

This transcript appears as received from the commercial transcribing service after inclusion of minor corrections to typographical and factual errors recommended by the Technical Point of Contact.

ADVANCING SCIENTIFIC AND REGULATORY APPROACHES
FOR USE AND DEVELOPMENT OF BIOMARKERS
FOR PREVENTATIVE VACCINES
MEETING

MONDAY, SEPTEMBER 16, 2019

8:34 A.M.

5601 FISHERS LANE

ROOM 1D13

ROCKVILLE, MARYLAND 20852

1 APPEARANCES

2 SPEAKERS:

3 MARION GRUBER, PHD - DIRECTOR,

4 OFFICE OF VACCINES RESEARCH AND REVIEW CBER/FDA

5

6 DEBRA YESKEY, PHARM.D. - HEAD OF REGULATORY AFFAIRS,

7 NORTH AMERICA CEPI

8

9 JEFF ROBERTS, MD - ASSOCIATE DIRECTOR FOR MEDICAL

10 COUNTERMEASURES AND SCIENTIFIC AFFAIRS OVR/CBER/FDA

11

12 BARNEY GRAHAM, MD, PHD - DEPUTY DIRECTOR,

13 VACCINE RESEARCH CENTER, CHIEF

14 VIRAL PATHOGENESIS LABORATORY AND TRANSLATIONAL

15 SCIENCE CORE VIRAL PATHOGENESIS LABORATORY

16

17 THEODORE PIERSON, PHD - CHIEF,

18 VIRAL PATHOGENESIS SECTION CHIEF,

19 LABORATORY OF VIRAL DISEASES

20 LABORATORY OF VIRAL DISEASE, NIAID

21

22 JULIA LEDGERWOOD, D.O. - CHIEF MEDICAL OFFICER AND CHIEF,

23 CLINICAL TRIALS PROGRAM VACCINE RESEARCH CENTER NIAID, NIH

1 KATRIN RAMSAUER, PHD - CHIEF SCIENTIFIC OFFICER,
2 THEMIS BIOSCIENCE GMBH
3
4 LONG WANG, MD, PHD - DIRECTOR,
5 GLOBAL REGULATORY TEAM LEADER,
6 VACCINES &INFECTIOUS DISEASE MERCK INC.
7
8 NANCY SULLIVAN, PHD - CHIEF,
9 BIODEFENSE RESEARCH SECTION VIRAL PATHOGENESIS LABORATORY, NIAID
10
11 JENNY HENDRICKS, PHD - HEAD BIOMARKERS,
12 VIRAL VACCINES, JANSSEN VACCINES ID&V
13
14 RONG XU, MD, PHD - DIRECTOR OF IMMUNOLOGY,
15 PROFECTUS BIOSCIENCES, INC.
16
17 PROFESSOR MILES W. CARROLL - DEPUTY DIRECTOR,
18 HEAD OF RESEARCH & DEVELOPMENT INSTITUTE NATIONAL
19 INFECTIONS SERVICE, PUBLIC HEALTH ENGLAND
20
21 JOHN MASCOLA, MD - DIRECTOR, VACCINE RESEARCH CENTER, NIAID
22
23 JOSH REECE, PHD - SENIOR DIRECTOR, VACCINE RESEARCH & DEVELOPMENT,

1 VACCINES & ANTI-INFECTIVES BUSINESS UNIT, EMERGENT BIOSOLUTIONS

2

3 RAFFAEL NACHBAGAUER, MD, PHD - ASSISTANT PROFESSOR DEPARTMENT OF

4 MICROBIOLOGY ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI

5

6 KARIN BOK

7

8 CRISTINA CASSETTI

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

1 **SCIENTIFIC MEETING**

2 **MONDAY, SEPTEMBER 16, 2019**

3 **8:34 A.M.**

4 DR. GRUBER: So good morning. My name is Marion Gruber. I was just
5 informed that I need to tell people that we have to wait another couple of minutes before we can
6 start because there's a long line of people outside waiting to get their badges. And I just wanted
7 to make this announcement because I think we have many, many participants that are connected
8 through webcasts and they're waiting. So let's just give it a couple more minutes. I mean, I hate
9 to be sort of behind schedule right from the get go, but maybe another, what, four or five minutes
10 and then we get started. Okay, thank you.

11 DR. GRUBER: Well, so folks are still coming in, but I think in the interest of
12 time I would like to get started so that we don't get too far behind. Good morning and welcome.
13 My name is Marion Gruber. I'm directing the Office of Vaccines, Research, and Review at the
14 Center for Biologics, FDA. And on behalf of the FDA and the National Institutes of Health and
15 the Coalition for Epidemic Preparedness Innovations, I really would like to welcome you to this
16 workshop entitled Identification and Use of Biomarkers to Advance the Development of
17 Preventive Vaccines.

18 And I don't know if my slides are up. I think I'll need to give it a minute here.
19 Ten seconds. Yes, so while they're looking for it, just I think as you all appreciate for preventive
20 vaccines for infectious disease indications, a well-recognized biomarker has tremendous
21 potential. It can guide basic research, facilitate vaccine development, guide the effective use of
22 these products, and of course in recent years biomarkers have become particularly important
23 because for some of the vaccine products that we regulate pre-clinical or pre-licensure, pre-

1 licensure or clinical disease endpoint studies are really challenging to perform because of low
2 disease incidents, et cetera.

3 So and biomarkers also help in just the very licensure pathways that the FDA has
4 and Dr. Jeff Roberts will discuss that a little bit more in the introductory session this morning.
5 Recent legislations including the 21st Century Cures Act and also the reauthorization of the
6 Prescription Drug User Fee encourages the use of biomarkers to enhance the development and
7 approval of new drug and biological products.

8 And that, of course, includes vaccines. And for these reasons all we have thought
9 of was timely and it was time to have a discussion of the use of biomarkers to advance the
10 development of vaccines and so we partnered with our colleagues from the NIH and C.E.P.I. to
11 convene this workshop.

12 The purpose really is to have an information exchange with our stakeholders from
13 industry academia and other government agencies about the scientific, the clinical, and the
14 regulatory challenges that are encountered in the discovery, the characterization, and
15 qualification of biomarkers for preventive vaccines.

16 And so the objectives of the workshop that you also can find, you know, as part of
17 the agenda is really to provide the context and understand the importance of biomarkers in
18 vaccine discovery and development. And we're going to use a couple of successful case
19 examples over the next couple of days to illustrate that.

20 We want to clarify the regulatory framework that informs the use of biomarkers
21 and vaccine development. We want to assess the quality of the evidence for biomarkers to
22 support decisions, that is regulatory decisions, programmatic decisions, and other decisions
23 regarding candidate and licensed vaccine products for specific infectious diseases.

1 We also want to explore how new technologies and innovations can be applied to
2 advance the science of vaccine associated biomarkers and then of course understand the
3 institutional perspectives and priorities for vaccine development and deployment.

4 So I think we have put together a very exciting agenda and we are very pleased to
5 announce that there is a webcast and personal attendance combined over seven hundred people
6 have registered and attend this workshop. And that sort of is a testimony to the really importance
7 of this topic.

8 And so I would like to take the opportunity here to thank all speakers who have
9 accepted our invitation to present their data and findings and to inform our discussions over the
10 next couple of days.

11 We have divided the workshop into six sessions and they are listed here. In the
12 interest of time, I don't want to read through this because you can see it from the agenda. We
13 want for this to be a very interactive workshop, which is why every session except the
14 introductory session that I will be moderating includes a Q and A session at the end.

15 And there participants are invited to ask questions and contribute this way to the
16 discussions. And for these sessions we also have what we refer to as virtual moderators who also
17 will field questions and accept questions from people that attend through webcast.

18 In addition, we put together a survey or survey questions that we invite you to
19 respond. And the purpose is really to solicit feedback from you on specific issues and what you
20 see as priorities or challenges associated with the development of biomarkers for preventive
21 vaccines.

22 And we would want to ask you to respond to these questions tomorrow morning.
23 So what we're going to do, we're going to hand these survey questions out using good old paper

1 format and it's going to be we collect them before you go to lunch tomorrow.

2 And then during lunch we will compile the responses, pull out some themes if
3 they emerge, and then inform the panel discussion this way. I would also like to mention that
4 this workshop is being transcribed and the plan is to publish a meeting report.

5 A few housekeeping items. We really need to keep on time and I'm already way
6 over my allotted time. But there is this little traffic light here, green, yellow, and red button.
7 And the moderators have to play traffic cop. So when you see the yellow light flashing, that
8 means you will have another five minutes for your presentation to wrap it up.

9 So in the end I would like to really thank the organizing committee for their
10 enthusiasm and effort and the hard work that went into putting this workshop together.

11 And they're listed on this slide. From the FDA, Sara Brown, Jeff Roberts, Valerie Marshall, and
12 myself. From the NIH, Karin Bok, Cristina Casseti, John Patterson, and Barney Graham. And
13 C.E.P.I., Debra Yeskey and Dr. Don O'Connell.

14 And last but not least, there are many, many people who worked behind the
15 scenes to make such attendance and such workshop possible. They are in charge of making the
16 hotel accommodations, the travel arrangements, all the logistics that have to be taken care of here
17 to run this workshop smoothly.

18 And all these people really deserve a special shout out and they have been
19 participating from the FDA our Office of Communication, Outreach, and Development and the
20 many people in the Office of Vaccine Research, and of course the NIH. So thank you to all of
21 you.

22 And without further ado I think we should get started. And I am now turning into
23 moderating the introduction session. And it's my pleasure really to invite to the podium Dr.

1 Barney Graham who's going to make a couple of welcoming remarks on behalf of the NIH. And
2 then launches right into his presentation. Thank you.

3 DR. GRAHAM: Welcome. And greetings on behalf of Dr. Fauci. This would be
4 his job if I, if he was here. He's doing the dual mandate that I'm going to be talking about in just
5 a minute. And is in the Congo with the Secretary and with Cliff Lane [phonetic] talking about
6 the recent Ebola outbreak, the ongoing Ebola outbreak.

7 So NIAID has a dual mandate. It not only does the basic research, let me do this.
8 It not only does basic research on microbiology and infectious diseases and immunology, but it
9 has to respond rapidly to emerging and reemerging infectious disease threats.

10 And there are many, and this is a map that Dr. Fauci often shows that, of
11 emerging infections, reemerging infections that have occurred just since HIV started this map
12 back in 1981. And not only are there emerging diseases, but there are many diseases that are old
13 and that still haven't been solved.

14 And so as we go forward looking for vaccine solutions for these old and emerging
15 diseases, NIAID balances the scientific opportunity and public health needs and looks for
16 opportunities to make impact. Especially we're talking today about vaccines.

17 So this is a very timely meeting and I'm really glad Marion thought of doing this.
18 And NIAID is glad to help and I know C.E.P.I. is glad to help. It's part of their mandate. And
19 so having better ways, easier ways, faster ways to get vaccines into common use is I think in all
20 of our best interest.

21 So I work with the Vaccine Research Center, Dr. Mascola is here, the director.
22 He's also part of this welcoming committee. And the Vaccine Research Center was founded on
23 the basis of developing an HIV vaccine. And we still don't have an HIV vaccine, but the

1 technology that has been developed to try to make an HIV vaccine has stimulated what we think
2 of as a new era of vaccinology in which a lot of new things are possible.

3 And so I'm going to spend my time talking about things that we might use in the
4 future. Not that we're quite ready to use them yet and we won't press that too hard. But there
5 are many things coming that I think could be used as biomarkers in the future.

6 And so the technologies that have been developed for HIV vaccine research have
7 led to new solutions or partial solutions for many of these viruses. The VRC has used a number
8 of different platform technologies to address these things.

9 And it is in a unique position to carry things from discovery up through clinical
10 trial and advanced development, because not only is there basic research program but process
11 development capacity, pilot plant, GMP, manufacturing capacity, clinical trials, and GLP
12 analysis. And then many, many partners within NIAID, especially to advance these concepts
13 into use.

14 So over this last ten years this is the balance of this challenge we have from
15 emerging viruses and the need to improve licensed vaccines. And really this is an influenza
16 slide, but the only thing we had to battle back against all these new emerging infections has been,
17 you know, egg grown vaccines.

18 But it's changing. And we now have a number of new technologies that are
19 changing what we can do not only to make vaccines but to evaluate vaccine responses. And so
20 vaccines are typically developed in an opportunistic way. These first four licensed viral vaccines
21 were made often just through will, the will of an individual.

22 But after that these clusters of vaccines were developed because of new
23 technologies. Here because subculture allowed growth of viruses in high titer. And here

1 because molecular biology allowed reassortants and molecular clones and things like that to, and
2 recombinant technology to make new vaccines.

3 And we hope that this new era of technology like structural biology is one of
4 these new tools, we hope that it may have created a solution for RSV. We'll see. But there's
5 many other technologies including multi-parameter flow cytometry and index sorting, rapid
6 human monoclonal antibody development and sequencing of even single cells that has created
7 these new opportunities.

8 And so for RSV where you have a protein on the virus that is unstable and flips
9 into this form and generates these kinds of immune responses, if you can learn how to stabilize
10 that you can then get immune responses that are much different. Here measured this by
11 neutralizing activity.

12 So there's, the technologies that in addition to making better vaccines through
13 structural biology, we can make things faster using concepts like synthetic vaccinology or
14 sequences from a remote place can quickly be brought into places where synthetic DNA and then
15 proteins and antibodies can start being generated within days or weeks.

16 And platform technologies have allowed us to make things faster. This is just an
17 example of DNA vaccine technology from the time of decision to make a product to the time of
18 first clinical trial. And with the Zika product from the time we chose the sequence to the time it
19 went into humans was around a hundred days.

20 And there's now technologies that can make it as short as fifty days from
21 selecting a sequence to starting clinical trials. The problem is, at least for emerging infections,
22 we're still often not fast enough. Even in the case of this Ebola outbreak, for us at least using the
23 Chimp Ad3, a vaccine that had already been in a vial, it had not been in Phase I trials yet but was

1 in a vial.

2 Despite getting it into West Africa through the Division of Clinical Research at
3 NIAID within about six months, it wasn't fast enough in Liberia to get an answer in the efficacy
4 trial. Now the VSV vaccine did get an answer, at least a partial answer in Guinea. And that was
5 good.

6 But in general we aren't getting there fast enough. And even with the Zika
7 vaccine, rapid development, getting into a Phase IIB efficacy trial within a year wasn't fast
8 enough to get a full result for efficacy in this Zika outbreak.

9 And so we need ways of getting new vaccines through the system that don't
10 require necessarily full field trials. And we need ways of evaluating vaccines that can make the
11 whole process go faster through the regulatory process.

12 So I'm going to focus on the role of monoclonal antibodies and the future role of
13 monoclonal antibodies in helping us solve some of these problems. And antibodies in our new
14 way of thinking about vaccinology are critical for antigen design, for understanding vaccine
15 formulation and regimen and schedules.

16 But today I'm going to talk about mostly their role in end point analysis. And not
17 only using functional properties of antibodies to do, to develop assays, but understanding the role
18 of antibody lineages that are important for getting to the right place with a vaccine program.

19 So influenza has used an antibody surrogate endpoint for many years and this is
20 1942 data just showing that HAI correlated with neutralizing activity. Of course everybody
21 really wants neutralizing activity, but here since HAI was a simple assay and correlated, that
22 became a standard.

23 And by the mid-40s, HAI, which had been correlated with neutralization and now

1 is correlated with disease outcome, showing that titers are around sixty-four, maybe a hundred
2 and twenty-eight, people with those kinds of titers had much lower illness during influenza.

3 And so HAI forty years later is still something we think about as a criteria for
4 licensing influenza vaccines, but the new H3N2 virus doesn't hemagglutinate very well. And
5 hemagglutination and the types of the red blood cells you need for different flu viruses are, vary.
6 And we're learning more and more how unreliable just this hemagglutinin is.

7 And so the question is do we need to go back to, do we need to go back to
8 neutralizing activity? And so for influenza the key molecule is a target for vaccine is the
9 hemagglutinin as you see here in red. The variability in the head is much greater than the
10 variability in the stem.

11 And of course if you're trying to target these conserved stem epitopes that can
12 mediate neutralization, they don't, they will not perform the hemagglutination because the
13 hemagglutination is based on an epitope up here in the head.

14 And so if you want to have a stem program or if you want to have a program that
15 targets antibodies like this one that come in just below the classically defined epitopes on the
16 head of hemagglutinin, but this does not hemagglutinate, then you need a different approach for
17 influenza correlative immunity. Especially for these types of antibodies. Either on the stem or
18 the head.

19 So we've been working on reporter viruses. We had had a group of lengthy virus
20 reporters, pseudo-type viruses, but the sensitivity of those at the neutralization was so much
21 greater than a normal virus because of the density of HA on the surface that we made an effort to
22 recreate a panel of viruses that could be reporter viruses for neutralization either by replacing the
23 PB1 gene with a fluorochrome or the HA gene with a fluorochrome in the case of pandemic

1 strain viruses.

2 So these can now be single round assays that read out in a high throughput
3 manner and you can do large studies, for instance with all these different monoclonal antibodies
4 with twenty-three H1 viruses, twenty-six H3 viruses, and groups of relevant pandemic strain
5 viruses shown here. And start getting readouts that may be more consistent and reproducible
6 than HAI assays.

7 So, for instance, just as an example, the Crucell antibody for group two viruses is
8 shown here. And it neutralizes all the group twos but not the group ones. The group one
9 antibody 6261 is just the reverse. So this type of assay can be developed in high throughput.
10 And I think it's time to start considering whether HAI is really relevant or not.

11 But the question for this talk and looking into the future is whether we can target
12 instead of functional assays for antibodies, can we target antibody lineages that we know will
13 turn into functional assays and can we use this instead of functional serology.

14 This, these kinds of concepts were developed mostly by studying HIV and
15 looking for antibodies like the VRC01 that was initially developed and shown to have broad
16 neutralizing activity by binding the C4 binding site in a class specific reproducible recognition
17 mode.

18 These are just crystal structures showing that many antibodies within this class
19 can do this. And then using sequencing data, keying in on this VH1202 heavy chain ileal and
20 being able to find antibodies within this lineage just by sequence analysis that have
21 characteristics that span between much higher neutralization or less with different degrees of
22 somatic hypermutation, et cetera, that can then be connected back to their neutralizing capacity.

23 So this idea of antibody lineages was shown with VRC01 initially but then

1 applied in HIV analysis as, because VRC01 requires so much somatic mutation, you wonder how
2 did it get there. And if you track infection with HIV back to its origin and then study how the
3 antibodies and viruses co-evolve, you can show how these antibody lineages evolve into
4 something that could have broad neutralizing activity but link it back to its unmutated common
5 ancestor at the beginning.

6 And so there is a program now in fact that Bill Sheaf [phonetic] is using this outer
7 domain of GP120 on lumazine synthase and immunizing humans to see if he can elicit that
8 unmutated common ancestor of VRC01 and then see if you can then boost it and evolve it using
9 other kinds of envelope antigens to become the kind of broad neutralizing antibody that we want.

10 So is there a time in the future where perhaps just being able to induce or bind the
11 right antibody precursor would be sufficient? We have assays that can do that. This is a Ramos
12 cell in which you can insert different types of B cell sequences.

13 And so for instance if you inserted this heavy and light chain of unmutated
14 common ancestor from a cell, you can then probe it with different antigens and ask do they cause
15 calcium flux. So you can even before you immunize a person find out if the human germ line
16 version of the antibody can be, can recognize your antigen of interest.

17 We've done this with influenza because as I said in the stem of influenza there are
18 highly conserved sites that can be defined within group, at least. The group twos have lycans up
19 here, the group ones have lycans down here. So the footprint of a group two or a group one
20 antibody is reciprocal in terms of where it is on the stem.

21 But there are other antibodies that combine both group one and group two viruses.
22 And they can be defined with footprints that look like this. And so you can find these lineages
23 by using hemagglutinin probes with flow cytometry and single cell sorting and sequence

1 analysis. And for instance this group of cells would bind both H5 and H1.

2 You can look at the sequences and you can find things in people for instance
3 immunized with pandemic strains of flu that both group one cross reactive or antibodies that
4 cross react between group one and two have defined antibody lineages. And group one is cross
5 reacting mostly this VH169 heavy chain.

6 But there's at least a dozen now lineages and motifs that can be identified as
7 antibodies that can become broadly neutralizing and effective against influenza. And you can
8 show that immunizing with either a group one or a group two can give you preferentially some
9 of these either multi-group, cross group, neutralizing lineages, or within group one cross group
10 one, neutralizing lineages.

11 And you can map these now, not by serology but by sequencing flow cytometry
12 sorted B cells. And the question is, is there a time in the future when just knowing the direction
13 of your antibody lineages would help you know whether a vaccine would work or not.

14 And so these types of vaccines with the headless stem, a trimer on a nanoparticle
15 representing a group one antigen can give heterosubtypic protection for instance here against
16 ferrites. And we have group two stems now on ferrite nanoparticles that can do this Ramos cell
17 calcium flux in using human germ line versions of antibody genes.

18 So the unmutated common ancestors of the lineages we're interested in can be
19 activated by this antigen so we know at least that human antibody lineages of interest are going
20 to be relevant for this particular type of vaccine.

21 These can also have cross reactive heterosubtypic protection and we know that
22 they're immunogenic now in humans. We don't have a lot of data, but this went into clinical
23 trials in April and these are plasmablast response from a single individual with quite a large

1 thirty-eight percent response to stem specific epitopes.

2 So in summary, new technologies are transforming vaccinology and providing
3 solutions for longstanding problems or emerging viral diseases. We think that targeting
4 structurally defined sites of vulnerability and defining specific antibody lineages and advances in
5 protein engineering will provide new options for influenza vaccines.

6 And new surrogate end points may require, be required to guide this iterative
7 process that we'll need to achieve universal vaccine immunity, especially for stem based
8 antigens that do not induce HAI and other more common types of activities.

9 So I will stop there and thank all these people who are in our flu lab, the flu
10 program at the VRC and many other groups within VRC who do this work. Thank you very
11 much.

12 DR. GRUBER: Thank you very much. That was a great kickoff to this
13 workshop. I think we have time for one or two questions. I think you can step up to the
14 microphones if you want to ask a question now. Unfortunately we don't have a Q and A at the
15 end of this session, so.

16 DR. GRAHAM: I think it might be too early in the morning.

17 DR. GRUBER: It is. Or maybe people are just stunned by, you know. But again,
18 the panel tomorrow afternoon also gives some opportunities to ask questions. So thank you
19 again, Barney, it was great. So the next speaker is Dr. Debra Yeskey from C.E.P.I. who will give
20 her remarks regarding C.E.P.I. and their portfolio when it comes to development and
21 identification of biomarkers.

22 DR. YESKEY: Thank you. Good morning. I'm, as Marion said I'm from
23 C.E.P.I. I'm the head of regulatory affairs for North America for C.E.P.I. And we are excited to

1 be collaborators with FDA and NIH on this very important topic. And just excited to hear that
2 seven hundred other people think that this is a very exciting topic, because we sure do think it's
3 important at C.E.P.I.

4 So the cost of emerging infectious diseases is vast, both in human and economic
5 terms. And recent economic work has shown that roughly \$570 million could be spent annually
6 for moderately severe to a severe pandemic, which could equate to 0.7 percent of the global
7 economic burden.

8 So this is important. Epidemics affect all of us in one way, shape, or form, either
9 directly or indirectly. And they don't respect borders and because we live in dense cities and the
10 travel is so quick, that as we know these diseases can rapidly travel across the globe.

11 And we know that vaccines protect us, and this is one of the most potential tools
12 that we have in our toolbox. We talked a little bit about the role of vaccine already, but just this
13 doesn't have to pertain to biomarkers specifically, but just to say how important the role of
14 vaccines are in outbreaks and epidemics.

15 Ebola, the West African outbreak, was the launch of actually C.E.P.I. in January
16 2017 in Davos at the World Economic Forum. And it was as a result of a consensus of
17 coordinated international inter-government plan needed to be in place for the development and
18 deployment of new vaccines against future epidemics.

19 So just a little bit about C.E.P.I. We are an innovative, global partnership
20 between private, public, philanthropic, and civil society organizations put together to stop future
21 epidemics. Our mission is to stimulate and accelerate vaccines against emerging infectious
22 diseases to enable equitable access to these vaccines in affected populations during outbreaks.

23 In our few short years of existence, we have made substantial funding

1 commitments upwards to the amount of \$456 million for our current portfolio of five priority
2 packages from the WHO R and D blueprint list as well as the Watch list. And we have three
3 rapid platform technology projects as well.

4 To support our vaccine development, we are funding a number of enabling
5 science initiatives. For example, funding epidemiology studies for Lassa, vaccine safety, and we
6 just recently posted an RFP for high containment animal facilities.

7 So historically vaccine development has been a long, risky, and costly endeavor.
8 Planning for emerging infectious diseases is also especially challenging because of a number of
9 things that are on the slides here. The disease itself, the frequency and the number, the number
10 of outbreaks, and the occurrences. As well as understanding the disease to the human condition
11 or the disease to the animals if we're using animals for efficacy and then bridging it back to the
12 human condition.

13 Layered in there of course is the all-important regulatory pathway and whether
14 we're using traditional, accelerated approval or animal rule. And then layered even deeper there
15 is running efficacy trials in lower- and middle-income countries as well as dealing with ministers
16 of health, ethics committees, national regulatory agencies, all have to be in sync for things to
17 happen.

18 It's daunting for sure and that's why this conversation and conversations that have
19 already started and new conversations that need to happen, the transfer and, of ideas and new
20 technologies is critical for moving forward.

21 So I didn't get this slide, so now things come up on its own. So the other night
22 my son, my thirteen-year-old son was having a stress attack because he had so much homework.
23 He had just come home from a football game and I said to him, well, calm down. And I asked

1 him, I said, well, how do you eat an elephant?

2 And of course he's thirteen, he looked at me like, Mom, you're being so
3 ridiculous. I said, well, you eat it one bite at a time. And he rolled his eyes. He didn't get it, but
4 anyway. This is what we have to do here as well. And we're peeling back the onion, whatever
5 you want to call it, is that we're having this conversation and I really, you know, think this is so
6 valuable to have this information exchange.

7 Biomarkers help us to enhance basic research to help de-risk and expedite vaccine
8 development programs and help to guide their use. So while we're waiting for real world
9 clinical data, can biomarkers help us expedite much-needed vaccines?

10 As an example we know that in the cancer field that the patient, patients and
11 clinicians have demanded a more precise medicine and targeted medicine for the cancer patients.
12 And biomarkers have been used for this and we can hope to aspire to have biomarkers in the
13 vaccine of emerging infectious diseases work as well.

14 So again, I'm so heartened to have the spotlight on biomarkers and the proactivity
15 of the agency to open this very important discussion. To explore new technologies, to
16 understand biomarkers in vaccine development, and look critically at the regulatory framework
17 to support their use.

18 And for C.E.P.I., ultimately our goal is to accelerate vaccine development and to
19 reduce the funding needs for these programs so they can be sustained to provide early access to
20 stop epidemics. Thank you, and enjoy your workshop.

21 MS. GRUBER: So I want to thank Debra for really catching us up on time.
22 Great, thank you. So our next speaker is my colleague from the Office of Vaccine, Dr. Jeff
23 Roberts, who will help you understand the use of biomarkers in regulatory decision making in

1 vaccine development and licensure. Jeff, take it away.

2 DR. ROBERTS: Thanks Marion. I want to echo the things that you talked about
3 this morning. I talked a lot with a lot of the speakers and we really appreciate them making the
4 time and effort to be here.

5 So I can't speak to C.E.P.I.'s perspective. But as they're loading up my slides,
6 I'll just sort of tell you that what I'm hoping to do is to frame this discussion from the beginning
7 to give you some insight about how we think about biomarkers from a purely regulatory
8 perspective.

9 And what I'm going to come back to again and again is context of use is the
10 critically important variable here. And what I'll do is I'll go through several case scenarios that I
11 hope are going to give some insight in these different contexts. So I'll just issue a bit of a
12 disclaimer here.

13 These case scenarios that I've chosen to go through, each one represents in many
14 cases decades of clinical and scientific work and annualizing evidence from many different data
15 sources. And they're tremendously complex. And I've boiled them down in many cases to one
16 slide.

17 So I'm leaning on the sophistication of this crowd. I know that there's
18 tremendous expertise and knowledge in this crowd, so, but having said that there's still the risk
19 that this is not going to be clear as I try to boil this down and make these, suss out these points
20 from these different case scenarios.

21 So that's just to say that if this goes by you and the point's not coming home,
22 we're going to have time to discuss these things. We will all, most of the colleagues here
23 welcome questions in any of these panel discussions to further help you understand the

1 regulatory perspective.

2 So this is good, this is most of what I was going to say on this slide. And these
3 are the case scenarios that I've chosen, which is traditional approval, accelerated approval,
4 bridging effectiveness, something about non-immune biomarkers, and the fact that we use
5 biomarkers to evaluate safety as well.

6 But I'm briefly going to touch on the mechanisms by which vaccine associated
7 biomarkers are developed and we'll come back to this concept of context of use, which is so
8 important.

9 Okay. So it's interesting if you think about it, if you go back to essentially the
10 beginning of modern vaccinology, you could make the case that the first vaccine associated
11 biomarker was developed at the same time as the first vaccine. When I went back and looked at
12 this story again about Edward Jenner, it was clear that his colleagues were tremendously
13 skeptical that this was going to work.

14 And it was because virtually everyone who milked cows on a regular basis
15 developed lesions on their hands. And many of these were bacterial infections that are known as
16 milker's nodes, but it wasn't clear the difference and not everyone was protected.

17 So everyone was highly skeptical. Well what Jenner was able to do is to show
18 that this characteristic ulcerative lesion that he then associated with vaccine tape was highly
19 associated with protection from smallpox. So really this vaccine tape was maybe the first
20 biomarker in vaccinology.

21 Now we're obviously using much more sophisticated techniques to evaluate the
22 immune response and to associate those with protection from disease. But it just illustrates how
23 important biomarkers have been from the beginning.

1 And this is, Marion touched on this, that really biomarkers are used from the
2 beginning to the end to enable the basic research and discovery. They can inform how the Phase
3 III trials should be conducted. They're used in post-licensure to determine how to implement
4 vaccines. All across the board biomarkers are tremendously important.

5 And as Marion mentioned, we are recognizing that less and less we're going to
6 see these big, randomized, placebo-controlled trials with a disease in point. It's not going to be
7 feasible for a number of reasons. So that's why I think we're seeing such interest in this because
8 biomarkers are going to become so important. Increasingly important.

9 Let's talk about terminology briefly. I thought I would start with this joint
10 FDA/NIH working group that created this glossary of sorts. And in this glossary they defined a
11 biomarker as a defined characteristic that is measured as an indicator of normal biological
12 processes, pathogenic processes, et cetera.

