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FOOD AND DRUG ADMINISTRATION  
CENTERS FOR DISEASE CONTROL AND PREVENTION

FILOVIRUS ANIMAL MODEL

WORKSHOP

TUESDAY  
SEPTEMBER 11, 2007

The Workshop met in the Main Auditorium in the  
Natcher Conference Center, National Institutes of  
Health, Bethesda, Maryland, at 8:30 a.m.

PRESENT

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<p>TABLE OF CONTENTS</p> <p>AGENDA ITEM PAGE</p> <p>Introduction. . . . .4</p> <p>SESSION 1: Filovirus Pathogenesis and Animal Models</p> <p>Introduction to Filoviruses . . . . . 10</p> <p>Comparison of Epidemiology, Clinical Course and Pathogenesis of Filovirus Infections. . . . 31</p> <p>Biomarkers in Human Pathology and Host Response to Filovirus Infections. . . . . 54</p> <p>Small Animal Models of Filovirus Infection. . . . 67</p> <p>Non-Human Primates as a Model for Filovirus Infection . . . . . 92</p> <p>Aerosolized Filoviruses in Three Species of Non-Human Primates. . . . .125</p> <p>SESSION II. Vaccines: Correlates of Protection and Relevant Functional Assays</p> <p>Filovirus Vaccine Design and Rationale. . . . .149</p> <p>The Role of Antibodies and Cell-Mediated. . . .167</p> <p>Immunity in Conferring Protection Against Filoviruses</p> <p>Initial Assessments of Correlates of Protection for and Ebola Vaccine. . . . .183</p> <p>Regulatory Perspectives on Use of Animal Models to Study Vaccines for Filovirus Infections. . . . .210</p> <p>Panel Discussion. . . . .235</p> <p>Adjourn</p> <p style="text-align: right;">Page 2</p>	<p>1 the opening remarks.</p> <p>2 DR. KURILLA: Thank you, Ping, and let me</p> <p>3 take the opportunity to welcome everyone and thanks</p> <p>4 for coming out. We look forward to a productive</p> <p>5 meeting. We're a little sparse at the moment. Peter</p> <p>6 Jahrling is giving traffic updates at the weather. I</p> <p>7 guess the weather is making 270 a virtual parking lot.</p> <p>8 So we'll probably get a flood of Fort Detrick people</p> <p>9 and those north of us coming in very soon.</p> <p>10 It's interesting, I was e-mailed yesterday</p> <p>11 by Mike Bray who told me that had very well-timed</p> <p>12 event for this workshop and I thought that he</p> <p>13 initially was referring to the fact that it turned out</p> <p>14 to be September 11th, which is today, the sixth</p> <p>15 anniversary of 9/11 and I think it's significant in</p> <p>16 that regard that six years later, while there's still</p> <p>17 a lot to do, we have, in fact, made quite a bit of</p> <p>18 progress.</p> <p>19 But what Mike was actually referring to is</p> <p>20 the fact that only within the last 24 or 48 hours has</p> <p>21 there been confirmation of an ebola outbreak in the</p> <p>22 Congo that has been going on. Last reports that I'm</p> <p>23 aware of about 160 people have already died with --</p> <p>24 out of about a total of anticipated of about 400 cases</p> <p>25 and we're fortunate that our CDC colleagues who are</p> <p style="text-align: right;">Page 4</p>
<p>1 P-R-O-C-E-E-D-I-N-G-S</p> <p>2 8:34 a.m.</p> <p>3 DR. CHEN: My name is Ping Chen. I'm the</p> <p>4 Program Officer in the Drug Development Section of</p> <p>5 Office of Biodefense Research Affairs in the Division</p> <p>6 of Microbiology and Immunology -- Microbiology and</p> <p>7 Infectious Diseases and I, in the past about a month</p> <p>8 or so I have exchanged e-mail with many of you and</p> <p>9 have phone conversations about this meeting and now</p> <p>10 we're all here. I'm looking forward to meeting you</p> <p>11 all. So on behalf of our meeting organizers, which</p> <p>12 are the DTRA, Department of Defense, FDA and CDC and</p> <p>13 NIAID, welcome you all to the Filovirus Animal Model</p> <p>14 Workshop and we have very -- the public response has</p> <p>15 been great to this meeting.</p> <p>16 We have -- over 100 people have registered</p> <p>17 for this meeting. I just want to have one thing to</p> <p>18 say on agenda, I e-mailed you a copy of a draft</p> <p>19 agenda. Now, you should pick up a copy in the front</p> <p>20 when you check in. On the last page of the agenda, we</p> <p>21 listed questions for the panel discussions which will</p> <p>22 be at the end of today and end of tomorrow.</p> <p>23 Without further delay, I would like to</p> <p>24 introduce Dr. Michael Kurilla, the Director of the</p> <p>25 Office of Biodefense Research Affairs at NIAID to give</p> <p style="text-align: right;">Page 3</p>	<p>1 here today are actually leaving right after this</p> <p>2 workshop to fly over to the Congo and attend to that</p> <p>3 issue.</p> <p>4 Let's see if I can manage -- so just to</p> <p>5 give you some context, I like this slide because it</p> <p>6 sort of highlights the fact that we have a habit in</p> <p>7 this country of premature declarations of victory on</p> <p>8 a lot of things, but significantly it's, you know,</p> <p>9 over 40 years and in fact, we're still struggling</p> <p>10 quite a bit with infectious disease and what we'll be</p> <p>11 talking about in this workshop, filovirus, has</p> <p>12 certainly been something that has been a longstanding</p> <p>13 issue that we hope we're finally beginning to</p> <p>14 seriously address.</p> <p>15 Again, it's the sixth anniversary of 9/11</p> <p>16 and importantly our 9/11 sort of began not with a bang</p> <p>17 but more with a whimper that started us down this</p> <p>18 course. However, since 9/11, we've seen a number of</p> <p>19 other diseases that have come to the forefront, wholly</p> <p>20 on anticipated SARS in this case or probably one that</p> <p>21 we should have a -- we have been anticipating, maybe</p> <p>22 not in this form but pandemic flu. All of these have</p> <p>23 the issue that is the crux of what the discussion of</p> <p>24 this workshop revolves around and that is the use of</p> <p>25 animals models in terms of product development. So if</p> <p style="text-align: right;">Page 5</p>

<p>1 we look at what we really have faced before us in  2 terms of emerging and re-emerging infectious diseases,  3 we see that the problem is not getting better. We  4 seem to be gradually adding more and more names to  5 this list on an annual basis.</p> <p>6 I want to highlight the fact that recently  7 with -- this year we have seen the broader context for  8 the strategy towards approaching how we can be  9 prepared for these sorts of events is the strategy for  10 chemical, biological, radiological and nuclear threats  11 that was released by HHS earlier this spring and then  12 was followed up with the implementation plan that came  13 out in April. What I want to highlight for those of  14 you who are not familiar with this, is to look at the  15 section specifically dealing with filoviruses.</p> <p>16 There's a recognition within the plan that we do not  17 have any specific counter-measures and that,  18 therefore, parallel efforts on both vaccines as well  19 as therapeutics will continue forward in order to  20 address this considerable unmet need.</p> <p>21 So one of the focuses of what we're  22 dealing with here throughout this workshop is the  23 implementation of the "Animal Rule," and I'm not  24 trying to steal any of the FDA's thunder in terms of  25 it, but just to sort of set the ground rules on how</p> <p style="text-align: right;">Page 6</p>	<p>1 HHS activities that have evolved over time. Now the  2 BARDA office where the real advanced development or  3 late stage, end stage development goes on as well as  4 the acquisition mechanisms, either for BioShield or  5 under Flu.</p> <p>6 At NIH we have basic applied and then the  7 product development schemes go through an early to an  8 advanced. What I've highlighted here is that there's  9 a number of different animal model work that goes on  10 at various stages and product development and we need  11 to be careful not to confuse the animal efficacy  12 models with animal infection models that maybe have  13 other relevant applications in terms of screening  14 potential products and in weeding out and refining  15 certain product development, but in terms of there's  16 quite a bit of animal model development going on.  17 What we need to do is to understand how we can take  18 the animal model development and develop animal  19 efficacy models that will in fact, be valid in terms  20 of submitting "Animal Rule" efficacy studies to the  21 FDA.</p> <p>22 And so with that, I will close because in  23 the end, this is what we're trying to avoid, another  24 occurrence such as this, although I should add, in the  25 interest of full disclosure, that this is actually</p> <p style="text-align: right;">Page 8</p>
<p>1 these -- how these discussions will be approached,  2 specifically the implementation of the "Animal Rule"  3 is where human efficacy evaluation is not technically  4 possible or ethically feasible and clearly in the case  5 of filovirus, that is certainly a valid assumption.  6 That being said, it does not eliminate the need for  7 human data specifically with vaccines. You still  8 require immunogenicity data and for therapeutics you  9 still require PK data in addition to an adequate  10 amount of safety data in humans that will be critical.</p> <p>11 When you do get approval under the "Animal  12 Rule" it does come with substantial post-marketing  13 studies and use restrictions and that needs to be  14 adequately discussed in terms of how we can best put -  15 - enter that into product development plans, but the  16 real crux, the final one here, is that we require an  17 appropriate -- the "Animal Rule" requires appropriate  18 animal model efficacy data and the subject of our  19 discussion will be on how we will go about generating  20 that animal efficacy data.</p> <p>21 What I have up here is a little  22 complicated but it is an integrated research and  23 development scheme that covers product development and  24 it tries to highlight both the NIH activities -- it  25 highlights a number of NIH activities as well as the</p> <p style="text-align: right;">Page 7</p>	<p>1 dealing with Senate and House ethics reforms in terms  2 of accepting political contributions. But it's just  3 as applicable to bio-hazards.</p> <p>4 So with that, I will turn the podium back  5 over to Ping who will introduce the first session  6 speakers and get us going or is it Marty going to take  7 it over? Okay, I'm sorry, Marty Crumrine will take  8 over. Thank you.</p> <p>9 SESSION I: FILOVIRUS PATHOGENESIS AND ANIMAL MODELS</p> <p>10 DR. CRUMRINE: Well, as you all know, I'm  11 not Peter Jahrling, but I'm standing in for him as  12 best I can and I probably won't have the same flowery  13 introductions that Peter might have planned but I do  14 want to welcome each of you to the meeting and thank  15 you for your attendance. Please be aware that this is  16 an open meeting, a public meeting and if any of your  17 comments -- you're concerned about any of your  18 comments from a product development point of view and  19 proprietary point of view, the meeting is being  20 transcribed and just for your information.</p> <p>21 Our first speaker comes to us from Canada  22 and we're very glad to have him here, he's Dr. Heinz  23 Feldmann. And we wish to thank you very much and we  24 appreciate your patience with us as we have progressed  25 through the process of getting you here.</p> <p style="text-align: right;">Page 9</p>

<p>1 DR. FELDMANN: Okay, good morning,  2 everyone. First of all, thank you very much for the  3 invitation to come here and to speak. It's a pleasure  4 to be here this morning and my -- or the task that was  5 given to me was the introduction to filoviruses. It  6 sounded a little bit strange first to me when I looked  7 at the participants and the other speakers to  8 introduce filoviruses so I hope some of you don't know  9 too much about them so they will enjoy a little bit of  10 the talk. And for those of you who know this all, you  11 may either want to take a break for coffee or just  12 take a nap.</p> <p>13 The first thing I would like to quickly  14 address is the taxonomy. Not that I want to change  15 it, but I just want to reemphasize that we have since  16 a couple of years, I think a new taxonomy that is not  17 very welcome in the field and not very much used by  18 people. I have the unfortunate job to be the  19 Chairperson of that group and I would like to get rid  20 of it to be honest. I get more complaints than  21 positive comments but I just wanted to make that  22 comment.</p> <p>23 Keep in mind that most of you in the field  24 have at least, at one point, had a say on this and  25 that's what we at least for now have to live with.</p> <p style="text-align: right;">Page 10</p>	<p>1 high as up to 90 percent and I think we've learned  2 over the past years that Marburg is not -- doesn't  3 show very low virions as we always were told by the  4 first outbreak back in '67 looking at Durba-Watsa as  5 well as Angola case fatality rates are coming into the  6 range of ebola or even exceeding it depending on what  7 numbers you're looking at.</p> <p>8 In terms of the particle structure,  9 filovirus are typical mononegavirales. They have an  10 unsegmented negative stranded RNA genome. Gene order  11 is typical starting with the nuclear capsid protein at  12 the three prime ends and phosphor protein equivalent  13 VP 35, matrix protein, VP 30, glycoprotein, VP 30, 24,  14 and the polymerase. If you look at differences, one  15 of the obvious differences are gene overlaps so each  16 gene has its inscriptional stop and start signal,  17 those are highly conserved and there is one pentamer,  18 UAAUU, on the negative sense genomic sequence, that is  19 coming in on both of those signals and when we talk  20 about about a gene overlap, this pentamer is  21 overlapping.</p> <p>22 Marburg has one of those overlaps, between  23 VP30 and 24 and Ebola has two or three depending on  24 the species. They might be involved in regulation of  25 transcription. It's not really fully understood but</p> <p style="text-align: right;">Page 12</p>
<p>1 We're within the order mononegavirales. There's a  2 known family, filoviridae. We have two genera Marburg  3 virus and ebola virus and I think so far most people  4 are fine with it but the problem comes with the  5 species designation and the abbreviations, but this is  6 what's currently published, at least in ICTV and which  7 actually never appears in almost any of the  8 publications because people don't like it.</p> <p>9 So we have one species, Lake Victoria  10 Marburg virus in the genus Marburg virus and we have  11 several strains in the meantime but no further species  12 at this point and I'm sure Stuart Nichol and the CDC  13 group could talk more about it. They have most of the  14 sequencing data. And we have four species of ebola,  15 Zaire ebola virus being the type species here. Sudan  16 ebola virus, Cote d'Ivoire and Reston ebola virus and  17 these are the official abbreviations. Actually,  18 there's one e too much here, sorry for that.</p> <p>19 And so the only way to change this is a  20 new taxonomy proposal and maybe we should go ahead  21 with it as everyone is so unhappy about it. In terms  22 of public health concern and it was just that, also in  23 terms of bio-terrorism concern, I think the ebola  24 virus, Sudan and Marburg, the major concerns coming up  25 is case fatalities ranging from as low as 23 and as</p> <p style="text-align: right;">Page 11</p>	<p>1 this is one of the main differences as well as the  2 expression and I come back to that, of the soluble  3 glycoprotein with the ebola which we don't have with  4 Marburg virus.</p> <p>5 Particles are filamentous in shape,  6 they're envelop, they have a central core and the  7 central core consists of the RNA genome and four  8 proteins and I will come back to that in a second.  9 Along the membrane we have the matrix protein and  10 VP24, a membrane associated protein. Even so, that's  11 being redefined. There's a lot of new data coming out  12 to VP24 location and function and we may have to  13 revisit that and there's a single glycoprotein on the  14 surface either called GP or GP12. There are several  15 designations our for this particular protein.</p> <p>16 This list of the proteins and their  17 potential function is not inclusive. There's new data  18 coming out almost every month, particularly from  19 Reverse Genetic Systems. So we have these four  20 proteins that are important or that are part of the  21 RNP nucleoprotein complex so they are associated with  22 the RNA to make that transcriptional and a replication  23 complex. The nucleoprotein, its main function is the  24 encapsidation of the genome and perhaps it's involved  25 in switching from transcription to replication.</p> <p style="text-align: right;">Page 13</p>

