gut component so we can't really look at the effect on the GI abnormalities in any of them. But we do see consistency between those three models, the effect on the behavioral components, at least the behavioral components as they are shown in the phenotype of that particular model. So, that much we know.

But, you know, in answer to the last question of this, are humans still the most reliable model. I don't know if they're the most reliable model but to me they're the most important model. So, as soon as we can extrapolate safely beyond the mouse and get it in humans then I think the story starts over again and then we can start learning new things and there's so much (inaudible). We'll certainly learn more in our exploration. Whether it results in effective treatment, we hope it will, but (inaudible).

DR. PANIGRAHI: I think, so my view is

for matters of safety PK/colonization, humans have worked best for us and we haven't really relied on the results of the animal models for these two considerations for a number of reasons. I think for matters of (inaudible) mechanism, in general is a screening tool when you have to go through a number of different possibilities. Humans for obvious reasons are not usable or appropriate. So, for that we've relied in animals and I think for those uses they can be very helpful.

Some of the models that have obvious limitations like germ free models or antibiotic treated animal mouse models of SPF background, can actually be very useful to understand causality to learns things about mechanism. So, I think depending on the use that you give them, even fermentation models can be useful if, I think, if you're using -- if you're trying to explore simpler questions that aren't really where the immune system doesn't really play a very obvious role, you just want to understand microbiome interactions. It really depends on what question

you ask.

SPEAKER: I'm Lawrence Royce and I wanted to know, there was one mention of lysates used and I was wondering, has anyone done any work that killed species and what kind of successes have you had. I know there have been quite a little bit of research that was done in the former Soviet Union using lysates and very successfully not stimulating but modulating the immune system and it was very interesting results. I think, one of the first discoveries in the Soviet Union was in 1976 with, I think, with lactobacillus rhamnoses, a lysate that had some interesting results. And I was wondering, does anyone else, who has done work in this area?

PANEL MEMBER: We've tried a lot of either bacterial lysates, heat killed organisms or a variety of end points. And I think it depends on the organism and the end point as to whether it shows an effect or not. So, for some things it works, for other things it doesn't. I think figuring out the why and when can you predict when

to work or not still needs to get resolved partly to define sort of what the molecules and what's the pathway of that interaction. Does it really require colonization or not, does it require a certain threshold that we're not giving, you know, a concentration that we're not giving lysates in dosing regimens and things of that sort. But I think that for certain aspects or certain phenotypes, either lysates and/or killed organisms, at least in our hands, have been successful.

PANEL MEMBER: The only other thing I would say is, you know, I mean I think with regards to AST and the part that we've done so far, I think the organism is important. As far as what would be in a lysate that might have an effect, well we certainly looked at the metabolomics and we looked at the metabolites that are different and there certainly could be situations where a metabolite might be beneficial. But then, you know, when we're going to have a continuous source of the metabolite, otherwise the

metabolite itself might be a drug. And then I think we would want to know what component of that lysate is responsible for the effect and then really focus on that particular component.

DR. PANIGRAHI: Now, I would respond the same way. I mean, we have tried but only with very specific bugs. Only (inaudible) bacteria whether that would do the same thing in our in vitro and animal models and they didn't. That doesn't mean that the components, if we're now thinking that it's not the whole bug that is doing 100 percent of the thing if it is even a modulation, maybe it would have the component would have done something or that the module is in place even if it didn't help against bacterial (inaudible) and (inaudible).

It all, I guess, boils down to the physiology and what you are trying to study. I think in future years we will see different components and how they really interact with each other or with host cell and the ultimate physiologic effect. Those will be done in future

years.

PANEL MEMBER: Well, there is PSA that also came out of Sarcuses work I believe when he was at UCLA. And there was a company, I can't remember the name of it, Symbiotic or something like that, that's working on specifically PSA and its effect on the immune system.