13 The important word here in this definition is indicator. In other words, we return
14 again and again to the concept that we are trying to predict a clinical outcome. We're not
15 necessarily measuring clinical benefit itself. And if you look at the list of biomarkers they've put
16 in this glossary, just to take the first one.

17 Whether a patient has a blood glucose of 80 or 180, that laboratory assay per se
18 matters virtually nil. Whether or not they're going to go into diabetic ketoacidosis does matter.
19 And whether they have long term outcomes associated with diabetes, that does matter.

20 And so the extent to which a lab assay that doesn't have any clinical relevance per
21 se can predict clinical benefit, that's what we're talking about here.

22 Okay. I had a series of slides where I laid out the Prentice criteria. I talked about
23 the different frameworks and included things like absolute and relative, correlates of protection,

1 mechanistic and non-mechanistic. But I'm going to dispense with all that because those
2 semantic discussions add a lot of heat but rarely light.

3 So instead I'll just put up this quote from this WHO White Paper of sorts, where
4 they convene a meeting of experts and publish this overview of definitions and methods in the
5 area of immune markers and vaccination protection.

6 And what they said is that all these different terminology frameworks, et cetera,
7 are followed by prominent contributors to the literature and major regulatory agencies who use
8 the terms differently. Until the subject matures appreciably beyond its current state, readers need
9 to be aware of the variety of usages of the terms and authors need to clarify their terms carefully.

10 So I thought that was a reasonable place to start. And the reason this workshop is
11 entitled biomarkers is because that term is not freighted with the way something like correlates
12 of protection is. You can start with the concept of biomarker and then talk about the concept, I
13 mean the context of use to define what you intend to do with the biomarker.

14 And in terms of what is intended to, you know, the goal or the objective, at least
15 from the regulatory perspective this is just a small partial list of all the different regulatory
16 decision making that might be associated with the use of biomarkers.

17 It's everything from establishing that it can accommodate use, doesn't cause
18 interference, to bridging manufacturing processes. The list could go on and on. So amongst all
19 those different things there's a substantial difference in the source of the data and the strength of
20 the evidence necessary to support whatever that regulatory decision is.

21 And I've listed some of the elements that form this context of use. And just I'll
22 list it at the end the intended goal or the objective in terms of the regulatory outcome is what
23 defines this.

1 So to try to flesh this out a little bit I chose these few of the regulatory decision
2 making sort of case scenarios. For the first one we'll talk briefly about the Hepatitis B vaccines.
3 So to start with this, I think we need to talk about what traditional approval is and means, at least
4 to us.

5 And I've put quotes around the word traditional because that word does not
6 appear in the Code of Federal Regulations. It distinguishes it from something like accelerated
7 approval, but we use that as shorthand just to talk about approval, period.

8 And typically the evidence that we need to see in order to support an application
9 for a traditional approval typically has come from these large, randomized placebo-controlled
10 trials with the clinical disease end point. And the rotavirus vaccines are a really good example of
11 how that was done.

12 But in many cases and as we've talked about increasingly we may need to use
13 what we're referring to as a scientifically well-established biomarker that predicts protection
14 from disease.

15 And so in the case of Hepatitis B vaccines, all of this evidence accrued over
16 several decades and I'm just briefly summarizing conceptually what these different sources of
17 evidence were. The first one just illustrates that Hepatitis immune globulin has been used both
18 in adult studies and in these infant studies to demonstrate that via passive transfer you can
19 prevent Hepatitis B infection. Illustrating at least that antibodies are mechanistically associated
20 with protection.

21 And then a large series of studies using the first generation of Hepatitis B
22 vaccines illustrated that if you responded by virtue of getting above this level of ten million
23 international units one-month post-vaccination, even if we couldn't measure it later on, that there

1 was reliable and durable protection from disease.

2 And that was the body of evidence. That was the evidence necessary for at least
3 for us to conclude that this is a scientifically well-validated biomarker that predicts protection
4 from disease. And so based on that I won't go into details. I think many of you may be familiar
5 with the licensure of HEPLISAV in 2017.

6 Just to point out that we used this biomarker of Hep-B surface imaging antibody
7 demonstrated to be noninferior to a licensed product is the basis for licensure rather than clinical
8 input disease.

9 So for the accelerated approval example, we'll talk about the maternal
10 immunization to prevent Group B Strep. That series of candidate products that are coming
11 forward. First, I just need to lay out what we mean when we talk about accelerated approval, and
12 these are basically the criteria which include that the product has to be developed, being
13 developed to treat a serious or life-threatening disease, treat or prevent.

14 And it has to provide meaningful therapeutic benefit over existing treatments.
15 But the really important thing here is that licensure can be based on an effect on a surrogate
16 endpoint that is reasonably likely to predict clinical benefit.

17 So that criteria is dramatically different than a scientifically well-established
18 biomarker that predicts protection against disease and can support traditional approval. This is a
19 different bar, reasonably likely to predict clinical benefit.

20 So in the case of the GBS vaccines, one of the manufacturers came forward and
21 proposed to use a GBS antibody titer as that biomarker to support accelerated approval. And this
22 is a long, complex story. And the reason I chose it is because we're going to hear a lot about it
23 tomorrow. This is really going to be fleshed out well tomorrow.

1 But the punch line here is that we convened an advisory committee and asked
2 them to weigh all this evidence and the result is that we agreed with them that accelerated
3 approval could be the licensure pathway. And that represents an important regulatory decision.
4 So those products are going forward based on that basis. And we'll talk more about them
5 tomorrow.

6 Okay, how about something that we do very commonly, which is to abridge
7 effectiveness from one group to another. Age groups, demographics, this sort of thing. We
8 thought that the cholera vaccine was a really great example of this because as you may know this
9 product was licensed I think three years ago now.

10 It was licensed on the basis of human challenge studies in which adult subjects
11 were enrolled and vaccinated or given placebo and challenged with wild type cholera. So this
12 was really a clinical disease end point study, but it was unique in that it was a human challenge
13 study.

14 But the important point is that those volunteers that were challenged were in the
15 eighteen to forty-five year age range. So measuring the immune response to cholera vaccine is
16 very difficult because it's probably mediated at the mucosa. Secretory IgA and probably
17 mucosal resident cell mediated immunity.

18 So really the best way to measure that the sponsor path they took forward was to
19 look at serum fibrocyte, all the antibody or SVA titer. And what they could show is that there
20 was a robust response at ten days post-vaccination. But that response waned and was essentially
21 undetectable in most subjects by three months.

22 The way this human challenge trial was devised is that people were challenged at
23 ten days and again at three months when they had undetectable SVA titer. So clearly SVA titer

1 is not mechanistically associated with protection.

2 So in spite of that I think because that was the best way to measure the immune
3 response, and because it was so well associated with protection at ten days and at three months,
4 that evidence was good enough to bridge efficacy from the population in whom efficacy was
5 demonstrated, eighteen- to forty-five-year-olds, up through sixty-five years of age for which we
6 had safety and imaging data.

7 So I hope that makes clear that this context of use required a different level of
8 evidence, and that's the recurring theme. Okay, so briefly I don't know how much everyone
9 knows about the immunology pathogenesis of HPV infection and how HPV vaccines work.

10 But I think the take home from this slide is just to illustrate that there is a
11 spectrum wherein on the left side of this slide you see HPV infection is demonstrated by PCR.
12 Virtually everyone who is sexually active is at one point infected with one strain or another of
13 HPV.

14 But obviously the vast majority of those are cleared. Some of those go on to
15 CIN1. Most of those are cleared. Some of them go on to advanced dysplasia and a very small
16 number go on to cervical cancer. So it's the spectrum that's an upside down pyramid.

17 So when we convened an advisory committee in 2001 to determine the licensure
18 pathway for HPV vaccines. And obviously what the manufacturers hoped to claim in the label is
19 prevention of cervical cancer. But I think it's very obvious that there are a number of ethical and
20 feasibility issues that dictate that you couldn't do a trial of an HPV vaccine, placebo controlled,
21 with a cervical cancer end point.

22 So we had to choose something on this spectrum and there was a lot of skepticism
23 about whether the immune response as measured by serum IgG would be effective to prevent an

1 infection that is limited to the mucosal surface.

2 So what we settled on was CIN2 and essentially we were making decision that we
3 agreed that this is a scientifically well-established biomarker that's going to predict cervical
4 cancer. And, you know, with all the data that had come in from all the vaccine trials we have
5 pivoted to PCR proven infection.

6 We've gone further to the left on that continuum. The reason I raise this is we're
7 going to talk a lot about CMV. In many ways that's sort of comparable in that many infections
8 occur among neonates. Many fewer of them lead to disease. So we'll talk about CMV
9 tomorrow.

10 And the last example is just to illustrate that we use biomarkers for all sorts of
11 other regulatory decision making. Sometimes it includes safety. I don't know if any of you are
12 familiar with this story of how Fluzone Quadrivalent we approved in 2013 for use in individuals
13 six months of age and older.

14 And that initial approval was for a single dose in six- to thirty-six-month age
15 range of 0.25 mL. And then 0.5 mL is the adult dose at three years of age and older. And that
16 was a carryover from Fluzone TIB which had that same dosing regimen. And that was really a
17 carryover from the 1970s where we were using whole inactivated virus that was much more
18 reactogenic.

19 So the bottom line is that the manufacturer proposed to change the dosing to 0.5
20 mL six to thirty-six months to make it consistent with the rest of the dosing. And I'll just boil
21 this down to say that they proposed a descriptive end point in the safety, but we agreed together
22 that safety and reactogenicity are so important in this age group, six to thirty-six months, that we
23 should construct this statistical analysis plan to be hypothesis testing rather than descriptive.

1 And this study went forward as a noninferiority study on a fever end point. And
2 they were successfully on that and we've now licensed this dosing for this vaccine.

3 Okay, I'll be brief about this because Sarah Brown is going to talk in more detail
4 about these issues tomorrow. But just to briefly lay this out, these are basically the ways that
5 biomarkers are developed in the vaccine space. Most of it initially happens under I and D. It's
6 confidential, it applies to a specific product for a specific manufacturer.

7 And really it only becomes publicly available later in development and sometimes
8 after licensure by virtue of appearing in peer-reviewed literature. And one thing that's
9 interesting and important about relatively new program the FDA, this biomarker qualification
10 program, is that potentially we could develop these biomarkers and make them publicly
11 transparent and usable by all sorts of different groups.

12 So that's really it. I think it's clear by virtue of the attendance how important
13 biomarkers are and how important they are going to be going forward. I hope I've kind of laid
14 out some of the complexities and the nuance in terms of the regulatory perspective for the use of
15 biomarkers.

16 And as we will keep returning to this issue of context of use is the critically
17 important variable from our perspective. And I just want to acknowledge several of our folks for
18 help putting this thing together. So thanks.

19 DR. GRUBER: Thank you, Jeff. I think the use of biomarkers in regulatory
20 decision making is not perfectly clear to everybody, right? I mean, I thought, I mean at least it's
21 clear to me now, so.

22 DR. ROBERTS: I didn't mention that we sometimes talk about correlative
23 protection internally and we can't even agree with each other about what we're saying when we

1 say correlative protection.

2 DR. GRUBER: Right, so actually we're going to move ahead and I would like to
3 invite the last speaker of this session to come to the podium. And that's Dr. Dean Follmann from
4 NIH who will speak on new technologies and computation capacities in the future of vaccine
5 biomarker development. Thank you.

6 DR. FOLLMANN: Well, thank you again. Thanks for inviting me to speak here
7 today. I'm really excited to be here. I think this is just a very intellectually interesting enterprise
8 we're getting involved in and very important now, so I'm happy to be here.

9 I'm going to be focusing more I guess on some of what Jeff Roberts talked. I
10 think the use of computational methods, statistics, and so on in early phases of vaccine
11 development is relatively straightforward. It can be complicated but I don't think it's
12 controversial.

13 I think where the rubber hits the road is when we're trying to, when we're not
14 able to do Phase III studies and how can statistical thinking help us in that regard. So why
15 biomarkers and vaccine trials. I'll be focusing on licensure type decisions.

16 So as we all know, the best study to do would be to randomize humans and show
17 benefit of the vaccine on disease in humans. This has been pointed out for many studies you can
18 do that, but for certain tough diseases it's not really feasible.

19 And I've got a few examples here. One is rare congenital diseases for which the
20 sample size to do such a study would be enormous until it's not feasible. These include CMV,
21 Zika virus disease, and Group B strep as was mentioned earlier.

22 Another difficulty is for bioterror threats which don't occur naturally and so we're
23 left with the conundrum of how to proceed with those types of threats. And then as was just

1 pointed out with Jeff Roberts, HPV is another example we can't really do Phase III studies. In
2 this instance it just takes too long and it's too rare to develop cervical cancer.

3 So biomarkers can help us with accelerated approval and an animal path to
4 licensure. And my perspective is that, you know, if you're not going to use disease, if you're
5 going to use a biomarker in place of disease, that entails a certain risk and we should understand
6 that risk. We should try and minimize it.

7 But also understand the risk in terms of the potential benefit. It's not just enough
8 to say, well, we have to have strong evidence. We have to imagine the benefit that licensure
9 would achieve.

10 So just to sort of give an example of potential risk, I'd like to talk about a vaccine
11 trial, the first vaccine trial I got involved in which was Vaxo-04. This was a Phase III
12 randomized trial of an HIV vaccine, the first Phase III trial, and they randomized about five
13 thousand individuals, two to one to vaccine placebo.

14 There's the immunization schedule on the slide there. And overall it was a well-
15 conducted trial. They had a large number of infections, 370 infections or so. And yet the
16 infection rates were nearly identical in the two groups. So nothing really going on.

17 The study was pretty controversial, though, because of a potential benefit in non-
18 whites. And as part of the analysis of that, we also looked at immune response. Let's see if I can
19 get that. Right, so one of the many immune responses they measured was blocking of antibody
20 using ELISA.

21 And in the vaccine group where you can measure this, right, in the placebo group
22 you can't measure the vaccine induced immune response because there is no such thing. But in
23 the vaccine group you can do this. And in this slide, we've broken down the relative risk of

1 infection by quartile of immune response in the vaccine group here.

2 So these are the twenty-five percent of the patients who had the worst immune
3 response, and these are the twenty-five percent of the patients that had the best response. So if
4 you look at these relative risks there's about a seventy percent reduction for the best responders
5 compared to the worst. And it's significant.

6 But remember this is a placebo controlled study, so we have another comparison
7 group. And if you redo the calculations with now using the placebo group as the reference, you
8 see there's about a seventy percent increase in the efficacy or the risk of infection and the
9 vaccine group for those who had the very worst immune response.

10 So what's going on here was pretty confusing overall. Nothing was going on and
11 it seemed like there were two hypotheses which could explain it. One is that vaccine induced
12 antibodies are identifying volunteers who have better immunity. Or it could be that the vaccine
13 has a very complex causal effect where if you have a poor response it increases your risk of
14 infection but if you have a good response it decreases your risk of infection.

15 And really that's all we could say. We had this curious result and if we didn't
16 have a placebo group we would be just on a study giving people vaccine, we might have thought,
17 hey, antibody looks great, let's view this as a success.

18 So that's always in the back of my mind when I think about biomarkers and
19 correlates, that antibody might be completely unrelated to a causal mechanism. Because in this
20 study if you believe hypothesis one, there was no causal mechanism.

21 So biomarkers can be used as a substitute for disease in certain situations and I'll
22 try and get into the Tower of Babel that Jeff Roberts mentioned where there's a lot of different
23 terminology. Briefly there's two main schools of thought about how you can use biomarkers as

1 substitute.

2 One is the Prentice criteria which basically sees that vaccine explains all, if
3 antibody will explain all of the vaccine effect. Implicit in this is that there's a vaccine effect to
4 start with, unlike the VaxGen [phonetic] study.

5 The other main school is called principal stratification and it has the daunting task
6 of trying to replace those question marks I showed in the VaxGen study with actual relative risk,
7 which is hard to do. Because like I said, you don't measure the immune response to vaccine in
8 the placebo group. They don't have any response because they didn't get a vaccine.

9 So these standard ways of looking at things require large Phase III studies
10 involving vaccine biomarker and disease. And even if you can do that, it's important to note that
11 substitution is not universal.

12 So, for example, you have the same disease, but different vaccine technologies
13 might require different surrogates. Or even if you're focusing on antibodies, it's not a given, say,
14 that half of antibodies will act the same or give you the same result as vaccine induced antibodies
15 with the associated sort of ecosystem that a vaccine will give you versus antibodies from natural
16 infection.

17 But for tough diseases, these Phase III vaccine trials are not really practical. We
18 can't really dig into those in any great detail.

19 So in the remainder of my talk I want to go through a couple of examples that I've
20 been involved in where we've wrestled with how to proceed with licensure or try to get licensure
21 evidence in these tough cases. So I'll start with anthrax vaccine.

22 So as you know, inhalational anthrax, which is what we're concerned about with a
23 bioterror threat, doesn't occur naturally and there's no human challenge model. And so if we're

1 going to license new vaccines or give extended indications for existing vaccines, it has to be
2 based on the animal rule.

3 So one way to proceed with this is to try and examine a relationship between a
4 putative correlate such as antibody and disease and then try and do it in as many different
5 situations as you can think of and hope that there's a common thread that supports the idea that
6 antibody is really the causative mechanism.

7 And so NIAIA did a number of studies looking at different anthrax vaccines, RPA
8 and AVA, in different animal species with different dilution, dilulence [phonetic], doses, and
9 times of challenge. And they were looking for hopefully a similar story that would support use
10 of antibody.

11 And intuitively if you have a similar relationship across all these different
12 settings, the antibody is a good proxy or better proxy or more supported proxy for human
13 disease.

14 So here is a summary of that data analysis. There were hundreds and hundreds of
15 animals studied with three different species, rabbits, cynomolgus, macaques and rhesus
16 macaques. Each one of these settings is for a particular vaccine species, diluent dose setting
17 where, well, excuse me. The only thing that was varied was doses.

18 So if you look at these, what we have on the X axis is antibody is measured by a
19 toxin neutralization assay and on the Y axis is the probability of survival. These circles up here
20 are animals that died, I mean animals that survived. Circles down here are animals that died.

21 And you see the probability of survival tends to go up with more antibody with
22 each one of these different settings across all these different things. Some of them are not so
23 reliably estimated, but some of them are relatively small studies.

1 So let's drill into that setting three which I focused on that I circled which was for
2 RPA vaccine in rabbit. So here's the overall dose response curve, this black line. And then we
3 were also interested does this relationship hold for different doses.

4 And what I've graphed here is it's hard to see but these different shades of yellow
5 correspond to the perturbation in the overall dose response curve for different doses. Using a
6 statistical model. You can see that these are relatively similar.

7 And so this sort, this kind of analysis supports the Prentice criterion for surrogacy.
8 As you change things, do you get the same relationship? Does antibody explain the vaccine
9 effect? And so we did analysis and in depth looked at that and found that the Prentice criterion
10 for surrogacy was met.

11 So great. Antibody could be a surrogate for monkey, but we don't want to, we're
12 not so interested in preventing disease in monkey, we want to prevent disease in man. So
13 another thing we wanted to do was to try and imagine, we're going to try and make the leap from
14 monkey to man.

15 How can we get some evidence indirectly of that? So we can make the leap from
16 monkey, from rabbit to monkey. So what we've done in this slide is to look at the estimated
17 curve, the probability of survival in the rabbit species is here. Then we looked at monkeys who
18 were immunized with AVA, figured out their antibody value, and these are those values here.

19 And then for each monkey using rabbit, we can come up with a predicted
20 probability of survival. So that's what these things are. You average those, it's about seventy
21 percent survival. Based on this monkey, based on using the rabbit model. And when you
22 actually compare it to the actual data, the monkey survival was about seventy-six percent.

23 So this is close. So we have two sources of information here. One we see the

1 same relationship with antibody across many different scenarios and this sort of comfort us in
2 making the leap from monkey to man or animal to man.

3 So I also want to talk, my next example involves Zika. Zika vaccines. The ideal
4 vaccine here I think would prevent congenital abnormalities. That's what I think we're most
5 concerned about. But a perfected trial for this is not really possible.

6 So how might we proceed? Well, we could conduct a field trial to look at the
7 vaccine effects in adults. Not necessarily women of childbearing age. Look at the vaccine effect
8 on disease or infection. This can be difficult to do because the episodic nature and difficult, it's
9 difficult to predict Zika outbreaks.

10 Or another path that I understand is being followed is to conduct human challenge
11 studies. So this would, either of these would provide some evidence that the vaccine works in
12 humans, but it's not really direct evidence on congenital abnormalities.

13 So what might we do? So Zika, like Dengue and other viruses of that type, can
14 episodically sweep through an area and after a while you get more susceptibles and then it can
15 sweep through the area again. So let's suppose like in 2025 we deploy a Zika vaccine in an
16 endemic area or an area where outbreaks might occur and there's incomplete coverage.

17 And let's suppose there's an outbreak in Puerto Rico in 2032. The outbreak ends.
18 We've had the vaccine deployed and we'd like to know what happens really in terms of
19 congenital abnormality outcome.

20 So what we could do is a case control study where cases would be children with
21 the Zika-like birth defects and controls would be children without Zika-like birth defects. So this
22 would be the kind of data we could get. Vaccine with birth defects, no vaccine with birth
23 defects, no birth defects with vaccine, and no birth defects with no vaccine.

1 And you can look at the odds of vaccine amongst those with birth defects over the
2 odds of vaccine for those without birth defects and estimate vaccine efficacy using this one
3 minus the odds ratio. So this is a standard metric for assessing vaccine efficacy in a case control
4 study.

5 One little thing I've shown here is maybe the people or the mothers who have
6 children with birth defects have been vaccinated a long time ago relative to those without, who
7 don't tend to get birth defects. And so this would allow us to go further than the standard case
8 control study.

9 We could construct a curve like this that looked at the vaccine efficacy as a
10 function of when they were vaccinated. So this would be an important thing to do. Not only
11 might we get a signal on whether it works, but also is there a period of time in which the vaccine
12 efficacy is waning.

13 And I'll also talk briefly now about Ebola vaccines. Another thing I'm currently
14 thinking about. So the VSV vaccine is currently being deployed in the Democratic Republic of
15 the Congo using ring vaccination, though it's not been licensed as far as I know.

16 There's also another vaccine by Janssen [phonetic] which may be deployed. I know that
17 there's been discussions of that in a protocol, though it's also not licensed as well. So we have
18 these things, we have these vaccines that are going to be out in the field. We could eventually
19 get evidence about efficacy in humans which is really what we want.

20 How could we do that? Well, with Ebola it's really kind of, it's difficult. But one
21 thing we can do is attest negative design. So this is a special kind of case control study where
22 you take individuals who are ill with an Ebola-like illness, they come to a transit center where
23 they're diagnosed as either having Ebola or not having Ebola.

1 These four are the cases and the controls. And then you look for the vaccine rate
2 amongst the cases compared to the vaccine rate amongst those controls. So here's an example
3 where the vaccine is relatively rare amongst Ebola cases, suggesting the vaccine works.

4 And as a comparison, the vaccine rate is similar in the controls, you know,
5 supporting the idea that the vaccine works. So once again, we can calculate an odds ratio to get
6 at the vaccine efficacy for this Ebola vaccine.

7 So that design, the test negative design, has mostly been for influenza. So you're
8 thinking older people in the U.S. and so on so it's relatively easier to conduct. In the Democratic
9 Republic of the Congo there are a lot of complications to that design.

10 One complication is that it's being deployed in a ring type deployment. So if
11 someone has Ebola, they'll vaccinate all his contacts and all the contacts of contacts. This leads
12 to a statistical issue of correlation and it leads to sort of complications in how you make a
13 confidence interval.

14 Another thing is that the vaccinated people have regular visits with the vaccinated
15 team and they have more, presumably more awareness of where the transit centers are and where
16 they can go. In contrast to those who don't, who aren't vaccinated, they tend to be, they won't
17 have visits with a vaccinated team. They're probably fewer at this transit center and they might
18 wait longer to come, all of which raise complications.

19 So this design I talk about requires certain assumptions, equal exposure, the ill
20 vaccinees [phonetic] go to the transit center with a certain probability and so on. We can try and
21 address these issues statistically using more sophisticated measures and try to deal with the bias,
22 but there's still risk of bias.

23 But one thing you could think about like often we're interested in an unbiased

1 estimate of vaccine efficacy. Sometimes maybe we just want to know like in standard Phase III
2 studies we want to rule out a lower bout in efficacy, maybe know for sure that the efficacy is
3 greater than twenty-five percent of fifty percent supporting the disease.

4 But anyway, maybe a test negative design while it might have a potentially biased
5 estimate, we might know for sure that the vaccine efficacy is really greater than twenty-five
6 percent. So this could add something in principle to information about licensure decisions for
7 Ebola vaccines.

8 So in conclusion, I think it's a real exciting time to marshal this new technology
9 and new thinking. I think it's exciting as a statistician to be involved in this. Deep knowledge of
10 immunology and vaccinology coupled with statistical kind of thinking is very helpful I think to a
11 path forward.

12 And one thing I wanted to know if we're not using disease at end point there's
13 certain risks involved in that, but we want to understand the risks, try and minimize them, and
14 also think about the risk in terms of the potential benefit. And that concludes my talk. I'd like to
15 thank a few people who worked with me on this over the years and there's a few references.

16 DR. GRUBER: Thank you so much, Dean. So now as the moderator I am facing
17 an interesting dilemma. According to my phone it is 9:59. The break is scheduled for 10:10 and
18 so we gained eleven minutes. So I'm asking the audience because I said in the beginning that
19 this is going to be a very interactive workshop. What do you want to do?

20 I can ask for the speakers to join me here and then we can have a ten-minute
21 discussion or you can use thirty minutes of your break to contact the speakers and ask your
22 questions. So what is it going to be? Let me ask you this a little bit more efficiently, more clear,
23 right.

1 So would you like for me to invite the speakers to join at the podium so that you
2 have opportunity to ask them some questions? Can I have a raise of hands for all of those in
3 favor. Then obviously you would have to ask questions. Okay, so I didn't see that many hands
4 going up.

5 So you want to really use the opportunity to just mingle here right now and say
6 hello and use the extra ten minutes and then join us again for the next session at 10:30? Can I
7 see a raise of hands? Yes, I guess so. So thank you very much and I hope you will stay for the
8 next session.

9 (WHEREUPON, a brief break was taken from 10:00 a.m. to 10:32 a.m.)

10 DR. GRUBER: So hello. I think we need to get started. We are really again a
11 couple of minutes behind. If I can ask people to take their seats and I'm looking for the chair of
12 the next session. There she is. Cristina, you want to take the podium.

13 DR. CASSETTI: Good morning. My name is Cristina Casetti and I am the
14 deputy director of the Division of Microbiology and Infectious Diseases here at NIAID. And it's
15 a pleasure for me to share the next session which is highlights from selected case examples,
16 lessons learned, and next steps. And I'm really happy to share some of my close friends and
17 colleagues.

18 And we're going to do the presentations and if there is time we're going to take
19 some qualifying questions at the end of the presentation, but we're going to roll the discussion at
20 the end. So the next presentation is by Ted Pierson and he's going to talk about mechanistic
21 approaches to developing biomarkers for Zika vaccine development. Ted.

22 DR. PIERSON: Great, well, I'd like to thank the organizers for the opportunity to
23 be here today to share some of our work on trying to actually to share NIAID's contribution to

1 the Zika vaccine development, Zika vaccine development and trying to understand aspects of the
2 immune response that may be useful predictors of protection.

3 So my laboratory study is Flavivirus. The Flaviviruses. And Flaviviruses are a
4 group of positive stranded RNA viruses that are transmitted to humans principally through the
5 bite of mosquitoes or ticks. And there are many clinically important Flaviviruses that include
6 dengue virus, West Nile virus, and yellow fever and Zika virus, which will be the subject of my
7 talk today.

8 Flaviviruses have a global distribution and are really a public health problem in
9 many parts of the globe. There are more than three hundred infections by Flaviviruses occurring
10 each year. And these viruses cause in humans a variety of clinical diseases that span a lot of
11 different problems from encephalitis to hemorrhage and shock to Guillain-Barre and then as was
12 discovered with the explosion of Zika in the Americas, congenital disease in women infected
13 while pregnant.

14 So it was these unique characteristics of Zika virus and the large number of, the
15 large and rapid increasing number of infections that generated a lot of interest in the
16 development of countermeasures. And these challenges included the congenital defects I
17 mentioned, Guillain-Barre.

18 Zika was the first Flavivirus for which sexual transmission was demonstrated and
19 may be an important part of its epidemiology. The presence and persistence of Zika and the
20 reproductive tissues raise the possibility that Zika may have an impact on fertility.

21 This, there is poorly understood persistence and then as a challenge for the
22 management and study of disease, there's poor, poorly specific diagnostics. So there was a lot of
23 problems that came about with the emergence of Zika.

1 Fortunately there was a lot of interest in solutions. And this manifests in a lot of
2 different spaces, including the development of many different platforms for Zika vaccines. And
3 these included live attenuated viruses by multiple groups and activated viruses by multiple
4 groups, a variety of different viral vectors that express certain portions of the viral genome that
5 have shown promise in preclinical studies, and several different synthetic DNA vaccine
6 approaches.

7 Today I'm going to talk to you about just one of these approaches and that is a
8 vaccine candidate developed by NIAID in collaboration between the Division of Intramural
9 Research and the Vaccine Research Center, so this would be my group and many different
10 groups at the VRC led by Barney Graham.

11 And this vaccine is a DNA vaccine that really leveraged the VRC's investment in
12 DNA vaccines for viral infections and emerging diseases. And the platform really was based on
13 work done by Barney, John, and Julie Ledgerwood on West Nile virus many years before.

14 So what we did was we designed a vaccine construct that included two of the viral
15 structural genes, a gene called PRM and a gene called E, and this is the E protein here. And we
16 made two different constructs. And one construct there was the wild type form of the genome of
17 the envelope and PRM proteins. And then the second construct we made a chimera that's shown
18 here in red that really amounted to about thirty or so amino acids that were introduced into the
19 Zika that corresponded to the, to residues in a related Flavivirus.

20 And the rationale for this antigen was the safety of the platform, the speed with
21 which it could be developed, and the fact that this type of vaccine produces something called a
22 subviral particle that had been studied in multiple preclinical studies before.

23 Today I'm going to show you some data from my laboratory that will be, that

1 will, that is the application of a technique that we developed many years ago called reporter virus
2 particles. These are essentially reporter viruses that allow an infection to be scored as a function
3 of reporter gene expression.