<p>1 That's not fully understood and shows for filoviruses.  2 The virion protein 35 is the phospho protein  3 equivalent, is a cofactor of the transcriptase and  4 replicase and is shown to be an interferon antagonist  5 in the production involving blocking of induction of  6 interferon.</p> <p>7 Virion protein 30 is a part of the RNP  8 complex and it's a transcription activator for ebola  9 whereas that function for Marburg is not really well-  10 understood. Many genome systems say that replication  11 can occur without VP30 but rescue of the full length  12 chromosome was only working with that VP30 so that is  13 still open, so there might be a difference between the  14 two viruses here and then the RNA-dependent RNA  15 polymerase is the act of transcriptase and replicase.</p> <p>16 VP40 is the matrix protein equivalent.  17 It's very important for particle formation and  18 budding. And VP24 is another membrane associated  19 protein. It's an interferon antagonist involved in  20 blocking signaling of interferon so there's two ways of  21 interfering with the interferon system here with these  22 viruses. It might have a role in host adaptation. At  23 least it's involved in the adaptation to the rodent  24 models, the mouse model as well as the guinea pig  25 model and very recently, a paper came out saying that</p> <p style="text-align: right;">Page 14</p>	<p>1 transcription and then the switch to replication via  2 a full length encapsulated plus sense genome and then  3 the glycoproteins go through the ER Golgi pathway  4 whereas the other proteins go through ribosomes in the  5 cytosol and then budding takes place at the plasma  6 membrane driven by VP40 and mainly the glycoprotein  7 and other proteins might be involved in this.</p> <p>8 It should be mentioned that the anti-  9 replication cycle occurs in the cytosol and that is,  10 with the exception of the bornaviruses, the normal  11 situation for mononegavirales. So there are several  12 ways that one could imagine you could interfere based  13 on literature work with other viruses and other  14 systems. Of course, interfering with the attachment  15 is the first and one way to go. Whether this could be  16 achieved with neutralizing antibodies, I'll leave this  17 up for discussion. We all know that there is a lot of  18 dispute about the efficacy of neutralizing antibodies.  19 Do they exist? I think we know now that there are  20 neutralizing antiviral, neutralizing antibodies but  21 that they are -- have any effect or could be used as  22 treatment modalities has to be shown in the future and  23 there's also, once we have the receptor, there might  24 be receptor specific antibodies.</p> <p>25 In terms of diffusion, interferon</p> <p style="text-align: right;">Page 16</p>
<p>1 it might be involved in primary transcription and  2 binding to the nuclear protein complex and I think  3 Gary Nabel's group has alluded to that first, some  4 years ago already.</p> <p>5 But glycoprotein is the receptor binding  6 partner, the fusion mediates fusion and is an  7 important immunogen, just to recap this. Looking at  8 the life cycle, filoviruses seem to enter by receptor  9 mediated endocytosis. There have been several  10 proteins described that mediate or function in binding  11 or filoviruses bind to but none of them have really  12 been described as a receptor, so I think the bottom  13 line right now is there is no identified receptor or  14 maybe there are several receptors. We don't really  15 know whether they function as co-receptors but the  16 literature is quite controversial in this area. But  17 we know now the domain that is involved in receptor  18 binding that has been published not too long ago.</p> <p>19 Then endosomal effusion needs pH -- it's  20 pH dependent and cathepsin cleavage might be involved  21 in activation of fusion but aside of a couple of -- or  22 one or two papers there hasn't been any real follow-up  23 on this so this is an area that needs to be looked at.  24 Uncoating is an event that is, I think, not understood  25 at all for many viruses. Then we have primary</p> <p style="text-align: right;">Page 15</p>	<p>1 particles, interferon peptides or maybe cathepsin  2 inhibitors might be something to look at. There are  3 several approaches here that would target  4 transcription and replication, SAH hydrolase  5 inhibitors, nucleoside analogues, interfering  6 peptides, antisense, oligonucleotides, and I'm sure in  7 the second day we'll hear more about this. I just --  8 I look at my task here just to describe and introduce  9 you to some of these events.</p> <p>10 Processing inhibitors of the glycoprotein,  11 serine protease inhibitors, glycosylation inhibitors  12 might be a ways to go and then assembly inhibitors and  13 an example, interfering peptides might be something to  14 look at. So there's several ways in the replication  15 cycle of these virus and other viruses that we could  16 interfere with and hopefully develop antivirals or a  17 combination of antiviral strategies.</p> <p>18 What I want to do in the next, I think,  19 four slides is to just very briefly go through some of  20 the tools, and I think over the last decade or even  21 longer, we have developed in several of the labs,  22 tools that will be very helpful to go and look for  23 antivirals and screen antivirals. I'll start with the  24 pseudotype viruses. They were very instrumental for  25 looking into virus entry and this is just an example.</p> <p style="text-align: right;">Page 17</p>

<p>1 In this case it's the VSV system. What are these  2 viruses. These viruses, VSV in this case, could be a  3 retrovirus and other viruses that lack the  4 glycoprotein in the genome. They usually have  5 reported genes, and then by generating these viruses,  6 and infecting cells that express your glycoprotein of  7 choice. You can make pseudotypes whereas the  8 glycoprotein is not incorporated into the genome.  9 This makes it a VSL-2 a workable system and you can do  10 single staph infections with these, so you can mimic  11 virus entry and transcription and all steps of  12 replication and virus entry, you can't mimic really  13 transcription replication because this is dependent on  14 the vector system in this case.</p> <p>15 So powerful tools to study virus entry but  16 definitely need confirmation with live virus system  17 and I think Tony is always addressing this issue  18 because these are not filoviruses. They usually, in  19 the case of VSV, this is a VSV particle and so there  20 might be issues with this. For the filoviruses, we  21 have pushed it a little further. We have recombinant  22 viruses based on VSV. That system was developed in  23 Jack Rose's lab where we actually replaced the -- this  24 ebola or Marburg virus genome genetic information of  25 the glycoprotein. We create VSV particles that have</p> <p style="text-align: right;">Page 18</p>	<p>1 you need those four proteins that basically are  2 important for the RNP complex. So with these systems  3 you can look into transcription and replication but  4 you can't go further. And then the development of  5 the full reverse genetic system where you have the  6 same setup in terms of the support proteins, but you  7 have a genomic copy and Victor Walskoff and Hans-  8 Dieter Klein's group were the first to describe it for  9 ebola. There is a system for Marburg out and many  10 genome systems, as far as I know we have for Marburg,  11 ebola Zaire as well as Reston at least in a published  12 version.</p> <p>13 There might be in labs other systems under  14 development. We're working on the Reston full length  15 clone but we don't have it fully established at this  16 point. So minigenome systems are restricted analysis  17 of transcription and replication. Infectious clone,  18 a very powerful tool that needs high containment and  19 I think the generation of the infectious clone also  20 opened up a lot of discussion towards the potential  21 misuse of these technology and these viruses.</p> <p>22 Just one quick example, the first of  23 course, is a marker expressing ebola virus that would  24 be very, very helpful for working or antiviral  25 screening. I think Stewart Nichols' group was the</p> <p style="text-align: right;">Page 20</p>
<p>1 these proteins on the surface but also have it as  2 genomic information so these particles are replication  3 competent but they are attenuated, if you compare them  4 to VSV wild type but again, it switches their cell  5 tropism towards that of the donor virus from where the  6 glycoprotein comes. This is in jurkat cell so this is  7 the VSV wild type. It can replicate whereas these  8 recombinant viruses cannot replicate in these jurkat  9 cells just as one example.</p> <p>10 So again, there are powerful tools to  11 study virus entry that need again, confirmation. They  12 have the same issues than the pseudotypes except they  13 can bring it a little bit further because they are  14 replication competent and at the very end, if I have  15 time left, I will address very quickly the use of this  16 as vaccine vectors and in case of that, you need to  17 address the safety issues with this attenuated -- live  18 attenuated vectors.</p> <p>19 One of the hallmarks was the development  20 of the reverse genetics systems, starting with the  21 minigenome system, I think Elke Newberger and Stephen  22 Baka were the first to look into this. This basically  23 brings in a short version of the genome with just the  24 flanking trailer and leader sequences and a reported  25 gene, for example, green fluorescence protein and then</p> <p style="text-align: right;">Page 19</p>	<p>1 first describing a GFP expressing ebola virus. We  2 have done two versions; one where the ebola -- where  3 the GFB is expressed as a separate transcriptional  4 unit after DNP and one where it's expressed after the  5 VP24. So these viruses are very stable in tissue  6 culture if you use Vero cells. They don't lose  7 expression at all and for several passages. But if  8 you go in vivo, they are attenuated, and that has to  9 be taken into consideration. This shows your data on  10 Step 1 knockout mice because our system is based on  11 the wild type ebola virus and you can clearly see,  12 particularly with the one that has it further, the  13 three prime and the clear attenuation in virulence and  14 I hope Tom is not mad, but I think he put this virus  15 here into nonhuman primates and it was highly  16 attenuated there as well.</p> <p>17 So keep that in mind. They might be good  18 for in vitro systems but they have -- they're most  19 likely attenuated in vivo. So I think I'll skip this  20 one because this is the VLP systems. All of you know  21 that people are working very, very intensively on  22 enhancing the virus like particle systems,  23 incorporating minigenomes to mimic replication steps  24 of ebola in -- under BSL 2 conditions and I think this  25 is a very promising approach but currently not as</p> <p style="text-align: right;">Page 21</p>

<p>1 advanced and you can't make as much particles with  2 most of the systems at this point as you would need  3 for some of the studies.</p> <p>4 Very quickly, into the gene expression  5 strategy of the glycoprotein because there's a big  6 difference between ebola and Marburg. For ebola the  7 primary product of that gene is the precursor of a  8 soluble glycoprotein that does cleave by furin or  9 furin-like antiprotease into two secreted proteins,  10 sGP and delta peptide, delta peptide being about 40  11 amino acids of the C terminal part of the precursor.  12 And those are secreted.</p> <p>13 Ebola needs RNA editing and Tony Sanchez  14 and Mick Divalshkoff describe that first in order to  15 express the precursor of the transmembrane  16 glycoprotein that's then cleaved by furin or furin-  17 like endoprotease in GP1 and GP2, GP2 being the  18 membrane anchoring part. At least in cell culture,  19 this is unstable and GP1 can be released and Plank and  20 Moshkoff show that there is also a metalloprotease  21 cleavage which basically produces a transmembrane  22 anchor minus GP12 so ebola has four potential soluble  23 glycoproteins and the important transmembrane  24 glycoprotein. Marburg can only go this way. It  25 cannot produce these proteins.</p> <p style="text-align: right;">Page 22</p>	<p>1 cleavage, at least that's one of the theories. And  2 this is being supported -- Ud Stroher in my lab did a  3 lot of experiments with the furin inhibitor alpha 1-  4 PDX and that has only limited effects on in vitro  5 replication if you compare it, for example, to West  6 Nile virus where it has a dramatic effect and reduces  7 titers by up to four locks, and this reduces titers by  8 close to one lock but less than one.</p> <p>9 John put this virus into non-human  10 primates and to our big surprise, there was no  11 difference in disease progression and outcome compared  12 to wild types so that we concluded that the furin  13 cleavage is not important for infectivity and  14 virulence in this system. Victor Volchkov looked at  15 the editing side and he found if he knocks out the  16 editing side, he produces a virus with a very  17 cytopathic phenotype but the titers go down quite a  18 bit in tissue culture as a result of this and he  19 concluded that the editing is important as an  20 evolutionary event to maintain this virus in  21 replication and not kill the target cells too quickly.</p> <p>22 Generating this virus and putting it in  23 non-human primates resulted in no difference to wild  24 type virus so these viruses didn't kill any faster but  25 also didn't kill slower in terms of the non-human</p> <p style="text-align: right;">Page 24</p>
<p>1 I have already alluded to the functions of  2 these proteins. In regards to the soluble proteins,  3 I think the bottom line at this point and please don't  4 take any offense in the audience because I know there  5 are some attempts to define functions but at this  6 point it's very vague what these soluble proteins do  7 and in what way they function and if there is a  8 function, so I would like to leave it with this at  9 this point but structural analysis of most of these  10 proteins are either on the way or have been completed.</p> <p>11 Just as one example, cleavage and  12 infectivity, with the reverse genetic systems, Yoshi  13 Koboka's group together with us, we generated a furin  14 cleavage site mutant which generates a virus that  15 doesn't have a cleave GP. You lack GP1 and GP2 and  16 when you grow this virus, you can see a slight  17 attenuation in Vero cells but it still grows quite  18 nicely in these cells and definitely can infect and  19 replicate. So we believe that this may be due to the  20 unusual location of the fusion domain. It's not at  21 this end terminus of GP2 as with many comparable  22 viruses in terms of diffusion event and we believe  23 that disulfide bridging here might actually oppose  24 after structural changes and pH changed diffusion  25 domain which will allow it to fuse without the</p> <p style="text-align: right;">Page 23</p>	<p>1 primate model and we saw a complete reversion to the  2 volatile situation. So there seems to be a lot of  3 pressure to keep these editing site function in vivo  4 and this is data generated by Tom and I hope he  5 doesn't mind that I just bring this up here.</p> <p>6 One other thing in terms of the sGP, what  7 we've been working on, is a potential anti-  8 inflammatory function of the sGP. If you treat  9 endothelial cells with TNF you get a permeability  10 increase. If you concomitantly use sGP, you can  11 actually block that effect. So which would make some  12 sense in terms of the lack of infiltration that we see  13 in the host, in the infected host, and this function  14 is clearly structure dependent as we have recently  15 shown but we don't know at this point whether this has  16 any meaning for the virulence in the host and in the  17 pathomechanistic way that has to be shown in future  18 studies.</p> <p>19 Pathogenesis model is highly complicated  20 and I think whomever you ask you would come up with a  21 different kind of model. I think main events are  22 target cell activation or impairment in the case of  23 the dendritic cells that will have different effects  24 on the immune system as well as inflammatory kind of  25 storm situation that then have different events of</p> <p style="text-align: right;">Page 25</p>

<p>1 vascular leakage on most likely pushing T cells into  2 apoptotic pathway as well as tissue culture up  3 regulation with the consequence of disseminated inter-  4 vascular coagulopathy. So if you want to summarize  5 that maybe in very, very vague terms, it's highly  6 immunosuppressive with an inflammatory system that  7 comes to some extent a little bit similar to a SARS  8 syndrome or a sepsis syndrome even though it's  9 different from that.</p> <p>10 Target cell activation, here is some data,  11 leads to secretion of mediators in this case, TNF and  12 we've shown earlier that in an in vitro model system,  13 that it can increase permeability that can be  14 completely blocked by an antibody that blocks the --  15 or neutralizes TNF, so TNF seems to play a major role  16 here. And this target cell activation in terms of the  17 macrophages and monocytes, can be blocked by MEK 1 and  18 2 inhibitors, very potently in vitro but that hasn't  19 been tried into any animal model.</p> <p>20 Inter-vascular coagulopathy I think Tom is  21 going to speak about this, he found out that rNAPc2  22 might have an impact on ebola or has an impact on  23 ebola in the non-human primate system and that I will  24 let him allude to that and this is my last slide. And  25 I would just briefly mention coming back to the VSV,</p> <p style="text-align: right;">Page 26</p>	<p>1 replicate at least in vitro a little bit faster than  2 ebola and Marburg would do, so there might be an  3 establishment of an antiviral state. rVSV vectors are  4 likely to activate dendritic cells and might prevent  5 the activations for Marburg and ebola infection.  6 These are just some ideas. We're still working on the  7 mechanisms here.</p> <p>8 And in conclusion, I think tools and  9 systems to investigate antivirals and other  10 therapeutic intervention are established. Animal  11 models, I didn't speak about that, but others will do,  12 have been developed and characterized so we're happy -  13 - fortunate in that regards. Effective therapy most  14 likely needs a combined approach targeted distinct  15 mechanisms, effective vaccinations seems achievable.  16 Even so, I haven't spoken about vaccines, but that  17 will be the next session and if we ever want to put  18 treatment into the field, we may have to think about  19 onsite laboratory support to control it. I think  20 that's one other measure. But I think Tom will maybe  21 talk to that and I would like to stop here. Thank  22 you.</p> <p>23 (Applause)</p> <p>24 DR. FELDMANN: I don't know if I'm  25 supposed to take any questions but I may have been too</p> <p style="text-align: right;">Page 28</p>
<p>1 the recombinant VSVs, we're shown over the years  2 together with Tom that the VSV system can be potent in  3 pre-exposure prophylaxis but recently, or more  4 recently, we have also shown data that it can be used  5 as an exposure treatment modality. This -- in both  6 cases here, ebola here and Marburg, animals were  7 challenged with the high dose, challenged 1,000 plaque  8 forming units that uniformly kills these rhesus  9 macaques and then 30 minutes later they were treated  10 with the VSV expressing ebola GP or Marburg GP and in  11 case of ebola, four of the eight animals survived that  12 treatment.</p> <p>13 They were sick but they survived and the  14 survivors developed medium levels of viremia, so the  15 level of viremia is one of the parameters that we have  16 to look for in terms of survival and I think Tony will  17 come back to that in his presentation. And very  18 surprising in terms of the Marburg, all animals in  19 this case, five survived. We didn't find any viremia  20 in these animals and none of them got sick. This was  21 a very, very surprising result. Keep in mind this is  22 30 minutes after. We haven't pushed it any further.</p> <p>23 What might be happening here is that rVSV  24 vectors are likely to infect the same target cells  25 because they have ebola tropism. These virus</p> <p style="text-align: right;">Page 27</p>	<p>1 long anyway. Sorry.</p> <p>2 DR. CRUMRINE: If you have questions,  3 please use the microphones.</p> <p>4 PARTICIPANT: Actually, just a comment;  5 when you were talking about trying to figure out what  6 sGP actually is for, I think we need to remember  7 there's a maintenance host out there somewhere and  8 that if it does have a physiological function, it's in  9 that maintenance host. So once that's tracked down,  10 then maybe we can figure out some of these things.</p> <p>11 DR. FELDMANN: That is a well-taken  12 comment. We haven't really looked into that  13 particular issue. Yes, we may not see a function.  14 Even so, it's very abundant in the blood of patients.  15 I think Tony has shown that in one or two  16 publications. So yes, I agree with you, that would be  17 another possibility.</p> <p>18 PARTICIPANT: Have you been able to show  19 whether sGP can bind to the TNF or not?</p> <p>20 DR. FELDMANN: No, we haven't. We're  21 doing this and we haven't been able in the first set  22 of experiments to directly show binding to TNF but I  23 don't want to say that these experiments have been  24 finished at this point.</p> <p>25 PARTICIPANT: Heinz, as you were talking,</p> <p style="text-align: right;">Page 29</p>