PANEML MEMBER: I'm going to interject, sorry, as moderator. I'm going to take my prerogative and ask kind of one last topical question. It really has to do with the fact that clearly, we've seen the historical use of probiotics and now we see this upsurge and rationally selected and based on human commensal colonization and causal association with diseases. So, that's a series of questions here but I'm going to skip through a little bit.

And I think it came up, I didn't mean to or I was going to bring it up but Elaine mentioned this recent study and it has to do with the idea of what our high resolution assays, what are our assays. Is colonization, even in a human model,

what is that telling us? And so, we have some recent papers that have just come out and they have suggested there may be more to the story of just pass through and detection in stool. And so, I want to ask, you know, essentially a question here. I mean, we've heard about high resolution assays to detect that this specific strain, that actual organism that you've given, Burnette talked about that. What is the role for actually looking within the intestine, within other communities to assess the efficacy of your strain?

Because, you know, in the paper, one of the things that they did correlate is mucosal, host transcriptional response is what correlated with mucosal colonization. And I think that's an interesting concept and I'm wondering how each one of you take that idea and move forward with it or not. I mean, whether or not colonization through stool detection is sufficient.

DR. PANIGRAHI: Well, I would fully agree with you and I won't say that especially if I'm thinking about the organism that I used. I

think because I did in vitro experiments and animal experiments, I know that they had to be in contact with mucosal cells. And now we have extra non-GI impact. And so, if they wouldn't be there, they are not associated with mucosa. I would be surprised if they're going to do their job. Doesn't mean that other probiotics, other components won't do it.

So, I think that if I can take IFC every three days for my baby, I'll be more than happy to do it. And many people have complained that oh, stool has nothing to do with it. They come get in and get out, the real ones are inside so you're not looking at it. But that's the best surrogate we currently have and I'm sure and that's why we use animal models and that's why we have to have some other models to have some idea.

PANEL MEMBER: I totally agree with you and actually we really need better diagnostics than we currently have. That paper that you mentioned, it's where the rubber meets the road. The epithelial microbial interface, it totally

makes sense that there's going to be stuff happening at that interface. And stool is kind of a crude measure, it's just kind of passing on through. So, we're missing a lot of very useful information but biopsying is difficult. As you know, we can't always put that into clinical trials and it's complicated ranging from, you know, colonoscopy time and the colonoscopy suite, being able to get the biopsies. But there's no question that we have to come up with better diagnostic tests.

I mean, if you think about it, when we give antibiotics, we measure creatinine. Why aren't we measuring what it's doing to the microbiota as part of, you know, we measure serum levels of the immune glycosides, we do all these things with antibiotics. We don't even measure that. Like there's a lot of diagnostics that need to be developed and I think that whole area is being completely overlooked.

PANEL MEMBER: I'll disagree a little bit in that I don't know that colonization itself

is always required. To use the example from bacteroid fragilis either polysaccharide A or other bacterial single lipids, those products by themselves can still exert effects on the host and in disease models. So, it's not clear that, clearly you don't need bacterial attachment because there's no bacteria in those experiments but then there's a question of how does those molecules interact with the host.

And so, there's still some host recognition of those molecules in some capacity. But whether or not you need bacterial colonization as a starting point, you know, in your study that was sort of a prerequisite to move forward is that the organisms had to colonize. But if one can identify the molecules themselves that then have an effect, you may be able to bypass that stuff of bacterial colonization itself.

PANEL MEMBER: Yeah, I would agree. Particularly with B. frag, I don't think we expect that it's going to colonize (inaudible). We aren't anticipating it will.

PANEL MEMBER: I still have to read the paper. I skimmed the abstract and looked at the summary but I'd be skeptical about throwing away all what we have learned from fecal samples because everything that this field knows is from fecal samples. And I think we've learned very useful things about what happens to immune phenotypes, for example, based on information you can gather from stool. What happens to colonization resistance based on information that's in the stool and then acted on these predictions to learn other things.