4 The, what's under the hood of this technology is a modified form of the genome
5 that is a self-replicating RNA that once introduced into cells can express a reporter gene, but
6 itself cannot be propagated from cell to cell. And so you can introduce this replicon into cells by
7 complementation where you just package it up in the structural genes of other Flaviviruses
8 including Zika.

9 And so this assay format works in the following way where you can use multiple
10 different cell types, most of what I'll show you today was done with a cell type called Raji cells
11 that express a known attachment factor for these viruses. But it's a fairly typical virus
12 neutralization format where you mix virus and antibody together and you add these immune
13 complexes to cells. You wait some period of time, you fix, and in these cases read out by flow
14 cytometry.

15 This is a very quantitative assay. It's very reproducible and something that can be
16 done at pretty high throughput. So with this technique and animal studies done in collaboration
17 with the VRC, we performed dose de-escalation studies of these two DNA vaccines in
18 nonhuman primates according to the following schedule.

19 So that, animals got two different doses of vaccine and then were challenged at
20 week eight, which is four weeks past the second dose of vaccine. What I'm showing you here is
21 the neutralizing antibody response that develops over time where this is the amount of infection
22 in our neutralization assay. So more infection is less neutralization. So this is complete
23 neutralization here. And the dilution of serum.

1 And so you can see the development of a neutralizing antibody response elicited
2 by both of these vaccines. What I'm showing you here is that both vaccines at all of these doses
3 both elicited a neutralizing antibody response of similar magnitude.

4 And this is a bit easier to appreciate here where I put the vaccines at each dose
5 next to each other. And this was true when we used two additional neutralization assay formats.
6 So the take home message here is that these two vaccines that are very similar in sequence
7 elicited similar neutralizing antibody titers in an assay independent way.

8 But what's really remarkable is when these NHPs were challenged the level of
9 protection by these two vaccines was not similar. And that the vaccine that encoded all Zika
10 sequence protected all of the animals in this experiment and the vaccine that encoded these thirty
11 amino acids in the stem and transmembrane region of this envelope protein had breakthrough in
12 each of these groups.

13 This is measured as a function of viremia days post challenge. We could look at
14 the outcome of this challenge experiment in a different way and in this experiment what I'm
15 showing you is the anamnestic response to infection. So this is looking at the antibody response
16 several weeks after challenge to look to see if you can detect the response of the immune system
17 to challenge.

18 And what you see is in the animals that were the control animals, all of them had
19 very robust antibody responses after challenge because they were naïve before and this chimeric
20 vaccine group most of the animals had an anamnestic response as defined by greater than a
21 fourfold change in neutralizing antibodies at either two or four weeks post-challenge. Whereas
22 only three animals in this 5283 vaccine group that had all of the Zika sequence.

23 So this was very surprising to us because it basically said that neutralizing

1 antibodies were elicited by both vaccines, but they conferred different levels of protection.
2 When we took this data and we estimated an antibody titer that would correspond to protection
3 from infection, what we determined with Martha Neeson [phonetic] and Dean's group was that
4 there was a different level of neutralizing antibodies that correlated with the protection by these
5 two very, very similar vaccines.

6 So this was a challenge. Because what we learned was that these vaccines were
7 not equally protected despite similar neutralizing antibody titers and these were the, this was the
8 thing that we thought we would measure as a predictor of vaccine efficacy.

9 So the fact that we had these two vaccines that were very similar that neutralizing
10 antibodies didn't protect, we realized that first broadly speaking that neutralizing antibody titers
11 may not be easy to bridge from vaccine to vaccine. And we were of course very curious about
12 what the difference in response of 5283 and 5288 was that corresponded to this.

13 So the first thing we had to figure out was of course vaccine elicited immunity
14 does not have to be antibodies. And so what we did was we focused our efforts on the antibody
15 response. And to do this Tracy Ruckwardt and Barney Graham's group developed an AG129
16 mouse model for Zika.

17 And we demonstrated first in this mouse model that these two vaccines would
18 protect these animals from infection. So these are mice vaccinated with these two DNA
19 constructs. And this is their neutralizing antibody response. This is the viremia post-challenge
20 and you can see both vaccines protected these mice against infection as measured by viremia or
21 survival or weight loss.

22 So then what we did next was to identify the role of antibody we immunized mice
23 with these vaccines. We took the antibodies out of the mice. We normalized for neutralizing

1 antibody activity and passively transferred neutralization active normalized quantities of
2 antibody back into mice.

3 And so you can see now these groups have the same level of neutralizing
4 antibody. We then challenged them, and as you can see quite clearly here despite the fact that
5 these groups of animals got the same quantity of neutralizing antibodies, so the same activity,
6 this corresponded to differing degrees of protection.

7 So this suggested that the antibody response was qualitatively different between
8 these two very similar vaccines. So there's a lot to unpack here and there's a lot of different
9 things that could be going on.

10 The first could be is that there's aspects of the antibody biology that's not related
11 to neutralization that's going to be important for protection. It could be that antibodies
12 neutralized by different mechanisms. Or it could be that the antibodies elicited by these two
13 vaccines are actually two different kind of populations of antibodies that have different types of
14 biology.

15 We've explored all three. I'm not going to show you the data for number two, but
16 I'll just tell you that antibodies elicited by both of these vaccines block infection, block
17 attachment as the mode of activity.

18 We started to look collaboratively with Gleed Altar [phonetic] at effector
19 functions of these antibodies. And what I'll say is that these antibodies have similar levels of
20 effector function capacity to the extent that we've looked so far.

21 The other thing I'll share is Gleed Altar (phonetic) that these antibodies, these,
22 excuse me, these vaccines both elicit antibodies that have similar degrees of capacity to bind
23 viral antigens, either soluble forms of the antigen or the very complex arrangement of antigens

1 found on the surface of the virus shown here in open bars and colored respectively.

2 And so they make similar levels of antibodies. These antibodies can attach
3 relevant forms of the virion and there's really no difference in the relationship between binding
4 antibodies and neutralizing antibodies that we could see so far.

5 So we started to think about what we knew about the complex biology of these
6 viruses and what I would like to share with you today is that despite the very elegant structural
7 biology of Flaviviruses, the reality is that these viruses exist in a dynamic state where the viruses
8 are actually moving around in a process that we're showing schematically here called viral
9 breathing.

10 And that when released from cells, they are released in a structurally
11 heterogeneous way. And we started to think about whether this complex biology would
12 complicate our ability to analyze the antibody response to vaccination with respect to
13 understanding protection. And that's the story I'm going to tell you now.

14 So Flaviviruses including Zika assemble at the ER of cells and are released from
15 cells like many viruses through the secretory pathway. And during this journey they travel
16 through the ER and the Golgi, and during this process there is a proteolytic maturation step that
17 results in the cleavage of this purple protein on the tip of a spiky form of the virus.

18 And in this spiky form of the virus the envelope proteins sort of are arranged up in
19 the air like a trimer or a TP. And during this process, once this is cleaved and the viruses are
20 released, they assume this very beautiful herringbone arrangement. This is called the mature
21 virus and we know this one is infectious. And this is called the immature virus and we know
22 genetically this one is not infectious.

23 But several years ago we clouded the field a little bit by demonstrating that not all

1 of the infectious viruses released from cells fall into this mature or immature state. In fact,
2 they're these viruses that have structural features of mature viruses and immature viruses that we
3 know from four different lines of evidence can be infectious.

4 And you can see an example of this here where there's a smooth surface and a
5 spiky surface. And what's clear is that this extent of virion maturation at the level of an
6 individual virion impacts many features of its biology including its capacity to be bound by
7 antibody or sensitivity to neutralization.

8 And I'm just going to show you this with antibodies against the envelope protein
9 or against PRM itself. And essentially if you just look up here, as we make viruses more mature
10 they become refractory to neutralization because of the steric constraints imposed by that very
11 dense herringbone arrangement shown here.

12 As you make viruses less mature so there's more of that chimeric structure,
13 generally speaking they become more sensitive to neutralization. And this is when you look at E
14 protein reactive antibodies or PRM reactive antibodies.

15 So an interesting thing about Zika biology is that infection by Zika results in a
16 neutralizing antibody response that is not markedly impacted by this very different change in the
17 structure of the virus. So very mature forms of the virus are neutralized equivalently or better
18 than formed virion that have this partially mature character, partially immature character.

19 And you can see this in six convalescent samples where we've measured
20 neutralization with the standard form of virus used in everyone's assay and a more homogeneous
21 mature form of the virus that we produce in our lab using specialized techniques. So there's
22 really nothing exciting to see here.

23 But you are surprised when we look at NHPs vaccinated with our two constructs.

1 What you can see is that the antibodies elicited by vaccine are markedly less capable of
2 neutralizing that mature form of the virus. And you can see that for 5288 and 5283 where you're
3 comparing in each case the titers against the standard preparation or the mature preparation. And
4 in each case the mature preparation is less sensitive to neutralization by antibody.

5 So this is different. This is a case where the vaccine elicited responses different
6 than the response of natural infection. And it's a case where these two vaccines differ with
7 respect to this property where this is to a lesser degree observed with 5283 than 5288.

8 Interestingly, this property of antibody response goes away very, very quickly
9 once the host sees a real virus. So the immune response very quickly addresses whatever's
10 missing here that confers an inability to neutralize mature viruses. And this is true for humans,
11 so this is data for subjects enrolled in a Phase I clinical study of both of these vaccines and
12 human beings make a maturation state sensitive response to these vaccines.

13 In fact, for that 5283/88 vaccine that is that chimeric vaccine, most of those
14 humans have an antibody response incapable of neutralizing mature viruses at all. So what does
15 this mean?

16 So what this hypothesis, well, our hypothesis is then is that mature antibodies that
17 neutralize the mature form of the virus may be particularly important to understanding
18 correlative protection. So to investigate this we did an experiment similar to the one I've already
19 shown you.

20 We vaccinated animals, we took the antibodies from these vaccinated animals and
21 we normalized them for functional activity. But this time we normalized them for their capacity
22 to neutralize mature forms of the virus. We passively transferred this activity normalized
23 antibody back into mice, challenged these mice, and now you can see when you explore directly

1 the capacity of these two vaccines to make antibodies that neutralize mature forms of the virus,
2 you now can see that they are similar in protective.

3 So in summary, vaccine elicited antibody is sensitive to the varied maturation state
4 in contrast to the antibodies elicited by natural infection. And our data suggests that neutralizing
5 antibodies that, measurements of neutralizing antibodies against the mature form of the virion
6 may better predict protection.

7 And this of course raises some interesting questions including whether
8 vaccination will protect against the mature form of the virus and what it, and is the mature form
9 of the virus the relevant one with respect to what is produced in vivo and delivered to you by the
10 bite of a mosquito.

11 There's of course implications for these data for the assay development and
12 support of vaccine development because every other assay that is used to date in support of
13 vaccine development is using standard preparations of viruses. And then furthermore all the
14 challenge experiments done in preclinical studies to evaluate vaccines also use standard forms of
15 the virions.

16 And then we're very interested in trying to understand what is the molecular basis
17 for this maturation state sensitivity in vaccine elicited antibodies because it is our hope that if we
18 can identify the molecular feature that confers this maturation state sensitivity we could develop
19 more straightforward assays that would be predictive of protection.

20 So I'll end on the most important slide. There's a lot of people in my group that
21 have contributed to this, but this has just really been a wonderful collaboration between my
22 group and Barney Graham. In my group most of the work was done by Sonia Maciejewski.
23 Tracy Ruckwardt produced some of the really important mouse data shown here today. We

1 collaborated with John Mascola, Julie's group at the VRC, and many other shown here. Thank
2 you.

3 DR. CASSETTI: We're going to keep the questions for the end. The next
4 speaker is Julia Ledgerwood and she's going to talk about Zika vaccine candidates and also talk
5 about different biomarker influence.

6 DR. LEDGERWOOD: Thank you to Marion and the organizers for inviting me
7 to talk about Zika. It's a nice follow on to what Ted talked about. I'll kind of go bigger picture
8 with what we did in response to the vaccine that was created and how we're thinking about
9 utilizing everything we've done for that trial in the most effective, efficient way we can and
10 looking at those samples in any way we can that might be useful. And so that really gets to the
11 heart of the biomarker question.

12 And I'll just say I'm present this from the scientific standpoint that we have about
13 the use of these samples. I don't know that any of the biomarkers we're considering would
14 actually apply to a regulatory pathway. But they are useful to think about scientifically, and
15 that's how I'll focus on them.

16 So let's see. There we go. And so when you hear this presentation you'll see that
17 we did a lot of things in terms of trial planning at risk, because we went so quickly. And the
18 point here is to just show you how quickly we did move as we get into this discussion.

19 Ted talked about the design of the vaccine and how that was brought forth. But it
20 was really done in a most efficient way I think anything has been in this area of work. So the, so
21 in 2015/16, late '15, early '16, Barney and Ted and the rest of us at VRC and in Ted's group
22 began to work on this.

23 Hundreds of constructs were evaluated in the lab and in animal models. This was

1 an early response to the outbreak. We submitted our pre-IND before we knew what the vaccine
2 would look like. The only thing we knew is that we were going to use the West Nile PRM and E
3 encoded DNA vaccine as a model and this would be a DNA vaccine.

4 And so we submitted a really brief pre-IND document to provide heads up to
5 CVR, and that was the launch of a collaborative approach to getting this first inhuman trial done.
6 So when the final DNA sequence of the best candidate was selected or what we thought was the
7 best candidate on April 24th, we started the process development and the manufacturing and we
8 submitted the IND in July and started vaccinating on August 2nd, the day after Labor Day.

9 So it probably could have been earlier if it hadn't have been for the holiday, but
10 that's how it works out. So then the second candidate which Ted talked about, 5283, turned out
11 to be the best one but it just happened that it was the second candidate we tested starting in
12 December.

13 Now why did we do that? It wasn't just to get a publication. It was because we
14 thought that maybe we could get a vaccine into play in the outbreak in time to get an answer,
15 which is almost impossible and I'll show you why. At least in this case.

16 So we started the Phase II trial with this candidate in March. So the first
17 vaccination was in December for the Phase I and the first vaccination for the Phase II was in
18 March. And then we completed all the vaccinations at the end of 2018 and the last follow-up
19 visits are in a few weeks.

20 So what that looks like is really summarized here. We went from sequence
21 selection to first human injection in 3.25 months. So I don't think you can really go faster than
22 that. And that's applicable when we look at the timing of how we were able to get to the
23 outbreak.

1 Even that fast, this is how it turns out. So biomarkers are important. So this is
2 what we did. We went forward with an adaptive design. It was the first time we had done that,
3 truly adaptive. And I think people use the word adaptive to mean they amend a lot. We do that.
4 But in this case we actually planned to implement the second part of the trial, the efficacy
5 portion, based on what we learned in part A without an amendment. And all of the IRBs and the
6 FDA agreed to that.

7 So we started part A with four, or sorry, three different options, randomized
8 people to receive open label because it's faster to get data open label than with placebo control
9 and it's more efficient. So we went open label. We gave four milligrams divided by two with
10 the PharmaJet device, or four milligrams divided by four, or eight milligrams divided by four.

11 At the same time that we were analyzing data from Phase I. So then we took all
12 available data from here, all available data from Phase I, all available NHP data, and we said
13 what should we immunize people with in July for this efficacy question?

14 And we chose for multiple reasons group one, four milligrams, two injections.
15 Partly because of the logistics and partly because of the immunogenicity data. Those two things
16 together prompted that decision.

17 So we evaluated almost thirty sites and selected seventeen in eight countries. And
18 that was based on site qualification, but also largely based on modeling efforts that were
19 performed by a group of about fifteen organizations. It was an all hands on deck effort.

20 So we modeled the outbreak, we went to sites, we started the trial. And the
21 reason we started the trial was to accomplish these three things. So we said we want to do this
22 because we want to get into this outbreak and assess efficacy of this vaccine.

23 Now like Dean told you, assessing efficacy of the vaccine couldn't really mean

1 congenital deformity, although that's why we were doing this. It meant do people get infected
2 with Zika when they're vaccinated or when they're not.

3 And then the way that we defined that initially when we launched the trial was
4 pretty strict, because based on what we knew about assays and based on what we knew about the
5 disease, we thought it was a highly symptomatic disease and the assays needed to be done
6 essentially in real time to get the best answer.

7 So we said people needed to have a symptom, really any symptom. The case
8 definition was really big. I think what we said in the protocol was any symptom that could be
9 representative of an arbovirus would prompt a virologic assessment.

10 And then in addition we said, well, it's great to know that Zika RNA can be
11 detected in urine because that's an easy sample to collect frequently. So we had people coming
12 in every two weeks, so twenty-four hundred people, seventeen sites, people came in every two
13 weeks to give urine and every month, so every other visit, to give blood.

14 The goal there was in parallel to the trial conduct, we would be searching for the
15 best PCR, most sensitive PCR we could find to use on these stored samples. So we're going to
16 look for cases in two ways, symptomatic real time and retrospective based on samples that we
17 have stored.

18 And then if there was any signal for efficacy, we wanted to be able to define the
19 correlate as best we could for this vaccine knowing that may or may not be generalizable to
20 others. And of course when you don't know what the correlate is, you don't know exactly what
21 assay you need to conduct at what time point.

22 So we had to take lots and lots of samples at lots of time points, because we don't
23 know and we need to write the protocol in the consent pretty vaguely so we can do other kinds of

1 assays besides the one we knew we would do, which was Ted's RVP.

2 So here's the thing, though. Chasing an outbreak is really hard because outbreaks
3 wane. These types of outbreaks wane. Which is good, it's good that the outbreak waned, but it's
4 hard to get scientific answers in that setting. So this is an outbreak diagram starting in 2015
5 through 2017 when we launched part A of the Phase II trial.

6 So just remember we did the first Phase I trial in the middle of 2016, in August.
7 And then part A and B of the Phase II trial here. So we weren't looking at really being able to
8 find very many cases, but Dean told us after much evaluation that we only needed ninety. And if
9 we got thirty we would still potentially get an answer.

10 So we thought it was possible, so we went to these sites where we thought we
11 might find an answer and then those sites were hit by three different hurricanes and an
12 earthquake. So it really felt like the world was against us and we just persevered.

13 What turns out to be important is this idea of risk management. So we hadn't
14 planned for hurricanes. We didn't know what to do if a site was hit by a hurricane, what do we
15 do with vaccine, what do we do with sample, how do we ensure subject safety?

16 But we figured it out rapidly and now that's on our risk mitigation list for future
17 trials. Truly. So that's what we're dealing with. So what we decided to do in the setting of a
18 waning outbreak and a difficult enrollment period because of natural disasters, was to re-look at
19 the modeling data, the current epi data, and the design of the trial.

20 And we modified the trial greatly. We reduced the time for septic follow up to no
21 more than, it was never more than two years, but for any remaining subjects who hadn't met two
22 years of follow up, they would only be followed-up up to one year.

23 And that allowed us to get safety follow up completed and to prevent the

1 collection of samples and potential case evaluations at a time when there was unlikely to be an
2 outbreak. So we also think that it's important to message this clearly, because we didn't stop the
3 trial and we're not stopping the trial for futility.

4 We really hope that the answer is in the stored samples, and that's what we'll talk
5 about. It just is that there's low potential value in continuing to look aggressively when there are
6 no cases. The economics law seems to play here.

7 So how do we think we can look for cases? Well, I told you the clinical diagnosis
8 would be our preference. People have symptoms, they come in, they're evaluated in real time.
9 And if they can't do that, the stored samples may give us the answer on subclinical or
10 asymptomatic cases.

11 If we don't see it there, how will we know if this vaccine is potentially protective?
12 Based on what we know about it in animal models and the assay data, maybe we can assess a
13 vaccine response for the potential to predict whether there would be an efficacy with the vaccine.

14 And if we really need to know how the vaccine performed in the setting of an
15 outbreak and we think there were cases we didn't detect by items one and two, maybe we could
16 look at samples and distinguish between natural infection and vaccine-induced immune response.
17 Which is really hard to do, but we think that's a possibility.

18 So clinical diagnosis we said to the sites bring people in with any symptom,
19 collect three days of samples on day zero through thirteen of onset of symptoms. And then send
20 them to the primary diagnostic CLIA lab in Washington. And we ran this Altuna [phonetic]
21 assay.

22 We've so far evaluated thirteen hundred possible cases and diagnosed two. So if
23 we can't do it that way, can't get to thirty or ninety cases that way, we'll do it by looking

1 hopefully at retrospective stored samples. So what we, also at the time we launched the trial
2 didn't know what the assay would be or the sample type of interest would be.

3 So we collected a lot of samples like I told you, every two weeks for urine, every
4 month for blood. When we collected blood we collected whole blood, serum plasma, and RBCs.
5 So that's a big endeavor at seventeen sites.

6 We knew that one of the assays in development would turn out to be the winner
7 and then we would apply that to use on the stored sample. Also an important reason why your
8 protocol and your consent needs to be written with the availability to answer questions using
9 samples that are collected in the most efficient way you can.

10 So here's the thing. The assay improvements were significant. During the course
11 of the trial the relative sensitivity of the assays that were available to us increased by, increased
12 by a magnitude, an order of magnitude that we didn't expect when we launched the trial.

13 And so we said, well, initially we thought that we had a pretty good assay option
14 with every two week sample collection, but what we realized was if we used that initial plan we
15 were really going to miss about fifty percent of possible cases. So we've adapted our plan using
16 data from the zip trial. Christine has been very helpful. And we plan to use a more sensitive
17 assay now depicted here.

18 So we changed our plans in that regard. But then we had to think about do we
19 need urine every two weeks? It turns out it's easy to collect urine every two weeks but it's really
20 hard and expensive to store it. Because we're talking about a million samples here, literally
21 almost a million samples of blood and urine.

22 So the other data that came out during conduct of the trial was regarding what
23 sample types and how long you could detect Zika RNA in them. And it turned out what we had

1 thought was that urine would be the most important and easiest to collect, but it wasn't. And it's
2 also highly variable.

3 It turned out whole blood was probably better. So we designed the trial to collect
4 everything and then we amended the trial to collect less and focus on the whole blood at fewer
5 time points. Because we knew we could really get an answer if we had collections about every
6 three months, we should be able to.

7 So we reduced the number of visits, we eliminated urine collection. We clarified
8 that an efficacy end point for infection could either be a real time symptomatic diagnosis or a
9 retrospective PCR diagnosis. So we clarified that and amended an approved version of the
10 protocol that is in play now.

11 And that also helps us to maybe get to thirty cases or ninety cases when we get
12 through the sample collection assessment. So what all of that did, that adaptive amending style
13 did, was it reduced the sample collection plan from 900,000 to 300,000 and the number of those
14 we intend to assay to 30,000.

15 So it's still a lot and it take a while and it's really expensive, but it's much less
16 than we had designed the trial for. So we gave ourselves that flexibility and then we efficiently
17 changed our plan.

18 This is an example of how it affects the sample collection plan. So we had, you
19 know, a number of samples for visit zero in the freezer compared to number of samples for the
20 later visits in the freezer. So it's just graphically telling you that it makes a big difference in
21 what you have to store and assess.

22 So the other thing we wanted to be able to look at was a vaccine response by
23 antibody and we planned to use Ted's assay that he just described. So we want to know if people

1 got a vaccine response. We also want to know if they got Zika, and we also want to know if they
2 got other Flaviviruses.

3 And there are ways to do all that and we worked closely with Ted and others to
4 make sure we'll have the right samples and plan to do so. And so the data on part A using Ted's
5 assay that he described today does represent an interesting picture.

6 So in the Puerto Rico site, this is in part A where we studied three different
7 regimens, which are shown here. We see a different picture. We see evidence of endemic
8 background immunity to Zika, which makes sense in Puerto Rico, but not at Baylor.

9 And we can see that there is a potential effect on the endemic nature of the
10 population to the vaccine response, but we're still seeing a vaccine response. So we remain
11 hopeful. And then fine tuning assays and understanding more about the correlate in parallel
12 should help us.

13 So then lastly we wanted to say that there would be samples in the freezer if an
14 assay were developed that could distinguish between vaccine and natural infection. And so we
15 wrote the protocol and consent broadly enough that we could look at that.

16 This assay wasn't developed at the time we launched the trial, so there was no
17 way that we could plan exactly how we would use it. It wasn't even known if it would be
18 possible to do such a thing. So as a reminder, our vaccine encodes for this PRM and E structural
19 proteins only.

20 So if there were an assay to one of these other proteins expressed by Zika that are,
21 if the protein was shown to the immune system and there was a highly sensitive specific assay to
22 detect it, maybe we could differentiate it if it weren't PRM or E.

23 And it turns out that kind of work did move forward in the broader assay

1 development portfolio that Cristina and others launched for us with lots of grant and contract
2 money. So there are assays now. One looks pretty good, that we could potentially distinguish
3 PRM and E response from vaccine to NS1, nonstructural protein response, that would only be
4 induced by infection.

5 So this would allow us to look at the serum we collected monthly and look for a
6 spike in NS1 protein in either the vaccine or placebo group and then compare the rate of that. So
7 that's another option that the flexible protocol and sample collection plan allows for. And we
8 plan to do that if we have any evidence that infections did occur.

9 So in conclusion, I think it's important this case highlights the importance of
10 casting a wide net when you design a trial and you don't know exactly what your biomarker will
11 be. Because that's often the case when you're planning something quickly.

12 And if you're not planning it quickly, maybe you do know what your biomarker
13 will be. But you have to assume that multiple approaches might be needed to get to an answer
14 that's bridgeable to something that you know. And so you have to plan for the unknown.

15 Assays may be developed that are more sensitive. Assays may require different
16 sample types. So flexible design in your protocol and consent is really key. And I also aim to
17 have the most complex acknowledgement slide of the entire conference, so we'll see how that
18 works out.

19 But, no, this is a huge effort. It was a very expensive and complex effort to
20 launch this trial so quickly during the outbreak. So many of you are probably named up there or
21 should be. So we'll take questions at the end I think.

22 DR. CASSETTI: Yes, thank you. The next speaker is Katrin Ramsauer and she's
23 going to talk about evidence from animal studies for an antibody baseline marker to support

1 effectiveness of chikungunya vaccines.

2 DR. RAMSAUER: Thank you. I want to thank the organizers very much for
3 inviting us. I'm speaking here as a manufacturer. Of course we are developing a vaccine to
4 prevent chikungunya. We are very close to our Phase III trial, so the discussions on how we get
5 the license is what I do every day.

6 We have quite intense discussions with many people in the room, from FDA,
7 from AMA. What I will do is I will not spend so much time under biomarker per se but on how
8 we think we can use an animal model to show clinical benefit of this biomarker infused by a
9 vaccine or also by infection.

10 A few steps back, I know it's a broad audience. People have different pets they
11 work on, so some words on chikungunya. So chikungunya is a mosquito transmitted disease
12 very closely related to Zika, so the two speakers before me can give additional talks I think about
13 chikungunya as well.

14 So the disease is a febrile illness. It causes a substantially severe, severe,
15 substantial viremia in the patients. It causes fever and it causes joint pain in also to patients in
16 the acute phase. And then the viremia declines very rapidly and then in many patients the
17 symptoms stay on for months or years.

18 So this fortunately is a disease with a very low mortality rate, but the morbidity is
19 quite substantial. So these people are unable to work and if a broad population is affected by an
20 outbreak this has quite an impact on the economy of the area.

21 So what we have done is we have developed a vaccine against chikungunya that is
22 based on a measles vector vaccine. So we at Themis, this is what we do. We use the measles
23 vector to insert foreign trans genes and express in this case chikungunya structural genes to

1 generate an immune response against the trans gene.

2 We have run a quite substantial clinical development program in the last couple of
3 years. We have now more than five hundred subjects vaccinated and analyzed some of the data
4 occurring in the final analysis. But we show the vaccine is very safe and immunogenicity is
5 measured by neutralizing antibodies. It was very convincing.

6 I have to add here that a substantial part of these clinical development program
7 was funded by U.S. government organizations. Cristina's group here, they funded a Phase I
8 clinical trial in the U.S., not only funded but conducted a Phase I trial that is currently being
9 analyzed. And the Department of Defense is funding two clinical trials that are currently
10 ongoing to show the safety in pre-exposed chikungunya subjects.

11 So with the data we have now in hands with the safety data, we prepare a Phase
12 III trial and this will be starting very soon. Now why am I here, why do I talk biomarkers, why
13 don't I just go into my field efficacy trial? Chikungunya was diagnosed in many countries, more
14 than a hundred countries worldwide.

15 It keeps coming up in outbreaks, very recently in Thailand and in parts of Brazil.
16 So it's recurring. But the last fifteen years the outbreaks got more and more over the world.
17 And what is quite important to say here, this is a picture of where Chikungunya was diagnosed,
18 but it's not in all of these areas at the same time.

19 And so what makes the whole epidemiology complex of chikungunya is that the
20 outbreaks are, can be explosive like in 2014/2015 when a completely naïve, immunologically
21 naïve, sorry, continent was hit by a chikungunya where in waves the whole continent was
22 effected.

23 But the individual outbreaks in each country didn't take very long. It was ten

1 months or twelve months. So impossible to really start early clinical trials. And I think Julie
2 could give an additional talk on how they tried, NIH had an internal chikungunya program and
3 they tried to do an efficacy trial in the midst of this outbreak. And also this trial started too late.
4 Not because it was too late but just because it's impossible to start it on time.

5 Now the currently there are small outbreaks reported worldwide, but the outbreak,
6 the attack rates of these outbreaks are very low if you calculate it to the whole population. So
7 also with these numbers of attack rates we find in places like Rio de Janeiro 0.04 percent. A
8 clinical trial to show efficacy would need at least 120,000 subjects in a very short period of time.
9 We are not talking about five years, we are talking about two to three months. So this is not
10 possible.

11 So, and there are other things I don't have to go into detail and explain to you how
12 difficult it is to set up clinical trials in lower middle income countries and other hurdles that can
13 affect it.

14 So what is the biomarker, what can be used as a biomarker for chikungunya? So
15 chikungunya is an Alpha virus and as for many other Alpha viruses that were old vaccine studies
16 have started for us real virus, for example. It became apparent that neutralizing antibodies really
17 do correlate with protection against disease.

18 So this, there was cumulative evidence in different animal models in small and
19 large animal models that showed neutralizing antibodies can protect against infection, can
20 protect against disease. Small animal models like joint swelling models in the mice show, with
21 antibodies who can prevent the joint swelling.

22 With our vaccine development we've done passive transfer of sera into mice and
23 showed protection against challenge. So this has been well-established. On the other hand,

1 cellular responses are sort of ruled out to play a role here because if you use adoptive
2 transference in immune cells, those cells alone do not confer protection. So really antibodies do
3 the trick.

4 And what are the antibodies raised? This is a nice picture of a very recent paper
5 from the Fremont's lab is this the chikungunya surface where the main glycoproteins, only two
6 are exposed, and the antibody response to those glycoproteins really are the ones that confer
7 protection. So mainly these.