1 it occurred to me that there's one area where we're  
2 notably lacking in information, that's on the  
3 polymerase gene. You know, for so many viruses,  
4 that's the first place we go when we try to develop  
5 anti-virals. So I'm wondering -- I know the bio-  
6 safety reasons and the bio-defense reasons why we  
7 limit access to it, but what do we know and should we  
8 be looking more at polymerase antagonists?  
9 DR. FELDMANN: Well, I think in terms of  
10 antivirals targeting the replication which to my view  
11 are the, you know, more classical antivirals, I think  
12 definitely we should, and starting with modeling. We  
13 know the most likely functional domains of this and  
14 some people working, many genome systems have been  
15 working with some of these aspects but I fully agree  
16 with you that that is one of the targets that should  
17 be targeted and may be also the co-factor or the co-  
18 factors in terms of ebola. But I haven't seen much.  
19 But I think with the systems that are now developed,  
20 if they can be put in to more high throughput  
21 screening systems and facilities being developed and  
22 I think that will come.  
23 I mean, I'm actually -- I mean, we heard  
24 that we don't have anything at the moment that we can,  
25 you know, stockpile or put forward. On the other  
Page 30

1 hand, I think filovirus is really a field that has  
2 advanced in knowledge so much over the last decade or  
3 maybe 15 years. I think this is one in -- my view one  
4 of the topics that needs to be discussed at a meeting  
5 like this, how can we push what we have further, but  
6 we also have to look into new avenues.  
7 DR. CRUMRINE: Thank you, Dr. Feldmann.  
8 I'd like to now introduce the second speaker, Dr. Tom  
9 Ksiazek, from the Special Pathogens Unit at CDC. Tom.  
10 DR. KSIAZEK: I certainly won't be as  
11 technical as Heinz, because I'm going to attempt to  
12 introduce what we have known from the field from at  
13 least the perspective of special pathogens. I'm from  
14 the Centers for Disease Control and we're involved, I  
15 think in response and emergency preparedness but our  
16 branch has tended to be focused more on the real  
17 disease and I'll give you a brief introduction to  
18 that.  
19 Filoviruses do cause real diseases in real  
20 people and in introducing the meeting, there was the  
21 suggestion again, that there is an outbreak ongoing  
22 right now. It's been ongoing for a couple or three  
23 months already before it finally gets discovered in  
24 terms of the ebola etiology of the outbreak, so it  
25 tends to highlight one thing, that these outbreaks  
Page 31

1 generally occur in pretty outback or far away places  
2 that are remote and logistically difficult to deal  
3 with.  
4 We, I think, Special Pathogens was created  
5 largely to deal with these serious diseases, to deal  
6 with high containment agents that have some threat of  
7 importation, I think at the time of the creation of  
8 the branch, but now we tend to dwell more on the bio-  
9 threat from adversaries than anything else. But what  
10 we know about the human disease actually comes from  
11 investigation of these outbreaks and it's  
12 unfortunately not as advanced as what we know from  
13 laboratory investigations and I think we're fortunate  
14 in that non-human primate models have provided, I  
15 think based on the casual observation of human disease  
16 and the very scientific investigation of non-human  
17 primates, a very good model with perhaps a couple  
18 limitations that the people are going to talk about in  
19 non-human primate models will tell you about.  
20 So what we do know is based on largely  
21 these limited experiences in outbreak situations and  
22 a couple of individuals that have been unfortunate  
23 enough to get or acquire laboratory infections.  
24 Again, I want to point out that outbreaks, when they  
25 do occur with rare exceptions have been at remote  
Page 32

1 locations. Can somebody reset that so it's not  
2 flashing at me all the time?  
3 And largely the primary objective becomes  
4 that of control of the outbreak and there is some  
5 conflict sometimes between that goal and trying to do  
6 an increase in scientific knowledge about these  
7 diseases. It very much limits our ability to take  
8 advantage of that opportunity and it's becoming  
9 somewhat more limited in recent outbreaks because of  
10 conflicts between different groups that have different  
11 roles in the response. And right now, we're  
12 attempting to sort out some of those differences in  
13 responding to the outbreak in Congo.  
14 So what I think probably the best way to  
15 review what we know from human outbreaks for the  
16 filoviruses, both Marburg and ebola is to, perhaps,  
17 look at the historical perspective of outbreaks as  
18 they've occurred so that we can sort of acquire the  
19 knowledge in the way that we have in responding to the  
20 outbreaks. And the total number of outbreaks of these  
21 agents is still not large by my count in the table  
22 that I'm going to use to present this it's still  
23 something you can count on your finger and toes but  
24 the last outbreak is going to break that prediction on  
25 my part.  
Page 33

<p>1 So first of all, we'll look at Marburg 2 which historically is, of course, the first outbreak 3 that did occur and I'm going to bring up some tables 4 which isn't up yet. Okay. These are on the CDC 5 Special Pathogens website and of course, the name 6 Marburg virus takes its name from an outbreak that 7 occurred in Germany in 1967 at a polio vaccine 8 manufacturing plant when primates that were imported 9 from Uganda infected the individuals that harvest the 10 kidneys for the production of tissue culture for the 11 production of the vaccine. And it was a relatively 12 small outbreak. It did occur in a developed country 13 where even though at the time you would consider this 14 a pretty advanced state of infection control and 15 critical care provided, there were secondary cases in 16 the individuals. So this sort of gave the virus group 17 an Andromeda strain-like appearance, in terms of the 18 nature of the outbreaks and sort of set the tone, I 19 guess for what followed.</p> <p>20 Not very long after that, the second 21 occurrence. And Marburg is marked by a number of very 22 small outbreaks and only a couple of any size at all. 23 I guess the first one would be much smaller than 24 perhaps, historically one of the last ones occurred 25 which was in Angola in 2005. However, the next</p> <p style="text-align: right;">Page 34</p>	<p>1 in 1987, another outbreak occurred which was also 2 associated with the Kitum cave. And this was a 15- 3 year old Danish boy coming back from school in Europe 4 visited the cave, became sick and was hospitalized in 5 Nairobi. He died giving a mortality, a grand total of 6 100 percent, one out of one.</p> <p>7 Then in 1998 through 2000 there's what's 8 called the Durba or Durba-Watsa outbreak, but this is 9 actually a series of small outbreaks where there are 10 primary cases that emanated from a gold mine in the 11 area and then had short-change of transmission that 12 added up to a fairly substantial number over this long 13 period of time. Unfortunately, the way, in general, 14 the outside public found out about this is that the 15 physicians, two of the individuals giving medical care 16 in this particular location, became sick and died and 17 that's really what brought the attention of the 18 authorities and the response that eventually got 19 mounted in 1999.</p> <p>20 And this was quite interesting from an 21 epidemiologic perspective because we were able to make 22 isolates from a number of these transmission chains 23 and we found that these were unique events. So that 24 the source of the infection, a miner who went into the 25 mine, each was infected with a unique virus and that</p> <p style="text-align: right;">Page 36</p>
<p>1 outbreak was in Johannesburg, South Africa when a 2 traveler, an Australian, and his girlfriend returned 3 from Zimbabwe. He became sick and during the course 4 of his illness, infected a nurse and his girlfriend. 5 He died. The two other individuals survived. So you 6 can see the mortality in the Marburg incident was 21 7 percent and in this instance, one out of three, 33 8 percent. So these numbers sort of begin to 9 accumulate.</p> <p>10 Then there was an outbreak involving a 11 French engineer who had visited the Kitum cave which 12 is on the border between Kenya and Uganda, and not 13 really very far from where the original monkeys had 14 been collected that were the source of the Marburg 15 outbreak and that individual then infected a physician 16 in the hospital in Nairobi where he was treated. He 17 died and the physician survived giving a mortality in 18 this instance of about 50 percent.</p> <p>19 The clinical information probably from 20 Marburg and there's a book by the folks who work with 21 this, "The Blue Book," that sort of documents what was 22 accumulated in terms of the clinical observations that 23 you should refer to and there's a set of references 24 you can get from this table or website that goes with 25 each one of these outbreaks that summarizes it. Then</p> <p style="text-align: right;">Page 35</p>	<p>1 short chain then carried on. So you could find much 2 has been described with ebola that you have an 3 individual who's introducing the virus into the 4 community and with Marburg the chains have generally 5 been short, whereas, with ebola they've been a little 6 bit longer and the outbreaks a little bit more 7 sizable. So that's one of the things, I think, that's 8 accumulated and I think it's also interesting that the 9 -- this outbreak essentially has genetic variation in 10 terms of the individual viruses that covers the entire 11 spectrum of genetic diversity that we find in the 12 ebola viruses, so it's interesting, I think, from that 13 perspective as well.</p> <p>14 And then the largest outbreak on record is 15 one that occurred in 2005 in northeastern Angola in a 16 town called Uige, which unfortunately in this country, 17 which had been through a long revolution, was an area 18 that was on the side that lost the war and was a 19 little bit politically neglected. That may have had 20 something to do with the poor response that led to the 21 outbreak actually being more sizable than these 22 official figures that I reported in the table. These 23 are the numbers that the Ugandan government has 24 reported. The databases at the end of the outbreak 25 were at about 420 cases. So and the mortality was</p> <p style="text-align: right;">Page 37</p>

<p>1 pretty much the same. So they cleaned up the data  2 base and excluded a fairly large number of cases for  3 reasons that I'm not entirely clear on myself.</p> <p>4 And then just very recently in the last  5 few weeks, we had the team members from our branch  6 that have come back from investigation of a very small  7 incident also associated with a mine in Uganda and the  8 common thread of these mines and caves is that there  9 are creatures that inhabit these that we and Eric  10 Leroy's group in Gabon have just recently published a  11 finding that we believe that there's one particular  12 species of bat, a fruit bat, that's found through a  13 wide range in Africa that has evidence of infection  14 with this. So this is beginning to make, perhaps a  15 little bit more sense over a long period of time. So  16 that outbreak involved only two miners. One case was  17 fatal, the other one survived, a mortality of 50  18 percent.</p> <p>19 So as Heinz pointed out, the mortality is  20 quite variable and I think probably Tom Geisbert will  21 talk about there are differences, also in the primate  22 lethality for these viruses with the Angola outbreak  23 which had the highest reported human mortality  24 seemingly being quite more virulent for non-human  25 primates as well.</p> <p style="text-align: right;">Page 38</p>	<p>1 protect medical care personnel well beyond what's  2 seen. But unfortunately these outbreaks occur in  3 resource poor areas where even that degree of  4 protection is not afforded to the medical personnel on  5 a daily basis.</p> <p>6 Unfortunately, after those two outbreaks  7 occur and during some of the initial investigations in  8 animal models, there was a lab infection that occurred  9 in England at Porton Down which is the third row of  10 this table. And then a single case with Zaire  11 occurred in 1977 in a mission station in northern  12 Zaire which is documented there and then Sudan there  13 have been a number of repeats now, the first of which  14 occurred in 1979 in the same area, associated with the  15 same cotton factory for the index cases and once  16 again, involving some dissemination through the  17 community through its occurrence at medical care  18 facilities again without lack of or with lack of  19 resources to support adequate infection control.</p> <p>20 And then something that, while I was at  21 Fort Detrick, occurred which put ebola back on the map  22 and in Time magazine and Newsweek and everything else  23 was an outbreak in a primate importing center that  24 occurred in 1989 in Reston, Virginia, just down the  25 road from here. And that outbreak which involved one</p> <p style="text-align: right;">Page 40</p>
<p>1 And ebola is a little bit longer story.  2 It did occur after the initial Marburg outbreaks, the  3 first two actually, with simultaneous outbreaks that  4 occurred in Sudan and Zaire which give their name not  5 to the species, although from my perspective, in a  6 backwards manner. And the outbreaks were pretty  7 sizable and I would suggest were investigated very  8 much from a retrospective point of view. A few of  9 these patients were seen in Kinshasa but most of the  10 investigation was after the outbreak had actually  11 already occurred and the sort of outside western  12 groups that went in did largely tote this up by  13 counting cases after the fact.</p> <p>14 It started in both instances and was  15 spread as ebola has been prone to do at least in early  16 outbreaks in medical care facilities where inadequate  17 infection control practices actually lead to that  18 being sort of the center of the outbreak and actually  19 serving as the vehicle through which infection occurs.  20 And the thing that's been demonstrated in later  21 outbreaks that the degree of infection control need  22 not be so severe as some of the photographs you might  23 see suggest. Just probably something we might call  24 standard precautions actually is probably adequate to  25 really greatly reduce the number of cases and probably</p> <p style="text-align: right;">Page 39</p>	<p>1 of the four species newly described at the time ebola  2 Reston appears to be quite pathogenic for non-human  3 primates and there were a couple of studies done both  4 by Peter Jahrling and also by the then residents of  5 Special Pathogens Branch.</p> <p>6 It's not as severe or pathogenic as the  7 prototype ebola Zaire and ebola Sudan strains for  8 primates but still does a pretty good job. There were  9 several people infected at the facility who were  10 giving care, none of which became ill, one of which  11 was documented because he had gone in because of his  12 diabetes to have a toe or the bottom of his leg  13 removed as I recall.</p> <p>14 There were several repeats of that so I  15 wouldn't count these actually in terms of the 1990 and  16 '89/'90 outbreaks in the Philippines as separate  17 outbreaks. The outbreak did issue forth from the  18 Philippines where the macaques were imported from.  19 There were some suggestion of human infections  20 associated with export facilities in the Philippines  21 but they used the IFA test which was a little bit  22 prone to nonspecific reaction to do that diagnosis.</p> <p>23 There was a repeat of the Reston episode  24 that involved a polio vaccine manufacturing plant  25 again in 1992 in Italy. The primates that were</p> <p style="text-align: right;">Page 41</p>