So, I'm sure that other types of data are useful and when we can all have them with the tools available then let's all have a party. But until then, you know, especially, you know, realistically it's (inaudible) to ask healthy individuals to go through a colonoscopy plus anesthesia plus whatever they had for no benefit for the healthy individuals to ultimately get this information. We're never going to get that. But we are going to get a colonization from fecal

samples if we ask for fecal samples and the patients are nice enough to give them to us. So, I think there's a lot we can do with the information from stool samples.

PANEL MEMBER: So, I didn't mean to suggest that stool sampling is not worth doing. And, in fact, in that cell paper they do sample stool in addition to doing the biopsies but they have more ends of sampling from stool samples then they do biopsies. And certainly, I think that it's important, I guess, for certain diseases though like for recurrent C- diff, for example, I think it's a diversity issue. Because actually I think less of a host immune component here maybe then say for IDD, for example. And so, for conditions like IDD or ulcerative colitis, maybe we will need that additional information from biopsies. And I think a lot of it is going to depend on the disease entity that we're talking about.

PANEL MEMBER: Okay with that, sorry. Last question, sorry I forgot, please.

SPEAKER: Hi, my name is Joan Holly, I'm from Data RI, LLC. It's a regulatory consulting firm in Maryland. I have a very general question. So, in some of your studies, you're using naturally existing strains as a drug to treat diseases. And if one day you found it's an efficacious drug and it's being proved is there IP protection on this drug and who's the IP? That's my question.

PANEL MEMBER: Boy, that's a question for our IP attorney who is not here. Yeah, well I think we believe that we'll have protections. Certainly, we'll have the protections associated with the drug approval with a biologic approval. But, you know, also method of use patents and such as that, I think, will provide some level of protection as well as the fact that it is a specific strain that we're talking about. And we believe that we'll be able to turn that into a therapeutic that's easy to take a lyophilized preparation that's easy to take and we'll have specific knowledge around the manufacturing of it.

DR. PANIGRAHI: So, the bottom line, I would say that naturally our current strains are not patentable in general. But if you show that it works for sepsis you can patent it. But if you show that it's working against cancer, you can patent it too. And that's the general take home I have learned in the last few years. But it will be really interesting when you find out if there is a component.

Like in our bug, we see that it does something, it secretes something. Then it again comes very close to the cell intimately attaches. So, then if we can find out what it is what is that piece, what is the component that's doing the job, then that can be a drug, that can be a separate idea altogether. But until then, the live bug, the whole bugs, I think it is yes and no, you can patent and you can trademark, you can do all different things but it may not be as robust as having a chemical component.

PANEL MEMBER: Yeah, again I should say if you're developing an organism that's not

currently available as probiotic and you get into a drug development process with it, you file and IND and you start generating data. My understanding, and I don't know if the people from CFSAN are still here. But my understanding is that once you go down that pathway for something that hasn't been commercialized as a nutritional you can't do that anymore. You can't start marketing it as a nutritional once it has been shown to be a drug.

SPEAKER: I was just curious about the strains that are not genetically modified, that are naturally existing. So if, for example, the strains you found can be used to treat one disease and it's been approved and then in another study have been found to be effective for another disease but yet you didn't patent for that use. Can consumers just use it for another treatment without, you know?

PANEL MEMBER: They'd have to source it. They'd have to get it from somewhere and I would think if it's part of somebody's clinical program

and they started seeing evidence that it might work in another condition, they'd jump on those patents right away to try to cover that from an efficacy use standpoint.

PANEL MEMBER: I think the other complicating piece there is what is the idea of a biosimilar in the field of probiotics. How far away from Bacteroide fragilis or any of the eight strains that (inaudible) has you have to be. Is it a different B. frag strain sufficiently far enough?

Everyone has patents written to be incredibly broad but until this goes to the courts and having courts adjudicate how narrowly those really have to be drawn, how many snips away from the genome sequence they submit do you need to be to be infringing on their IP? So, you'd still be able to make some money off this even if they did all the leg work.