8 Those two proteins are of course contained in all vaccine construct, but there are
9 many other vaccine projects ongoing, the VLP construct from VRC, live attenuated viruses,
10 formal and inactivated viruses, all of those contain these antigens of course.

11 Another advantage chikungunya has as opposed to for example dengue is it
12 occurs worldwide in four genetic lineages but really in a single serotype. So if we get protection
13 against one serotype, we can also protect against other serotypes. This has been shown in vitro,
14 also for the VRC project to show cross neutralization against all the four lineages and we have
15 shown that also with our clinical trial samples and primate samples that we get protection against
16 all four genetic lineages.

17 So chikungunya is more or less the easy one. It seems so. But also from the
18 literature there are some human data on what are, what neutralizing antibodies do and there are
19 some prospective coed studies that is, were done following outbreaks or during outbreaks where
20 it was found that people who had a very low level of neutralizing antibodies at the start of the
21 outbreak, those people were protected against symptomatic disease.

22 So also this was from this example I mentioned here is from the Philippines.
23 There were additional studies done in Cambodia and India that came to the same result.

1 Now what we are trying to do is not to set up an animal model where we can
2 show, so in our vaccine studies we find high levels of neutralizing antibodies in the, in our
3 clinical trial participants.

4 Now what we want to do is correlate the antibody level those participants raise
5 against our vaccine to protection against disease. And for this purpose we are using an animal
6 model.

7 And so there are several animal models available, but we ruled out the small
8 animals because this is a really artificial animal model for chikungunya. You basically need
9 interferon deficient animals or you have to inject virus directly into the joint.

10 So both are useful tools to study pathogenesis or some early vaccine studies, but
11 not for this kind of study. So a natural host for chikungunya are nonhuman primates.
12 Chikungunya is maintained in a sylvatic cycle in parts of southeast Asia and Africa.

13 In nonhuman primates, so chikungunya infects those animals, replicates in those
14 animals, and these animals also reflect some of the chikungunya symptoms and some of the most
15 important chikungunya symptoms. And these can be used to show protections.

16 We have used this animal model in a collaboration with Scott Weaver at UTB.
17 We've immunized his monkeys with our vaccine. They received two shots and we showed
18 protection so the animals raised antibodies against the vaccine and we showed complete
19 protection against challenge in this model.

20 So what symptoms do you find in humans and in nonhuman primates and why do
21 we believe this is a suitable model? So a quite characteristic symptom is the acute and strong
22 uremia you get early in the infection during the acute phase of the disease.

23 And this is seen in the same kinetic in humans and in nonhuman primates caused

1 by this strong replication of the virus in the system. Of course inflammatory cytokines are
2 increased in the circulation. The, there is a strong effect on immune cells, on liver enzymes that
3 can all be measured, and this is quite well recapitulated, reflected in the nonhuman primate
4 model that's quite comparable to the human model.

5 It gets more complex looking at the chronic phase, so in humans patients who
6 report still joint pain more than two months after the onset of the disease are chronic cases. And
7 this is of course more difficult to reflect in nonhuman primates.

8 But so these animals get arthritis but only if we use a ten thousand-fold higher
9 challenge dose than you would usually get by a mosquito, so it's a very artificial challenge
10 model. And probably the pathogenesis causes the arthritis in that case is a different mechanism
11 than you would induce with the viremia that comes, that peaks at day two or three.

12 But then it's also for humans. There are several effectors that contribute to the
13 development of the chronic disease and those cannot be reflected in an animal model. So age is
14 one of the things, so people who are older than fifty are more susceptible to develop chronic
15 disease.

16 Pre-existing joint problems, so pre-existing arthritis or injuries in the joints can
17 contribute to development of chronic disease. We could hurt the monkeys in the joints before,
18 but I think you don't want to go that route. So and then there are many other factors that
19 contribute that cannot be reflected in animal model.

20 So what we have then done is we looked into using this model now to do passive
21 transfer to take actually the sera or plasma we collect from human clinical trial participants and
22 passively transfer them into nonhuman primates and show protection against symptoms of acute
23 disease but also symptoms of chronic disease.

1 So therefore to set up the model we had several things to consider. One was
2 obviously what material do we transfer. Do we transfer serum or plasma from individual
3 subjects to an individual monkey? From an individual subject to a number of monkeys? How
4 many subjects do we look at? Or do we use serum pools? Which challenge strain do we use?
5 Which challenge dose do we use? And of course how do we measure in the end what is the
6 protective level?

7 So what we ended up choosing was a pool of plasma, so we used samples we
8 collected from the ongoing clinical trial in Puerto Rico where we had the opportunity to have the
9 subject pre-exposed to chikungunya, so actual chikungunya patients. And the other half was
10 naïve at baseline and received a chikungunya vaccine. And then a group that, a group of
11 monkeys that received only naïve plasma.

12 We passively transferred those samples and then challenged within twenty-four
13 hours and looked at some of the disease parameters, what you can manager in the nonhuman
14 primates. There was viremia, cytokine levels, hematology blood biochemistry levels, and also at
15 time of necropsy the joints and viral load in major organs.

16 And so these are quite fresh data and so what I cannot tell you is the actual end of
17 the story. So this is really work in progress. What I can show you is how the model works for us
18 and how we believe we can interpret the data we get from the small pilot study.

19 So what you can see is reflected, that's the animals that received naïve sera and
20 they present a very good, high viremia of chikungunya, but all animals that received antibodies
21 either convalescent or vaccine recipient antibodies were completely protected against viremia.

22 We then also looked of course at the time of necropsy and it was quite an
23 important finding. Do we find virus in the joints? And so remember so there is a very high and

1 strong curve of viremia that ends already at day seven.

2 And a week later we look for virus in joints and major organs and what we find in
3 the knee and ankle in the naïve plasma recipients, we get quite some, as in all the animals we
4 find virus. But in none of the animals that received antibodies we found any virus. So this
5 already shows that the antibodies can protect the infiltration of the joints. And probably by
6 preventing the viremia completely.

7 Then of course we looked at, and this is the eventual goal of this whole model is
8 we want to find out what is the threshold, what antibody level do we need to induce in our
9 human subjects to confer protection? And this we want to demonstrate in the animal model.

10 And so we looked at the pre-challenge titers and so obviously the naïves were
11 naïve. The convalescent had a higher level, but the vaccine, so the animals that received vaccine
12 recipient sera had a titer that was quite low at a level of ten to twenty. So this was really low
13 levels and all, remember all of the animals were completely protected.

14 Quite importantly, also here, this here shows the immune response to the
15 chikungunya challenge. Similar to what Ted has shown before for Zika in the mouse model. So
16 we find anamnestic response to, of course not in this one, so these, the naïve animals, they
17 respond to the challenge to develop their own immune response. But the animals that received
18 antibodies did not respond to the virus at all. So they completely prevented a response too to the
19 challenge virus.

20 So to summarize this is how we think we can move ahead. So we can prevent,
21 completely prevent acute disease in this animal model, even with very low levels of antibodies.
22 In addition to what I've shown you, we also found that we can prevent virus infiltration of
23 spleen, liver, and joining lymph nodes.

1 We can prevent transient fever, increased serum cytokines, and changes in blood
2 biochemistry or white blood cell counts. And we induced the sterilizing immunity so this is
3 already very convincing data to show that this neutralizing antibodies are a biomarker and in this
4 animal model is also suitable.

5 What do we show about prevention of chronic disease? We do find that we can
6 prevent viral infiltration of joints. We cannot find inflammation of the joints. We looked at it,
7 we looked at joint sections, and we couldn't find any signs of inflammation at that time post-
8 challenge.

9 But coming back to this figure and maybe to summarize what I said is so this
10 animal model shows a lot of features you can find in humans. We can prevent the acute phase
11 and we are quite convinced that by preventing the acute phase of the infection we can also
12 prevent the chronic phase of the infection.

13 But this is really work in progress again and of course work in progress in
14 discussion with regulators. So again coming back to the chronic manifestation, so there is room
15 between the challenge dose we have used, so we have chosen a challenge dose that is close to
16 what you would receive through a mosquito.

17 A dose that would induce arthritis is ten thousand times higher. But there is room
18 in between, so there is still room to work on the model to also show inflammation and maybe
19 prevention from inflammation. Now the points that need to be discussed is what antibody level
20 then protects against very, very high challenge doses.

21 Our antibody levels that I need to protect against, ten to the seven infection, a
22 threshold of protection that is needed for a vaccine, I don't believe so. But this is something that
23 has to be worked on. But with this model and with the data we have we are quite convinced that

1 this can be done.

2 Okay, and I think I've already summarized this, so thank you very much for
3 inviting us. And, oh, I forgot to thank one organization. C.E.P.I. has very recently also invested
4 in your chikungunya vaccine program and they've substantially contributed to our Phase III
5 clinical trial which is quite important. So thank you very much.

6 DR. CASSETTI: Thank you, Katrin. We'll move on to the next speaker, Dr.
7 Long Wang is going to talk about end points for prophylactic congenital CMV vaccine
8 development.

9 DR. WANG: All right, so, first of all thanks for the organizer for giving this
10 opportunity here. So as Christina just mentioned, I'm Long Wang from Merck Vaccines. And
11 I'm going to talk about here something not related to mosquitos. It's called end point for
12 prophylactic congenital cytomegalovirus, CMV vaccine development.

13 So I'm going to briefly talk to you about what is CMV and what's the disease
14 mode and economic impact it has. And also going to explain a little more about what is called a
15 primary CMV infection. And then I'll summarize a workshop that was convened by FDA and
16 NIAID and the CDC back in 2012. And then I'm going to share some of the challenges that we
17 have run into in clinical trials. I'm going to compare, so CMV with rubella before bringing back
18 to the theme of this workshop which is biomarker.

19 CMV, so cytomegalovirus, so is, so beta host virus, it's a DNA virus and we use
20 envelope as any other host virus once you get infected with CMV you get lifelong latency. And
21 CMV is also quite ubiquitous in humans and so which accounts to about sixty to ninety percent
22 of the worldwide population already infected. But mostly SO without symptom, it is for healthy
23 population.

1 And why we're interested in CMV is because it can cause congenital CMV
2 infection and disease in infants, which could have some severe consequences. And congenital
3 CMV actually is the most common congenital infection, which actually counts to .6 to .8 percent
4 of the live annual births in the US.

5 And if we break that down into say infants born to CMV negative and CMV
6 positive mothers, so for CMV negative women at the baseline who got infected for the first time
7 during their pregnancy, which we also call primary infection. There is a thirty to forty percent of
8 chance that infection will be transmitted to infants.

9 And for the CMV positive women, that is reduced to one to two percent.
10 Congenital CMV disease itself has many different forms and the most common form is sensory-
11 neuro-hearing loss and long term neurodevelopmental disabilities. And an annual cost to care
12 for that is estimated to be more than \$2 million. And currently there's no vaccine to prevent the
13 congenital CMV infection. There's some treatment options, but they are suboptimal.

14 So and that's why it's a huge medical need. On this slide I also mentioned
15 besides congenital infections CMV can also cause severe disease in immune compromised
16 individuals. But for the purpose of my talk I'm going to focus on the congenital infection.

17 I already explained the primary CMV infection is basically there, so seronegative
18 women get infected for the first time during pregnancy and that infection is virulently transmitted
19 to, via the placenta to infants. And ten to fifteen percent of the infants will be born with
20 symptoms and ninety percent of them will go on to have long term E.coli.

21 Even for those eighty-five to ninety percent of infants who are born without
22 symptoms, they can still develop, I said long term E.coli, about five to ten percent of them will
23 have that. So that's why it's a huge public health issue and that's why I think back in 2012 there

1 was a workshop convened by FDA, NIAID, and CDC.

2 And so to discuss the priorities for CMV vaccine development. I was not at that
3 workshop at the time, so my understanding was they discussed the target populations and the end
4 points to use in different population. And for the purpose of my presentation I'm going to focus
5 on the adolescent girls and women of childbearing age.

6 And for those populations, there are three possible end point that we can study.
7 So one is the CMV infection in the girls and women themselves, or the congenital CMV
8 infection end point in their infants, or the congenital CMV disease in their infants. So
9 fortunately, so in the 2012 workshop it was broad consensus that studying the congenital CMV
10 disease prelicensure is impractical due to the low incidence rate and also long follow up as
11 needed.

12 And at the time there was also acknowledgement for the adolescent girls, you can
13 just study the rate of CMV infection in those girls and for women of childbearing age, besides
14 the CMV infection in women themselves, we also need to look at the congenital CMV infection,
15 CCMVI, in their infants.

16 The problem with that is that still comes with a lot of challenges. We as a
17 company actually shared in the 2012 workshop that one of the challenges is we will need a very
18 large sample size to study that. And here this table just illustrates some of the different scenarios
19 we look at.

20 And I'm going to just draw your attention to the two rows that was in the middle.
21 Here we estimate or we assume the congenital CMV infection incidents at .8 percent with a
22 pregnancy rate of ten percent. If we want to demonstrate it as vaccine efficacy using the
23 conventional criteria, which is to compare the lower find of ninety-five percent confidence in the

1 estimate to twenty-five percent, we will need thirty-eight thousand seronegative women. And
2 even if we moved the goal post to zero percent, we will still need twenty-five thousand
3 seronegative women.

4 So as I mentioned earlier, sixty to ninety percent of the population are already
5 seropositive, so that means we need to screen a lot more subjects and based on our estimation we
6 will need to screen about eighty to one hundred thousand women to get those twenty-five to
7 thirty-eight thousand seronegative women, which I'm sure you will agree is a very large sample
8 size.

9 And besides the sample size, we also learned other challenges which here I tried
10 to categorize into five different buckets. One is recruitment. As we all know, trying to recruit
11 women who are planning pregnancy into any trials can be challenging.

12 Not to mention here we are trying to strike a very delicate balance. We are trying
13 to find women who are both at risk for CMV infection and also has a high pregnancy rate. Even
14 if we were successful in finding those women and there could be potential behavior changes that
15 might affect the outcomes of the trial.

16 For instance, by just being in the trial, being exposed to the increased education of
17 the CMV, that might, so decrease the acquisition rate already. And on top of that, there might be
18 some interesting dilemma.

19 For instance, if in a trial we find a woman is infected with CMV, do we tell her or
20 not? If we do, and will that affect their decision to carry the pregnancy to term? And also
21 obviously studying the congenital end points, we need to follow those babies.

22 So and the vaccine that's in development my understanding is pretty much all
23 targeted at let's say women before they get pregnancy, so that means we give them vaccine, we

1 have to wait for women to get pregnant and give birth to a child. And you can figure how long
2 that waiting can be.

3 And also for sample babies, so try to get urine samples from babies without
4 contamination can be a practical challenge. And also there is a very narrow window post to get
5 the samples for congenital CMV detection because many of those babies might get infected
6 postnatally.

7 And also many of those babies, particular babies with symptoms might be ill and
8 hospitalized. Therefore it's very challenging to get the samples in the time period that we want.
9 And last but not least, there could be some retention consults.

10 What I meant by that is let's say if we start a vaccine and find out the vaccine is
11 efficacious in preventing the infection in women, there might be concerns that for IRBs or ethic
12 committees to let the trial go on with half of the subjects randomized with placebo knowing that
13 they might go on to deliver a baby with congenital CMV infection and disease.

14 So with all those challenges, we basically and kind of scratch our head a little bit
15 and try very hard to figure out how can we do better. So for that we turn to the history book of
16 vaccine development. We try to compare CMV to rubella. The reason we choose rubella is
17 there are some remarkable similarities between rubella and CMV.

18 So those virus can cause mild infection in healthy population. But if women get
19 infected, particularly for first time during pregnancy or especially the first trimester, that can
20 cause very severe congenital infection and disease.

21 In the case of rubella it's called congenital rubella syndrome which can manifest
22 as a triad, so skinny nose, congenital cataract, and heart disease, et cetera. And congenital CMV
23 disease I already mentioned, so it can manifest as sensory neuro hearing loss, vision loss,

1 developmental delays.

2 So before rubella interesting back in 1960/70's there were more than one rubella
3 vaccine developed. So based on the protection for clinical rubella in the vaccine needs. And for
4 instance women of childbearing age. And there was no study to look at the outcomes in infants
5 before the rubella vaccine was licensed.

6 I'm not an expert in Zika. Anyway, so I'll try to continue. All right, thank you.
7 Even the, so the med count is really infants. Anyway. But if you look at the figure on the right-
8 hand side, after the rubella vaccine was implemented, as you can see there was a dramatic
9 decrease in the congenital rubella syndrome in parallel to the clinical rubella.

10 So this is not surprising at all because the rubella infection in women is a
11 precursor in the congenital infection in infants. So if you brought the infection you women
12 shouldn't be surprised that you can prevent or at least reduce the congenital infection in infants.
13 And we ask well why can't we do the same for CMV, right?

14 And because after all the CMV infection in women is also an obligatory precursor
15 for congenital CMV infection in infants. And this was very nicely demonstrated in a publication
16 of Karin Bok published in 2003. Basically she looked at the babies with congenital CMV
17 infection and went back to look at the samples from their moms.

18 And it turned out all the congenital CMV infection cases came from seronegative
19 women who were seroconverted within pregnancy. And for those seronegative women on the
20 top that were never seroconverted and there was zero case of congenital CMV infection.

21 So with that said, I think it's a fair question to ask, well, can CMV infection in
22 women be a valid biomarker for congenital CMV infection and disease? Again, so bring back to
23 the 2012 workshop, there was already consensus that congenital disease is impractical. It was

1 acknowledged that congenital infection can be a valid biomarker.

2 But what about CMV in women? So here we tried to compare the present counts
3 of these two potential biomarkers, congenital CMVI and CMVI in women. For congenital, so
4 infection obviously it's a more feasible endpoint than the congenital disease. And also we see
5 the same in infants as the congenital disease.

6 But the problem is we need very large sample size as I discussed earlier. Also
7 there are other practical challenges associated with recruitment, retention, and also sample
8 collections. For the CMV infection itself, so obviously it's a much more visible end point than
9 both the congenital infection and congenital disease.

10 As I mentioned, it's an obligated precursor for the congenital infection. So this
11 potential can enable much earlier access to this important vaccine that can address this huge
12 medical need. The only problem with that is it might be actually a higher hold out than the
13 congenital infection itself. Because in theory you can't have a vaccine that does not completely
14 prevent the infection in women but is still efficacious enough to prevent the transmission to
15 infants.

16 And on the flip side, you need a very highly efficacious vaccine to completely
17 rule out the possibility of transmitting to infants. Anyway, so with that I'm going to summarize
18 what I just mentioned.

19 So congenital CMV infection disease is a huge medical need and vaccine
20 obviously is the most promising strategy to control that. And the end point that we need to study
21 in clinical trials depends on the target population and also visibility.

22 There was consensus that congenital disease is not practical. The congenital
23 CMV infection is already acknowledged as a biomarker. But we believe the CMV infection in

1 women itself can be a potentially valid biomarker as well, particularly if you look at the
2 remarkable similarities between rubella and the CMV and the fact that that rubella vaccine.

3 So even though it was licensed based on protection in the vaccinees themselves,
4 but were also very successful in bringing down the congenital rubella syndrome after
5 inflammation. And with that I want to thank a few colleagues. I don't have as busy
6 acknowledgement slides, but I will thank, but I do want to thank Dr. Kevin Russell, Dr. David
7 Gutsch, Paula Annunziato, and Ercem Atillasoy from Merck. And particularly Dr. Russell.

8 So he and I were initially planned to co-present this, but he graciously agreed to
9 let me to present solo due to twenty minutes limitation. But he and I had a gentleman agreement
10 that said I would give him all the difficult questions. I will answer the easy ones. Anyway, all
11 right, thanks.

12 DR. CASSETTI: Now we'd like to invite the speakers to the table for the
13 discussion and questions. So this session is really meant to involve you all in asking questions of
14 speakers and initiating discussions. So if there are questions, please come to the microphone.

15 PUBLIC: I want to ask one question about you doing the passive transfer study
16 on the animal model. So how did you define how much plasma or, you know, you would
17 transfer before you do the experiment?

18 DR. LEDGERWOOD: You mean the amount of plasma or the antibody levels
19 you need to transfer?

20 PUBLIC: Yes, antibody levels before you do the experiment. I mean, you
21 determine that you know how much you need to transfer, but how when you do that experiment
22 design and how do you know how much antibody transfer you can provide for the result to come
23 out. I mean, yes.

1 DR. LEDGERWOOD: So, I mean, one of the things I didn't mention is we are
2 using a really stellar production utilization test to determine levels of antibodies in the humans
3 after vaccination. And we use the exact same assay for the monkey primate samples. And this is
4 facilitated for both species. So this was one of the purposes when we did these studies to find is
5 this the level we are starting with, a level that protects all the animals. And so this is the starting
6 levels to protect all of them and then what we plan to do is to dilute down to find what is the
7 minimum level of antibodies we need to protect these animals.

8 PUBLIC: Okay, so the level which you determine on animal seems low, so that
9 will come out on your clinical trial?

10 MS. LEDGERWOOD: No, no. So what we, the titer actual was not the level
11 from the pre-transfer series is what we really measured in the animals. So the pool we used had
12 a tenfold higher titer level at least.

13 PUBLIC: Thank you.

14 PUBLIC: So this is more directed at Ted. You highlight some real issues and
15 challenges around what type of virus to use in neutralization assays. So I was just wondering if
16 you have an answer to the optimal virus challenge material. Is it something that you can grow in
17 vivo and then use that in your in vivo assays and in vivo challenge assay as well?

18 DR. PIERSON: Yes, so thanks for the question. So I think the first important
19 point I wanted to convey is this is worth thinking about in all of the viral systems and others that
20 are involved in vaccine development. With respect to the Flaviviruses in general, the issues that
21 I raised are experimentally manipulatable, so we could change the virion maturation state for
22 viruses and we do both in our RBP system and infectious viruses.

23 So it can be manipulated. The question is, what is the right type of assay that will

1 predict protection. And what I have tried to convince you of is the virion maturation state is
2 something that very well may be one of those parameters. Now obviously as Julie said, we will
3 have to continue to study this problem in the context of clinical trials to see what actually
4 correlates with protection beyond the preclinical studies that we've done.

5 PUBLIC: Yes, I mean, I think it's particularly important in ethically challenged
6 studies if you are challenging with the wrong type of virus that your vaccine happens to work, it
7 obviously doesn't translate very effectively when they're naturally infected in humans later on.

8 DR. PIERSON: Yes, if I can just add, and I should have made this point. But one
9 of the procedural challenges with this feature of Flaviviruses is that the host enzyme that does
10 virion maturation may differ between mosquitoes and mammals. So the challenge virus that
11 comes out of a mosquito may look very different than the type of virus that the host then
12 generates immediately after infection. So this could be a situation of problems on top of
13 problems. Thank you for the question.

14 PUBLIC: I did want to congratulate Julie for an enormous amount of work and
15 you kind of put everybody else to shame with what you were able to achieve at the VRC. But
16 my question is for Ted also. And you showed a lot of data and I don't know that I've got it right,
17 but I think your vaccines actually showed that you do get when you vaccinate, and you just
18 follow the animals, you do get a hundred percent protection, right? No mortality upon direct
19 vaccination? Was that correct?

20 DR. PIERSON: The degree, so we, in the NHP model we were protecting against
21 viremia in that context. And our capacity to protect against viremia differed between the two
22 vaccine contexts that I showed. The vaccine that is in the advanced clinical development was
23 able to protect against viremia at all of the relevant doses that I showed. And in most of those

1 animals protected against an anamnestic response.

2 PUBLIC: Right, so but your animals are protected, but when you do the path of
3 transfer studies your antibodies are being transferred. Are you capitulating a hundred percent of
4 the protection? So have you considered viremia responses to be contributing to the overall
5 protection?

6 DR. PIERSON: Yes, I'm sorry, I was talking way too fast and didn't even use all
7 the words I should have. The mouse model we used is a mouse called an AG129 mouse. It is a
8 mouse that lacks its entire capacity to do an innate immune response, which is a very important
9 predictor, or contributor to protection. So it's an exquisitely sensitive animal model which
10 makes it markedly different than a wild type mouse which is replicating.

11 So the mouse model in the NHB is a little bit apples and oranges. The value of
12 the mouse model was it allowed us to pull the antibody response away from the other potential
13 contributors. For Flaviviruses, it is very clear that cellular immune responses contribute to
14 protection.

15 So, you know, obviously post-vaccination you have a blend of things. But our lab
16 was focusing on this antibody marker because both it has functional significance and is
17 something you can track as progression of a clinical dominant.

18 DR. LEDGERWOOD: Just to add, I didn't get into it because I don't think it's
19 the most feasible biomarker, but we do detect T cell responses by ICS in the trial.

20 PUBLIC: Yes, and with so many people, it'd be hard to look.

21 DR. LEDGERWOOD: Yes, it'd be tough.

22 PUBLIC: I just wanted to say for transparency we are also developing the CMV
23 vaccine. But I have a question for Dr. Wang. In one of your slides I think you make the point

1 that in order for congenital infection women to be used as end points, you would need a high rate
2 of vaccine efficacy to, for the prevention of congenital infections.

3 On the other hand, you also mentioned that it's possible for a vaccine to have less
4 than, to be relatively less efficacious and yet still be able to prevent general infection in the
5 infant. So I was wondering do you have any direct evidence that you really need a very high
6 efficacy rate versus against, a vaccine against infection in women to translate into prevention of
7 congenital infection?

8 DR. WANG: That's an excellent question first of all, thanks. Thanks, that's an
9 excellent question. So what, obviously, I was talking about at least from the slide perspective
10 was mostly ready for obviously there are a lot of research there. But I think the transmission via
11 the placenta, its mechanism is not well understood. So, and again I think from one perspective,
12 and at least from the medical perspective, you need a very high efficacious vaccine that can
13 prevent transmission to infants. But no vaccine is one hundred percent efficacious.

14 And also, there is also an aspect I want to mention is obviously there are a lot of
15 debate in the field about this and I want to remind the audience we don't necessarily have to look
16 at IVCI cell or the CCM cell in isolation. They are a combination of those. Does that answer
17 your question?

18 PUBLIC: Okay, thank you.

19 PUBLIC: This is a question for both chikungunya and CMV. You both have
20 similar problems, I think because what you want to do is either immunize and protect women
21 and hope that that protects the embryo, or have a new titer that you think will protect so that you
22 don't have to immunize many, many people in many places to get an efficacy outcome.

23 So the CMV has the problem of having to have a large population because of low

1 incidents, and chikungunya has to have a large population because of sporadic nature. And you
2 probably have to immunize lots of people in lots of places.

3 So the question is, let's just say that those two biomarkers gave you a regulatory
4 pathway to get in and then you were asked to do post-marketing studies. You do a post-
5 marketing study without a placebo group and I just want to know if you've thought through how
6 much it would cost or how long it would take to get an answer in a post-marketing study for
7 either of those two disease conditions and how certain you would be that it was actually going to
8 work since you wouldn't have a placebo group.

9 DR. RAMSAUER: All right, that's a very good question and it's also the subject
10 of the discussions we currently have with FDA and EMA. We do think about that. I think one,
11 the only thing I can say to this is that it's, as we have presented it is very unlikely to be at the
12 right time at the right place, show efficacy for the chikungunya vaccine. I think this doesn't
13 change if the vaccine was licensed. So there can't be a placebo-controlled trial before licensure,
14 it cannot be done after licensure. However, the side of whatever case control study would be
15 after licensure that is ongoing, how much it would cost to launch would be that's...

16 DR. WANG: Again, I think that's definitely the million dollar question we are all
17 trying to figure out, right. And post-licensure comes with challenges as usual to use placebo
18 control and also there are some creative designs that we potentially can look at. How we can
19 look at the impact on the congenital infection post-licensure and as we have seen from the
20 congenital various symptom cases. So while it's complicated to do, it's not impossible.

21 DR. CASSETTI: Next question.

22 PUBLIC: My question is also for CMV with the low incidence issues that you
23 are facing in adult women. Would it be worth considering to conduct a trial in kindergarten-aged

1 children where you have arguably a high attack rate and then essentially try to extend the
2 findings into adults? So potentially assuming that you can protect children from infection, that
3 you would see similar protection in adults?

4 DR. WANG: Yes, so that's a great question as well. So as I mentioned in 2012
5 workshop there was broad discussion about a potential of having populations but for the purpose
6 of my talks on the adolescent girls and women. And obviously if you want to start with the
7 children because iteration would be different and there was a lot of, I'm sure there was a lot of
8 discussion in the community about potentially vaccinating children.

9 But one potential variable that is as I mentioned CMV infection itself doesn't
10 have much impact. So if you want to vaccinate children for a disease that doesn't manifest as a
11 symptomatic disease and that carries some challenges as well. Does that answer your question?

12 PUBLIC: I too have a CMV question, but maybe it goes to Kevin Russell. It
13 may make rational sense to use CMV infection as an end point in the CMV, predominantly CMV
14 sero-negative population, but what we know from CMV is that CMV sero-positive women also,
15 they're running this, particularly in low- and middle-income countries.

16 A lot of the population is CMV sero-positive. So what's the biomarker, right, for
17 a CMV vaccine in a CMV sero-positive population?

18 DR. WANG: Yes, that's definitely a tough question. I'll take a stab at this. If
19 there's anything that Dr. Kevin or so please feel free to come and chime in as well. So obviously
20 for CMV positive, right, and currently, well, my group talk was focused on sero-negative
21 because it comes with a complete different set of challenges as I'm sure you know well. And
22 one of the biggest challenges is end point, so what you have in the study and that's even more
23 challenging, in a sense. But I saw Dr. Russell already at the podium, you want to, yes.

1 DR. RUSSELL: No, I certainly agree. Kevin Russell, a colleague of Long Wang.
2 No, the end point that you might look at in sero-positive is really the challenge again is made up
3 of B virus what are you going to look at. It's going to depend a lot perhaps on what your vaccine
4 design is, what the construct of that vaccine is.

5 But also there's the huge challenge in the fact that those sero-positives to shed
6 periodically. That's why there is a burden in the children within the sero-positive population.
7 But the other challenge, and I think that the answer to this is likely post-marketing and how it
8 might impact in those countries that might have a little bit of uptake.

9 But the other challenge that is partly in answer to a previous question is with
10 cytomegalovirus there isn't universal testing, so how do you really have an understanding of
11 what the disease burden is in the children, is one of the challenges that we're struggling with.

12 Fortunately there's increased recognition of this need, just within the CMV
13 community. But true uptake of universal testing is still controversial in babies and how much we
14 can engage with that is questionable. But I think that would help both in sero-positive long term
15 efficacy in infants as well as in the sero-negative populations.