<p>1 imported into Italy issued forth from the same  2 Philippine facility which after this outbreak was  3 eventually closed for good. So the source of that  4 particular outbreak and where the Reston virus came  5 from is still a little bit of a mystery. However,  6 having been a number of times to the facility in the  7 Philippines, it happened to have an orchard and there  8 were a great number of fruit bats that frequented the  9 primary care facility, the primate care facility so  10 that perhaps, given more recent data as the source of  11 that virus but that's unconfirmed at the moment.  12 And there have been a series in Gabon and  13 Congo in Central Africa, the first of which was noted  14 in 1994. I think it was diagnosed initially as the  15 yellow fever outbreak and shortly after that there was  16 the fourth species discovered in a single case in a  17 investigator who did a post on a dead chimpanzee in  18 the Tai Forest where there had been some excess  19 mortality in both gorillas and chimps and that  20 individual became sick, was treated in Switzerland,  21 did recover but the virus was recovered and sequenced  22 by Bernard Legino who was at the Institut Pasteur at  23 the time.  24 And then the reoccurrence of Zaire after  25 almost 20 years occurred in 1995 in a large outbreak</p> <p style="text-align: right;">Page 42</p>	<p>1 professional, a physician left Gabon, traveled to  2 South Africa and there infected one critical care  3 nurse, who I think was involved in intubating him.  4 She died, he survived, giving a mortality of 50  5 percent for that particular small incident. These  6 outbreaks, as you can see as you go down through the  7 Zaire outbreaks are pretty commonly with mortalities  8 that vary from usually around 75 up through 90  9 percent.  10 And there was yet another Reston, small  11 Reston episode that occurred in 1996 when one building  12 half of a shipment was contaminated with ebola Reston  13 virus which was associated with once again, the  14 Philippines. And more recently, a small Sudan  15 outbreak that occurred in Southern Sudan was  16 investigated and -- I skipped over one here. The Gulu  17 outbreak also provided a fair amount of opportunity  18 because of a mission hospital that had fair logistics  19 to do some followup and some look at basic immunology  20 of some of the survivors was afforded by that  21 particular outbreak, this being a little bit less  22 remote than some of the other outbreaks.  23 However it was in sort of a high risk area  24 in terms of the security, there being rebel forces  25 that were active in the area, and travel at night and</p> <p style="text-align: right;">Page 44</p>
<p>1 in Kikwit, centered around two hospitals there. This  2 outbreak afforded a little bit more opportunity to  3 actually observe patients as teams from WHO, CDC, and  4 various MSF Medecines Sans Frontieres were able to  5 respond and there was with great effort, some  6 logistical support for the collection of clinical  7 specimens. It also was an outbreak in which there was  8 some effort to use immunotherapy in that a small  9 number of patients, six or seven, I can't remember the  10 exact details, were given whole blood from individuals  11 that had had the disease and recovered and were in  12 convalescence and the numbers were too small to  13 perhaps make anything out of it but it looked like  14 this may have had some promise but the ability to  15 retain whole blood and use that for immunotherapy  16 other than from patients in the immediate area is  17 probably a difficult thing to carry out. But it did,  18 at least, I think at that time, attempts to protect  19 primates, non-human primates, from infection using  20 various types of vaccines had been unsuccessful at  21 least light a spark that perhaps re-interested people  22 in ebola vaccines.  23 Again in -- there's a series of Gabon and  24 Congo outbreaks which includes an exported/imported  25 case that occurred in Johannesburg when a medical care</p> <p style="text-align: right;">Page 43</p>	<p>1 other things that did impede somewhat the outbreak  2 investigation. And then again, central Africa with  3 Gabon and Republic of Congo, small outbreaks some  4 maybe medium sized, all of them individually  5 associated usually with exposure to a primate or an  6 animal found in the forest probably not the primary  7 reservoir which has now been suggested to be bats as  8 more recently Marburg has been as well.  9 This is just a series of those that  10 occurred until more recently the Southern Sudan  11 outbreak that was relatively limited and was mixed up  12 with a measles outbreak that was occurring  13 simultaneously and that brings us to the current  14 outbreak which the numbers are not very solid in terms  15 of what's been reported so far.  16 So I make the totals in terms of outbreaks  17 if one totes up the Philippine and Reston and South  18 African Gabonese outbreaks, seven individual Marburg  19 outbreaks, excluding also lab infections and 20, now  20 21 with the recent ongoing outbreak in DRC occurring.  21 At this point, I'd just like to open it up for  22 discussion or questions based on what the experience  23 has been. Again, I would sort of hasten to add that  24 most of the information we have about pathogenesis is  25 more based on the non-human primate model than it has</p> <p style="text-align: right;">Page 45</p>

<p>1 been on that derived from field investigations of  2 outbreaks that have occurred. And also I'd just like  3 to acknowledge the founders, supporters and subsequent  4 members to Special Pathogen Branch and our long-term  5 collaborators at USAMRIID and the South Africans and  6 WHO, who's in the field investigations is always a  7 partner as well.  8 (Applause)  9 MS. CLAUSEN: Tom, I don't recall seeing  10 it but that outbreak, not the outbreak but the patient  11 or the lab worker that was infected in Russia, was  12 there ever any writeup about that?  13 DR. KSIAZEK: I haven't seen other than a  14 news report. You're talking about the one last  15 summer?  16 MS. CLAUSEN: Yes.  17 DR. KSIAZEK: I haven't seen any  18 scientific publications about that. Heinz, have you?  19 No, I mean, it sort of made the news and the  20 community, it bounced around in.  21 DR. FELDMANN: Tom, you briefly touched  22 the issue of the bats and in your paper in terms of  23 Marburg you basically if I -- I should know it because  24 it just came out but you basically had one species of  25 fruit bat that was positive. I know that Botswana has</p> <p style="text-align: right;">Page 46</p>	<p>1 actually found antibody in some of the same species in  2 the investigation from Uganda. Those bats were all  3 collected in '99 and 2000, I believe, or maybe all  4 even in 1999.  5 PARTICIPANT: Tom, you nicely summarized  6 the mortality in the various different outbreaks. I'm  7 curious though as to the prevalence of exposure and  8 seropositivity in various outbreaks. In other words,  9 do you have asymptomatic infections and what -- how do  10 they vary among different outbreaks. Do you have any  11 data on that?  12 DR. KSIAZEK: Well, we've done, as other  13 people have with previous technologies, antibody  14 surveys and I find it to be a little bit confusing  15 because first of all the populations are difficult to  16 test in that they're usually very sticky sera which  17 makes it a little bit harder to sort out the signal  18 from the noise. But we don't find high prevalences.  19 We certainly do find a few individuals that are  20 reactive which also can be made to react in a Western  21 blot but we sometimes are a little bit confused about  22 the results even having gone that far. But there's  23 not a lot of indication that there are many infections  24 without subsequent disease, particularly in people  25 that have a high degree exposure like medical care</p> <p style="text-align: right;">Page 48</p>
<p>1 done, I guess with you, I'm assuming with you in Durb-  2 Watsa. Those results are not published but I heard  3 they are basically under review. Is that -- can you  4 comment? Is that the same bat species because in  5 ebola, we seem to have several versus Marburg, your  6 study really focused basically, I think with one  7 exception on one particular species, didn't it?  8 DR. KSIAZEK: Yes, I mean, the story is a  9 little strange in that the Marburg results actually  10 came from an ebola investigation that we were doing in  11 conjunction with Eric Leroy in Gabon and they also  12 were screening the bats, which in our hands have all  13 been negative for ebola actually in more recent  14 collections rather than the published paper, but they  15 did get a couple of hits with Marburg virus, sequenced  16 it, found that these were all from one particular  17 species, the Egyptian tomb bat, Rosettas egypticus,  18 and the antibody results in this instance did also  19 support that.  20 Bob has, I think three or four species  21 which I'm not even sure that Rosettas is really  22 represented in those species and it was at the time  23 that we were involved in it, PCR results only but  24 we've had a bat conjugate made which has increased the  25 efficiency of doing antibody assays and he's now</p> <p style="text-align: right;">Page 47</p>	<p>1 personnel.  2 If -- we have done surveys after outbreaks  3 in those individuals and really don't find that there  4 are many individuals that have had a reasonable degree  5 of exposure who have seroconverted but have not had  6 the disease.  7 DR. ADBY: Sir, Tony Adby from USAMRIID.  8 In that most recent outbreak or sporadic outbreak,  9 consistent of the two individuals that you referred  10 to, on PubMed they had mentioned that one of the  11 individuals, and I don't know if it was the one that  12 survived or not, had skinned a colobus monkey several  13 weeks prior and the hide was recovered and I believe  14 sent to the CDC and I was wondering about the results  15 of that.  16 DR. KSIAZEK: Yes, I mean, it was a black  17 and white colobus which I would describe as a red  18 herring. There were a number of issues with it. It  19 was actually skinned at a date that was probably  20 incompatible with the onset of disease from what we  21 know about Marburg to begin with and then we did test  22 the specimen but the hide, essentially, of the monkey,  23 was all that survived. It had been treated. We  24 couldn't derive any PCR signal. We did make isolation  25 attempts and also looked for antigen in the skin by</p> <p style="text-align: right;">Page 49</p>

<p>1 you know, histochemistry. There's no suggestion the 2 monkey was involved at all, I guess to the extent the 3 tests were valid given the conditions.</p> <p>4 MR. NEWSOME: Ed Newsome, NIAID. Tom, I 5 know we'll come back to this later today but I wonder, 6 since you're on the topic, if you could address route 7 of exposure. I mean, obviously, if there's dirty 8 needles and blood transfusion involved it's obvious, 9 but initial cases from people in mines and so forth, 10 it seems to me like aerosol transmission is possible.</p> <p>11 DR. KSIAZEK: Yes, our assessment is that 12 aerosol plays almost no role in the occurrence of 13 natural disease. It's not that it doesn't occur but 14 in the Kikwit outbreak there was a fairly thorough 15 look at that and there was no suggestion that people 16 that occupied common dwellings that were sort of a 17 size and nature that you would have expected -- if 18 there were aerosols to be created that it would have 19 led to infection of the individuals that were co- 20 resident.</p> <p>21 We believe that most of it is sort of 22 direct exposure to individuals, particularly in the 23 latter part of the disease when they're shedding or 24 secreting fairly large amounts of the virus by several 25 routes, probably mostly from mucosal surfaces and</p> <p style="text-align: right;">Page 50</p>	<p>1 transmitted?</p> <p>2 DR. KSIAZEK: Well, the epidemiology just 3 doesn't support that as a means of transmission, that 4 it requires individuals who have had direct contact as 5 opposed to being sort of present in areas where if 6 there were an aerosol being created, it would be 7 infectious. Certainly aerosols are infectious, if you 8 mechanically create them in a laboratory environment, 9 but I'm not at all saying that's not a route that 10 hasn't been demonstrated to be very infectious with 11 these viruses.</p> <p>12 But, we believe, for instance, that 13 medical care personnel needn't wear space suits or 14 full body respirator sort of protection things like N- 15 95 that really represent what we consider to be more 16 droplet protection is an efficient level of personal 17 protection in these outbreak situations and the 18 outbreak in Kikwit where essentially no medical 19 personnel save one individual who had sort of a funny 20 exposure that can be explained after the introduction 21 of these sorts of practices were infected, in spite of 22 the presence of patients almost continuously for 23 another couple of months during the outbreak.</p> <p>24 DR. CRUMRINE: Thank you, Tom. I think 25 it's time we move on and now I'd like to turn the</p> <p style="text-align: right;">Page 52</p>
<p>1 bleeding probably increases the amount of blood that's 2 present, although there is some, I would say 3 controversy in that immunohistochemistry particularly 4 for Zaire and Sudan, is a useful diagnostic technique 5 on cadavers and one of the places where the virus is 6 found in the skin is actually in sweat glands, so if 7 the virus secreted enough that actually the skin 8 itself may be a source of the virus.</p> <p>9 We did, I think in the Gulu outbreak, make 10 some attempt to swab skins and we couldn't find that 11 there was any recoverable virus and I think in one out 12 of many specimens only could we find it by PCR using 13 techniques that we reckon are pretty sensitive.</p> <p>14 DR. DONG: John Dong from GenPhar, Inc. 15 Actually, I would like to have a follow-up question 16 with regard to aerosol transmission. In animals, 17 clearly as shown by Tom Geisbert and others, the 18 aerosol virus can infect in animal models. And also 19 recent outbreak in the gorillas and one mode of 20 transmission suggested has been the animal sneezing. 21 That's also suggesting aerosol transmission.</p> <p>22 In human, the first symptoms of fluid 23 build-up in the lungs is coughing. So that also could 24 create a lot of aerosol containing viruses. And what 25 evidence suggesting those aerosol will not be</p> <p style="text-align: right;">Page 51</p>	<p>1 microphone over to Peter Jahrling.</p> <p>2 DR. JAHRLING: Thank you. I apologize for 3 being late. Those of you who know me know that nobody 4 expects to see me before 10:00 o'clock in the morning. 5 Even when I try to get down here early, two and a half 6 hours on 270 this morning was no picnic.</p> <p>7 I apologize for missing Heinz's talk which 8 I know is always extremely good. Mike Bray and I have 9 just been reading our Blackberries in the margins and 10 it appears that there's some 347 reported cases in the 11 DRC outbreak now with 140 something deaths, some 12 suspicions that the virus is less virulent than what 13 we've seen before but that could be because all 14 suspect cases are being counted as ebola right now, 15 but certainly a major outbreak. And that information 16 is coming from Karen Hawkins-Reed, who's the CDC 17 individual at the DRC.</p> <p>18 So moving right along now, we'll go to 19 comparison of epidemiology clinical -- no, that was 20 Tom wasn't it, Biomarkers in Human Pathology and Host 21 Response to Filovirus Infections by Tony Sanchez of 22 CDC.</p> <p>23 DR. SANCHEZ: Thanks, Peter for the 24 introduction. It's good to be here at the NIH and as 25 Heinz said in his talk, he was a little unsure about</p> <p style="text-align: right;">Page 53</p>

<p>1 what he was supposed to and so was I. So I had to 2 take a look at what exactly biomarker meant in terms 3 of this presentation. And I went to a site on FDA and 4 found this definition, "A characteristic that is 5 objectively measured and evaluated as an indicator of 6 normal biological pathogenic processes, or 7 pharmacological responses to therapeutic 8 intervention." So basically, one is looking at 9 substances that one can measure in terms of the 10 disease process and in vaccine and drug development 11 this would be very important.</p> <p>12 Well, I'm going to be echoing a lot of 13 what was already said, I think by Tom and Heinz but 14 moving on, we have an incomplete picture of human 15 pathology and events leading to severe disease but has 16 the characteristic similar to septic shock late in the 17 course of filovirus disease. As Tom said, there's 18 been few opportunities to study these infections, 19 mostly since the areas in which these outbreaks tend 20 to occur are very remote and ability to safely go in 21 and sample and isolate the materials one needs from 22 the patients to do the testing is dangerous and we 23 often times opt in favor of safety and not to perform 24 bleeds on patients if we aren't totally comfortable.</p> <p>25 So but there have been occasions where we</p> <p style="text-align: right;">Page 54</p>	<p>1 virus preparations for ebola virus of Zaire, Sudan, 2 Ivory Coast and Reston species as well as three 3 isolates of Marburg virus. And also shown here, as 4 Heinz talked about, was non-structural sGP. This is 5 a Zaire version of that. And as I said, proteins have 6 been characterized to a large degree but mostly the GP 7 has been focused on, as the greatest amount of work 8 done and Dr. Gary Nabel's group has identified a 9 cytopathic or a toxic effect associated with GP1 mucin 10 like region, at the C terminus of that molecule that 11 projects outward and if one deletes that sequence, one 12 shows a decrease in CP and may have an important 13 effect in the human patients. It's been verified in 14 other laboratories, but the details of how that might 15 work in human patients is still unclear.</p> <p>16 Again, Heinz talked about sGP, unclear 17 role in pathogenesis, antagonistic effect of VP35 and 18 of course, recent findings that VP24 inhibits 19 interferon signaling and is a virulence factor in 20 adaptation to animal models. I did not include in 21 this, and I apologize, GP2 also has an 22 immunosuppressive motif in it that may be important in 23 the immunosuppression that takes place very early in 24 disease and this is a very big question as to how this 25 occurs in setting up this pathogenesis, this severe</p> <p style="text-align: right;">Page 56</p>
<p>1 have collected specimens. Disease characteristics are 2 not identical to those of animal models. The closest 3 one is a non-human primate but that model, as we'll 4 hear from Tom Geisbert is more accelerated but is very 5 close to human. Studies of human infections, early 6 outbreaks of filovirus disease provided limited 7 information, mostly clinical observations and a little 8 bit of blood testing looking at liver enzymes but 9 there's no comparisons quantitation of those values, 10 which ones are real, what the prognostic value of 11 those were in terms of patient living or dying. 12 Recent episodes have provided better insights into the 13 disease process and I've listed some here. I would 14 point to the Kikwit, the Gabon and the Gulu episodes 15 as providing a large amount of information.</p> <p>16 Blood samples taken from patients and 17 tested in the lab for -- to see what substances one 18 can identify that would be associated with severe 19 disease and hopefully this new outbreak in DRC will 20 provide additional information. So in terms of 21 biomarkers, one can look at the virus or the host. 22 For filovirus the proteins and RNAs have been well- 23 characterized and I'll go into those.</p> <p>24 For the filovirus proteins, this is an SES 25 page showing the structural virion proteins, purified</p> <p style="text-align: right;">Page 55</p>	<p>1 acute infection.</p> <p>2 So all these proteins can be targets of 3 antibodies, polyclonal, monoclonal to monitor the 4 disease. And depending upon what aspect of it you're 5 looking at, one can use these reagents to follow. 6 Such assays as IFA are used in identifying 7 filoviruses, it's very subjective but in my 8 experience, it's very easy to perform. You can do an 9 IFA in 20 minutes, and if you want to check on the 10 presence of the virus, it's very easy to perform. 11 Given the right reagents, you can have a very clean 12 reactant. The problem is that you get non-specific 13 reactivity in diagnostic assays. It's much cleaner in 14 ELISA-based assays such as the antigen capture. 15 ELISA, the ebola version of that works much better 16 than the Marburg but we're looking to improve that 17 situation now.</p> <p>18 Immunohistochemistry is very useful in 19 handling specimens that have been fixed in formulants 20 and from the field, also if one is working with animal 21 models, being able to fix the carcass and later go 22 back and look at infections in various tissues. It's 23 very nice. It also gives the -- has the luxury of 24 having an indication of any pathology associated with 25 the tissue, with the filovirus staining.</p> <p style="text-align: right;">Page 57</p>