PANEL MEMBER: All completely untested. As you said, that's the wild west. And biosimilars are not as easy to get on the market

as a generic is. You do have to do some clinical work.

PANEL MEMBER: Okay so with that, we're going to close session three. Thank you very much, speakers, very interesting and entertaining talks, I really appreciate it. And we're going to round the basis on this workshop and I'm inviting Dr. Carolyn Deal who opened us up today to give some closing remarks.

DR. DEAL: Well, I know it's getting late and I will just take two minutes. First of all, I want to thank all of our speakers today. Really appreciate them putting time and effort into their talks and contributing this. And then really thank all of you in the audience.

You all were in this room, there is actually two and a half overflow rooms in this building downstairs that were also full. So, I want to thank everyone who participated in all of the discussions.

Because I think this has been something we really wanted to hear a broad breadth on input

on. I know my colleagues at CBER and CFSAN did also. But then last of all, I really want to thank the organizing committee which some of whom you've seen today who were the moderators for these sessions who put a lot of time and effort into coming up with this program. And so, I really want to thank all of them and all the colleagues from CBER and CFSAN who participated and all the other NAIAD participants.

And so, the last thing I wanted to leave with you in thinking about all of this is I think all of us have a lot of enthusiasm for the possibilities in the future for live microbium based products. I mean, this is a new, growing and evolving area and I think we're all learning. We're intrigued by a lot of the possibilities. I think we also know that there are cautions that need to be considered, some of which have come out today.

I think some of the things we've heard is there may be advantages to considerations for well characterized products in terms of

reproducibility of manufacturing and more importantly even to think about to ensure reliability of use. So, that's one consideration as we move forward.

I know there's been some debate about where there should be the lines between the regulatory considerations for probiotics and live bio therapeutics and I think there's always some evolution and thought of that as how we go forward. But I think then one of the other things is those are the product issues but there's also the clinical issues.

Many of these are complex infections and diseases, they're not all well-defined and I think the necrotizing enterocolitis has certainly shown us that that it's not always a well-defined infection. And I would even argue sometimes C. difficile infection is not also.

And so, it shows the points to the need for clinical studies with well-defined clinical endpoints and also with well-defined diagnostics in those studies. So there, I think, is the other

potential gap area that we see is some of the need for new and better refined diagnostics.

All of these are needed to support regulatory decisions from our FDA colleagues in the future. I think importantly for all of us in the public health and medical community what we all really want and most importantly is to be able to have reliable regulatory decisions and to provide informative, useful and reliable information to patients and to the providers.

And so, that's the thought I really want to leave you with is all of this as it evolves over the next years and as we get products that we can move into routine use. Because that's the ultimate goal is to be able to provide reliable, useful information not only to the providers giving these products but to the patients that receive them and hopefully that we can improve public health.

So, that's all the comments I wanted to make. Again, thank you all for coming and I hope you don't drown on the way home because the

hurricane I've heard has moved up here leaving our North Carolina colleagues ability to get home.

So, thank you all very much.

(Whereupon, the PROCEEDINGS were adjourned.)

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CERTIFICATE OF NOTARY PUBLIC

STATE OF MARYLAND

I, Thomas Watson, notary public in and for the State of Maryland, do hereby certify that the forgoing PROCEEDING was duly recorded and thereafter reduced to print under my direction; that the witnesses were sworn to tell the truth under penalty of perjury; that said transcript is a true record of the testimony given by witnesses; that I am neither counsel for, related to, nor employed by any of the parties to the action in which this proceeding was called; and, furthermore, that I am not a relative or employee of any attorney or counsel employed by the parties hereto, nor financially or otherwise interested in the outcome of this action.

(Signature and Seal on File)

Notary Public, in and for the State of Maryland

My Commission Expires: December 2, 2021

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