16 PUBLIC: I have a quick question for Ted. You showed in your transfer work
17 that when you transferred antibodies to normalize for neutralization that you saw a difference
18 when you normalized for neutralization of the trigger items you saw very similar. But when you
19 do the normalization you necessarily increase the amount of neutralizing antibodies and other
20 antibodies that can bind to the virus itself. So have you tried to focus more just on those specific
21 antibodies by pulling out antibodies that are not the ones you're interested in? To really just
22 focus on those that bind and mature on their own?

23 DR. PIERSON: Yes, so that's an excellent question. So, I mean, first we had to

1 demonstrate first that it was the mature neutralizing antibodies that were the one that would
2 confer equivalent levels of protection. And with that information in hand, our goals are to
3 identify what it is about that population, either that is present or as Carl perhaps alluded to, are
4 absent that makes that experiment work the way it does. With the long term goal of identifying a
5 way to measure protective responses that are not as complicated as the methodology I shared
6 with you today.

7 PUBLIC: And ten days ago or so we had a anabolizing TB workshop in this
8 room and I thought one really useful discussion was one around challenge dose in animal
9 models. And how challenge dose affected correlative protection of biomarkers but also the type
10 of immune response. So for instance in that chick model you said you had to go really high to
11 get joint pathology.

12 But is that the right approach, say, with Zika? Traditionally in animal models we
13 want a really high attack rate. So we go up in dose. But in the human often the inoculant is
14 small and you have the in vivo replication over several days or weeks. So just wondering how
15 you felt about dose versus detecting the biomarker.

16 DR. RAMSAUER: So the way we approached this in our model and the way we
17 studied it was to actually use the dose that is transmitted by mosquitos. That's why we started
18 with the small model. And we would get to very high areas. We get to the ten to the seven
19 within only two or three days after challenge.

20 And it's very likely that the whole mechanism then will affect the pathogenesis
21 and also the function of the neutralizing antibodies that we then applied to that model. So what
22 we really strongly believe that a lower challenge model that's a lower challenge dose that is
23 closer to the natural way of infection, by intradermal infection. Of course we can't do, I mean,

1 we could, but doing this whole complex setup it was key to the infection of the animals would be
2 I think too complex to interpret.

3 So going very low and showing that we have the good viremia is a very good
4 model we believe. The high challenge dose models can be used to generate additional data, I
5 believe. They can be used to generate data and like the more yes or no question, is the antibody
6 protective even against this type of the disease. And not as a tool to actually show a threshold of
7 protection. For this we should use a more natural way of infection. But the high challenge dose
8 would certainly help to find out more of the mechanism of how this antibody works.

9 PUBLIC: We have some questions online. I have, wait a second. The first
10 question is for Dr. Katrin Ramsauer. To follow up on the answered question, why not use
11 purified antigens instead of serum if you say that utilizing antibodies may be responsible for
12 protection?

13 DR. RAMSAUER: Yes, that's the other way to use it. We chose the more direct
14 and faster way to just use directly plasma. But, yes, purified antibodies is also a way and it's
15 also something we're considering for the confirmation. So we'll determine the threshold of
16 protection, confirmation of threshold of protection, and then we confirm with purified antibodies
17 from continued trial.

18 PUBLIC: Okay, another question for Dr. Ramsauer again. Do you see durable
19 antibody responses against chikungunya virus, could this also be a biomarker of vaccine
20 efficacy?

21 DR. RAMSAUER: Durability against infection or vaccine, so against infection
22 the antibody response is very durable. Its people are protected for the rest of their lives after
23 they've undergone infection. And this has been shown in cohort studies where people with very

1 low titers, neutralization titers were completely protected against symptomatic disease.

2 For the vaccine obviously it still has to be determined. For the vaccine durability,
3 antibody response of the vaccine we have data for six months and we have a very good durability
4 with the antibody response. And this has to of course be extended longer and is also something
5 we can address in the animal model to show to use long term follow up serum and show
6 protection by those types of antibodies and classes of antibodies.

7 PUBLIC: Another question for Dr. Wang for CMV. How can the need in CMV
8 sero-positives be met while this approach?

9 DR. WANG: Can you repeat the question? Can CMV positive be...

10 PUBLIC: How can the need in CMV sero-positives be met while this approach...

11 DR. WANG: So what do you mean by this approach? Do you mean the vaccine
12 that's being developed, right? So, well, currently so it's for the mock vaccine program that we
13 are developing currently for sero-negative population. And for reason that Dr. Russell
14 mentioned only for sero-positive the end point, so it's not, there's no wide consensus about what
15 is the most appropriate end point to study the sero-positive. Again as I said obviously the
16 community is really still looking at mechanism and also other immune mechanisms for
17 protection in sero-positive. With the advancing signs, it is something we keep a close eye on.

18 PUBLIC: Thank you.

19 DR. CASSETTI: Are there any other questions for the speakers? If not we are
20 going to adjourn for lunch and we are coming back here at 1:15.

21 (WHEREUPON, a brief break was taken from 12:13 p.m. to 1:17 p.m.)

22 DR. GRUBER: The first session, which was wonderful keeping time.

23 DR. BOK: Thank you everybody for coming back from lunch. And we're going

1 to start with another exciting area. It's a very exciting time for Filovirus right now. And we're
2 very interested in hearing this wonderful panel of speakers. So the session is entitled, first of all,
3 sorry, I didn't introduce myself. My name is Karin Bok. I work out of the Vaccine Research
4 Center, NIH. And I'm part of the advising committee.

5 I'll be moderating this session, which is entitled progress on the development of
6 biomarkers in animal models for hemorrhagic fever viruses. Our first speaker is also part of the
7 Vaccine Research Center.

8 She is going to talk, there's a little bit of change in the agenda. She's going to
9 talk about immune correlates of protection for recombinant adenovirus-based Ebola vaccines.
10 And she is a senior investigator in the Biodefense Research Section at VRC. Thank you, Nancy
11 Sullivan. I missed the name.

12 DR. SULLIVAN: Thank you, Karin. Okay, thank you very much. What I'd like
13 to do today is share some of the insights that we've gleaned over a couple of decades of working
14 on recombinant Ad-based Ebola vaccines and trying to understand mechanisms and correlates of
15 immunity.

16 So there are some key takeaways that I hope I can help you agree. One is that the
17 immune correlative of survival does not necessarily equal the mechanism of protection. I think
18 Jeff did a nice job this morning showing how the thinking has advanced on biomarkers and the
19 fact that they don't necessarily have to be mechanistic.

20 And then I think what we found now looking at many, many different vectors and
21 different regimens for giving vaccines is that correlate is, the significance of the correlate is
22 impacted by the variability of the endpoint and the ease and precision of measurement. And I'll
23 show you that when we're comparing antibody correlates to T-cell correlates.

1 This is the most important set of takeaways that I hope I can convey to you, and
2 that is that the correlate is context-dependent. So it will depend on the antigen composition in
3 the vaccine. It will depend on anti-vector immunity in the subject at the time of vaccination. It
4 will depend on the vaccine platform and also the regimen. So number of shots and then the
5 interval between vaccination and infectious exposure.

6 And then finally I'm actually going to start with the historical animal models of
7 Ebola pathogenesis and the fact that they really weren't designed for bringing vaccines into
8 advanced development in trying to predict responses in humans.

9 And so the first studies were really done by Sue Fisher-Hoch back in 1977 and
10 published also in 1995 where their aim was to infect monkeys and really have a bad outcome for
11 the monkeys. They were really looking at something that was highly lethal and also elicited
12 some of the hallmarks that we think about for Ebola virus infection.

13 The infectious route was intraperitoneal. That's not something that humans get
14 exposed to. And the dose of the exposure was ten to the fifth guinea pig lethal dose fifties. What
15 does that mean in terms of human exposure? We don't know. And in fact I'll argue just what
16 she did is that some caution in extrapolation of data from monkeys to humans should be used.
17 The dose of the virus is very high, the route of infection is unnatural, and clinical support is
18 lacking.

19 That was back in the '90s. I'll tell you in the ensuing decades that really hasn't
20 changed. So our animal model uses infectious doses of 1,000 PFU or 500 to 1,000 TCID₅₀ or
21 six PFU if it's intraperitoneal. We have no idea how that relates to LB₅₀, LD₉₉, or human
22 exposure doses.

23 So when we think about vaccine development, we're using those models and

1 we're trying to use the virus organization to decide what to target. And most of the vaccines
2 target the glycoprotein because that's the surface exposed viral protein. It's the only surface
3 exposed protein on the virion.

4 And then we use cynomolgus macaques to test these vaccines. So just going back
5 to this route and dose of exposure, the first study showing protection in macaques used a
6 challenge model where the route was intraperitoneal and the dose was six platforming units.

7 The timing was three months after the final shot. And this study was actually
8 highly criticized and the protection wasn't believed because they looked at that number six and
9 said, well, that's a really low dose exposure. So subsequent studies and then also advancing to
10 get to a single shot vaccine performed studies in an intramuscular challenge model where the
11 dose was 1,000 PFU.

12 The timing was one month after the single shot, also uniform protection. And this
13 protection was believed because that number 1,000 was thought to be high. I'll tell you that in
14 both of these models there was uniform mortality and controls and the monkeys died within six
15 to ten days.

16 So all of these measurements that we make for exposure dose and tissue culture
17 are somewhat artificial. You really need to know the LD50 or the LD99, which in monkeys we
18 don't really know.

19 So the widely used model for testing vaccines is the intramuscular 1,000 PFU
20 because it's a high dose and because everybody uses it. It really is historical. It has nothing to
21 do with estimating exposure doses in humans. It really is simply because that's the model that
22 killed the monkeys and showed the same disease.

23 And so bear in mind that that's about a hundred times the LD99, so we've done

1 challenges down below, 10 PFU and it kills all the monkeys. So it's a really high dose. The
2 disease is accelerated compared to humans. And then the NHP mortality rate also far exceeds
3 what we see in humans.

4 So we have to exercise caution when we think about using this model to bridge to
5 human immune responses after vaccination.

6 Okay, so in the course of doing all these studies we generated a lot of data and
7 were able to show with recombinant Ad-based vaccine that we saw a relationship between the
8 binding titer to Ebola GP. This wasn't neutralization, it was simply binding. And the outcome
9 in terms of fatalities or survivors. So the higher the titer the lower the mortality.

10 And so Dean FOLLMANN helped us put this into a more quantitative model
11 where he did logistic regression of the titers and predicted survival outcomes. And it was a
12 highly significant relationship and predicted a survival titer of 3,000 for eighty-five percent
13 survival.

14 So that was really helpful to us, but it was also about the time that we realized that
15 preexisting immunity to the vector itself in humans was quite high and could compromise the
16 potency of the vaccine. So we really needed to move to a nonhuman adenovirus and we did that.

17 It gave us an opportunity to ask does the titer predicted for Ad5 vaccine protection
18 hold when we move to a new vector? And in fact it's close, but it's different. And so when I
19 talk about the context of the antibody correlate, this is the first takeaway, and that is that the
20 vector can determine the correlate of survival and you can't extrapolate from one vector to
21 another. You can't say that because this is protective for this vaccine it will also be protective
22 for another vaccine.

23 That was shown also in a paper in 2018 where there were a number of groups

1 vaccinating with different combinations of different recombinant adenovirus vectors. And they
2 compared the titers to an Ad5 immunized group. And they showed that the antibody titers in all
3 of these groups, they were prime boost groups, were quite high.

4 However, when they challenged these groups, only the Ad5 group was protected
5 despite the fact that the antibodies were high. So it just tells you that that protective correlate
6 doesn't predict protection by other platforms.

7 Okay, we know why that is, these different vectors use different receptors.
8 Because they use different receptors they target different cells. And it turns out that the CAR
9 receptor, the Ad5 and Chimp Ad3 use is very widely distributed. Sorry, is very restricted in its
10 tropism compared to the receptor four, these vectors that's widely distributed. And that can
11 impact how a vaccine elicits immune responses.

12 So in addition to the vector being an important context, the composition of the
13 vaccine is also critical. So we've had the opportunity to test monovalent vaccines against just
14 Ebola Zaire. And then bivalent vaccines against Zaire and Sudan.

15 And when we compare the eighty-five percent survival titer here, we see a more
16 dramatic difference than we saw when we compared vectors. So even though it's the same
17 vector, simply the addition of another antigen changes that titer that predicts eighty-five percent
18 survival.

19 Okay, so when we think about that, we also think about this preexisting immunity
20 problem when you have populations that might have immunity against the vector that you're
21 using for vaccination. And we did a study in monkeys to ask the question, you know, can we get
22 protection in subjects that are pre-immune and what does that mean for the immune correlate?

23 And so what I'm showing you here is a naïve monkey vaccinated with Ad5 and

1 then four monkeys that were previously made immune to Ad5 and vaccinated with the Ad5
2 vector. And what you can see is that they all have really good antibody titers despite this
3 preexisting immunity to the vector.

4 However, when we looked at protection we lost protection in those animals that
5 were pre-immune against the vector. So their immunity against that vaccine actually
6 compromised the response. And we didn't see that in the antibody.

7 In fact, when we looked at CD8 T-cells, that's where we saw the big difference.
8 So that tells you that in pre-immune monkeys, you lose that antibody correlative protection that
9 was previously observed.

10 So this got us thinking a little bit more, and along with a lot of other studies that
11 suggested CD8 T-cells were important for protection by Ad vectors, got us thinking a little bit
12 about this notion of mechanistic versus non-mechanistic correlate.

13 And so one experiment we did was we generated vaccine serum from vaccinated
14 animals, purified the immunoglobulin, and then transferred that to naïve monkeys just before
15 challenge. So if the antibody is a mechanistic correlate, it should protect monkeys that are naïve
16 and not vaccinated.

17 And what we found was that most of those monkeys were not protected by the
18 immunoglobulin alone. It's difficult to transfer T-cells to see the effect of the T-cells, but we can
19 flip the experiment where we vaccinate the animals and then just before challenge deplete all
20 their CD8 T-cells.

21 And when we did that, we lost protection. So this told us that antibodies play a
22 role and correlate with but are not sufficient for vaccine protection. And CD8 T-cells are
23 required for protection. So this is that difference between mechanistic and non-mechanistic that

1 Stan Plotkin talked about years ago and we understand that better now I think in a number of
2 model systems.

3 So then it got us to ask can we identify a T-cell correlate? So if T-cells are so
4 important, can we actually measure immune correlates of protection in T-cells? So like
5 antibodies, T-cells are complicated. They're not all alike. And because of the work of Mario
6 Roederer and Rick Cowp [phonetic] and Bob Cedar [phonetic] and others outside of the VRC,
7 we've identified pretty powerful methods for looking at these different T-cell subsets. And we
8 distinguished them by their cytokine expression patterns.

9 And so if you measure interferon gamma by ELISPOT, it's not coming from all
10 cells that are identical. Some of them make interferon gamma with two cytokines, with one
11 cytokine, with, by themselves. And the important thing here is that each of these populations is
12 functionally distinct.

13 So this side tends to be more effector, these are very short-lived. This tends to be
14 more memory with these having good memory potential because of the IL2. We can represent
15 these proportionally by asking in a vaccinated subject what proportion of their cells make three
16 or two or one cytokine.

17 And we can even tease that apart even further. So if we antigenically stimulate
18 these PBMCs, we can ask about the total cytokines. But then we can ask how many express
19 three cytokines, how many express two cytokines, in which combinations and in which, which
20 are making just single cytokines.

21 And when we were testing an alternative to Ad5, another human vector Ad35,
22 that gave us the first opportunity to see a key difference in T-cell quality. So when we compared
23 the protective and nonprotective vaccines, the total CDA responses were basically the same.

1 When we teased this apart and looked at the T-cell quality, now we could see a
2 difference that the protective vaccine had this large population of double cytokine producing
3 cells in contrast with the nonprotective vaccine had single cytokine producing cells.

4 And it wasn't just any population. Those double cytokine producers made
5 interferon gamma and TNF, which is a key effector CD8 T-cell functional subset. Makes sense
6 for Ebola that you'd need effector cells very rapidly.

7 Now how do we measure an immune correlate in our Chimp Ad vaccine? It's a
8 little bit difficult, and this is where the precision of the measurement is important. So for
9 antibodies they increase gradually and they stay pretty constant. You don't have a lot of
10 fluctuation, so it's pretty easy to measure.

11 And so we're usually measuring them just prior to challenge and that's turned out
12 to be a very powerful as I showed you correlative protection. T-cells on the other hand are
13 dynamic. And even during this time that we want to picture them, they're all over the place,
14 they're fluctuating.

15 And when we add to that that the animals are outbred where each animal could be
16 in a different point of its T-cell response makes it very difficult to capture any T-cell qualities.
17 So we worked with Dean Follmann who helped us come up with a couple of different strategies
18 for increasing our power to detect a correlate if it is there.

19 So we screened for twenty-three T-cell parameters. So I won't go through these
20 in detail, but you can see there are lots of combinations. And we're measuring not only the
21 magnitude of each of those combinations but the proportion of those combinations.

22 Now nice that you can measure a lot of things, difficult when you have multiple
23 comparisons because your P value for significance now is a lot more difficult to reach. We had

1 two data sets, and again this is really Dean drove all of this analysis where we used one data set
2 and identified this double, triple, and double down here set of what we call tri-bifunctional T-
3 cells as being a significant correlative protection in a small study.

4 And then we went ahead and tested that in a larger set of animals and asked if that
5 correlate still held, and it did. The problem is because of those multiple comparisons, the
6 threshold changes. And while we don't reach statistical significance, I think it's really important
7 to see this and also consistent with what we had seen previously in a much, much smaller study
8 with this effector T-cell phenotype being important.

9 Okay, so the bottom line is how predictive is this? So if we use a rock analysis to
10 evaluate the sensitivity and specificity of that prediction, you can see, and one is a perfect
11 predictor. The antibody's pretty close. So the area under the curve is .88.

12 CD8 is not as good, but it's not nothing. What Dean asked was can we improve
13 the antibody predictor by adding in the CD8s, and in fact you really can't. So that antibody
14 correlate is so strong partly because of the ease of measurement that it really dominates any
15 immune correlate that we would see.

16 Okay. So very quickly just in the remaining time, for the Ebola vaccines we're
17 asking the vaccine to do a lot because we want it to protect quickly but we also want some long-
18 term immunity from this vaccine. And when you think about immune responses, you're really
19 looking at two different kinds of processes for rapid immunity and immune memory. And I
20 show here just by differences in the antibody and T-cell characteristics.

21 We had an opportunity to evaluate this difference between peak and memory
22 immune responses when we did a Chimp Ad3 or Chimp Ad68 MVA boost. So when we primed
23 boost with Chimp Ad3 MVA and challenge about a year later, we get uniform protection. When

1 Chimp Ad68 is the prime, we get no protection.

2 And that was interesting to us because when we looked at the antibody responses
3 this post-boost antibody response in Chimp Ad63 was about twenty-five times the protective
4 titer, what we would call the protective titer.

5 So this is either a difference in the vectors, remember context is important, or the
6 inability to predict long term protection from that acute correlate. It says that you need to
7 determine your correlate at the time that you're trying to predict protection.

8 When we looked ten months post-vaccine, that protective titer was still higher
9 than what we would predict to be protective at the acute time point. So really those correlates
10 determined in the acute model don't predict durable protection. You can't extrapolate from
11 those acute models into long term protection.

12 Okay. So then just quickly I'm looking at the T-cells. When we look at this
13 development from naïve to effector to memory, you can have a situation where you
14 overstimulate T-cells and they crash very quickly as I'm showing here with this particular
15 interferon gamma effector phenotype.

16 What we want is this effector memory phenotype that I showed you, but we need
17 some memory with that to keep that going for a year. And when we look at these responses in
18 the Chimp Ad3 and Chimp Ad63, when we look just at CD8 magnitude, Chimp Ad63 is actually
19 got reasonable responses just after the boost.

20 When we look at memory, it's quite low. And it's beyond that magnitude when
21 we look at the quality. Chimp Ad3 has this nice effector phenotype that's more than three after
22 the prime. What we see is a big difference is in the memory population where Chimp Ad63 has
23 those populations. They might be a little bit less dominant than Chimp Ad3. But the fact that

1 the magnitude is so low means that we're probably not reaching a threshold for that critical T-
2 cell quality.

3 So for Chimp Ad3 MVA we've got this nice memory and effector T-cell
4 response. For Chimp Ad63 it's a lower magnitude and also lower proportions of these cells that
5 are important.

6 Okay. So that just says inferior CD8 quality for priming can associate with a poor
7 survival outcome long term. And again it nullifies extrapolation of the antibody correlate across
8 platforms and across intervals of vaccine infectious challenge.

9 And so in summary the dose and route of the challenge model don't reflect natural
10 exposure. We don't know what the scale factor is. The fact that the VSV vaccine is being
11 widely used should help us answer some of those questions about scale factor, I hope with some
12 real-world data.

13 The main takeaway is that the antibody correlate is context-dependent and it's
14 empirically derived for every vaccine. All of these things can impact it. And for the Chimp Ad3
15 vaccine antibody titer was significant, but it's a non-mechanistic correlate and the T-cell
16 correlates were not helpful in improving the strength of the antibody predictor.

17 Thank you very much.

18 DR. BOK: Thank you, Dr. Sullivan. So next we're going to hear from Jenny
19 Hendricks. She's the head biomarkers for viral vaccines at Janssen Vaccines. And she's going
20 to tell us about candidate biomarkers to support clinical development of adenovirus 26 Zaire
21 Ebola vaccine and MBA BM5 vaccine.

22 DR. HENDRICKS: Thank you. And I have to say I thoroughly enjoyed Nancy's
23 talk just now. It seems that we are very nicely placed back to back. I hope you will find that my

1 talk is complementary to hers and not redundant. Of course we have a different vaccine and as
2 she said some of these effects might not be extrapolatable to other vaccine platforms.

3 I want to thank the organizers also for inviting us and providing us with the
4 opportunity to speak today. For me especially personally it's quite thrilling to be here. One of
5 the first experiments I did work at Purcell. Now he owns the vaccines. When I started there I
6 was actually nonhuman primates protection experiments.

7 So to stand here and I've taken leaps into the clinical development being much
8 closer to bringing these vaccines to the people who need them is very, very exciting.

9 So since a couple of years already, we are experiencing severe outbreaks of
10 Ebola. When the outbreak of 2014 happened, our company decided to significantly accelerate
11 the program that we had ongoing at that moment by jumping into clinical development phases,
12 about all the phases just around the same time.

13 Also scaling up manufacturing to a level where it would be launchable. And now
14 with the ongoing outbreak in DRC, this seems more relevant than ever. Why do we take an
15 immune-bridging approach, however? We know that these Ebola outbreaks, although there have
16 been two severe outbreaks in the last decades, they are not very predictable in terms of location
17 and size.

18 And if you are unlucky you just might hit the end of an outbreak, unlucky in the
19 sense of a vaccine development program, you might hit the end of an outbreak and you would
20 not have sufficient ability to extract proof that your vaccine is protecting.

21 So in this situation it would be possible to use license under the animal rule or
22 exceptional circumstances as they use in the EMA. And for this we use the methods that were
23 actually already used for development of an anthrax vaccine, which Dean FOLLMANN

1 discussed this morning.

2 When we started selecting and implementing these biomarkers for bridging, we
3 encountered several challenges. Of course you have to use a standardized and relevant animal
4 model that is a good model for the human situation. And this standardization helps you interpret
5 the results in a reliable fashion.

6 We have to identify those immunological biomarkers that we want to examine
7 and then we have to devise a statistical model to describe the immune-bridging approach.
8 Making sure that we can actually extrapolate from one species to another is very important.

9 We have to assume that both these species have similar responses to the vaccine
10 as well as those responses having similar associations with protection. And of course the animal
11 model has to have clinical relevance. So its clinical and disease course has to be relevant for the
12 human situation.

13 So we sought to bridge immune responses in people to survival after an Ebola
14 virus challenge in a nonhuman primate model. So we did that by establishing an immunological
15 parameter that correlates with survival after Ebola challenge in a fully lethal nonhuman primate
16 model.

17 We want to measure this same immuno-parameter in people after vaccination and
18 then infer a clinical benefit based on the comparison with protective responses in nonhuman
19 primates. This is the model that we have chosen. It's a Fang endorsed model that uses the
20 EBOV Kikwits 1995 B3 challenge virus which has similar clinical symptoms as the human
21 situation as well as disease kinetics.

22 What we do is use synose [phonetic] to do a heterologous prime boost
23 vaccination. We come first with our Ad26 ZEBOV vector followed four to eight weeks later by

1 the MVAB and Filo vector. And then after four weeks we challenge with 100 PFU
2 intramuscularly. One week before that we take blood for immunological measurements. And
3 then follow the monkeys up.

4 So Nancy has touched upon this as well. Just to compare the nonhuman primate
5 model with the human situation. So on top you see the nonhuman primate model which is the
6 100 PFU IM injection, so they have a time until they display symptoms of roughly five and a
7 half days.

8 And then after one and a half days you already have monkeys dying, so this is a
9 fully lethal model. And it is quite comparable with the human situation that have been described
10 for needle stick accidents. So also there you see medium time to onset of symptoms of six days
11 and then there it's a little bit longer until death sets in, but also there have not been any survivors
12 described.

13 In contact situation, human Ebola disease has a little bit longer kinetics, so there's
14 a spread of time to onset of symptoms and also a lot of, much wider spread of time to death.
15 And this might even be very relevant of course. Then you have time to seek assistance.

16 So this comparison shows that this nonhuman primate model was actually quite
17 suitable to use as an immune-bridging model, because it does have all these clinical parallels.
18 However, it is indeed on the more quicker and severer side, so possible clinical benefit would be
19 considered at least minimum outcome.

20 So this is our clinical regimen that is under development. We used Ad26 Cboff
21 [phonetic] followed by an MVABM Filo vaccination spaced two months apart. And when you
22 do this as the clinical dose, this is top dose, so five times ten to the ten virus particle for Ad26
23 and one times ten to the eighth infectious units for MVA. You find that your monkeys, all of

1 your monkey survive. Even when you go down in dose with Ad26, still we find a hundred
2 percent survival in the monkeys.

3 If we now use shorter intervals or much, much, much lower doses, we do see
4 lower protection rates. So when we started out identifying relevant immune markers that could
5 be associated with protection, we look for those immune responses that are associated with
6 protection from the Ebola. So antibodies and T-cells.

7 And we looked at those immune responses that we know are listed by our
8 platform. So for these challenge models, we focused on those binding antibodies measured by
9 GP ELISA and on neutralizing antibodies measured by a cytovirus VNA as well as specific T-
10 cells producing interferon gamma.

11 What we didn't consider were an Ebola outside VNA, detailed ICS analysis,
12 because we didn't have full data sets. So in the end we wouldn't have enough data to actually
13 devise a useful model out of it.

14 So as the 2014 outbreak was happening, we had to accelerate our program and
15 also think about how to bring this to a full development, this immune-bridging. So what we did
16 was take all the data that we had available at the time, and that data was actually from early
17 studies. It was made out of different components, different spacings, different sequences of all
18 different nonhuman primate studies.

19 The Ebola Cbof, actually 6 Cbof was actually part of a multi Filo vaccine, so that
20 we also incorporated in this data set just to have as much data as we could look at as possible.
21 And if you then start ranking it and look at which level of antibodies or T-cell responses were
22 actually correlating with survival, you can see that there is not a real easy limit to distract from
23 these graphs.

1 But it is quite evident that most of these immune parameters do have an
2 association with protection if they are at higher levels. So as said, we used the same statistical
3 approach as for the anthrax vaccine, so we built logistic regression curves. We look at the raw
4 curves for discriminatory capacity and we explored multivariate models, so really a nice parallel
5 with the preceding talk.

6 What they do is plot non-survivors at the bottom and survivors at the top and then
7 you get this relationship as drawn by the logistic regression curve, which is the orange line.
8 Then you can use your human data to actually plug into this function and derive your probability
9 of survival estimates out of it. So this is the anthrax vaccine example, by the way.

10 So when we now do that with our data, you can see that here we have the ELISA
11 data, so again the deceased monkeys at the bottom, the surviving monkeys at the top, and you get
12 this very nice relationship with binding antibodies.

13 We see actually a very similar curve with the neutralizing antibodies and the
14 ELISPOTs also does the trick although this curve is slightly less steep. And what you can also
15 see in this ELISPOT is that there's a, that there's even one monkey that has no ELISPOTs but
16 still survives.

17 So the classification of the ELISPOT for survival is not as good as some of the
18 other assays. If we examine the rock curves, we can see that the humoral [phonetic] assays in
19 general was a better discriminatory capacity than the T-cells.

20 And what we next explored was dual models. When we looked at dividing
21 antibodies and neutralizing antibodies to get a reaction we found that they significantly correlate
22 with each other, so therefore they are not useful to use in a multivariate analysis. So we chose to
23 proceed with the ELISA based on the robustness of the assay.

1 We added ELISPOTs to the model as well and we saw actually that the T-cells
2 only marginally add to the discriminatory capacity of the model based on the ELISA. And
3 therefore we thought that the ELISA model was necessary and sufficient to proceed.

4 So while the initial set was made out of these five studies that were made out of
5 these different regimens and different spacings, we did want to test whether there were any co-
6 variants that would influence the model. So we tested for those doses, those lots, the
7 directionality in the spaces and we saw that all of those co-variants have negligible influence on
8 the discriminatory capacity of the model.

9 We did want to have some more data in the lower end of the curve just to get a
10 better prediction of the entire logistic regression. So we did two dose down studies, really to
11 generate more data there. And that even improved the fit further using the ELISA in the model.

12 So when you use that to bridge from nonhuman primates to humans, you want to
13 also be sure that you eliminate as much variation as you can from sources that you can avoid. So
14 what we wanted to do was use the same assay for nonhuman primates as humans in the same lab.

15 So what we did was a parallel investigation to see if we would have sufficient
16 robustness out of the ELISA for the nonhuman primates similar to the humans. And what you
17 see here is what we did was take the ELISA titer curves and put the slopes in the upper isotope,
18 and you can see it both for nonhuman primates and humans, those slopes and isotopes, those
19 curves look very, very similar. So we concluded that there is sufficient parallelism to move
20 forward.

21 We then proceeded to validate according to the bioanalytical method validation
22 from the FDA both assays, human and nonhuman primate separately. And then remeasured all
23 our nonhuman primate samples in the validated assay in order to construct the final immune-

1 bridging model.

2 So what you then have is your final model and then you can proceed to using
3 human data and trying to derive clinical benefit from inserting the human immunogenicity into
4 the nonhuman primate logistic model. And this predicted survival probability is likely a
5 conservative estimate given the differences between a nonhuman primate and the human disease.

6 In addition to survival, we can also look at disease progression in monkeys. So
7 what you see here is two different measurements. One is after challenge time to death and the
8 other is the time of, the onset of symptoms to death.