<p>1 Immunoblot or Western blot assays in flow 2 cytometry are easily performed. Well, this RT-PCR 3 assays for filoviruses have been around for a long 4 time, since the genetics started from I'd say the 5 early '90s and the conventional Agarose 6 electrophoresis method detection of PCR products has 7 been displaced by real time detection, FRET 8 quantitation using either TaqMan primarily or 9 molecular beacons as probs. Now, virus load is very 10 important in assessing the state of a patient and what 11 we found is that quantitation of either viral RNA or 12 antigen, the virus loads are associated with fatal 13 outcomes.</p> <p>14 Lower levels of antigen are associated 15 with mild disease and that's very logical. And I 16 should point out that the amount of antigen in very 17 severe cases is massive. It's not very hard to detect 18 in terms for ebola Zaire. For Marburg and Sudan 19 they're not as much but it's very easy to detect.</p> <p>20 Moving on to host biomarkers, and one 21 thing that I forgot to include was fever in this 22 presentation. One can monitor the fever. If there's 23 an acute infection, one can see a rise that 24 corresponds to the severity of the disease and the 25 decrease as the patient recovers. Human immune</p> <p style="text-align: right;">Page 58</p>	<p>1 virus clearance. That's not to say that late in 2 infection they don't assist but there is -- it doesn't 3 seem from all the studies that have been done, 4 patients that have been looked at, that it has a very 5 prominent role. And as Heinz pointed out, filovirus 6 particles are resistant to neutralization by antisera 7 and antibody binding. Some studies have shown that it 8 may actually enhance virus entry.</p> <p>9 Okay, cellular immune response are 10 critical to the efficient clearance of virus from the 11 body through cytotoxic T cells. PBMCs from patients 12 that survived ebola virus infection had higher numbers 13 of T cells than those that did not and these surviving 14 patients also had increased numbers of CD8+ and 15 activated CD8+ T cells. Gabon ebola Zaire patients 16 showed a similar result in that patients fatal cases 17 had decreased expression of CD3 perforin and 18 interferon gamma. In ebola Sudan patients, fatal and 19 non-fatal outcomes are linked to an HLA-B profurin and 20 we were able to take a look at a subset of the ebola 21 Sudan infected patients in Uganda and look at their 22 HLA-B locus and these are the alleles that we found 23 throughout those patients and what we were able to 24 show statistically is that B7 allele is associated 25 with survival while 15 and 67 are associated with</p> <p style="text-align: right;">Page 60</p>
<p>1 response, these are delayed in filovirus patients. 2 This little box shows the appearance of RNA and 3 antigen in the blood and the thick areas indicate 4 where one would see the highest incidence of a 5 positive result. Actually antigen should be extended 6 a little bit more to include the Sudan from our Gulu 7 experience but we seen that post-onset from, oh, for 8 the RT-PCR about minus one out to 20 or so. It's hard 9 to -- 15 or 20, we can get a very strong signal.</p> <p>10 Antigen peaks out at this region. The 11 mean time of death for patients post-onset is from 12 about seven to 10 days but we see that IgM antibody 13 and IgG turn up after this time. And these are ELISA- 14 based assays which we've tacked down the antigen on 96 15 full plates and can bind specific antibody to those 16 antigens. And in the case of the Gulu outbreak, some 17 of the patients did not develop antibody until after 18 the antigen cleared from the blood and we were holding 19 patients until they seroconverted to release them into 20 the public and some of them we had to hold onto for a 21 long time because they weren't seroconverting. So 22 antibody may not be perhaps a good biomarker in 23 following the disease but it's -- there you have it.</p> <p>24 Okay, during the acute phase of infection, 25 antibodies do not appear to have a prominent role in</p> <p style="text-align: right;">Page 59</p>	<p>1 fatality.</p> <p>2 Cytokine production, cytokines are 3 expressed in patients but the role in stemming or 4 enhancing the progression of a disease is unclear. 5 Prolonged exposure to virus antigen may actually 6 induce an unresponsive state. And the amount of 7 antigen in the blood is massive in the virion fatal 8 and at the peak time of disease. It's not difficult 9 detecting antigen in the blood. Apoptosis is a 10 component of the pathology. Leukopenia and appearance 11 of atypical lymphocytes is likely due to unregulated 12 apoptosis that is taking place. How this takes place 13 is unclear in terms of ebola virus and Marburg virus 14 infections. And apoptosis is associated with fatal 15 outcomes in ebola Zaire patients.</p> <p>16 I would like to now move onto some blood 17 chemistries that were performed on the Uganda patients 18 in 2000/2001 that we use a portable Piccolo system 19 where we could do clinical chemistries and back in CDC 20 we're able to look at those specimens and analyze them 21 further for a couple of other compounds but what we 22 found out from looking at those serum specimens is 23 that in fatal cases compared to non-fatal, there was 24 a higher level of nitric oxide and also looking at D- 25 dimers, were much higher also than in non-fatal cases.</p> <p style="text-align: right;">Page 61</p>

<p>1 And these two substances, nitric oxide 2 will cause a dysregulation in the homeostasis of the 3 cardiovascular system and the D-dimers will disrupt 4 the micro-circulation and cause organ damage and 5 failure. So these are very bad markers once we reach 6 high levels for these patients. We see an increase in 7 the AST over the ALT but AST is not restricted to the 8 liver. And we believe that a lot of the elevated AST 9 may come from tissues other than the liver, the heart 10 or muscles and ebola virus is affecting a wide range 11 of organs. So that may not represent a problem with 12 the liver. We also see that the glucose and total 13 bilirubin are not disrupted over normal over non- 14 infected patients and non-fatal cases, so the liver 15 seems to be functional and not in that great a 16 distress, although it is highly infected.</p> <p>17 Amylase is elevated, pancreatitis is set 18 up in these patients, BUN and creatinine, there's some 19 renal problems taking place. And albumin and calcium 20 is down and the calcium may be due to -- in part to 21 this pancreatitis that is set up. So we have a very 22 unhealthy picture here in these patients. And it 23 leads to a syndrome, a shock syndrome that's similar 24 to septic shock. And these are -- this table shows 25 the thresholds of these various compounds that when</p> <p style="text-align: right;">Page 62</p>	<p>1 DR. JAHRLING: Questions for Dr. Sanchez? 2 DR. CHOW: Gary Chow from DTRA DoD. 3 That's a pretty good presentation. I wonder whether 4 CDC or someone else in the audience here, you know, 5 have good list of the biomarkers to correlate the 6 animal model and the human case, if we have subject 7 data or we don't have.</p> <p>8 DR. SANCHEZ: We have a lot of information 9 from the non-human primates and then guinea pigs that 10 correlate with these. In fact, the D-dimer testing 11 was suggested by Tom Geisbert and we added that and we 12 were able to show that DIC is a component of the 13 disease in humans as suspected but that was kind of 14 like the nail in the coffin. So those parameters that 15 we looked at are present in at least non-human primate 16 models so there's no problem following them.</p> <p>17 DR. JAHRLING: Several more times during 18 the discussions in the next couple of days, I think we 19 know a whole lot more about these things in animal 20 models than we do in the humans, which is great but it 21 does beg the issue when we're trying to cross -- when 22 we're trying to get things through the FDA under the 23 Animal Efficacy Rule, if we can't make paralance to 24 what's going on in humans, we've only got one side of 25 the equation solved. I'm really pleased to see some</p> <p style="text-align: right;">Page 64</p>
<p>1 one reaches, one can expect a very poor prognosis for 2 the patient.</p> <p>3 Okay, conclusions and observations, 4 there's an assortment of filovirus in host biomarkers 5 that can be measured during human infections as well 6 as can be imported over to animal models. Recent 7 events in the study of filovirus entry, replication, 8 pathogenesis and host responses should provide an 9 impetus to the development of therapeutics and 10 vaccines. Relevant animal models will be key to this 11 development to say the least. I'd like to finish with 12 a slide that I threw into the very end. It involves 13 Uganda 2001 -- 2000/2001 outbreak and it was a stamp 14 that the government issued commemorating the efforts 15 of volunteers that took place to take care of this 16 outbreak and some of those volunteers died and with 17 the new facilities that are being opened in the United 18 States and elsewhere, I think it's incumbent on the 19 researchers to really think about the studies that 20 they'll be performing because you're going to become 21 volunteers in this effort or you're going to get 22 volunteered, so it's incumbent on us to perform these 23 studies as safely as possible. With that, I'll end 24 and entertain any questions. 25 (Applause)</p> <p style="text-align: right;">Page 63</p>	<p>1 progress that you guys have been making in 2 accumulating that data, recognizing how difficult it 3 is under field conditions and difficulties in 4 obtaining any kind of blood and then processing it in 5 any kind of meaningful way is a continuing challenge. 6 But I think with you know, increased emphasis on 7 getting into these outbreaks in a timely fashion and 8 the establishment of field stations in West Africa, 9 and what have you to facilitate the processing of 10 these samples, maybe those kinds of data will be more 11 easily achieved in the future.</p> <p>12 DR. FELDMANN: Tony, this is Heinz 13 Feldmann. In terms of the last slide, maybe I missed 14 that. When we do animal experiments, we also look for 15 platelets. Did you look for platelets, and you don't 16 consider that a marker? That was the first part in 17 this -- answer first, okay, sorry.</p> <p>18 DR. SANCHEZ: Yes, the platelets are 19 reduced, but in terms of assays that can be done 20 easily, you know, I just kind of -- I didn't include 21 all of the assays or markers that one could look at 22 but platelets, there's a thrombocytopenia that occurs 23 in these animals.</p> <p>24 DR. FELDMANN: And the question to the D- 25 dimers, if I -- Tom, you have to correct me if I'm</p> <p style="text-align: right;">Page 65</p>

<p>1 wrong on this, but when you look at the non-human 2 primates, D-dimers are almost as early as RNA or even 3 before. How is that in the human situation? Do you 4 have any data on this? When D-dimers come up, is this 5 at a later time point or could that be a very early 6 marker?</p> <p>7 DR. SANCHEZ: Yes, we see that in humans 8 as well. And in fact, in these human specimens, it's 9 -- we're much higher than the monkey values that we 10 were seeing. So it's incongruence a little bit trying 11 to understand. You read the literature, how these D- 12 dimers are associated with severe consequences where 13 we see massive amounts of these in the patients and 14 how they're surviving this long is mysterious, but 15 yes.</p> <p>16 PARTICIPANT: Could you comment on the 17 biomarkers in terms of type specifics, type 18 specificity and whether it's related to ebola, Marburg 19 and which serotypes would be that also important in 20 that prevention scenario, vaccination or even drugs 21 maybe have differences for different viruses.</p> <p>22 DR. SANCHEZ: All this information comes 23 from ebola patients. We have very little information 24 in our -- in the Reston, perhaps the Russians may have 25 on the incidents that they had with their laboratory</p> <p style="text-align: right;">Page 66</p>	<p>1 mean, we have -- it's a tremendous luxury in the 2 filovirus field that excellent results, I mean, 3 incredible results have been attained using non-human 4 primates. Very largely the work of Tom Geisbert, Lisa 5 Hensley, Peter Jahrling. That group at USAMRID have 6 obtained data that I think 10 years ago nobody would 7 have dreamed of that we would know as much about 8 filoviral hemorrhagic fever as we do today, and it's 9 come from that work.</p> <p>10 What I'm going to be doing is trying to 11 compare what we see in small animal models to what we 12 know at this point about non-human primate disease, 13 and the comparatively little bit that we know about 14 the disease in humans, which Tony reviewed for us. So 15 first of all, I'm going to be talking about just the 16 development of rodent models. I should probably say, 17 actually before even getting into rodent models, just 18 the fact that, in terms of what are the animal models 19 that we have available, what can we choose from in 20 studying filoviruses that non-human primates are very 21 good models. Every species that's been tested so far 22 has been found susceptible and developed severe 23 disease. There are no ebola resistant non-human 24 primates that I've ever heard of. If you take virus 25 straight from a human and put it into a guinea pig, at</p> <p style="text-align: right;">Page 68</p>
<p>1 infections but so far there's very little data with 2 respect to, with Marburg. The Reston is out because 3 it only infects monkeys and there's only that one 4 Ivory Coast specimen. So hopefully we can get a good 5 look at the Zaire cases and the DRC and perhaps 6 perform some similar testing and get a broader 7 picture.</p> <p>8 DR. JAHRLING: Okay, well, we're scheduled 9 for a break. We're running about five minutes late, 10 but if everybody could be back by 10:40 that would be 11 terrific. There's goodies outside for refreshments.</p> <p>12 (Whereupon, the above-entitled 13 matter went off the record at 14 10:14 and resumed at 10:40 15 a.m.)</p> <p>16 DR. BRAY: Sorry about the delay there. 17 I'm going to be talking about small animal models of 18 filoviral hemorrhagic fever. It's a lot easier to do 19 this now that Heinz has talked and Tony, and we've 20 heard a lot more about the basics of the disease. I 21 do wish that, actually if I'd been in charge of 22 putting this -- the order, I would have had non-human 23 primate models coming before my talk so that I'd be 24 able to compare what we see in guinea pigs and mice to 25 non-human primates, which is -- primates really -- I</p> <p style="text-align: right;">Page 67</p>	<p>1 least in the Marburg outbreak of 1967, they reported 2 seeing mild disease. This led then to adaptation to 3 guinea pigs by serial passage, I'll describe this 4 later on. So guinea pigs became a model fairly early 5 on.</p> <p>6 Mice, once they're over the age of about 7 one week, I'm talking about suckling mice, these are, 8 you know, one week after birth, become resistant to 9 filovirus infection. So you do the same thing that 10 they did in Marburg with a human isolate. In a normal 11 adult mouse, you don't see any sign of disease there. 12 They become totally resistant, and this happens quite 13 early in life. The only other animal models, there 14 has been Botswana's work putting ebola Zaire virus 15 into some bats, and documenting that there was a 16 chronic or persistent infection. They didn't get 17 sick. Otherwise, this is it.</p> <p>18 I think there was some work done early on 19 putting virus into hamsters, probably putting virus 20 into rats with negative results, and those models have 21 never been used. So in guinea pigs, during the 22 Marburg outbreak, '67, as I mentioned, they found that 23 they could make a guinea pig become mildly ill with an 24 isolate from patients, and then if they passage the 25 virus, that is if they took the first generation of</p> <p style="text-align: right;">Page 69</p>

<p>1 sick animals, killed them, removed liver, made a 2 homogenate of that, injected it into a next round of 3 healthy animals, that if you used that strategy and 4 continued it, roughly four times, that they found that 5 the animals developed lethal disease. This is a 6 process called adaptation.</p> <p>7 What's really happening here is natural 8 selection is taking place. You're putting a mixed 9 population of virus into an environment where those 10 particular viruses that are able to replicate well 11 have an advantage, and then you end up, after you've 12 done this for several generations, with a virus 13 population that is largely able to replicate well in 14 this new host.</p> <p>15 And it turns out, again, with guinea pigs, 16 if you do the same sort of trick with ebola Zaire, 17 with Sudan, it works well. It's been done with other 18 viruses as well. The lesson really is that, in guinea 19 pigs, these animals are somewhat susceptible to 20 infection, and that adaptation has been done a number 21 of times. It's not a difficult task, so these guinea 22 pigs have been used as an animal model for quite 23 awhile. Without going into the details of 24 pathogenesis, once you have a stock of virus that's 25 lethal, you put it into a guinea pig, and you can do</p> <p style="text-align: right;">Page 70</p>	<p>1 four days and die in five to seven days after 2 injection of wild type virus.</p> <p>3 And some of the findings also that I did 4 at that time were simply a repeat of what was found 5 earlier in Marburg. So here you've got a combination 6 of findings that SCID mice are susceptible. If you 7 inject them with the filovirus, they can't get rid of 8 it, and they will eventually die, but they are 9 resistant, but if you take a normal mouse and remove 10 its Type 1 interferon response, they become very 11 susceptible. So what seems to be happening is, in 12 SCIDs, the Type 1 interferon system is able to resist 13 infection but can't get rid of it, and clearly Type 1 14 interferon is playing a central role in resistance.</p> <p>15 If you think about what happens in non- 16 human primates, animals that by any route of 17 infection, by apparently any dose of any filovirus, 18 become severely ill within a matter of days, less than 19 a week. Clearly whatever their Type 1 interferon 20 response is doing, if anything, it's ineffective, 21 whereas in mice and probably in guinea pigs as well, 22 Type 1 interferon is very effective.</p> <p>23 This is work that I did in, starting in 24 1996, sorry '95, adapting ebola Zaire virus to mice. 25 This is just by a classic sequential passage of virus,</p> <p style="text-align: right;">Page 72</p>
<p>1 this by any route of infection, they'll become ill in 2 three to four days, and they die in about seven to 10 3 days.</p> <p>4 In mice, it's more difficult. As I said, 5 they tried this in Marburg in '67, didn't get 6 anywhere, dropped the question. Nobody really 7 examined filovirus infection in mice until the 1990s. 8 Here, actually, I should probably refer to this first, 9 John Huggins found that if you put wild type ebola 10 virus into SCID mice, the animals would get sick, but 11 they didn't get sick right away. They wouldn't even 12 show any signs of illness until probably 12 to 15 13 days, then they developed sort of a slow wasting 14 disease, dying at three to four weeks, so looking 15 nothing like filoviral hemorrhagic fever. This is, 16 you know, not the sort of thing that you would say is 17 a good model.</p> <p>18 Later on I started working for John and 19 was able to, because of the availability of knockout 20 mice and all sorts of variants and reagents that you 21 can use in mouse research, found that if you got rid 22 of the Type 1 interferon response, mice became very 23 susceptible to wild type filoviruses, both Marburg and 24 ebola, and that a receptor, interferon alpha/beta 25 receptor knockout mouse would become sick in three to</p> <p style="text-align: right;">Page 71</p>	<p>1 starting in newborn mice, and then doing the same sort 2 of thing they did in Marburg with guinea pigs, take 3 livers out of sick mice at day five, day seven, make 4 a homogenate, put it back into mice, and just keep on 5 passaging, and it worked. I ended up with virus that 6 was lethal for adult mice, had a change in phenotype, 7 which is very useful. I could recognize plaques of 8 mouse adapted ebola virus, because they were clear 9 plaques rather than sort of traditional bulls eye.</p> <p>10 I was able to grow that up, make a large 11 prep. It's been distributed, and people at USAMRIID, 12 at CDC, in Winnipeg and actually now in the Galveston 13 lab, have this virus and have been using it. I'd like 14 to report Kelly Warfield is here in the audience, and 15 she's allowed me to say that she and her colleagues 16 used a strategy that I wish I had used back in the 17 '90s of how to adapt a virus to mice. She started out 18 with SCIDs, and worked with Marburg, and found that if 19 you sequentially passage virus in SCID mice, the time 20 to death goes down and a process of adaptation takes 21 place and in fact, their time to death is reduced 22 drastically.</p> <p>23 If you then take the virus out of SCIDs, 24 put it into normal mice and continue to passage it, 25 you could end up with a highly virulent virus for --</p> <p style="text-align: right;">Page 73</p>