9 And here we have used again the data of all different regimens including lower
10 doses. And you can see that we have a slightly delayed onset to death and a better survival time
11 after symptom onset in vaccinated monkeys. And this, there is a trend for correlation with the
12 ELISA titers.

13 In addition to that, we have measured, looked at viral load. We know that viral
14 load is highly associated with outcome, both in humans and nonhuman primates. Here's a plot
15 for nonhuman primates, so you have a much lower survival probability at higher viral loads, both
16 at peak and at day five/six that we measured.

17 And also here there's a trend that these viral loads are lower if you have higher
18 ELISA titers. In thirty-four of the vaccinated nonhuman primates we didn't have any detectable
19 serum viral load in these monkeys at any time point.

20 So taken together our clinical regimen under development can protect against
21 Ebola virus disease in a stringent nonhuman primate challenge model. We have shown that both
22 antibodies, neutralizing and binding antibodies as well as cellular mediated immuno-responses
23 correlate with outcome after challenging nonhuman primates.

1 And that the binding antibodies are a strong predictor and therefore useful for
2 outcome after challenge. The, there's also impact of vaccination on symptoms, disease
3 progression, and viral load. And the antibodies that we detect in humans suggest a high
4 likelihood of protective effect.

5 The predicted survival probability at peak is perhaps a conservative estimate
6 given the differences between the nonhuman primate disease and the human disease.

7 And last, I want to thank everybody. And I don't have the names here, so that's
8 always safer because then you cannot forget anybody. So in general organizations, the partners,
9 our funders, and the people that worked so hard on this. Thank you.

10 DR. BOK: Thank you, Dr. Hendricks. We'll have questions at the end of the
11 session. So our next speaker is Rong Xu, the director of, she's the director of immunology at
12 Profectus Biosciences and she's going to talk about the development of quadrivalent Filovirus,
13 Lassa vaccine, and considerations for use of biomarkers. Welcome.

14 DR. XU: Thank you, the committee invite me here. It is my great honor to be
15 here to present the data we generate from Profectus for this quadrivalent vaccine development.
16 This work was support by the NIAIA contract. So Profectus Biosciences is the vaccine clinical
17 stage vaccine company and it was released from Pfizer Vaccine Research to give out the vaccine
18 for the acute and chronic viral disease and their cancer.

19 We have multiple vaccine platforms including plasma DNA vector vaccines and
20 also vascular vector vaccines and prime boost system for the vaccine technology. And using the
21 DNA prime vascular boost technology we can generate very strong TCR responses and that can
22 be used in both our T-cell vaccines for the chronic viral disease and the cancer treatment.

23 And using the vascular vax prime and the vascular vax boost technology they can

1 generate very strong B cells and antibody responses, and that can be used for prophylactic
2 [phonetic] B cell vaccines for the infectious disease antibody defense indication.

3 So vascular, why we use vascular virus vectors vaccines. The vascular virus
4 vaccines is a single strand non-segmented virus and because it's very small genome and only
5 coding five protein genes. But it has the capacity to insert multiple foreign genes and also can
6 be modulation of the antigen expression by controlling the gene location and position.

7 We also can make the attenuated mutation synergistically by relocate the N gene
8 shuffling and also the G protein cytoplasmic tail [phonetic] truncation. Because it's replicates
9 competent vectors and also is tied to the microphage, the antigen presenting cells and that's making
10 the immunogenicity responses very good.

11 And plus attenuated mutation also can increase immunogenicity. So how about the
12 manufacturing? We had developed the facility that corrected effectively progressing in the
13 company-certified product cell line and we also developed the GMP manufacturing and
14 purification process in place.

15 So we're talking about a lot of viral vector vaccines has anti-vector immune
16 responses for the vascular virus vectors. And very little pre-existing immunity in the human
17 population. And we did a clinical trial already demonstrate that the effectively homologous
18 prime boost working very good produced antibody responses.

19 So while the most advanced developed the vascular vector is the VSV vectors.
20 And the wide spread VSV genome had these five genes. And on the sequence of the nuclear
21 protein and the first four proteins and the matrix protein and the glycoprotein and the large
22 proteins.

23 So the VSV backbone was used for the Merck's Ebola vaccines. And that

1 vaccines we have here. In that vaccines they had to replace of the VSV glycoprotein by the
2 Ebola GP. That vaccine had been used on the most recent outbreak on Phase II or Phase III trial.
3 And it's proved very good protecting efficacy.

4 However, it has numbers of adverse events happened for this virus, for this
5 vaccines. And especially has a low presentation of people has showed transit viremia on the
6 joint as arthritics. So in order to reduce these undesirable side effect and Weiss and Profectus
7 had been, sorry, had give up this new attenuated VSV vector called N4CT1. And was
8 developing in the Weiss and the carry on Profectus.

9 So in this vectors we had relocate on the GN gene in the fourth position and also
10 for the glycoprotein cytotail had been truncated. And also we put on the E-BOB GP on the first
11 position which enhance of the gene expression and also enhance immunogenicity of the vaccine.

12 So this N4CT1 vector backbone had been used in our HIV vaccine as well as
13 Ebola vaccine. We already complete the Phase I trial for both and is showing pretty good safety
14 and immunogenicity.

15 So this attenuate, double attenuate of this vaccine had been tested on the very
16 sensitive of the murine rubella studies and in this study we had testing various VSV vector
17 vaccines. And in the very young mice and follow the IC inoculation.

18 And here we can see the on the Y type of vaccines, Y type of the backbone. And
19 we see the LD50 has less than ten PFUs. And in the N4CT1 backbone and vaccine we can see
20 there's LD50 is over seven logs.

21 Okay. So Lassa is the, Lassa fever is transmission by rot and the virus was first
22 identified or discovered on the Lassa, the town of Lassa in Nigeria in 1969. It was majority
23 dominantly had outbreak happened on the West Africa and also you can see the map has these

1 outbreak of the location as well as serological evidence of the human infection is also located
2 around Africa area.

3 Ebola, Lars, and is also first identified on the river of the Ebola, which is located
4 on the Democratic Republic of Congo. And from 1976. From then there's multiple infection
5 and emerging happen on the West Africa. It's very similar epidemic area.

6 So both of disease had on the WHO blueprint of primary disease list and
7 overlapping of the region for this nature outbreak of Lassa fever and the Filovirus these lists and
8 all highlight the need for the prophylactic vaccine to develop this quadrivalent to prevent both
9 the Filovirus and the Lassa virus infection for Africa.

10 So that's why the Profectus has started to think about working these quadrivalent
11 vaccines. In our quadrivalent vaccine we have combined three Filo vaccines as well as one for
12 the Lassa vaccines. And using our VSV backbones and three Filovirus vaccine has using the
13 VSV N4CT1 backbone and the each, each vaccine has the Filo TP, Filovirus TP is located on the
14 position one.

15 So we have the E-BOB GP from the myangle [phonetic] strain and the Sudan GP
16 from the bonaface [phonetic] string and the Marburg from Angola strain. In the Lassa vaccines
17 we're using this VSV info 30t backbones and this Lassa vaccine, Lassa GPCs from lineage for
18 Josiah string.

19 So we have making these individual vaccine and we planned it as the trivalent
20 vaccines. This is the first our quadrivalent prove concept nonhuman prime studies. This is the
21 randomized partially blind and placebo controlled study and it was done on the UTMB.

22 And the keys for that. We have thirty-two Chinese macaque and divided into the
23 four arms according randomly by the sex and the age and the body weight. So in each arms we

1 are including five active group monkeys and three placebo. And the active group the monkeys
2 received the quadrivalent vaccine four time ten to seven which one time ten to seven for each
3 components.

4 And in the placebo group we gave them VSV, N4CT1, HIV-GAG vaccines. The
5 vaccine was given on the arm, injection on the date zero and fifty-six. And on day eighty-four
6 the monkeys received the challenge, about 100 PFU on the arm. And with all the heterologous
7 and the virus and except the Marburg.

8 And this virus is low passage virus and in the Lassa group you notice that we gave
9 this Lassa is from Nigeria has lineage two as genetic from the lineage four. Weakened for
10 twenty-eight hours, twenty-eight days for observation and in the end of study all the vaccine
11 group monkeys, a hundred percent survival. And all the control group vaccine was cured from
12 the disease.

13 So after immunization before the challenge, we also collected the sample serum
14 and also PBMCs for immune response assess. And this is data showing you that the
15 neutralization antibody response is, we see the significant antibody, neutralizing antibody was
16 detected after the single dose immunization. And for all of four antigens and this neutralizing
17 antibody titer was boost after second immunizations further for more than one logs.

18 And this neutralization assay was developed in the Profectus using the chimeric
19 virus which you, VSV 30t expression all the different antigens, GP on the first position. And
20 with a GFP as reporter gene. So the assay can be used for high throughput and there is a
21 Florence based assays.

22 This data showing you that we also did test the T-cell responses by interferon
23 gamma ELISPOT response using peptide, GP peptide pool to cover the Lassa or each Filovirus

1 GP. And we see here after single immunization detectable T-cell responses was can't see but is
2 on the less percentage of animals. But after second dose we see significant higher of the
3 antibody response for each of the antigens. And it's about seven to eight percent of animals has
4 response except Marburg has fifty percent.

5 I remember this assays also work on the frozen PBMCs. Sometimes people know
6 the frozen PBMCs is not that sensitive. So probably fresh PBMC is much better.

7 This is post challenge survival data. We can see it from the control group and in
8 the Filo or post-Filo challenge and on day six to nine the animals succumbed from disease. And
9 in the Lassa challenge group and the control group has died between day thirteen to fifteen post
10 challenge.

11 But all the animal from our quadrivalent group has all healthy and survive until
12 the end of the study. So this is the post challenge viremia data by plat assays. So you can see
13 here from all the control group animal and showing the very high viremia and peak on the, and
14 Lassa is peak on the day, about day ten. And the Filovirus peak up about day six.

15 Looking for the quadrivalents immunized group and post Lassa challenge there's
16 only one from five animals has shown transient viremia on the day ten. And all other group post
17 challenge Filovirus and all the quadrivalent vaccine the animals all do not show any viremia.

18 This is test by RTPCI tested genomic RNA for the challenge virus. And here you
19 can see post Lassa challenge we do see a high RTP, higher RNA from control. But we do see
20 three from five animals detected RNA and there's one, two of them has only transient RNA,
21 genomic RNA on the day ten. And one has a little bit longer. And you can detect until day
22 twenty-one it disappeared.

23 For the post, it's a year challenge and there are two from five vaccine group has

1 detectable RNA and the one of the animal has a little bit longer RNA level until day twenty-one.
2 There's no RNA detected from post Sudan challenge on the quadrivalent group. And in the
3 Marburg challenge in the vaccinated group and one from five have the transient RNA detected
4 on day six.

5 So we also see the vaccine, vaccinated group has a different, significant difference
6 of the clinical chemistry result. And here we see on the control group post challenge, you see
7 very high peak of the liver enzyme elevated on the ALT/NAST level. And all the control
8 animals post Lassa or Filo challenge. But you do see almost now a very low the enzyme
9 elevated on the quadrivalent group. And on the creatinine level you also only see the control
10 animals has a level peak. But in the quadrivalent animals you don't see anything.

11 This is the gross pathology summary and you see on the control group animals,
12 you do see the enlarged, the lymph nodes, spleen, and also damaged liver. But in the
13 quadrivalent vaccine animals there's no significant lesion was found. So suggests that the
14 vaccine protect of the organs damage as well.

15 So we have followed up of this vaccine develop and further to the preclinical
16 safety studies. And this showing you the murine and rubella studies which test for different
17 construct of the, with similar backbones and all Lassa GP vaccine.

18 So we see here from the N4CT1 within the HIV-GAG as insert gene and compare
19 the Y tag VSV and you see the PD50 and the LD50 is much, much higher required for the virus
20 injection. And the Lassa GP vaccine, we do see the same thing. So you need over seven logs of
21 the virus inject. This actually the maximum of the virus we can give them, so you need over
22 then that to reach to the PD50 or LD50.

23 We also tested the virus shedding on the immune biodistributed studies. In that

1 studies you, we do inoculation the young mice with a ten to seven PFU vaccines and then follow
2 the day zero to day ten to collect all the tissues and determine the virus shedding by the vaccine.

3 And we didn't see any of the virus shedding from the blood, brain, liver, lung, and
4 kidney and as well as the reproductive organs. And for the, for both the, and for actually here we
5 use the N4CT9-GAG1 as one of the examples. This virus, this vector actually has more
6 virulence than the N4CT1 because the truncation is a little bit longer. And even the cytoplasmic
7 tail a little longer. If you truncate it more then you get more attenuated.

8 Even with that one you can see it's on the, from day two, day zero to day ten you
9 don't see anything from spleen not over the LD. I only see the virus shedding residue on the
10 injection muscle of the lymph nodes. And on the Lassa N430G the same thing. But this virus
11 shedding residue was disappeared after day five.

12 We did also the rapid toxicology study to see the, how the safety and we do not
13 see by both for Filovirus we did the trivalent tox study and for Lassa we did monovalent tox
14 study. And we do not see any significant safety issues in the clinical sign. And we do see both
15 vaccine produced anti Filo GPO, anti-Lassa GP antibody responses, and there's no virus
16 shedding we found so far in the Lassa and the trivalent never see any to pinpoint.

17 And we do see transient increase on the CRP and the fibro concentration while
18 two days follow each dose injection but it's recurred after time.

19 So this is a five years of vaccine, quadrivalent vaccine development. So we are
20 on the track to deliver on the complete Phase I in year five. Now we are on the year three,
21 beginning of year three. We did a couple biomarkers and try to determine the biomarker
22 correlation protection on these vaccines because it's important we cannot do the Phase III trial in
23 the Lassa and the Filo. And we actually did a lot of study and different ones.

1 And we identified that the antibody response is pretty correlated. So some of the
2 biomarker also was subjected on the clean cut and both on Filo and Lassa. I'm not going into
3 detail, no time. So the summary is our quadrivalent vaccine has demonstrated efficacy in the
4 monkeys as well as safety in the animals model and immunogenicity proved also in the
5 preclinical studies. And the biomarker was considered for the vaccine immunogenicity with
6 antibody response or the safety and efficacy from clinical trial.

7 The first is working out the vaccine and work from Profectus and we have high
8 calibration with the UTMB for the monkey study. And we got funding from NIAID for support.

9 DR. BOK: Thank you.

10 DR. XU: You are welcome.

11

12 DR. BOK: Okay, we have the last presentation of this session. Professor Miles
13 Carroll from, he's the deputy director and the head of research and development Institute of
14 National Infectious Service of Public Health in England. And he's going to speak about
15 comparisons of naturally acquired immune response versus vaccine induced immune responses
16 to Ebola. Thank you, welcome.

17 DR. CARROLL: Thank you. So, yes, thank the organizers for the opportunity to
18 share this data with you. I had four fantastic years at NIH, so appreciate the funding, my post-
19 doctoral training, and I also appreciate the funding from the FDA which supports much of the
20 work I'm going to talk to you about today.

21 So I'm going to try, I'm going to concentrate on looking at the naturally acquired
22 immune response, which we think is probably something related to protective response. And
23 then see how that compares to vaccine induced immune response and maybe go back and have a

1 look at some of the early data in nonhuman primates.

2 But fortuitously that's been done for me by the earlier speakers. Our involvement
3 in Ebola virus research goes back to '76 and Nancy very nicely showed on the early studies on
4 the NHP model from Portland down. But more recently in the West Africa outbreak, we
5 provided a lot of the diagnostic support to WHO, MSF, and the Guinean government.

6 And we are actually, we're the reference laboratory for the phase, or for the ring
7 vaccination study with the VSB vaccine. So that gives an opportunity to work with the Guinean
8 government and do a lot of, a big follow-up study, a longitudinal study of immunology on
9 survivors and the permissions to transport a lot of the human samples back to the U.K. and
10 Europe for further analysis.

11 So perfect tutorial, Nancy, and I knew you were going to mention Portland down
12 so I made sure I mentioned you. So thank you very much. So the key of the adeno work would
13 appear that there is involvement and importance in both antibody and T-cell. I think that's the
14 conclusion there.

15 And a seven party functional phenotype that has been suggested from the early
16 work at NIH. We know that there seem to be a synergistic effect with their head to order prime
17 boost with addition of MVA with some pretty significant enhancement of both ELISPOT T-cell
18 response and ELISA antibody response.

19 But we haven't unfortunately had a talk from Merck on VSV today, so I'm going
20 to do my best to cover that vaccine as well. Just to say that in NHP studies and in human studies
21 that the T-cell responses are thought to be so strong, but we definitely see a T-cell response in
22 humans.

23 And looking at back at data by Andre Amazzi [phonetic] and Hinds Feldman's

1 [phonetic] lab at the Rocky Mountain lab in Montana, some great work done to highlight that the
2 immune response is CD4 dependent. And appears to be antibody mediated and it would appear
3 that CD8 responses are not so important on their own. But he doesn't say that they're not
4 involved.

5 But we're suggesting more an antibody mediated type response. So it's not
6 unusual that two different vaccine approaches will have two slightly different potential
7 mechanisms. And that may be the case with the data presented to us so far.

8 But we have to remember that the only vaccine that we have got a clinical
9 efficacy for was fortuitously that we're in Guinea at the tail end of the outbreak and worked with
10 WHO and MSF and the Guinean government to do the ring vaccination study. So it's important
11 to go back and look at humans that have been vaccinated with this vaccine as well.

12 And for many years people have accepted a naturally acquired immunity is
13 extremely powerful and I think the great example is smallpox. This is an outbreak in England in
14 Leicestershire in I think 1910 which illustrates that the boy on the right is the recently recovered.
15 And obviously because it's such a strong immune response they took the risk of putting next to
16 his roommate who's obviously highly infectious at that time.

17 So obviously there's a lot of dogma around that Ebola survivors are protected
18 against reinfection and there's some data showing that in outbreak settings a lot of survivors are
19 recruited back in to work in treatment centers. And many, many cases about PPE so it's already
20 been shown there, and some NHP shows that animals that have survived probably are likely
21 protected on reinfection.

22 Some interesting data from a Phase II study in Guinea during the West African
23 outbreak. They suggested that plasma from EVD, Ebola virus disease survivors did not treat

1 effectively those infected individuals. Not to say it wouldn't protect them, but I think there's
2 some discrepancy over the neutralizing titers in those survivors' plasma.

3 And we know the neutralizing antibodies appear to have therapeutic effect and
4 clear supporting data that antibodies are definitely important. When we started this study there
5 was relatively little known about the antibody and T-cell response in survivors and that's one of
6 the reasons that the European Union funded this work and subsequently funded by the FDA.

7 And again, we want to look at that polyfunctional T-cell phenotype that Nancy so
8 elegantly described to see if we can see that phenotype in survivors that we think are protected.

9 So working with the Guinean government and the survivor community, we set up
10 two cohorts, one in a place called Coyah just north of Conakry and one in Gueckedou, which
11 was the outbreak epicenter where we did the diagnostic testing. We did the diagnostics in both
12 Gueckedou and Coyah and that's why we had some good trusting relationships there and were
13 able to do these studies.

14 So took blood samples from survivors but were also really interested in people
15 that cared for their sick and dying relatives who were exposed to a lot of viremia and vomit and
16 diarrhea, et cetera, but never actually showed any classical signs of sickness.

17 So did they seroconvert and adding more support from the French group of Leroy
18 and Bays [phonetic] published in 2000 where they showed that there's definitely silent
19 infections.

20 So out of the two cohorts, about 120 survivors and about seventy contacts and ten
21 controls per year, but mounting up to about thirty controls in subsequent years. So ELISPOT
22 was done using overlapping peptide libraries the same as used in the vaccine studies.

23 And then T-cells and plasma shipped back to Europe where we did more in depth

1 analysis on a neutralization using live Ebola Mayinga and T-cell phenotyping and epitope
2 mapping. So the assays were aligned with those that they used in the vaccine immunology
3 studies in humans and also in nonhuman primates.

4 So this is N equals 1, but it's just a start. It illustrates the point really quite well.
5 And so this is the make a pool, make a pool one, make a pool two of the overlapping peptide
6 library. And we've got interferon gamma secreting units per ten to the six.

7 So this is a very good friend of ours, Sassa Bass [phonetic] who was very keen on
8 having his T-cell and antibody responses analyzed. And at a year after survival about 150 spot
9 forming units per ten to the six cells. And this is somebody who's had a primate boost, a very
10 unorthodox mixture of Ebola vaccines. And that's two weeks after his boost and then that's four
11 weeks after the boost. So you look at maybe memory, affected memory, at least a tenfold
12 difference in intensity.

13 But when you look at all of the hundred and twenty or so survivors, and this is a
14 live neutralization assay, we can see a pretty significant range in neutralizing antibody levels.
15 And very interesting is that you also see a subset of people with non-existing neutralizing
16 antibody levels. But you also, people that we know were infected because we have that PCI data
17 because we did it, we performed it on them when they were in the treatment center.

18 And then what's the evidence that these silent infections occur? It seems to be
19 pretty strong. We see relatively good but much weaker neutralizing antibody response in people
20 that were sub-symptomatic or the so-called silent infections really do seem to be there.

21 This is the person that had two unorthodox vaccinations coming about one in
22 thirty-two. But usually with a single vaccination you're getting a titer on average about one in
23 twenty.

1 And then looking at ELISPOT results we can see again a huge variation in
2 intensity and also looking at those sub-symptomatics, they also have a T-cell response as well.
3 Further evidence that this appears to be a real phenomenon.

4 We also are very interested in looking at the fingerprint of the neutralizing
5 antibody response. So using a used display system which presents fifty to a hundred amino acids
6 of different parts of the glycoprotein, we were testing high neutralizers, medium neutralizers, and
7 low neutralizers.

8 And there does seem to be a signature of different regions that the individuals
9 bind to. It's not just intensity, it's definitely where the immune response is directed to that
10 dictates neutralizing antibody response.

11 But if you look over the first year of, if you divide the survivors in a time post-
12 recovery and mumps here and a number in each group and then you look at their T-cell
13 ELISPOT result, their antibody by ELISA and by neutralization, there's no significant change
14 over that one-year period.

15 That then led us to look at subsequent years, and you can see that the neutralizing
16 antibody and the T-cell response stays pretty constant, at least three years where it can be four
17 years in some people after recovery.

18 And did some initial data analysis to look at the type of the effector memory
19 response because obviously in the literature the virus seems to reside in immune privileged sites
20 and could it be leaching back into the systemic environment and re-stimulating that T-cell
21 response.

22 So Tom Tipton [phonetic] agreed, looked at that phenotype, and it's a little bit
23 confusing but it looks like essential, sorry, a stem-like memory which means that it's seen the

1 antigen some time ago. But we also do see signs of an effector memory as well.

2 So next looking at the polyfunctional T-cell analysis that have been talked to
3 today about the NHPs for the adeno and the MVA prime boost work where they identified a
4 propensity of double positives or triple positives in after those vaccinations. And maybe a
5 suggestion that they're the ones that are important in vaccine protection.

6 So using peptides from the overlapping library of glycoprotein, looking at
7 survivors on average about two years after recovery, and then analyzing those responses. And
8 what we found is that the most significant correlation between survivors and controls is in this
9 double positive interferon gamma, T iO5 positive iO2 negative phenotype which again does
10 seem to have some correlation at least with the work done for the adeno and MVA in the
11 nonhuman primate studies.

12 Next want to look at what were the epitopes that were recognized by people that
13 have had natural infection and those that had received vaccination. So looking at about fifty
14 survivors, this was back in Coyah, done on fresh PBMCs, and then subdividing that, the different
15 pools of peptides.

16 We concentrated on this peptide pool 14 and found, were able to tease out a
17 number of different epitopes that appeared to be consistently seen by survivors. And as a recent
18 study by KTU and Adrian Hill's group, Jenner Institute, when they've done epitope mapping
19 from those people vaccinated with adeno MVA Ebola vaccine have also shown some similarities
20 in those epitopes.

21 So in summary, the Ebola virus survivors have extremely robust, long term T-cell
22 and antibody responses, on average about tenfold higher than you get in vaccination. But that's
23 not saying that vaccination isn't good enough. Obviously the VSV vaccine suggests it is good

1 enough. However, the titers and T-cell responses that induces, you get some similarities in the
2 polyfunctional T-cell phenotype.

3 You've got similarities in the epitopes that survivors see and vaccinees see. The
4 sub-symptomatics definitely seem to be there and has to be considered when you're vaccinating
5 a non-naïve population as well. And but really interesting to us is a number of these survivors
6 has no detectable neutralizing antibody or T-cell memory post six months survival.

7 Maybe there's another mechanism that we're not yet looking at that we need to
8 analyze. I've also done some IgG isotyping ratios compared to virus and vaccinees and both of
9 them are coming out similar with a lead of IgG1. And we're counting out cisco [phonetic]
10 analysis on all the data that we have to see if we can find correlations.

11 And we already have an initial analysis showing obviously correlation between
12 ELISA neutralizing antibody and CDA responses. And an area that hasn't really been looked out
13 so much is the variation in the Ebola virus sequence which obviously during the, when the virus
14 evolved during the outbreak it was quite different to the one at the beginning, and see how the
15 vaccine induced antibody response can cross protect against those variations in the glycoprotein.

16 And the most important slide, acknowledgements. Many, many thanks to the
17 European Mobile Lab led by Stephan Gunther by the Nocht Institute in Hamburg. A huge
18 support from the Guinea authorities, WHO, and MSF. And I would say a great group of people
19 that we worked with also volunteers who did a lot of the field studies and nothing can be done
20 without funding. And thank you to the European Union and the Food and Drug Administration.
21 And thank you for your attention.

22 DR. BOK: Thank you. I'll invite the speakers to come back to the table so we
23 can have questions from the audience. I would also ask if you can please identify yourself before

1 you ask your questions. Yes, thank you, go ahead.

2 PUBLIC: My name is Daniel LeRock (phonetic), Astra (phonetic). I'm just
3 wondering, it's really interesting, I think it's a question that could be related to all of you. But
4 the T-cell response with the CH response adeno, especially the adeno I would say primary versus
5 the natural infection, it seems that there's more maturation or that CD45 or RO and TM, TM T-
6 cells, effector memory T-cells, from the infection compared to the vaccination. Is that right?

7 DR. CARROLL: Well, we do have vaccinated PBMCs. We haven't done, had to
8 have anything come from the literature. But remember those studies on vaccinees is usually
9 done within so many months. These studies were done two years post survivor, so it might just
10 be when they actually saw the antigen as well.

11 PUBLIC: But do you think like, I don't know, a CD45 RO could be kind of a
12 biomarker, especially with this adeno based vectors approach?

13 DR. SULLIVAN: I think when you're comparing survivors and vaccinees it's
14 difficult because the vaccine response you're looking before exposure to the virus. And so
15 always the difficulty with doing the survivor studies is are you looking at the response that
16 protected them or are you looking at the response that is a result of massive virus replication and
17 T-cell expansion.

18 DR. CARROLL: You're talking about the NHP subject's vaccination challenge
19 was obviously the survivors. We're looking at humans that have never seen the vaccine.

20 DR. SULLIVAN: Right, and so my point is that when you look at survivors you
21 don't have what their T-cell response was at the time of infectious exposure. And so you've got
22 all of this virus replication that establishing the cause and effect can be difficult.

23 PUBLIC: Exactly. And if you look also off B-cells assessment such as

1 plasmablast memory B-cells assessment as well.

2 DR. CARROLL: So we are still doing those studies. We haven't done them all
3 yet, but that's something we are definitely very keen on completing.

4 DR. HENDRICKS: So maybe just to respond because we are not so fortunate
5 that we have a very detailed ICS on the monkeys, however we have some results on the humans.
6 We do see fully functional cytokine expressing responses, but we haven't looked into the
7 memory markers in so much detail.

8 DR. BOK: Anybody else? I have a question. So you mentioned, it was very
9 interesting that you're trying to study how the strain variation over time is going to affect your
10 correlate. And I was wondering if now that we have therapeutics that we know work if we can
11 do something like the RSV field where you measure competing or something competing to the
12 specific epitope that you know is constant and neutralizes, right?

13 DR. CARROLL: So the study we were doing is we've made some recombinant
14 glycoproteins that have at least six amino acid changes in the same glycoprotein. I remember
15 during the evolution of the virus in 2013 to 2016 picking up about two amino acid changes per
16 month. That was quite some structural changes.

17 We know that some of them then escape recognition by some of the monoclonal
18 antibodies that we use, but the work's not been completed yet. A polyclonal antibody response
19 hopefully will still be able to have a cross protection. But I think it would definitely affect the
20 neutralizing ability of a strain. A glycoprotein from 1995 and or 1996 compared to a virus that
21 we see today is a little bit different.

22 DR. BOK: Yes.

23 PUBLIC: So we heard a very compelling argument on the animal rule approach

1 by J and J and using 100 PFU challenge. And so my question is, is this a strategy and an
2 argument you're making or do you have prior approval of prior agreement with regulatory
3 groups that this is an acceptable way of doing the animal rule? So the question is does the FDA
4 or EMA agree or ever have agreed that 100 PFU challenge is the right way to do the animal rule
5 analysis?

6 DR. HENDRICKS: I can only comment of course on, I can only comment of
7 course on what we have discussed, so I don't know what the position is regarding the use of the
8 entire model for other people as well. But our situation was a little bit just the situation that it
9 was the model that we were using. So that was the data that we had and what we were further
10 building on. That is a reasonably well-established standardized model, so that was the data that
11 we had and that is the data that we are submitting. I guess you have to ask other people.

12 DR. CARROLL: Did you want to comment?

13 DR. SULLIVAN: Yes, I think, you know, it's been a topic that's been discussed
14 for many, many years. There was an interagency group that put together a consensus view that
15 1,000 PFUs should be the challenge. Unfortunately, it's not like the Supreme Court where you
16 get to write dissenting opinions. So while it might have been a consensus view it wasn't a
17 unanimous view.

18 So I think, you know, probably being open, hopefully the regulatory agencies are
19 going to be open to using a model that perhaps is closer to the exposure that humans get.

20 PUBLIC: And why not go down to ten, which is the LD99?

21 DR. HENDRICKS: So we have a dose down study done and you can go much
22 lower still with a hundred percent lethality. In the end it's difficult, and I think Nancy also
23 commented that six is a low number, but it doesn't mean it doesn't work in experimental setting

1 and it doesn't give you a hundred percent lethality, so.

2 DR. SULLIVAN: Yes, so but that six was IP, and so there's a big difference
3 between IP and IM, but your point is well taken, Barney. And so it could go much lower. And
4 in fact when you're trying to bridge to human immune responses, if you have a very high
5 challenge dose the amount of vaccine you need for protection is by definition higher. And so
6 that titer is going to be much higher. And so if you really want to bridge to humans, you have to
7 bridge not only the vaccine response but try to get closer to the challenge dose.

8 DR. CARROLL: I'll just say that many of the NHP modelers that I speak to in
9 the Ebola field think that 1,000 PFU is too high. The CFI in humans is around fifty percent and
10 NHPs is a hundred percent, and that's not so natural. And especially the group of Harvey
11 Rowell [phonetic] and the P4 lab in Leon [phonetic]. They titrate down to sub-ten and still get a
12 good hit rate.

13 PUBLIC: I have a very quick question to the challenge dose. How will other, is
14 it comparable from lab to lab? So it's 100 PFU your lab, 100 PFU in the other lab? We've been
15 through some challenges with comparing biological assays for different viruses from lab to lab.
16 How do those, how are those challenge doses identified?

17 DR. XU: We were discussing this question for a while in the group and, yes, I
18 think trying to use a similar you know result change for that. I think for the challenge dose and I
19 have another that is, you know, right now we try using high doses because it's easier for animal
20 studies to try to use less number of animals. So reach to the conclusion. And if you try to
21 change the clinical dose, infection dose, we might be ending with a huge amount of animals
22 because we couldn't be using 100% lethal dose for the infection. So, you know, it may be using
23 a big number.

1 DR. SULLIVAN: Just getting to the lab to lab comparison. So everyone uses a
2 different cell line with different fetal bovine serum and different assays to measure what their
3 titer is. It's not an LD50, it's not an LD99. So it's just not comparable. You can say it is, but
4 it's not.

5 Does it have to be? I don't know. I think you just look at the science and if you
6 do a study with a vaccine and all the control animals die and all the vaccinated animals live, I
7 think really you have to look at the totality of the data to make a decision. I don't know that it's
8 necessary to have every lab doing exactly the same thing.

9 PUBLIC: I have just sort of one comment on a few of these things. So I'm Bill
10 Down [phonetic], I've been involved with the interagency group, the FANG that's been involved
11 in a lot of these things. And just, we were trying to put together as Nancy is well aware of a
12 study on, we were trying to put together a consensus on the doses to use and that was difficult to
13 come upon.

14 But I think in data with different studies that people have done looking at different
15 doses, there wasn't very much difference between 100 and 1,000, I don't think. And it was
16 asked why not go down to ten or one. And some of that had to do with the ability to quantitate
17 and the variability of the plaque assay.

18 So that's why I think it was felt that 100, if you were off by an order of magnitude
19 goes down to ten, you know, you could measure that pretty easily. But if you were at ten you
20 could get down to the border of where you could measure if there was any variability in the
21 assay.

22 So that was why people were sticking with like 100 in some of the studies we did.
23 In terms of the assay, I would just say too that one of the things that the group did was to, and

1 Amy was right here was very involved in that, and standardizing the plaque assay and trying to
2 come up with everyone using the same cell line and the same conditions as best as possible.

3 But I will say there's a little bit of art in it. And some, you know for example
4 some of the labs that we were transferring they had the SOP on running that assay, they had the
5 same cells but they still were having difficulty getting the exact same results. So there definitely
6 is some, even though we've tried to standardize as best as possible, it's tricky, the plaque assay
7 that's been used by everyone. So I have to agree with those comments.

8 PUBLIC: Can you guys talk about route of administration for the mucosal
9 challenge and pluses and minus of that, too, and the ways that you've been talking about the
10 dose.

11 PUBLIC: Can you repeat the question? Sorry.

12 DR. SULLIVAN: So the question was can we talk about route of challenge. And
13 you asked about mucosal. So right now there's no standardized mucosal model, and that's
14 probably the primary exposure as humans. And I know people are working on that, but we don't
15 know anything about that yet or a lot.

16 Just thinking about the two different challenge models that are widely used, and
17 that's intraperitoneal and intramuscular, you really can't compare challenge doses because the
18 target cells intraperitoneal are much different than the target cells intramuscular.

19 And so it's just a very different infection and you could imagine that six PFU IP is
20 a much higher challenge dose than 1,000 PFU IM. It really just depends on the viral dynamics.
21 Anybody else want to take that?

22 PUBLIC: Jerry Johnson (phonetic). There was a lot of good comments just said
23 about the practical aspects of doing lower doses. But the disadvantage of the model we have

1 now at the higher dose, even ten or 100 is that it's good for acute but it doesn't, because the
2 animals die so fast.

3 It is not reflective of human disease and doesn't give the immune system a good
4 chance to have an amnestic response to the virus. So it's very difficult in this model to test
5 amnestic responses with the high challenge. If you could do a thousand monkeys and challenge
6 them with a tenth of a dose, which would be what is normal, then you might give the immune
7 system a chance to demonstrate amnestic response.

8 So the very nature of the model and its practical aspects limits the ability to
9 measure a very important component of the vaccine.

10 DR. BOK: Thank you. I've got a question for Dr. Xu. You were the first one to
11 address biomarkers for safety and I was wondering if you could elaborate on how you're
12 validating those from limited HP studies. So what's your process?

13 DR. XU: I just think about that, you know, if you are looking for a file so we
14 should really let Ecolab look at those markers if the lab has already started on that. We want,
15 you know, in country where so any marker lead to the outcome, clinical outcome. So I think
16 that's very useful information should consider. I think animal study might be to monitor it, and
17 this is and after challenge you could use survival of monkeys and see how that can with people
18 dying off. I mean, we're looking for the clinical pathology and we can just add them, but we
19 have to go to the similar clinical biomarker outcome.

20 PUBLIC: Okay there are questions online. Before I ask online participants to
21 please post the questions under the Q and A section of the connect. And I have one question for
22 Dr. Carroll. Have you ever seen clinically confirmed cases of PVD with low or no antibody
23 response or all of them are automatically high titers?

1 DR. CARROLL: So what we saw when we looked at 120 of them, we did see a
2 small subset that six months after recovery on average we couldn't detect an antibody response.
3 But we have the documentation to know that they were physically infected in the first place. So
4 the answer is yes.

5 PUBLIC: Thank you. Is there any other question online? Please post it under the
6 Q and A section.

7 DR. BOK: Okay, so we have a little bit more time if anybody wants to ask any
8 last minute questions. Nancy, you addressed really well that we were not able to generalize
9 correlates, not even sometimes to the same platform if we have different antigens expressed,
10 being expressed. So how do you interpret when studies compare immune responses of different
11 vaccines using the field as if one was better than the other when they are totally different
12 platforms, totally different Ebola vaccines? How do you address those conclusions from those
13 studies?

14 DR. SULLIVAN: I'm not sure which studies. I'm going to try to guess what
15 you're getting at here. But I think what you're saying is if someone reports on an antibody titer
16 for one vaccine platform and says, well, we think this is protective because it is for this other
17 platform, I think it needs to be empirically determined for every platform. And really at the two
18 primary intervals that we're thinking about.

19 So one interval is, you know a month after vaccination for rapid protection and
20 the other one is a year after vaccination. So you'd want to do studies in monkeys showing what
21 the protection is and what titer correlates with protection at that acute time point and then do
22 another study where you look at it for the durable time point.

23 And it may be that you don't get a correlate when you measure acute antibodies

1 for durable protection. Maybe you do get a correlate when you measure those antibodies late,
2 you know, at the time of challenge. I think we haven't done that yet, but it needs to be done for
3 each platform.

4 DR. BOK: Perfect, exactly what I asked, thank you. Okay, so unless we have
5 online or in person questions, we'll thank the speakers. It was a fantastic session and I think it
6 was very useful for all of us. Thank you and we're going to break. Yes, thanks.

7 (WHEREUPON, a brief break was taken from 2:55 p.m. to 3:17 p.m.)

8 DR. YESKEY: Okay, to stay on time I think we're going to go ahead and get
9 started on session three, updates on selected topics. My name again is Deb Yeskey. I'm from
10 C.E.P.I. We will start with our first speaker, hopefully he's here.

11 DR. MASCOLA: I'm here.

12 DR. YESKEY: Oh, you're here. Yes, I'm sorry. Dr. Mascola is going to talk
13 about the prospects of identifying correlates of protection in clinical studies of HIV monoclonal
14 antibody candidates.

15 DR. MASCOLA: Good afternoon, everybody. I'm not going to reread the title.
16 But as you can tell we're going to talk about HIV. And it's a real treat for me to be the only talk
17 on HIV at a meeting, it's pretty rare for me, so I'm happy about that.

18 So I'm going to break the talk down into three questions, all related to passive
19 transfer of HIV antibodies. So the first is can we expect monoclonal antibodies to protect
20 people? The second is what in vitro assays would predict that? And then third I'm going to tell
21 you about some ongoing efficacy studies and talk about what we can learn from correlates of
22 protection in those studies.

23 So I have to start with just a little bit of background even though many of you

1 know HIV. So there's only one virally encoded protein on the surface, so we think that that's
2 really the sole target for antibodies with regard to protection.

3 The envelope is a heavily glycosylated trimer, so it's a particularly difficult target
4 for antibodies. And then I borrowed a slide from Dennis Burden [phonetic] because he's drawn
5 the slide to scale so you can see the envelope here. I think everybody knows the primary and
6 secondary receptor.

7 But it's interesting when you draw it to scale to look at the relative size of IgG
8 antibodies with respect to the envelope. And the point of this is that there's been about twenty
9 years of pretty elegant structure function studies in HIV and they pretty uniformly show that if an
10 antibody can bind in high affinity to the trimer, you interrupt the entry process.

11 So at least for HIV, binding to the trimer, the surface trimer, is highly associated
12 with virus neutralization. And that's useful to keep in mind as we talk about measurements.

13 So in part as a consequence of the fact that it's so heavily glycosylated and a
14 difficult target, one of the things that we know in HIV is that broadly reactive neutralizing
15 antibodies come up pretty late. So you get binding antibodies quickly, you get some strain
16 specific neutralizing antibodies in months, but you don't get broadly reactive neutralizing
17 antibodies actually for a couple of years.

18 So we understand why that is pretty well. But for the purposes of this talk I think
19 the important point is people have found these donors that make broadly reactive antibodies and
20 isolated. So since about 2009 there are hundreds of broadly reactive neutralizing antibodies to
21 HIV.

22 And we know a lot about them. We know structurally exactly where they bind.
23 So now we're just looking at the FAB of the antibody. And so we define very specific regions of

1 what we'd call relatively conserved epitopes. So the apex of the virus has a glycan site here.
2 This is the CD4 binding site where the virus attaches to the cell. And this is the site where you
3 interrupt the fusion mechanism of the envelope protein.

4 So we know in a lot of detail about the antibodies. So that's all just background.
5 And then I guess finally to say because HIV is so diverse, literally, you know, thousands and tens
6 of thousands of strains worldwide, we represent these antibodies on a plot like this which is
7 potency on the X axis. So this direction is more potent.

8 And coverage, coverage meaning how many if we, and we do this, we make these
9 large panels of HIV strains, two hundred strains from different parts of the world, different
10 groups. And we ask how many, what percent of the viral panel is neutralized.

11 So you can see that you don't get a hundred percent because HIV is antigenically
12 so diverse. And some antibodies which get ninety-five or more percent of the viruses are not as
13 potent as some antibodies which miss some viruses but are highly potent when they do
14 neutralize.

15 And at the very end I'll just talk about how combining antibodies makes sense,
16 but it's kind of evident from a plot like this. So we have all these antibodies and many of them
17 are in the clinic, and I'll talk about that in a moment.

18 So can we expect them to protect and then what assays would we use to predict
19 that? So this is just a slide to say we've been studying passive transfer of antibodies since about
20 1990, so almost thirty years. And uniformly investigators can show in animal models and
21 monkey models that you can completely block infection, completely prevent infection with
22 certain antibodies.

23 So I won't go over the slide, but what we don't know is if this would happen in

1 humans. And that's really a critical gap in thinking about correlative immunity for HIV. But
2 with regard to the monkeys, given that there's so much data, a group of investigators got together
3 and this paper just came out actually this week online.

4 And the title is A Meta Analysis of Passive Immunization Studies Shows that
5 Serum Neutralizing Antibody Titer Associates With Protection Against Shift Challenge. So it's
6 pretty self-evident, but I'll show you the data from the meta analysis. So to the bottom line from
7 the summary is that the formal analysis indicates serum neutralization titer against the relevant
8 virus, the challenge virus, is a key parameter.

9 So how is this done? I think people know the SHIV model where it's the
10 envelope of HIV on SIV, so it replicates in monkeys. And the sort of simplest way it's done is to
11 passively infuse any given antibody and then one or two or five days later and now people even
12 do it out further than that, is simply challenge one time. And the controls would all get infected,
13 so people call this a high inoculant challenge, so a little bit analogous to the Ebola world.

14 There's some debate about whether one could use a high inoculant challenge or
15 other investigators have used a lower inoculant challenge where actually exposed the animals
16 weekly until over about four to eight challenges the controls get infected and you can look for
17 impact there.

18 But the meta analysis looked at a whole large body of data with this model to try
19 to, with this challenge model, to try to get a sense of what level of antibody is needed to protect.
20 So I won't go over it in a lot of detail. There's several different groups, my group included.
21 Scripps group, Harvard group, Al Martin at NIH, and Nancy Haguewood [phonetic] at Oregon.

22 Thirteen published studies, five unpublished studies, and a total of about 270
23 animals were included in this meta analysis. And again, it's all of this higher dose model, so we

1 tried to do a meta analysis of studies that were all done in a similar way.

2 And the bottom line to that and you've seen, I don't know where these slides
3 came from on the graph, but they shouldn't mean very much. But this is what you want to look
4 at. And you've seen these type of logistic models now several times. And if I quote from the
5 abstract in the logistic model that adjusts for antibody epitopes and challenge viruses, serum Id50
6 titer was highly, had a highly significant effect on infection risk.

7 So a couple of things here. Irrespective of the epitope or the antibody or the
8 challenge virus, we saw this relationship. And by highlighted in bold, you can see that fifty
9 percent protection was achieved when the serum neutralization titer at the time of challenge was
10 about one to ninety.

11 And the confidence interval was relatively tight, seventy-five protection as you
12 can see here. So it gives us an idea of what serum neutralization titer might be needed for human
13 studies. And this has been useful in the design and thinking of human studies until we actually
14 have human data.

15 But there's one more just really important background point. So I told you that
16 HIV is very diverse. And that impacts how we think of that SHIV model. So this is one
17 antibody, an antibody that I'll tell you about in a minute that's in a large clinical trial. And this is
18 viral sensitivity if we look at two hundred or so viruses.

19 So you can see some viruses are resistant, some are highly sensitive, and some are
20 less sensitive to any given antibody. And you see that pattern with different antibodies. So if I
21 model on this challenge SHIVs and these are actual SHIVs that are used, you could pick a SHIV
22 that's highly sensitive to this antibody and get protection relatively easier.

23 But if you use this SHIV you need a lot more antibody. And what we noticed in

1 looking at it before we did the meta analysis, so virologists eyeballing data rather than working
2 formally with statisticians to get an answer, was that protection was generally seen when the
3 serum concentration was well above the IC50 of the virus.

4 So in this case if the IC50 is .1, if we were at 100, or actually just 10 micrograms
5 per mil, you get protection. But if the serum level of the antibody was 10 micrograms per mil
6 here, you wouldn't necessarily see protection. So pretty obvious.

7 But what this means is a hundred times the IC50 is the same thing as saying a
8 serum neutralization titer of one to 100. So we sort of had this sense before we did the meta
9 analysis that we could convert antibody levels to neutralization. And because the virus is so
10 diverse antibody level is not so useful because the antibody level only really relates to the
11 sensitivity of the virus that one is challenged with, which varies quite a bit.

12 So it's, I think it's more useful and more uniform to talk about the serum
13 neutralization titer. So you may be asking what about the role of other Fc-mediate effector
14 functions. And indeed people have knocked out Fc-mediate effector functions and done these
15 studies and you do lower the impact of the protection, at least in some studies.

16 But it's pretty small, twenty or thirty percent. And then other, and these are
17 mostly done by Dennis Burden and Ann Hessel [phonetic]. Other studies where they've done
18 this, they don't knock out protection at all. But that's usually when the antibody is very potent
19 on its own.

20 So there is some role of ADCC but it's subdominant. If you passively transfer
21 just a non-neutralizing antibody into the macaque model, you don't see protection. But what I
22 think is fascinating and Nancy Hague [phonetic] has done this, if this is mostly in the neonatal
23 macaque model, if she challenges first, infects first, and even up to forty-eight hours later gives

1 antibody, she can actually eradicate infection.

2 So Fc-mediate effector functions clearly play a role, it's just that if we're talking
3 about classic immuno-prophylaxis, classic prevention, virus neutralization seems to be dominant
4 and a major factor influencing protection.

5 So the main thing I wanted to go over was a Phase II B study that's ongoing
6 because it should teach us something about protection. So this is not abstract. These are all
7 antibodies that have been made GMP and are in clinical trials. So a number of antibodies to the
8 CD4 binding site. This is the V3 glycan site, the apex of the virus.

9 There's actually a tri-specific antibody that has three different FABs on it that's in
10 Phase I, a bi-specific antibody that has an FAB and an anti-CD4, and antibodies here. So there's
11 a, these are all in the clinic so you will be hearing about and the FDA and the EMA will be
12 hearing about it. I guess they know they're hearing about these studies.

13 So the antibody that's most advanced is one actually that was just isolated early.
14 It does come from our center, the Vaccine Research Center. It's to the CD4 binding site. If you
15 plot a Filo genetic tree of viral strains, you see a lot of red which means it, the antibody
16 neutralizes irrespective of clade, and that's mainly because the site is pretty conserved.

17 It's moderately potent, although not as potent as some of the newer antibodies.
18 So a decision was made after a lot of discussion with a lot of people to move this antibody into a
19 formal Phase II B efficacy study, a non-licensure Phase II B efficacy study.

20 The study design was based in part on what we learned from Phase I studies. So
21 antibodies have a pretty commonly known half life. F1 intravenously infuses at twenty mgs per
22 kg. You get something that looks like this with regard to concentration. The antibody decays, in
23 this case with a half life of about two weeks.

1 And so we had to decide before we had the meta analysis how much antibody to
2 use in an efficacy human study. So we chose an interval of about two months because after two
3 months the levels of antibody were below ten and it seemed like that was probably below what
4 would be our guess of a protected threshold.

5 So the study that is ongoing is called the antibody, it's actually two harmonized to
6 very similar parallel studies, the antibody mediated prevention studies which are Phase II B,
7 proof of concept. And basically asking the question in a placebo-controlled manner, can a
8 passively infused antibody protect humans.

9 It's being conducted by two NIAID sponsor networks, HVTN and PTN, which
10 stands for vaccine and prevention networks. And many, many people involved. So I'm really
11 presenting for them because they have designed and conducted this study.

12 So the design that was decided upon after a lot of discussion was two different
13 doses, a relatively higher dose and a lower dose versus placebo once every eight weeks as I just
14 told you. Two harmonized studies, so one is done in high risk men in the Americas, North and
15 South America, and one is done in high risk women in southern and east African countries.

16 Forty-six hundred people enrolled, open for enrollment in 2016, and now fully
17 enrolled and in follow-up. So there actually should be an answer forthcoming within the year.
18 The study is very well conducted, the adherence amazingly is high. Because in this particular
19 case the antibody is actually given intravenously in a clinic and they worked very hard to get it
20 done quickly, but you see all the sites.

21 So this study should yield an answer and what might we learn. So I just wanted to
22 briefly review why the study designers decided on two doses. So here's the higher dose of thirty
23 mgs per kg. You can see what you'd expect. This is just a range of concentrations over eight

1 weeks.

2 Here's the lower dose. And so all told you see that there's a range of antibody
3 concentrations over the eight weeks. Now somebody can get infected at any point along this
4 eight weeks. So the study was designed specifically and powered specifically to detect an effect
5 on acquisition, but also to be able to associate antibody serum level with protection.

6 So participants are followed monthly and then we try to, they try to impute the
7 time of infection by the follow-up period by the serology and also by using molecular evolution
8 by sequencing the virus at the time of infection. And using a molecular clock.

9 So they'll probably be able to tell a time of infection within a window of a couple
10 of weeks. And then there's what's called formal siving [phonetic] analysis. So everything,
11 every single virus that is infected, every breakthrough virus, is isolated. Its envelope is cloned
12 and it's tested for neutralization.

13 So the study can formally ask if there's a dominant type of virus that's infecting,
14 for example, does the antibody exclude acquisition of the more sensitive strains versus the more
15 resistant strains.

16 So I borrowed a slide from Yunda [phonetic] Wong who's one of the statisticians
17 involved in the study at the University of Washington, and she made me promise to say three
18 times that this was completely a hypothetical scenario just as a teaching point for those of us that
19 aren't statisticians.

20 So one could see a prevention efficacy in theory overall in the study where the
21 study has two doses, antibodies decaying over time, of say forty percent. But if one compares to
22 the placebo group, it's possible you could find a very low PE if the viruses are resistant and a
23 high vaccine efficacy or high antibody efficacy for sensitive viruses. So that's a potential

1 outcome.

2 So one can see that we are poised to learn a tremendous amount about what the
3 role of viral resistance is and the level of antibody or the level of virus sensitivity as it relates to
4 the level of antibody.

5 So what are the implications of this study? First of all, it could be, if the antibody
6 works, proof of concept that antibodies can prevent HIV infection in humans the way we've seen
7 in monkeys for many years. And as I said, provide evidence on the level of serum neutralization.

8 So we can convert the level of antibody just as I explained to the level of serum
9 neutralization that might be needed to protect with some hope that serum neutralization can
10 convert or translate across antibodies the way it does in monkeys.

11 There are some limitations to this study. It's just one antibody to one epitope, so
12 we'll learn what we learn with regard to that antibody and we'll hope that it translates to some
13 extent to other antibodies.

14 Because of the populations at risk where it was possible to do the study, one study
15 is done in men where the clade is mostly clade B. The other study which has the same design is
16 done mainly, completely in women in a different continent where the clade of the virus is CNA.

17 So with regard to understanding if there are differences, gender differences, clade
18 differences, we may not be able to sort those out in this particular study.

19 So just to end, these are antibodies that are all in Phase I and we represent their
20 neutralization potency by these scatter plots where more potent is down. So the news is pretty
21 good in the sense that this is the antibody I just told you about.

22 These are all antibodies to the same site that are in the clinic, and you see they get
23 incrementally more and more potent. And the resistant fraction goes from thirteen percent down

1 to three percent. So we're making better and finding better and better antibodies.

2 And then you get antibodies to other sites that can be enormously potent, even if
3 they're not that broad. And this lends itself to combining. So I can show you a quick schematic.
4 So these are the same data on a heat map where deeper red is more potent.

5 So you can get an antibody like to the CD4 binding site where there's not a lot of
6 deep red, but it's very broad. And an antibody which has a lot more deep red but is not very
7 potent, and you can see that this antibody misses a lot. But it's picked up by this antibody and
8 this antibody.

9 So if one combines two antibodies, you get broad coverage and certainly with
10 three you can get near universal coverage at relatively good potency. So there is a lot of
11 discussion in the field about combining antibodies for a next study.

12 And the last thing I'll mention is that one of the other characteristics of antibodies
13 is that one can engineer the half life. So again this is the antibody that's in the Phase II B, given
14 every eight weeks or so. In this case it was given every month, just in this PK.

15 But a mutation that increases affinity to the FCRN just has this really impressive
16 effect on prolonging a half life. So one can measure antibodies out at six months, even with a
17 subcutaneous administration here.

18 So I think there is interest in the field in doing a follow-up study, a Phase II B
19 study, that would depend on what's found in the first study. But the considerations are to use
20 two or three antibodies in a follow-up study that would have the potential to show high efficacy.

21 That study could presumably verify that neutralization is strongly associated with
22 protection, now using not just one antibody to one epitope. And then that brings up the whole
23 discussion, and I'll just end here because these are early days for HIV, of the potential for

1 commercial manufacturers to bridge if they have a product to a serum neutralization correlate.

2 I'll stop there and look forward to the discussion later.

3 DR. YESKEY: Next we have Dr. Josh Reece, senior director of vaccine research
4 and development, vaccines and anti-infectives business unit at Emergent BioSolutions, talking
5 about the use of biomarkers to support an indication for anthrax post-exposure prophylactics.
6 Welcome.

7 DR. REECE: Thank you. First I just want to thank the organizers for inviting me
8 to speak today. Today I'll talk about anthrax vaccines. I know they've been mentioned a
9 handful of times earlier today, probably give you a little more detail on the biomarkers that were
10 used in a couple of different anthrax vaccines.

11 So the first part of my talk today will be around BioThrax. This is the only FDA-
12 licensed anthrax vaccine. So I will review in the context of a post-exposure prophylaxis
13 indication how we got that licensure.

14 The second part of the talk will be focused on AV7909, and that's our next
15 generation anthrax vaccine. And how we are leveraging what was done with BioThrax into
16 AV7909. And then at the end just very briefly I'll talk about mechanism of action and how we
17 think our adjuvant is being, is able to enhance the AVA.

18 So BioThrax or anthrax vaccine absorbed AVA, again I mentioned it's the only
19 FDA-licensed anthrax vaccine. It's licensed for two different indications. So the original one
20 was for pre-exposure prophylaxis and then we have our post-exposure prophylaxis indication
21 when combined with antibiotic therapy.

22 The post-exposure prophylaxis licensure was obtained through the FDA animal
23 rule, so this was actually the first vaccine to obtain a licensure using that FDA animal rule. I

1 think everybody here is very familiar with this, so I'm not going to go into any detail of all those
2 words on the slide, but just to say in regards to BioThrax, the models that we used were both the
3 rabbit model and the nonhuman primate model.

4 We correlated the PEP efficacy of the vaccine in those animals along with their
5 TNA titer, took that data, took that TNA data to see what would be predictive of protection in
6 humans, performed the clinical trial to get immunogenicity data on the vaccine, and then applied
7 for licensure.

8 So the only slide that I'll show you that actually has, and this is more proof of
9 concept which is post-exposure, is this slide. So this is just demonstrating in a rabbit model what
10 it looks like to have BioThrax in addition to an antibiotic therapy in a post-exposure scenario.

11 You can see the added benefit of adding the vaccine to the adjuvant, I'm sorry, to
12 the antibiotic. So if you just had antibiotics in a post-exposure setting, anthrax will be lethal.
13 Adding BioThrax to it is protective.

14 So moving on to a pre, I'm sorry, a post-exposure, no, pre-exposure prophylaxis
15 model. So this is the primary rabbit study that got BioThrax licensed for PEP. You can see the
16 survival curve here showing the titration with different doses of BioThrax and the survival that it
17 shows.

18 And then if you also take a look at this is the TNA titer, so the toxin neutralization
19 assay is the one that we used for protection. So the NF50 value is on the Y axis followed by the
20 on the X axis the day of sampling. You can see with the rabbits a nice dose titration showing the
21 TNA levels.

22 And then a logistic regression analysis of those gives you this. So this is again,
23 this is the main way that we got BioThrax licensed for PEP. Seventy percent probability of

1 survival is associated with a TNA NF50 of 0.56. That's become the gold standard for TNA
2 levels showing protection.

3 And then since that time of that initial rabbit study, we've done five additional
4 rabbit studies. And you can see as you do more of these studies that TNA threshold does shift a
5 little bit. So in rabbits the threshold goes from that .56 down to about .21. And then if you look
6 at it in macaques, the NHP data shows a BioThrax level of protection at about 0.29.

7 And then the last part I mentioned is taking a look in the clinic at what does the
8 immunogenicity of BioThrax look like in humans. And if you give BioThrax at two weeks apart,
9 three doses subcutaneously, this is the data that we got. So these are our geometric mean TNA
10 titers for the different days with their ninety-five percent confidence intervals in the graph.

11 At day sixty-three that TNA titer was 0.86. So if you take all of that data
12 together, this is where you end up. So in the rabbit model again the 0.56 was the TNA NF50
13 associated with seventy percent survival. In our clinical trial, seventy-one percent of subjects
14 met that threshold.

15 If you look at it in an NHP, TNA NF50 of .29 was the threshold for survival. And
16 in the clinical trial, ninety-three and a half percent of subjects met that threshold. Taken all
17 together into our submission package, again BioThrax licensed for PEP.

18 Moving on to the next generation anthrax vaccines, so AV7909, this is being
19 developed in conjunction with BARDA, so BARDA's funding this work. BARDA's
20 requirements were to get an accelerated immune response, reduce the number of doses, and also
21 it has to be of course used in conjunction with antibiotics.

22 So the AV7909 is really just AVA and we add CPG to it. So that's the additional
23 adjuvant. The indication is the same, post-exposure prophylaxis in combination with antibiotics.

1 But instead of three doses we're looking at two doses here. And instead of being subcutaneous
2 we're looking at IM.

3 So pathway to licensure, where are we? The red line that says today is where we
4 are and we are about ten years into this at this point, but the light at the end of the tunnel is there.
5 We're targeting a licensure at the end, or sorry, submission for a license application at the end of
6 2021.

7 You'll see there's no manufacturing on here because our manufacturing has been
8 completed. Non-clinically, a couple of studies left that I'll mention in a second. Then we have
9 two ongoing clinical studies.

10 All right, so similar to the model I showed for BioThrax but with a couple of
11 exceptions, this is the pathway we're targeting. Rabbits and CPG don't get along so we can't
12 test AV7909 in rabbits. So therefore the animal models we're using are nonhuman primates and
13 guinea pigs.

14 So again we'll establish our TNA titer associated with protection in those two
15 animal models, show that it's efficacious, take that data in combination with the existing rabbit
16 anthrax vaccine data, look at a TNA titer that is then predictive in humans, clinical trial, apply
17 for licensure.

18 So in nonhuman primates you can see here is our survival curves. Again, it's the
19 same thing from BioThrax. You titrate it out, you get varying levels of survival. If you don't
20 have the vaccine, you are not going to survive.

21 Then if you look at the TNA NF50 levels in the animals again, this is NHPs, the
22 data is right there, you can see it. This is again geometric mean TNA titers with ninety-five
23 percent confidence intervals. Take those together, do logistic regression, here's your survival

1 curve.

2 So you can see this TNA threshold keeps drifting lower, so this is 0.15 now. So if
3 you take again AV7909, seventy percent probability of survival, your TNA50 here is 0.15. And
4 this correlates to a clinical endpoint that I'll talk about momentarily.

5 All right, so development, where are we? I mentioned all those animal studies.
6 We've completed the guinea pig and the nonhuman primate studies. As of 2018 the FDA says
7 no additional confirmatory animal studies are required, so our non-clinical work is essentially
8 done on that side except for those two toxicology studies that we're completing this quarter.