<p>1 that kills mice. So she's been successful in doing 2 that. There's a manuscript accepted coming out about 3 this, and more work in progress. So for those of us 4 who really are interested in small animal models, 5 there's been a nice piece of progress recently. I'm 6 sure Kelly would be willing to talk about this at 7 lunch time.</p> <p>8 What I haven't mentioned about mouse 9 adapted virus, I said with guinea pigs that they're 10 susceptible to infection by any route. With mice, you 11 have the peculiarity that the virus only causes 12 disease after intra peritoneal injection. It would 13 probably cause disease after intravenous inoculation, 14 except I had never really got up the guts to inject a 15 tail vein of a mouse while in a space suit with ebola 16 virus, but if that were done, I'm sure it would cause 17 disease, as well.</p> <p>18 So there's something about mice, and this 19 apparently is true of the Marburg virus isolate as 20 well, that there -- the resistance to infection is 21 very potent, it's still -- even with the mouse adapted 22 virus, animals can resist infection if they see the 23 virus first by a subcutaneous or intramuscular route, 24 but they can't do anything about it if the virus shows 25 up intra peritoneally, or probably intravenously.</p> <p style="text-align: right;">Page 74</p>	<p>1 lymphocytes is a very prominent finding. In mice, 2 this has recently been shown to be the result of 3 apoptosis. Steven Bradford has done this work with 4 Tom Geisbert.</p> <p>5 In guinea pigs, I would expect the same 6 thing happens. Lymphocyte apoptosis seems to be 7 simply a feature of severe infections. Richard 8 Hotchkiss has reported on this in Sepsis. We had a 9 paper in EID a few months ago describing this and a 10 variety of other infections, and the more you look, 11 the more you'll find that lymphocyte apoptosis is 12 simply part of a severe infection. So not 13 surprisingly, it's present in both mice and guinea 14 pigs infected with filoviruses.</p> <p>15 Now, an interesting difference and one 16 that people tend to talk about a lot is the fact if 17 you look by microscopy, you find very little fibrin 18 deposition. Here's where I wish that Tom had given 19 his talk first, because he's going to show some photo- 20 mikes where there is a lot of deposition of fibrin in 21 the tissues and this goes along with DIC, it's part of 22 the -- you would expect to see this in an animal that 23 has a high level of D-dimers. It's just part of that 24 severe coagulopathy.</p> <p>25 And if you look in guinea pigs you can</p> <p style="text-align: right;">Page 76</p>
<p>1 So what does the disease look like? Here 2 just a summary. I think I've said these things 3 already. Yes, that's just a repetition. So I'm just 4 going to give some summary slides. I'm not really 5 going to go into the data. It's a short presentation. 6 In terms of kinetics and viral replication, you know, 7 if you're taking blood samples day by day after you've 8 infected animals in both guinea pigs and mice, 9 typically you see virus in the blood stream appearing 10 on Day 2 or Day 3. It rises very rapidly. You have 11 a plateau phenomenon, where it will reach a level of 12 107, 108 or so by Day 4 to 5 and stay there. And it 13 remains very high through the point of death.</p> <p>14 All of these models are uniformly lethal. 15 In terms of histopathology, I will show a few 16 pictures. Macrophages, as has been pointed out 17 earlier, Heinz's diagram showed this, macrophages seem 18 to be the primary site of viral replication. Virus 19 can replicate in many different types of cells, but 20 macrophages appear to be the central player. 21 Dendritic cells are also infected, so true now of both 22 guinea pigs and mice. The virus, when it gets to the 23 liver, infects hepatocytes, causes necrosis there, 24 infects perenchymal cells of some other organs, many 25 other organs, and again, causes necrosis. Death of</p> <p style="text-align: right;">Page 75</p>	<p>1 find some, if you look in mice, it's very hard to find 2 fibrin deposition. So this is a difference in the 3 model, and it's one that everybody is aware of. Just 4 looking again microscopically in terms of where 5 replication takes place, here we're at Day 2 in a 6 mouse, and you can see antigen here of mouse adapted 7 ebola virus, very prominently in macrophages. Other 8 cells here along the marginal sinus in a lymph node, 9 nothing showing up in lymphocytes.</p> <p>10 If you go to Day 4 in the spleen, now by 11 encyto, where is the virus replicating? It's here in 12 the marginal zone where, you know, looking at just the 13 holes, the way this tissue appears even by Day 4, 14 there's been massive destruction of macrophages, 15 dendritic cells. Cells other than lymphocytes are 16 being directly destroyed by the virus. I don't have 17 a good photo-mike, but if you could look closely at 18 these lymphocytes, you would find changes there of 19 lymphocytolysis, a breakup of cells, karyorexis things 20 like that, that are characteristic of apoptosis, and 21 I think Tom will show photos of that for non-human 22 primates.</p> <p>23 In the liver, here again, mouse-adapted ebola 24 virus, we've got a lot of necrotic cells, you see 25 inclusion bodies. It's hard for me to pick them out</p> <p style="text-align: right;">Page 77</p>

<p>1 right here, but anyway, here are some nice viral 2 inclusions. So you get this focal, multi-focal 3 hepatic necrosis that's very typical. This could be 4 a shot from a non-human primate or of a human infected 5 with a filovirus. In terms of changes in blood cell 6 counts, guinea pigs and mice both show an early rise 7 in total white cells. This appears to reflect 8 mobilization of immature granulocytes, you see 9 immature neutrophils showing up. Lymphocytes tend to 10 decline over the course of illness, which seems to 11 correspond to this lymphocyte apoptosis, and of 12 course, you get thrombocytopenia, and this is very 13 prominent in both species.</p> <p>14 In terms of blood chemistries, Tony talked 15 about some of these things. We see AST and ALT. 16 Interestingly, AST tends to be higher in mice and in 17 guinea pigs, just as he said in describing humans. 18 LDH tends to be elevated as well, as it often is in 19 severe disease. There are signs of hemoconcentration, 20 increased BUN, increased total protein. Hemoglobin 21 concentration also going up, presumably reflecting the 22 vascular leak that's going on. In terms of coags, the 23 bottom line on mice is we really don't know very much 24 about it. We did some limited testing of this back in 25 1996/'97 or so, tried to figure out what was happening</p> <p style="text-align: right;">Page 78</p>	<p>1 I'm going to mention in a minute that Type 1 2 interferon, suppression of Type 1 interferon probably 3 plays a major role here.</p> <p>4 But in any case, the virus disseminates 5 very rapidly, spreads to more macrophages, spreads 6 throughout the system, and because these macrophages 7 are producing pro-inflammatory cytokines tissue 8 factor, you get a systemic inflammatory syndrome. The 9 immune system, of course, should be doing something 10 about this, and the major mechanism is antigen 11 presentation by the dendritic cells to lymphocytes. 12 The bad news in filoviral infections is that the 13 dendritic cells are either being impaired by 14 infection, or are being outright destroyed by 15 infection. At the same time, you're losing your 16 lymphocytes through apoptosis, and in the majority of 17 cases, and in apparently all cases in mice and guinea 18 pigs, there's no effective adaptive response.</p> <p>19 I think that the -- what Tony talked about 20 a couple of things that some patients, obviously, some 21 humans do survive. The picture in my mind is that 22 those people who can generate an adaptive response 23 quickly enough, before their dendritic cells are lost 24 and their lymphocytes are all going down, are the lucky 25 few who are able to make it. In rodents, it simply</p> <p style="text-align: right;">Page 80</p>
<p>1 in mice, did a few PTs and PTTs, didn't really come up 2 with much. I believe Kelly's group is doing 3 considerably more work looking at these questions 4 about coagulopathy in filovirus infected mice.</p> <p>5 Guinea pigs, to date, is a little bit 6 better. We were, in fact, able to show that you get 7 a prolonged prothrombin and partial thromboplastin 8 time in guinea pigs. Inflammatory responses, in mice 9 you'll see elevations of TNF alpha, IL-6, MCP-1, other 10 pro-inflammatory cytokines. In guinea pigs, there's 11 very little data in this area, the problem there being 12 lack of reagents, although there may be data that I'm 13 not aware of in this question.</p> <p>14 So just looking overall at filoviral 15 hemorrhagic fever, you know, Heinz gave a very good 16 introduction to this and showed, you know, what the 17 basic pathogenesis is. I tend to think of the disease 18 as basically a race between viral replication and 19 spread, and the attempt of the immune system to do 20 something about it. Filoviruses are able to replicate 21 extremely well in macrophages. They produce a very 22 high yield. You get thousands of new virions coming 23 out. Probably a single virion plus a single human 24 macrophage is enough to produce fatal disease. That's 25 all it really takes, because the virus just takes off.</p> <p style="text-align: right;">Page 79</p>	<p>1 doesn't happen, and non-human primate models, this 2 doesn't happen. But there's simply this contest 3 between virus and host, and in the great majority of 4 cases, the virus wins.</p> <p>5 It's eliminating the cells that are needed 6 to produce an effective adaptive response. With 7 filovirus infections, you also have a lot of tissue 8 necrosis. All those hepatocytes going out, and a lot 9 of other cells being infected and dying. So this 10 increases the severity of disease. One question I 11 don't think has come up this morning in terms of how 12 important is hemorrhage. Filoviral hemorrhage fever, 13 after all, just by the name, you would expect patients 14 would be doing a lot of bleeding as their dying. I've 15 never been to an outbreak, I've haven't seen a 16 patient, but what I've read, what I've heard is that 17 hemorrhage isn't that extensive. You know, don't 18 believe everything Richard Preston wrote, that people 19 don't just melt and bleed from all orifices.</p> <p>20 In fact, in non-human primates, although 21 there is certainly hemorrhagic phenomenon, they don't 22 die from bleeding. It's really not the cause of 23 death. The cause of death has to do with shock. It's 24 the result of all those infected macrophages, and all 25 those cytokines and chemokines, and the effect on</p> <p style="text-align: right;">Page 81</p>

<p>1 vascular function, but that's how people are going 2 out.</p> <p>3         So this comes back to the question, well, 4 how about those rodent models where you don't see 5 fibrin deposition? Well, maybe that's not that 6 important. I don't know. I'd like to understand why 7 rodents don't have all that fibrin deposition, and 8 whether that's a worthwhile research question to 9 pursue, I'm not sure. Everybody has a wiring diagram 10 that they like for pathogenesis. This is mine here. 11 You've got the major players, virus in a macrophage. 12 Suppression of Type 1 interferon responses allows this 13 virus to disseminate very rapidly, and then you get 14 direct tissue injury, cells that are infected and 15 killed by the virus, and then the indirect effects of 16 mediators produced by infected macrophages.</p> <p>17         These have already been mentioned by 18 various people. You get a recruitment of inflammatory 19 cells that affects the blood counts, loss of 20 lymphocytes through apoptosis, the effects on vascular 21 function and production of tissue factor, which I know 22 Tom will be talking about.</p> <p>23         So a good animal model is one in which a 24 small dose of virus leads to these phenomena that I've 25 just been over. You would expect to see high viremia</p> <p style="text-align: right;">Page 82</p>	<p>1 response -- they are inherently resistant to filoviral 2 infection because of their Type 1 interferon response. 3 And probably the same thing is true of guinea pigs, as 4 well.</p> <p>5         This has a major effect when you start 6 testing drugs in vaccines, because you're now dealing 7 with an animal that's already resistant to infection. 8 Primates are helpless when they're inoculated with a 9 filovirus. They can't do anything about it, but 10 rodents can, so this affects the results that you get 11 when you test a drug or a vaccine. And this is very 12 well-known. Tom even published -- Tom Geisbert, 13 published an article documenting the fact that rodents 14 are poor predictors of vaccine efficacy, and this has 15 turned up as well with -- in drug testing. I'm just 16 giving a couple of examples here.</p> <p>17         It was mentioned very early, I think Heinz 18 said, you know, no one has ever yet shown that 19 neutralizing antibody can protect a non-human primate. 20 A variety of efforts have been made here. They 21 haven't worked, but you can easily protect mice using 22 antibodies, and you can protect guinea pigs using 23 antibodies, including monoclonal antibodies. So this 24 is a -- you know, if I had to give a very short 25 explanation of why that is, I would say it's because</p> <p style="text-align: right;">Page 84</p>
<p>1 and persistent viremia through death. You would 2 expect to see infection and necrosis of macrophages 3 and dendritic cells. You would want to see lots of 4 pro-inflammatory mediators. You would, if you could, 5 measure -- determine that there is increased vascular 6 permeability and shock, and you would expect to see a 7 lot of lymphocyte apoptosis. And these changes are 8 seen in guinea pigs and mice. I probably went a bit 9 too far in saying, you know, we don't really have data 10 on vascular permeability in mice, but I think the fact 11 that cytokines are produced, and the right source of 12 cytokines are being released, would imply that in 13 rodent models.</p> <p>14         So again, rodents, mice in particular, are 15 highly resistant to wild type filoviruses and this -- 16 if you knock out Type 1 interferon, you can make them 17 totally susceptible to infection, so clearly that 18 response is very important. Also, you can take a 19 normal mouse and treat it with anti-interferon alpha 20 antibodies and make it susceptible to lethal 21 infection. So this is a very simple demonstration 22 that, yes, interferon responses are protecting mice. 23 Also, you can give mice interferon and protect them, 24 or you can give drugs that induce interferon and 25 protect them. So in mouse models, the interferon</p> <p style="text-align: right;">Page 83</p>	<p>1 of this -- they are already inherently resistant to 2 infection, and they can respond very briskly with an 3 interferon response.</p> <p>4         Vaccines, similar results, the normal 5 expression is it's easy to protect mice, and this is 6 correct. So then the question is, what can you do 7 about it? Just briefly here, how do they -- the basis 8 of resistance, several people now have mentioned that 9 there are two interferon antagonists encoded by 10 filoviruses: one that prevents the initial production 11 of interferon beta, another that interferes with 12 signaling from both, actually the interferon 13 alpha/beta and the interferon gamma receptors, so this 14 is a very potent combination. Clearly, filoviruses 15 care a lot about interferon responses.</p> <p>16         So the only real suggestion I would make 17 in terms of exploring models would be, how about using 18 mice that don't have an interferon response? STAT-1 19 knockout mice have been described already. Heinz 20 mentioned these when he was talking about testing wild 21 type filoviruses in which various modifications have 22 been made through reverse genetics. You can infect a 23 STAT-1 knockout mouse with any of the filoviruses, and 24 maybe these are the animals that should be used for 25 further vaccine testing, and for drug testing. We</p> <p style="text-align: right;">Page 85</p>