9 And then clinically, so we've performed three clinical trials. The first one was
10 just a proof-of-concept back in 2005. Since then our two major ones were the Phase I B study in
11 2014 as well as the Phase II study in 2015. And we have two additional studies that will be
12 performed.

13 If you look at that 2015 study, that Phase II study, this is the key data that drove
14 us into our Phase III study that we're in right now. And you can see pretty dramatic change if
15 you add CPG to AVA. So the blue line there is our BioThrax with the standard PEP dosage two
16 weeks apart three doses.

17 The red line is AV7909 with the two-dose regimen. And you can see that it does,
18 the adjuvant impacts both the kinetics and the magnitude. So with AV7909 we see a peak
19 response at day twenty-nine. With BioThrax it's at day forty-three. And then you can just see
20 it's a much higher response as well.

21 At day sixty-four you can see that the vaccines kind of line up with each other, so
22 comparable amounts of TNA response whether it's two doses of AV7909 or three of BioThrax.
23 Important to note that Phase II study and the previous studies before it found the safety profile is

1 almost identical to BioThrax.

2 So here's what's left. So our Phase II study, our drug-drug interaction study, I
3 mentioned that BARDA had a few requirements, one being use in conjunction with antibiotics.
4 So we're testing AV7909 with both ciprofloxacin and doxycycline. That study is currently
5 enrolling. And then it's probably about ten percent enrolled as of this week.

6 The Phase III study has completed enrollment. We enrolled 3,864 subjects in it
7 and I'll tell you a little bit more about that study. First let's talk about the goals of it. So the
8 objectives of the study were to show lot consistency, manufactured at a production scale, make
9 sure our lots are being manufactured consistently, as well as making sure the product is
10 immunogenic.

11 And then of course the most important, safety. So those are the main objectives.
12 So how do we get to our endpoints? I've shown you these graphs before, so this is the rabbit
13 study in BioThrax showing the 0.56 threshold of protection for seventy percent survival.

14 So that's where we drove three of these endpoints, so again immunogenicity at
15 day sixty-four. We're looking for across all of our lots a protection level greater than or equal to
16 0.56 equivalence. So then we're going to compare lot to lot comparisons, again that day sixty-
17 four NF50 response, immunogenicity-wise.

18 So there's two here. The first one's based on that rabbit study again. So if you
19 pool all of our lots of AV7909 together, at day sixty-four is our NF50 greater than or equal to
20 .56. The second is not inferior to BioThrax, so I showed you a graph that showed BioThrax
21 given on that zero and twenty-eight-day schedule and it came up with that .29 TNA NF50 for
22 seventy percent survival.

23 So that drove that endpoint. Day sixty-four NF50 greater than or equal to .29 in

1 BioThrax versus AVA. And lastly there's a secondary endpoint. So with the anthrax attacks in
2 2001, we found that compliance to sixty days of antibiotics was really not great. I don't think
3 anybody really wants sixty days of antibiotics.

4 So if we can get a faster and more robust immune response with AV7909, it's
5 possible that you maybe wouldn't need a full sixty-day course of antibiotics. So to look at that,
6 that's when we put this secondary endpoint in here. So at day twenty-nine, and again this is the
7 data that I showed showing the TNA NF50 response with AV7909 at day twenty-nine it's a 0.15
8 threshold. So we're looking at that response as a secondary endpoint.

9 Phase III. So I mentioned we're done enrolling. We started enrolling in March of
10 this year, finished enrolling in August. That was about a little over three months early, almost
11 four months early. We screened 5,294 people, we enrolled 3,864 of them. Safety profile-wise,
12 we had a DSMV meeting in June.

13 DSMV said the study was fine to continue, so it is now in the follow-up window.
14 So the follow-up window will close in the third quarter of next year. We're currently analyzing
15 our TNA samples to get our endpoint data and that should finish in the first half of next year.

16 We'll lock the database at the end of next year and have a final study report the
17 first part of '21. And you may recall from that timeline slide, the goal is to apply for BLA in the
18 fourth quarter.

19 All right, so then just very briefly at the end here. Mechanism of action, so we
20 showed this kinetic and magnitude of the response being better by adding CPGs. So some of us
21 are wondering what is driving that.

22 So just historically looking back at CPGs, an article from about ten years ago
23 showed adding CPGs will create a larger proportion of memory B-cells. So we can see the data

1 on the left showing a higher number of B-cells when you add the first bar. It's probably hard to
2 read, but that first bar is AVA plus CPG. And the second bar being AVA alone.

3 So definitely more memory B-cells and they also have a higher affinity. So that's
4 the second graph there in the middle of the page.

5 Also T-cells, T-cell response is important. So this paper about five years ago
6 talked about biomarkers related to adding CPG to AVA. It showed again numerous markers
7 were elevated after adding CPG. T-cell effect was much higher due to the CPG adjuvant. It also
8 correlated with better humoral immune response.

9 And just two quick slides on some of the data that we have. So this shows in
10 guinea pigs that have been immunized four weeks apart and then cells taken out and challenged
11 at day sixty-nine, you can see an ELISPOT data here so the marks in red are those with CPG.

12 The first couple of bars there are just before challenge. But post-challenge in both
13 the lymph nodes and the spleen, you can see a significant increase in the amount of memory B-
14 cells. And then lastly those B-cells in addition to having a higher number of them, they also
15 have better avidity. So again the same type of setup, guinea pigs immunized four weeks apart.

16 Day forty-one we tested the KDs or dissociation rates, which as that number
17 drives down it shows a higher avidity. So it's significantly, barely, but significantly more higher
18 avidity antibodies.

19 Okay, so brief summary, TNA, gold standard for assessing protection from
20 anthrax disease. We're using it successfully with BioThrax and now we're using it with
21 AV7909. Bridging of clinical and non-clinical data, that's the pathway we're using for PEP
22 licensure.

23 Both vaccines tested in clinical studies showed really different levels of

1 protection, so that TNA NF50 value, whether it's .15 or .29 or .56 depending on the animal or
2 the vaccine, it does vary. Adding CPG as I mentioned again, your kinetics and your magnitude
3 of response are higher, probably better avidity enhanced B-cell populations. And they, those
4 differences may be why we're seeing the variation in level of protection and TNA thresholds.

5 Acknowledgements, there's a lot of people at Emergent, so there's a few names
6 there, but there's a whole lot more that did most of this work. BARDA of course, outstanding
7 partner for all of this. Original work was done through funding from NIH. And Patel Anarqvia
8 [phonetic], our main external partners that are working on the studies. Thank you.

9 DR. YESKEY: I'd like to invite our last speaker for today, Dr. Raffael
10 Nachbagauer from, he's an assistant professor at the Department of Microbiology at Mount
11 Sinai. And he will speak on the next generation of influenza vaccines, recent activities, and
12 identifying correlates of protection and biomarkers for next generation influenza vaccines.
13 Welcome.

14 DR. NACHBAGAUER: Thanks a lot for inviting me. And we've seen a little bit
15 about influenza this morning, but since this is not a pure influenza crowd I'm going to give a
16 brief overview of the issues that we're facing, what the current vaccination approaches are, and
17 then a general overview of the assays that are currently being considered for those novel
18 vaccines.

19 Influenza has a severe burden in the human disease. It manifests as a mild to
20 severe respiratory illness. It causes annual epidemics and sporadic pandemics. In terms of
21 seasonal influenza, we see three to five million cases and up to 650,000 deaths every year.

22 For pandemics, it can vary quite a bit. We saw in 1918 pandemic, which was the
23 most severe pandemic on record with estimates as high as 100 million deaths. And then on the

1 other end of the spectrum we have the 2009 pandemic which was more similar in severity to one
2 of the epidemic outbreaks that we've seen in recent years.

3 Influenza viruses have two major surface glycoproteins the AJ here shown in
4 green and the NA here in red. And the AJ is the primary target of current influenza virus
5 vaccines. And it constantly changes by antigenic drift. And sometimes viruses can reassort,
6 which is called antigenic shift and that can cause new pandemic outbreaks.

7 Influenza viruses are quite diverse. The majority of the influenza A viruses is
8 found in an animal reservoir and again if reassortment occurs this can lead to novel pandemic
9 outbreaks. We have two groups of Influenza A viruses, group one and group two, and then we
10 have Influenza B. Influenza B mainly circulates in the human population.

11 And what's really important to mention is that the current seasonal vaccines that
12 we have do not protect against those outbreaks from new avian viruses or even similar H1
13 viruses like in 2009.

14 In terms of what's circulating in the human population, since 1918 this was
15 mainly restricted to H1N1, H2N2, H3N2, and then again H1N1. Influenza B has been
16 circulating for at least 1940, but probably longer. As I told you it has been only isolated from
17 humans and on rare occasions in seals. So it presumably has been circulating for a long time in
18 humans.

19 This is the virus vaccine effectiveness data from the CDC. And as you can see
20 here the best year has sixty percent vaccine effectiveness and sometimes it goes as low as ten
21 percent, which clearly shows us that we need better vaccines than what we currently have.

22 In particular the current vaccines aim to elicit antibodies against the agent. And
23 we have heard earlier today that those antibodies are really highly neutralizing, but unfortunately

1 they are also strain specific because those epitopes are really rapidly changed by mutations in the
2 viruses.

3 And I also want to talk about the current correlate of protection, which is based on
4 hemagglutination inhibition. We've heard a little bit about it this morning. This is based on a
5 very simple assay. The virus can bind to sialic acid on red blood cells and if it binds to those red
6 blood cells it crosslinks them and it sustains essentially a mesh in a micro-titer well.

7 If you add antibody that interferes with the sialic acid binding, you can reverse it,
8 and that's called the hemagglutination inhibition. And as we heard earlier today, it correlates
9 quite nicely with neutralization. And therefore it was an assay that was good enough for the
10 longest time.

11 It has been first discovered in the '40s, '50s, and then further shown in 1970s
12 through challenge studies that a titer of one to forty is a correlate of protection. And this number
13 is generally used as just the correlate but there are a couple of flaws with the HI.

14 And the first one is that the one to forty number is essentially a fifty percent
15 protective value. That means that if you have a one to forty titer you only have a fifty percent
16 reduction in risk. Which is a problem if you consider that a lot of the studies aim to elicit this
17 one to forty titer, while you might have to go way higher than that.

18 The protective titers can actually differ quite a bit in different age groups. In
19 older individuals in severe seasons, you might require titers as high as one to six forty to get the
20 same protection as a one to forty in other populations.

21 And last but not least HI assays do not detect antibodies elicited with some of the
22 novel universal influenza virus vaccine approaches. So you really cannot use this assay
23 anymore.

1 What are those targets for novel universal influenza vaccines? First of all, the HA
2 is still being considered. In particular we now focus on more conserved epitopes in the HA. The
3 M2 is an ion channel which is not that abundant on the virion, but it also has been studied quite a
4 bit as a vaccine target.

5 M1 and MP are internal proteins and they're also being considered. And then last
6 but not least the NA is the second glycoprotein has also been considered as a vaccine target. So
7 I'm going to talk a little bit more about the specific targets.

8 As I told you and you heard this morning there are conserved epitopes in the
9 Influenza HA. The head domain is mainly variable and it's immunodominant, so the new
10 strategies have to really figure out new ways to elicit antibodies against this dopamine as well as
11 some conserved epitopes in the head domain. So there is still some conserved epitopes there as
12 well.

13 What's important for the stalk antibodies that bind this domain is that they're
14 broadly cross reactive. They can in most cases bind within one group of influenza viruses. In
15 some cases even all groups. They're generally only weakly neutralizing, but seem to mediate a
16 lot of their function through effector mechanisms, Fc-mediated.

17 And again importantly they're HI inactive, so they cannot be detected with the
18 current correlate of protection. For M2, as I said, it's very lowly expressed on the virion surface
19 itself. But it is also expressed on the surface of infected cells.

20 And it's highly conserved which made it an attractive target to get broadly
21 protective immunity. Antibodies against M2 have been shown and in particular to the external
22 domain have been shown to elicit protection through Fc-mediated functions. And it has also
23 been shown that T-cells targeting M2 can contribute to protection.

1 MP and M1 are also highly conserved. They are internal proteins only that are
2 not presented on the virion surface. And but they're also very abundant in infected cells. And in
3 general people who pursue M1 and MP as vaccine targets try to make T-cell based vaccines
4 where you can still target those internal proteins.

5 And the neuraminidase has been often and for a long time ignored as a vaccine
6 antigen. Currently the NA content is not standardized in seasonal vaccines. And for the most
7 part people have started now to think about introducing it into the seasonal vaccines as well.

8 Antibodies against the NA have been shown to be broadly cross reactive in some
9 cases. And importantly they have also been shown to be an independent correlate of protection.

10 When we talk about correlates of protection for influenza viruses in an antibody
11 context, we have to really consider the multiple functions that those antibodies can have. The
12 classical HI antibodies as I told you would inhibit attachment, but then stalk antibodies for
13 example can inhibit fusion on coding, which is essentially a downward step after virus entry.

14 Then some antibodies can inhibit the budding. So for example antineuronal
15 antibodies and to a less extent stalk antibodies can interfere with this step. And then through Fc
16 functions, antibodies can also attract complement or Fc gamma receptors on cells that cause
17 either ADCC or ABCP.

18 So all those different effects can be measured with different assays. And I want to
19 start out by talking about ELISAs. What's really nice about ELISAs is that it has been
20 established as a correlate of protection for multiple other infectious diseases.

21 The big plus and the big minus at the same time is that it detects pretty much any
22 antibodies. So for the most part you cannot infer from an ELISA titer if it's going to be
23 functional antibody or not. On the plus side, you get IgG and IgA also specifically measured in

1 this assay.

2 You can get some functional assessment by looking at avidity as well assuming
3 that a higher affinity avidity antibodies can be more protective. And you can also use
4 competition assays with known antibodies that where you know that they are protective to figure
5 out if you get similar antibodies.

6 The results depend highly on the choice of substrate, so if you use a whole virion
7 you're going to get very different responses in your assay than if you use recombinant proteins.
8 For the stock-based ELISAs there's also different ways of measuring them. Either you can use
9 one of those mini-HAs that have been stabilized or you can use the chimeric HAs that we have
10 used in our laboratory.

11 Overall it seems like there is some correlation, but in general you have to always
12 consider what you're coating and also how much you're coating on the plates. Importantly, we
13 have been able to show recently that antibodies measured by ELISA can actually correlate with
14 protection in vivo.

15 So those are, this was a study done in Nicaragua in baby cord. Not just baby cord,
16 but also adults. And we saw that antibodies measured by ELISA as well as HI as has been
17 previously shown correlated with protection.

18 A big caveat in this case is that this was naturally acquired immunity. The score
19 was mainly unvaccinated. So if these results would hold true in a vaccinated population where
20 this type of immunity is purely through inactivated vaccination, it's not entirely clear.

21 Next neutralization assays. We've heard a little bit about that earlier today, too.
22 They have been shown to correlate with protection, but for the most part in the context of HI
23 active antibodies. As I told you, stalk antibodies are less neutralizing, so it's not really clear if

1 the same neutralizing titer would be required to get protection.

2 Neutralization is not the primary mode of action for the stock M2E or NA
3 antibodies. And the results again depend very much on the choice of assay. We've heard a little
4 bit about the pseudo-type assays this morning which are entry only assays versus multi-cycle
5 assays where you have replicating virus in which case you even can measure NA antibodies
6 which keep the virus from budding.

7 So there's pros and cons in each of those assays. What's always important is that
8 you need to know what you're measuring when you're using a specific assay.

9 A big benefit of the pseudo-type particles is that they can express NAHA or NA,
10 including just mini-HA only if you wanted to measure only stalk antibodies. While for the
11 multi-cycle assays you really require functional HAs and NAs.

12 The neuraminidase inhibition assay has been widely used and has been shown to
13 correlate with protection independently. It can measure the functional activity of mainly NA
14 antibodies. However, stalk antibodies can also contribute to some extent to the inhibition that is
15 seen in the assay.

16 This really depends again on the virus that is being used, which the HA, you can
17 replace the HA and you get different types of stalk antibody inhibition. And as soon as you have
18 high NA antibody levels, you see less of an interference of those stalk antibodies.

19 And we've heard a little bit about Fc-mediated functions in other contexts. In
20 influenza it has also been more and more important recently, especially in the context of HA
21 stalk and M2E antibodies. There is a potential requirement of using multiple different assays to
22 cover all the Fc functions that those antibodies can actually elicit including ADCC, ADCP, or a
23 complement.

1 A big problem with this, with these assays is that they're fairly complex and it can
2 make it pretty hard to standardize them. And there are some reporter assays out there which can
3 simplify the setup, but it's really important to first show that those are meaningful results.
4 Because otherwise you have a nice assay that give you nice, consistent results, that might not
5 mean anything down the road.

6 But one assay that we're using in the lab was developed by Promega, which is
7 based on a Jurkat cell line that expresses a single Fc-gamma receptor. And triggers downstream
8 lucifer signal. And we actually have some preliminary data that shows that this actually
9 correlates with NK cell activation. So it might be feasible to get a simpler assay which is based
10 on a reporter that still gives you a complex answer.

11 The last serologic assay that I want to talk about, and we've heard a little bit about
12 it from the other pathogens, is the passive transfer model. A big plus for this is that it can really
13 test all the combined functionalities going from NI to HI to regular neutralization.

14 It's an in vivo model where we generally use pooled serum samples and transfer
15 them into mice, challenge them with influenza viruses intranasally, and then either take the lungs
16 on day three or day six. Or we just follow them for fourteen days for weight loss.

17 So this is a very useful model. A problem is that it may not capture the entire Fc
18 mechanisms because it's human antibodies in a mouse model. That said, in general non-
19 neutralizing antibodies from a human source have been shown to protect in a mouse model.

20 Those studies are kind of expensive and rather difficult to standardize, but we
21 have recently shown that serum antibody titers measured from humans transferred in mouse
22 predicted the level of protection that we get if we correlate it with weight loss.

23 So it seems like at least when you look at the serological readout and the in vivo

1 protection, you can get some correlation for the ADCC reporter assay and again the ELISA
2 assays.

3 I tried to make a list here in terms of the serological assays which go from the
4 ease of standardization to further difficult to the passive transfer model. At the same time the
5 functionality goes in the other direction. What's also important to mention is that there are
6 currently active efforts underway.

7 We're working with the Gates Foundation on generating a standard serum that
8 could be used by companies, by other academic institutions, to test those sera in their assays to
9 figure out how those different assays relate to each other and what type of responses those assays
10 measure.

11 And lastly, I want to talk a little bit about T-cells. To be honest, this is not my
12 expertise. But there's also not been that much work in influenza, but it's starting to ramp up
13 more recently.

14 Importantly, T-cells have been shown to contribute to protection from influenza
15 virus infection. And also to the clearance of virus. There are a multitude of assays, and I think
16 what we've seen earlier today in terms of Ebola virus, I think would be really useful to also do an
17 influenza to just look at the different populations of cells.

18 Because right now we don't really know what the optimal assay would be in the
19 specific cell population that we need to contribute to protection from the T-cell side of things.
20 And the question really is if there might be a simple assay out there which is not directly
21 responsible for the protection but could nicely correlate.

22 So, for example, there was a study a couple of years ago that showed that
23 granzyme B release of T-cells correlated with protection in the elderly population.

1 So my final considerations for the identification of correlates of protection for
2 next generation vaccines would be that the protective mechanisms may differ quite a lot
3 depending on the type of immunization, the vector that's being used, and pretty much mostly on
4 the target end you have to adjust the assays.

5 I think challenge trials at the vaccination with new vaccine candidates can really
6 accelerate the identification of correlates of protection. That said, I think there is to some extent
7 an artificial level of those challenge studies, so it might also be important to have cohort studies
8 that study natural infection in the regular population.

9 I think standardization is going to be really, really important to try to identify
10 correlates of protection by being able to compare results from different trials. Because otherwise
11 you're always stuck with the single trial that you are running yourself and you can't really go
12 back and figure out what did they measure in their trial where they had success.

13 And I think that's also really important point and that the current testing mainly
14 relies on serology. And it's a respiratory disease, the entry is through the mucosa. So I think we
15 should really think more about measuring mucosal immunity for antibodies, but also for cell
16 immunity.

17 And I think an important point to consider is that clinical trials that test those next
18 generation vaccines, even if you're not planning to immediately test every single assay, I think it
19 would be good to bank samples, serum, mucosal samples, as well as cell samples where you
20 could go back and test them at a later point if somebody else in the field discovered a new
21 potential correlate of protection.

22 I think there's a lot of progress in the field. And if we don't try to compare our
23 results and are open about sharing our findings, I think we're going to have a hard time really

1 making progress. Thank you.

2 DR. YESKEY: If everyone can come to the table for the Q and A session, please.
3 I'd ask folks to line up for questions.

4 PUBLIC: All right, the mic will intercept it. It's a question to Josh really. Your
5 animal rule study, lovely study, and you showed us quite nicely the brackets at a particular level,
6 and then you did that in humans, that was fine. How did you do it when you involved the
7 adjuvant? Did they say you had to hit a certain level of stimulation over and above what you got
8 with the antibody? It's just how did you get the formulation right under animal rule? I'm a bit
9 confused.

10 DR. REECE: You mean going back how did we derive the dose in humans?

11 PUBLIC: Yes.

12 DR. REECE: In one of our Phase II studies it was all about dose optimization.
13 So we looked at different schedules and different combinations, different levels of ADA,
14 different levels of CBG, and then tried to see what gave us the maximal response at the earliest
15 time point. That's how we derived that.

16 PUBLIC: But the regulator didn't say under that, the opposite showed, actually
17 over and above?

18 DR. REECE: To my knowledge, no.

19 PUBLIC: So I'm going to ask a question of my friend and colleague, Dr.
20 Mascola. We talk about these things all the time. You mentioned the idea that maybe if you got
21 a result on a passive antibody study you could apply that across other passive antibodies and
22 bridge to that. What if you did a passive antibody study with a stalk antibody for the flu or
23 another antibody for HIV and then used that as a biomarker for vaccines? Even though vaccines

1 are inducing CV4s or CV8s, et cetera, if they could induce that particular antibody function that
2 you know can work in an antibody only setting and not be interfered with by these other things,
3 would that be a biomarker? How can you envision that being a biomarker for a vaccine?

4 DR. MASCOLA: Yes, that's an easy question. You know, we had the discussion
5 today that it's all context prevalent, right? So it really depends on how confident the field is that
6 neutralizing antibody is a dominant enough correlate of protection. Within passive antibodies,
7 that's an easier step. To make that bridge to a vaccine to get a polyclonal response to various
8 epitopes that includes CD4 and CD8 responses, I think is a tougher step. I wouldn't rule it out. I
9 think, you know, you would need to present the package of a pretty broad set of data to a
10 regulatory agency and probably work with them. So I'm going to equivocate a little on that.

11 PUBLIC: I have a question for John also. HIV in the end is, as we know, a
12 sexually transmitted disease and except for IV drug users that's really transmitted locally. And I
13 know your group and the groups that are working on these monoclonals have put effort into
14 measuring antibody locally. And I wonder if you could just describe a little bit some of that
15 kinetics and whether or not the trials will be modified in relation, end points in relationship to
16 those kinetics at the end point, at the local level.

17 DR. MASCOLA: So I'd say a couple of things. One, I think we're on firmer
18 ground with passive antibody transfer than vaccines when you think about the mucosal response.
19 Because a vaccine that gives us active immunity including B-cells and IgM and IgA mucosa,
20 whereas here we're talking about fusion antibody it distributes and also it distributes to mucosal
21 surfaces.

22 And we understand that distribution a lot better now. We understand that it's not
23 transudative as much as active transport mediated by OCRN. And one can measure it, although

1 it's not being measured in Phase II study. But it's being measured in Phase I and Phase II
2 studies. So, for example, the HIV vaccine trials now is doing biopsy studies out as far as a year
3 after a single passive transfer of an antibody that does or doesn't have an LS mutation that gives
4 it the long half life.

5 And remarkably they can see antibodies in, for example, rectal biopsies, out at a
6 year. So there's ways to demonstrate that antibodies are present in mucosal surfaces. It just
7 turns out to be really hard. There's not a simple non-biopsy measurement.

8 Rectal wicks or mucosal samples tend to be hit or miss, so it's a harder thing to
9 measure and we're depending to some extent on the concept, and there are data for this, that
10 what you see in the serum does generally reflect a distribution more broadly.

11 PUBLIC: Thank you. I just wonder for all of this studies and research, do you
12 basically rely on the merits or do you go with PPP partnership? The base money you corral or
13 somebody support you and eventually the whole thing is covered?

14 DR. MASCOLA: I'm not sure we understood the question.

15 PUBLIC: I mean, is your studies based on friends, basically PPP partnership,
16 that's propaganda basically by the merits of many rather than yourself. In other words, which is
17 now is really propaganda, for all the crime which is an abuse. And it can destroy our society
18 with individuals and families is very serious and that's what I'm concerned about.

19 DR. MASCOLA: So I'll let them speak, but our work is funded by BARDA, so
20 the Biomedical Advance Research and Development Authority under HHS.

21 PUBLIC: What I mean it's not just grant, government grant is okay but it is such
22 things as PPP private partnership which is a totally different kind of thing. I want you to realize
23 that.

1 DR. NACHBAGAUER: Yes.

2 DR. YESKEY: I mean, I think where possible with government funding can be,
3 are you talking about access to the data once published? I mean, you know, there are certain
4 aspects of these relationships that these investigators have and these companies have to then
5 make data available to the public. It's different based on funding. Is that?

6 PUBLIC: I think most important is the starting point. You have to get the money
7 first and in order to get the funding you don't have a memories, you get money and then you ask
8 the government much, matching the fund and the whole thing is different again.

9 PUBLIC: Marion Gruber. I just wanted to make a comment. I really think, you
10 know, I appreciate all the different perspectives and opinions that are being expressed. But I
11 think we should realize that this workshop really focused on the scientific aspects of biomarker
12 development for preventive vaccines. And I think, you know, you're raising interesting points
13 but I think you should really, you know, consider this more appropriate for a different forum.
14 Thank you.

15 PUBLIC: May I ask whether the basic starting point where did they ask you to
16 get the money first and then they had to match it. And just a little bit our relation. Our relation
17 is there and the government funding and that's okay. But if they ask you to fund and collect the
18 money first and government has to subsidize all your funds then from the beginning the money,
19 money, money ...

20 UNIDENTIFIED SPEAKER: Ma'am, okay, again, I think this is very much
21 appreciated your perspective and opinion, but I think we need to really continue to focus on the
22 scientific aspects of the biomarker development. Thank you very much for your comment.

23 PUBLIC: But I think we have to know how to protect our society first. The

1 whole thing basically the research is to benefit our society rather than corrupt our society.

2 PUBLIC: I have a question.

3 DR. YESKEY: Please.

4 PUBLIC: My question is to Raffael. Raffael, I noticed that on one of your slides
5 you were talking about protective titers for influenza, you talk about the one recording being
6 common. In that one it showed different levels I think for different tiers or something. I was
7 curious about what data generated that, where you had different titers for influenza? Just a little
8 more explanation about that one interesting slide.

9 DR. NACHBAGAUER: Yes, I couldn't get into more detail on that one. So we
10 published the results early this year. This was done in a household over study in Nicaragua.
11 Those individuals were followed for multiple seasons. In this case we analyzed the results from
12 two H1N1 influenza seasons.

13 And those households are closely monitored and essentially followed at the time
14 of infection and then all the household members are automatically enrolled and followed for
15 transmission. So that's how we got a pretty high attack rate in that study.

16 What was shown in the columns were two different things. The bar of the column
17 itself represented how many individuals with certain titer range were in the specific group. The
18 points that we showed were the attack rates in the specific group. And then we had essentially
19 an extrapolation of how the correlate would go.

20 So eventually if you, we saw high attack rate in individuals with a very low titer
21 and a low attack in individuals with high titers.

22 PUBLIC: So this is a prospective study with serologies and then seeing who's
23 infected or not infected.

1 DR. NACHBAGAUER: So it was retrospective in the sense that the samples
2 were collected prior. But the analysis was done blinded and essentially, so all the measurements
3 were done blinded and then analyzed after the fact. The samples were banned from previous
4 sections.

5 PUBLIC: All right, if there are no questions we can open it up to questions
6 online. The first one is from Ketel Patel [phonetic] and this question is directed to Dr. Mascola.
7 The question is since the animals are smaller compared to humans, would maturation of
8 antibodies in animals and human make a big difference and thus does it affect the time point at
9 which we look at these biomarkers?

10 DR. MASCOLA: I think the answer is yes. There's a pretty big difference, so we
11 do adjust by weight. So we give antibodies as a dosage by milligram per kilogram of body
12 weight. But still the distribution and the active transfer point differ, which is why the human
13 Phase I safety data, the human Phase I pharmaco-kinetics data are really critical to considering
14 biomarkers. I don't think you can really do it in the animals.

15 PUBLIC: The second question is from Vijay Jelpa (phonetic) and this question is
16 for Raffael Nachbagauer. Is there value added in assisting antibody affinity and grants using this
17 tier for influence or other vaccine development studies along with a list of other assays?

18 DR. NACHBAGAUER: I do think so. And we're currently going into that with
19 serum samples from a clinical trial that is ongoing. We have no results from the specific
20 measurements at this point. The reason I think it might be important is that we saw quite drastic
21 differences between our research grade assay where we use low coding concentrations of antigen
22 versus a very optimized assay done at a zero. And if you have low coating concentration on your
23 plate you measure some affinity to some extent.

1 And all of our preclinical correlation data as well as those studies that have shown
2 were based on the research grade assay. So we're right now trying to figure out if it is the
3 affinity measurements that play a role in there and I think they might.

4 PUBLIC: Thank you. I have one quick question for you. You mentioned that
5 antibodies put a stop. Not from a neutralization. Do you think they prevent virus
6 communication or can you comment on?

7 DR. NACHBAGAUER: So they, depending on the assay they can be
8 neutralizing. It's just their neutralizing to a lesser extent than HAI antibodies. They have
9 multiple function as has been shown. They can inhibit fusion, they can inhibit the maturation of
10 the HA, they can have the assimilated effects. But they can also inhibit the cleavage of the virion
11 particle from the cell. So those are potentially multiple functions.

12 PUBLIC: One last question from the online participants. The slides that the
13 speakers presented, will those be available for distribution? So that is the last online question.

14 DR. ROBERTS: I think we're going to try to do that the best we can, recognizing
15 that many of these slides are going to be confidential. But we'll try to make them available as
16 much as possible on the same site where we offered registration.

17 PUBLIC: There are no questions from the room. Thank you.

18 DR. YESKEY: All right, thank you very much. Thank you to our speakers and
19 thank you all for participating today and for your attention and all of your comments and
20 questions. We will adjourn for today and we will meet again at 9:00 tomorrow morning. Thank
21 you.

22