<p>1 don't have the data, but it would be an easy one to 2 explore. Pick some drugs that have worked in normal 3 mice and haven't worked in primates. Pick some 4 vaccines that have worked in primates -- sorry, worked 5 in mice, but haven't worked in non-human primates, and 6 see what happens in that model.</p> <p>7 Otherwise, I think the bottom line is that 8 scientists have always used rodent models in 9 infectious disease research. When I started at NIH 10 back in the `80s, I was working on dengue, and we used 11 a model of mouse adapted dengue virus that Albert 12 Sabin had created. This was an intra cerebral 13 inoculation of dengue virus, and the mice died of 14 encephalitis. Well, that's not dengue, but everybody 15 used the model, you know. Scientists are sort of 16 addicted to used rodent models, and this will 17 continue. We just want the models to be as good as 18 they can be. Thank you.</p> <p>19 (Applause)</p> <p>20 DR. JAHRLING: Questions for Dr. Bray?</p> <p>21 PARTICIPANT: Thank you, nice talk. In 22 terms of the mouse adapted virus, what can you tell us 23 about genotype, and other phenotypic characteristics 24 that -- is it attenuated related to it's response to 25 Type 1 inteferon, to its replications, to its ability</p> <p style="text-align: right;">Page 86</p>	<p>1 PARTICIPANT: Right.</p> <p>2 DR. BRAY: I think there's potential 3 there. Now, I'm not a macrophage person. I know that 4 it's easy to say, just test things in macrophages, and 5 this is a science all to itself in terms of, you know, 6 trying to actually replicate what's happening in vivo. 7 But as I was showing from the diagram, I do think that 8 most of what happens in filoviral infection really is 9 the result of the interaction between virus and 10 macrophage, and this is something that should be 11 studied.</p> <p>12 I know Lisa Hensley published a number of 13 years ago, I mean, my study of the pro-inflammatory 14 cytokine response in isolated human macrophages. I 15 think that's something that ought to be pursued, and 16 particularly now that we can do reverse genetics, and 17 we can alter the virus, and then you can go back and 18 look at interactions with macrophages. So, I tink 19 that's a very profitable area.</p> <p>20 PARTICIPANT: Mike, that's a very 21 fantastic presentation, a lot of rich amount of 22 information. I have a comment, maybe a question and 23 then I have a question, little bit complicated. First 24 of all, I think that normally would believe the acute 25 virus doesn't have the opportunity to adapt. So with</p> <p style="text-align: right;">Page 88</p>
<p>1 to replicate in macrophages? Can you elaborate at 2 all?</p> <p>3 DR. BRAY: In terms of chemokine/cytokine 4 responses?</p> <p>5 PARTICIPANT: Yes, and ability in vitro to 6 replicate in the --</p> <p>7 DR. BRAY: We don't have -- there's not a 8 lot of data on cytokine responses in normal mice 9 infected with mouse adapted virus. The -- in terms of 10 interferon responses, there seems to be very little 11 induction of interferon. It shows up late in the 12 limited studies that we've done. You wouldn't be able 13 to detect interferon in serum until out to Day 3 or so 14 when animals are already becoming ill. So there seems 15 to be some evasion there.</p> <p>16 In terms of chemokines, I mentioned, you 17 know, there are some pro-inflammatory cytokines that 18 have been identified, but not a lot of data actually 19 on the model.</p> <p>20 PARTICIPANT: So what's your feeling about 21 using in vitro replication in macrophages to -- as a 22 prelude to doing animal model testing, since this is 23 the site of replication?</p> <p>24 DR. BRAY: You mean simply working with 25 primary macrophages?</p> <p style="text-align: right;">Page 87</p>	<p>1 the mice model seems the ebola actually quite 2 effective in adapting to host and it become new 3 viruses being capable to infect. So potentially in 4 the nature if the virus is not infecting primates, 5 which it doesn't very acute clinging of the host, if, 6 in fact, something like fruit bats and other animals 7 will -- there is an opportunity for the virus to adapt 8 and evolve into new subtypes of species. I think that 9 raise the concern if we're using any form of the 10 vaccine type that allow the virus replicate, that may 11 raise the concern the virus eventually will adapt to 12 the host and it become virulent. That's one first 13 comment.</p> <p>14 The second, I think very important what 15 you present the interferon pathway, there is two 16 possibilities in my mind, maybe wrong. One is the 17 virus less infectious to murine model, to mice, but 18 then interferon pathway is capable to protect mice. 19 In primates, the virus so pathogenic, so lethal, so 20 even with the normal interferon, it may not be able to 21 protect, but if that's the case, whether or not we can 22 -- giving the higher dose interferon, take a mechanism 23 to enhance the interferon production will not become 24 at least adjuvant assistant therapy for vaccines or 25 drugs. That's one question.</p> <p style="text-align: right;">Page 89</p>

1 And secondly, another possibility is the -  
2 - you're supposed to show interferon antagonist  
3 carried by the virus. Your words, the virus carries  
4 very much interferon. So there must be a reason. If  
5 the interferon antagonist mechanism doesn't work well  
6 against murine deferon, but work well in primates,  
7 there is maybe become a target for development therapy  
8 or vaccine was what you're thinking.

9 DR. BRAY: Okay, I think I can combine an  
10 answer to both of those last two questions about  
11 interferon responses. Most of your questions seemed  
12 to have to do with non-human primates, and I'm going  
13 to defer a lot of that discussion to Tom Geisbert,  
14 who's been doing some work on use of Type 1 interferon  
15 in primates. One thing that's very difficult in the  
16 area of interferon is that we don't know very much  
17 about interferon responses. Most of the data that we  
18 have are really based on serum levels of interferon.  
19 You know, interferon itself is, of course, acts  
20 locally. You've got this autocrine and paracrine  
21 effect of interferon, that is probably how it does  
22 most of its work in preventing local dissemination,  
23 and if all you've got is a plasma level, then it's not  
24 really telling you very much about what's happening at  
25 the level of the infected cell.

Page 90

1 It could well be -- well, you know, as I  
2 mentioned in mice, you can -- you measure interferon  
3 in the plasma, in non-human primates you measure  
4 interferon in plasma of infected animals. In the case  
5 of primates, these are animals that are dying of  
6 infection, and they have a high level of Type 1  
7 interferon. To me, what this means is the kinetics  
8 are wrong. The virus is outpacing the interferon  
9 response. Yes, they're making interferon, they aren't  
10 making it quick enough. They're not making enough of  
11 it. So it has to do with that.

12 The other thing is that, you know, when  
13 we're looking at these infections, this is not --  
14 these are not filoviruses in their maintenance host.  
15 We're looking at accidental infections. You know,  
16 human infection is irrelevant to filoviruses in  
17 nature. They replicate in some animal that we haven't  
18 identified yet, maybe it's bats, and it's that  
19 interaction with the Type 1 interferon response of the  
20 maintenance host that really explains what these  
21 interferon antagonists are for, what they're doing.  
22 So here we're only looking at the train wreck that  
23 happens if the filovirus gets into humans, and we can  
24 sort of speculate that we don't know why the virus  
25 really has these features.

Page 91

1 DR. JAHRLING: Okay, thank you, Mike, for  
2 a very interesting and provocative presentation. We  
3 need to stay on schedule and move on now. The next  
4 presentation is by Dr. Tom Geisbert, Non-human  
5 Primates as a model for filovirus infection.

6 DR. GEISBERT: Okay, good morning. First,  
7 I'd like to thank the organizers for inviting me to  
8 talk about some of the work that we've done over the  
9 last, oh, five, 10, 15 years on how using non-human  
10 primates as models for ebola and Marburg virus  
11 infection, and I want to point out right away that,  
12 while I'm not an employee at the moment, almost all  
13 the work that I'm going to talk about was done years  
14 ago and over the last, again, 10 or 15 years by Peter  
15 Jahrling, and myself, and Lisa Hensley at USAMRIID.

16 Mike saved me a lot of time here. I  
17 thought I was going to have to explain a little bit  
18 about the rodent models versus the non-human primates.  
19 Again, I just want to point out, if you take ebola or  
20 Marburg virus from the infected patient in Africa, put  
21 it into almost any species of monkey, they die.  
22 Historically, the macaques, rhesus and cynos have been  
23 used for filovirus research. There's been some work  
24 done with African greens, and the Russians actually  
25 used the hamadryad baboons, and Peter Jahrling tells

Page 92

1 an interesting story that when he was visiting ALEV  
2 and the guys at Vector, he was asking them, "Why do  
3 you use baboons?" And it turns out that they had an  
4 issue with food, and they adapted the baboons to eat  
5 sugar beets. The macaques wouldn't eat sugar beets,  
6 so they died.

7 And so that's kind of some of the story of  
8 why they used baboons, but we -- there's really no  
9 instance here in the states of anyone using baboons,  
10 so I'm going to kind of focus on the macaques and the  
11 greens for the purposes of this presentation.

12 Mike talked also about the differences,  
13 the discrepancies between the rodent models and the  
14 non-human primates, and when I started out in the  
15 filovirus field, and Peter and I were doing a lot of  
16 studies with different vaccines and different  
17 treatments, it was extremely frustrating. You would  
18 have a treatment or a vaccine that would protect the  
19 rodents 100 percent of the time, and then wouldn't  
20 work in a non-human primate.

21 So we spent a lot of time trying to figure  
22 out why that was. I think that Mike is right. I  
23 think anything that you do to induce the interferon  
24 response in a rodent will protect that rodent, whereas  
25 it doesn't seem to have much effect in a non-human

Page 93

<p>1 primate. And this is just -- Mike had eluded to this;  2 this is just -- the main difference that we found is  3 the fibrin deposition in spleen, and a lot of the  4 other tissues. This is very typical in the red pulp  5 marginal zone. You get a lot of fibrin deposition in  6 the macaques. You can look for days and try to find  7 very small deposits sometimes in guinea pigs and you  8 never see any in mice.</p> <p>9       So there's definitely differences in  10 fibrin deposition, and the coagulation disorders. Tom  11 Ksiazek and Tony Sanchez brought up a very good point,  12 and I'm glad to see Tony's talk, because we're finally  13 starting to get some human data. Historically,  14 there's been very little known about what happens in  15 human infections and, you know, working in the  16 pathology field for a long time, we didn't even have  17 access to tissues. There was just small dribs and  18 drabs from outbreaks, small handfuls, and probably in  19 maybe 18 total cases to date, where there was even a  20 histo block. Sharif Zaki has done some fantastic  21 work, but we just haven't had the tools and we haven't  22 had much to look at. In 1987, I was fortunate enough,  23 the Marburg Grabon case actually came to USAMRIID, and  24 this is actually a section through the spleen of that  25 case, and you can see virus particles in the red pulp</p> <p style="text-align: right;">Page 94</p>	<p>1 for biomedical research. The HIV field is really  2 focused on the Indians. They're better modeled --  3 better models. So actually, I went back to the  4 records. Almost everything we've used in our group at  5 USAMRIID has been Chinese origin. The human -- there  6 was a study published in Science recently where the  7 genome of the rhesus macaque was sequenced and  8 identified. So I think that's really important for --  9 I think they could have a lot of utility for  10 filoviruses and using the rhesus macaque. There is  11 about 93 percent sequence identity between rhesus  12 macaques and humans.</p> <p>13       Cynos, this is going to open a can of  14 worms. Actually, Joan, my wife, picked up on this a  15 couple of years ago when we were doing some work for  16 Nancy Sullivan, and we had been using Vietnamese  17 cynos, and I actually point out there's 10 different  18 sub-species of cynos. The ones that are primarily  19 used and that we've used over the years and most of  20 the suppliers have, are either Philippine, Vietnamese,  21 Chinese, Indonesian or Mauritius. And John was doing  22 some work with one of Nancy's monkeys one day and  23 said, "Something is different with these." And it  24 turns out that when Nancy went back and looked, they  25 were Philippine cynos, and traditionally on most of</p> <p style="text-align: right;">Page 96</p>
<p>1 marginal zone area, but you can see fibrin deposits  2 here.</p> <p>3       Now, fibrin is not -- we don't see this  4 quite as much with Marburg as ebola. It's a lot more  5 dramatic with ebola than Marburg, but clearly, this  6 does happen in humans, and Tony presented some nice  7 data this morning on the coagulation response and I'll  8 talk a little bit about that with monkeys. But  9 clearly, this is an important component of both the  10 human and non-human primate disease.</p> <p>11       Again, I'm going to focus on three  12 species, rhesus, cynos and African greens. Why use  13 one versus the other? A lot of this over the years has  14 had to have been done with cost and availability. I  15 called Primate Products a few weeks ago. They're  16 charging \$2,500 for a green, up to about \$4,500 for a  17 rhesus macaque. Now, that's without shipping charges  18 or overhead. At USAMRIID last year, Lisa and I were  19 paying about \$6,500 for a rhesus macaque, and then  20 about \$87 a day to keep it in a Level 4 lab. So these  21 are costly studies.</p> <p>22       Looking at rhesus macaques, there's two  23 sub-species, the Indian origin and the Chinese origin,  24 and this is out of about, I think there's six sub-  25 species of rhesus, but two have primarily been used</p> <p style="text-align: right;">Page 95</p>	<p>1 Nancy's studies we've used Vietnamese cynos.</p> <p>2       On most of the -- fortunately for us when  3 we looked at the records, most of the VSV work that  4 we've done with Heinz Feldmann has been with the  5 Indonesian. Probably the most similar would be the  6 Vietnamese and the Indonesian, Philippine a little  7 different. You can actually look at the animals and  8 tell, but by far the outlier by a landslide is the  9 Mauritius, and you can just physically look at a  10 Mauritius versus any of these other ones and tell,  11 they tend to have a darker, grayer coat. The eye  12 sockets are a little different. The facial structure  13 is different. They tend to be kind built like a wild  14 boar up front, real heavy in the top. It's very easy  15 to tell these from the others.</p> <p>16       And what was important is there's a study  17 in the Journal of Immunology that was published last  18 year that defines 66 MHC 1 alleles in cynomolgus  19 macaques of Chinese, Vietnamese and Mauritius origin.  20 Most of the MHC 1 alleles were found only in animals  21 from a single geographic origin, and this suggests  22 that cynomolgus macaques from different origins are  23 not interchangeable in studies of cellular immunity.</p> <p>24       So I think this is something that we all  25 need to think about in the future when we're looking</p> <p style="text-align: right;">Page 97</p>

<p>1 at using cynos and rhesus macaques for our vaccine 2 studies, in particular. African greens, some of the 3 original work that was done in Marburg and then by -- 4 at Porton Down with filoviruses in the early years was 5 done in African green monkeys, and they're not a bad 6 model. The problem is that there's a number of 7 reports here with Marburg and ebola where they do not 8 develop the macular rash. Now, you might think at 9 first, well, it's a darker face, more difficult to 10 see, but if you really look at the abdomen, chest area 11 of the monkey, there is light skin, and it would be 12 easy to see a rash. We've done some studies with John 13 Huggins back in the early 1990s with I was as 14 USAMRIID. We never saw any rashes. There was one 15 Russian study that reported a few mild rashes, but 16 I've never seen one yet in a green. And again, rashes 17 are seen in -- depending on the outbreak, depending on 18 the situation, maybe a half or so of human cases, and 19 we certainly see them to various degrees in the 20 macaques.</p> <p>21 If you look at the published literature, 22 what's been published using these three different 23 species, there's been a number, again the greens 24 mostly in the beginning, just pathogenesis model 25 development studies, 22 with the rhesus, 10 with the</p> <p style="text-align: right;">Page 98</p>	<p>1 monkeys, same exact seed, 25 rhesus monkeys, 100 2 percent mortality in both groups. Mean time to death 3 for the cynos is 6.6 days, 8.4 for rhesus, this is 4 ebola Zaire. And the range is about five to nine in 5 the cyno, and seven to 10 in the rhesus. So 6 everything being equal in the ebola Zaire model, it 7 takes a little bit longer to produce a lethal 8 infection in African greens. You do see some 9 variability among these different species of 10 filoviruses.</p> <p>11 Sudan, the Gulu, we only got about 50 12 percent mortality in cynos, where we got 100 percent 13 with the 76 boniface isolate. Tom Ksaizek had 14 mentioned the difference with Marburg Angola, and it's 15 very dramatic. We have a paper coming out on this in 16 JIV pretty soon but, historically, a lot of the work 17 we had done was with the Marsocci, the 1980 isolate, 18 and you can see, in rhesus, the mean time to death is 19 about 11 days, the range is 11 or 12 days. When we 20 put the Angola isolate, the Angola strain into rhesus 21 monkeys, most of these animals died on day seven. And 22 the disease course was much, much faster, and we don't 23 know why that is as the moment. We've talked about 24 passage history, but if you put Ravn -- Gene Johnson 25 put Marburg Ravn into a rhesus, and we had the</p> <p style="text-align: right;">Page 100</p>
<p>1 cynos, and you see a reverse with the vaccines and the 2 treatment studies. Most of the treatment work has 3 been done with the rhesus, where most of the vaccine 4 work has been done with the cynos, and I know that 5 when we started doing a lot of treatment studies in 6 the early days, a lot of the drug companies that we 7 were working with had done all their PK testing in 8 rhesus, and that's kind of why a lot of the work just 9 tended to go into rhesus, and then you want to compare 10 one study with the next, so you continue to use 11 rhesus. The same thing with cynos, a lot of the 12 vaccine studies are done with cynos, and you keep 13 using cynos.</p> <p>14 This just shows some of the work that Lisa 15 and I have done probably over the last five or six 16 years at USAMRIID. It's a table showing, just with 17 our own group, and I'm going to talk a little bit 18 about trying to compare data among different groups 19 and different studies. But one of the things I want 20 to point out, all of these studies were done with 21 1,000 pfu intramuscular injection. Until the very end 22 of my talk, everything I'm going to talk about was 23 intramuscular injection.</p> <p>24 And this is -- in the rhesus and the 25 cynos, we have a cohort of 36 positive control</p> <p style="text-align: right;">Page 99</p>	<p>1 original Ravn isolates. It's a very low passage, and 2 killed two out of three animals. So it's something 3 other than passage history. We just don't know what 4 it is at this point.</p> <p>5 I'm going to go -- this is kind of a 6 controversial topic, but I'm going try to address it 7 the best I can anyway. And if you look at the 8 literature and you look at some of the old Russian 9 literature, it's difficult to interpret. Then, you 10 know, with a lot of the new labs being built, and as 11 you start, even within the same laboratory, you start 12 trying to compare studies across different groups, 13 study end point becomes important, and you can see 14 with the previous slide, some of the groups of 15 animals, you're talking two, three, four animals in a 16 group. It's not like the 25 or 35 we have with the 17 macaques.</p> <p>18 And so, you know, if you're doing a 19 treatment study or an LD50 or some kind of a study 20 like that, when you decide to terminate the study 21 becomes very important, and there's a lot of 22 subjectivity. The Lab Animal Care and Use Committee 23 at USAMRIID has done a fantastic job of trying to come 24 up with correlates, things that would predict survival 25 versus death. It's very difficult. There's still a</p> <p style="text-align: right;">Page 101</p>

1 lot of subjectivity, and this is an animal, and this  
 2 is not really an unusual case. This is a rhesus  
 3 macaque that was on a treatment study that was  
 4 infected with ebola. This animal had a very prominent  
 5 macular rashes, necromotic rash that developed right  
 6 here between the lip and the nose. It had even more  
 7 dramatic rashes on the legs. Platelet counts severely  
 8 dropped, high liver enzymes. The temperatures went up  
 9 to 105 on day six, dropped to 93 on day 14. I think  
 10 most people would have probably terminated the study.  
 11 This animal survived and is perfectly healthy.  
 12 And so again, I just want to point out  
 13 that when you look at different studies and you  
 14 compare, well, this study had a mean time to death in  
 15 rhesus or cynos or Zaire of seven days or eight days  
 16 and well, this group got 10 or 11 days, there's going  
 17 to be a lot of variability depending on study end  
 18 point, because there is subjectivity.  
 19 I want to talk a little bit about the  
 20 intramuscular route of exposure, and I want to talk  
 21 about how this -- in our macaque model, I want to talk  
 22 about how this relates to human disease. In the 1976  
 23 outbreak in Kikwit, there were 85 documented needle  
 24 injections. They were reusing needles for vitamin  
 25 injections, 100 percent mortality in the needle

Page 102

1 injections, just like our macaque model. Mean  
 2 incubation period was about 6.3 days. There were 149  
 3 documented cases of contact exposure in this outbreak,  
 4 80 percent mortality, and the mean incubation period  
 5 was 9.5 days.  
 6 So while, on one hand, we think the  
 7 macaques are maybe exclusively sensitive, we also have  
 8 to keep in mind that, when humans are injected with  
 9 high doses of the ebola virus with needles, there's  
 10 100 percent mortality, and the mean time to death or  
 11 the incubation period or the window between infection  
 12 and death is very short, as well. This just shows how  
 13 the challenge dose in the injection model relates to  
 14 the disease course. We had a number of monkeys,  
 15 again, this is cynomolgus monkeys. There's 30 some  
 16 monkeys in this cohort. And the window is pretty much  
 17 six to eight days, mean again, 6.6 days.  
 18 If you drop the challenges from 1000 pfu  
 19 to 10 pfu, you move the window out about nine to 12  
 20 days. Now we had -- we had a couple studies in the  
 21 beginning when we were developing the model where we  
 22 were working with a dose where we had maybe 200, 250  
 23 pfu, and it's exactly like this. So it seems like you  
 24 have to drop down about two logs, in other words, 200  
 25 pfu really isn't any different than 1000, but if you

Page 103

1 drop down to about 10, then you can extend the window  
 2 out. See, the same thing with Marburg Angola. This  
 3 is Marburg Angola in a rhesus macaque, 1000 pfu, the  
 4 mean time to death is about day 7, window 6 to 8. If  
 5 you drop it to about 40 to 50 pfu, you move it out to  
 6 about day 10.  
 7 So again, challenge dose -- and the  
 8 Russians showed this with Marburg, as well -- does  
 9 have some -- it does matter. One of the things that -  
 10 - when Lisa came and joined our group a number of  
 11 years ago that Lisa and I were both interested in is  
 12 trying to develop these models to develop better  
 13 treatments and better strategies for treatments. So  
 14 in other words, if you can understand how the virus  
 15 causes disease, then, you know, that should give you  
 16 insight into developing more effective interventions.  
 17 And so we did two sequential pathogenesis  
 18 studies. One of these was published in the American  
 19 Journal of Pathology, and Lisa's working on the  
 20 Marburg one. And so this was with the cynomolgus  
 21 macaque model, again, 100 percent lethal, and the  
 22 animals were injected with 1000 pfu of ebola Zaire,  
 23 and then sequentially killed up to six days, you know,  
 24 three at day 2, four at day 3 and so on. And again,  
 25 so what we're trying to do here is get like a frame by

Page 104

1 frame view of what's going on in the ebola and Marburg  
 2 infection. And this is a study that Lisa and I did  
 3 with -- we used the Ci67. It's one of the 1967  
 4 isolates of Marburg virus, same study design for  
 5 Marburg virus.  
 6 This happens to be the organ titers from  
 7 the ebola study, and the only thing I wanted you to  
 8 look at here, is the virus first pops around day 2 in  
 9 the macrophage rich tissues, spleen and the lymph  
 10 nodes. The high titers are reached primarily in liver  
 11 and spleen. For Marburg, the liver is a little bit  
 12 more involved with ebola, and I'll talk about that.  
 13 This just shows how dramatic between, you know, day 4  
 14 and 5, particularly in these models, or maybe 5 and 6  
 15 in the Marburg model spleen -- and your antigen is the  
 16 red orange color -- just incredible amounts of virus  
 17 between, say, day 4 and 5, within a 24-hour period you  
 18 go from, you know, scattered virus to just an  
 19 overwhelming infection. As Mike said, for most of  
 20 these models the target cells are primarily the  
 21 monocytes, macrophages, dendritic cells. This is true  
 22 for both ebola and Marburg. You get a prominent loss  
 23 of lymphocytes, lymphopenia. Mike talked about this  
 24 a little bit. Lymphocytes, interestingly, are not  
 25 infected by the virus. In other words, you don't get

Page 105

<p>1 -- the virus doesn't bind to a lymphocyte and  2 replicate, you know, assemble, mature. You don't get  3 progeny virus from lymphocytes, yet they die in  4 massive numbers. And the process for the macaque  5 models is apoptosis, is the process. And you see this  6 for both ebola and Marburg.</p> <p>7 Again, the liver seems to be a little bit  8 more involved with Marburg. This is a lot of fatty  9 degeneration. This is pretty typical for what we're  10 seeing with Marburg Angola. Again, there's something  11 different with Angola, we're not quite sure what,  12 versus Musoke. It gets to the liver involved with  13 Zaire, but not to the -- with the ebola viruses, but  14 not quite to the extent. We tend to get higher  15 titers, maybe 109, 10 -- I've even seen up to 109.8 with  16 Marburg where 107, 107.5 is pretty typical organ titer  17 per gram for ebola.</p> <p>18 And you also see this by  19 immunohistochemistry. You see, again, a lot more  20 standing of the hepatocytes and cooper cells, very  21 dramatic with Marburg. You see it with ebola, but to  22 a lesser degree. Liver enzymes, just as Tony and Mike  23 had pointed out, AST and ALT levels are both increased  24 for Marburg and ebola, probably AST a little bit more.  25 So one of the things, again, that I want</p> <p style="text-align: right;">Page 106</p>	<p>1 human cases. We also see this in the ebola in the  2 macaque models, and total protein remains relatively  3 constant. So, you know, your endothelium, it's not  4 like NEPA or rickettsial infection where you get just  5 the endothelium is wiped out and you're -- because at  6 that rapidity, you'd expect total protein to drop, but  7 it's a small molecular weight proteins like albumin.  8 But clearly there's vascular -- the vasculature is  9 damaged to some extent, and it's not functioning  10 properly.</p> <p>11 D-dimers, and Tony talked about this, and  12 Mike has talked about this, this is huge. And if you  13 look at the macaque models, this is ebola on the left,  14 and Marburg on the right. You can see, viremia  15 doesn't start in the macaque models until day 3 or 4,  16 but you've got very large increases at day 1 and 2 of  17 D-dimers. In the macaque models, you get a  18 thrombocytopenia. D-dimers are important because it's  19 a fibrin degradation product, but it shows that you  20 have activation of both the clotting and the  21 fibrilytic pathways of blood coagulation. And they're  22 seen in over 95 percent of cases of DIC elevated  23 levels, and particularly in sever sepsis.</p> <p>24 Coagulopathy, more than half of the non-  25 human primates, probably 80, 90 percent are going to</p> <p style="text-align: right;">Page 108</p>
<p>1 to come back to, and it's one of the primary  2 differences, and I think it's extremely important  3 between the rodents and the non-human primates is DIC,  4 and Tony Sanchez did a great job of showing how you  5 see this in human cases, and this is one of the major  6 aspects of disease in the non-human primate models,  7 and a number of years ago, we were trying to ask, why,  8 what causes this? Well, there's two things that could  9 cause it. One is widespread injury to the endothelial  10 cells. Then when we did the ebola serial sac study,  11 this is day 5, and these animals, again, they're dying  12 on day 6.</p> <p>13 And we're not seeing infected endothelial  14 cells. There's a vessel in a lymph node, and all the  15 antigens out here in the extra-vascular area, and Lisa  16 did a really nice stain for von Willebrand's factor,  17 so that you can the endothelium light up nice. The  18 endothelium is intact and all the antigens outside the  19 endothelium, this is an EM that I did from one of the  20 animals at day 4. You don't see any infection of the  21 endothelial cells. You see some activation, start to  22 see some fibrin deposits, platelets starting to  23 adhere, but really not a lot going on with the  24 endothelium.</p> <p>25 Tony showed earlier that albumin drops in</p> <p style="text-align: right;">Page 107</p>	<p>1 have various degrees, these happen to be severe. You  2 get some bleeding at the -- puddling at the vena-  3 puncture site, but very prominent in the macaque  4 models. You also see this in some of the organs.  5 This happens to be a classic lesion of the  6 gastroduodenal junction. You see how this develops  7 till about day 5. This is not pathognomonic for  8 ebola. There's a lot of congestion here. You see  9 this with a lot of, a number of diseases, and Mike  10 Bray pointed out that, while ebola is hemorrhagic  11 fever and you do get some bleeding, it's not like  12 rigid pressing in the hot zone. There's clearly  13 coagulation disorders, and I like the term congestion.</p> <p>14 You don't -- you do have this disorder,  15 but you don't really have frank hemorrhage in most of  16 the cases. What's interesting in the macaque models,  17 too, is you see the fibrin actually shows up as early  18 as day 4. This happens to be spleen. This happens to  19 be a vessel in the renal medulla and the kidney, and  20 again, we're seeing fibrin deposited before we see  21 infection of the endothelium. And so that caused us  22 to ask the question, Well, was it the second major  23 mechanism that triggers DIC, which is the release of  24 tissue factor, or other thromboblasic substances into  25 the circulation. And to make a long story short, yes,</p> <p style="text-align: right;">Page 109</p>

<p>1 when ebola infects macrofages or monocytes, it up  2 regulates tissue factor, and we don't think this is  3 the only thing, the only contributing factor or  4 trigger for the DIC. It's one of probably a number.  5 And we also see the same thing for Marburg. Marburg,  6 it just comes up a lot later. And so if we look at  7 the macaque models, I'm just summarizing some of the  8 similarities and differences between Marburg and ebola  9 in the cynomolgus macaque model. Early target cells  10 are the same. You certainly get the loss of  11 lymphocytes. I didn't show all of the pro-inflammatory  12 cytokine data, but you get increases in a number of  13 cytokines, IL-6, TNF alpha, MCP-1 with both of these.  14 Fibrin deposits do occur in Marburg, but  15 they are not quite as dramatic or as prominent as in  16 ebola. Thrombocytopenia, you also see that in  17 Marburg, not quite as dramatic as ebola. Most of this  18 is just temporal differences. It just seems for  19 Marburg everything happens a little closer to the end,  20 where ebola maybe it's just a little bit earlier, and  21 the liver is -- in our models, is a little bit more  22 involved with Marburg than ebola. This is a cartoon  23 that Lisa and I developed to kind of show what we  24 thought was happening with regard to coagulation,  25 where you get macrophages and monocytes that up-</p> <p style="text-align: right;">Page 110</p>	<p>1 first infected the monkeys with 1000 pfu of ebola  2 Zaire, and then one group of animals was treated about  3 10 or 15 minutes after challenge with NAPc2. Another  4 group of animals we waited 24 hours to treat. We got  5 the same result either way.  6 We got 33 percent protection. Again, this  7 is 100 percent lethal model. Now, I think it's  8 obvious with any of these post-exposure treatments  9 that we need to start walking these out past this time  10 point, but I think this shows the coagulopathy clearly  11 is important in these models, because clearly a drug  12 that blocked the tissue factor, factor 70 pathway had  13 a very beneficial effect, and protected about a third  14 of the animals, again, in a post-exposure regiment.  15 One of the other -- this is a natural  16 anti-coagulant, still continuing on the coagulation  17 theme, that Lisa and I were able to identify was  18 protein C, and in our ebola model, we can see very  19 significant drops in protein C levels, again by day 3  20 or 4, and this is important because there is an  21 association with a severe sepsis, and a drop of 40  22 percent of protein C in severe sepsis is a predicator  23 of poor outcome. And you can see that the levels here  24 are way below 40 percent, and you can also see in  25 human cases of severe sepsis, that almost all the</p> <p style="text-align: right;">Page 112</p>
<p>1 regulate tissue factor. You get budding of these, or  2 break-off of these membrane bound micro-particles.  3 You get a lot of membrane proliferation of cells when  4 they get infected with ebola and Marburg, and we've  5 actually found tissue factor in these membranes.  6 This, then, can trigger the tissue factor,  7 Factor 78 pathway through the extrinsic pathway, also  8 of blood coagulation, also feeds back through the  9 intrinsic through factor 9, and the bottom line is you  10 get too much fibrin. You get a lot of cross-linked  11 fibrin. And so one of the strategies that we had  12 early on was to try to block this pathway, and there's  13 a drug called rNAPc2 that Mike talked about. It's a  14 recombinant nematode protein that actually blocks the  15 -- specifically blocks the tissue factor, factor 70  16 pathway. And we published a paper in Lancet a number  17 of years ago, back in 2003, showing, in our rhesus  18 macaque model, that we had 33 percent protection, and  19 we protected three macaques, where there were two  20 different regiments.  21 I think one of the important points here,  22 because we repeated this study several times so that  23 we actually believe the result, but we had a  24 significant delay in death of all the animals that  25 were treated, but anyway, what had happened was we</p> <p style="text-align: right;">Page 111</p>	<p>1 cases have very low levels, below normal limits of  2 severe sepsis.  3 Now there's a drug that the company has  4 used, that Eli Lilly uses to treat severe sepsis, and  5 it's activated protein C. It's called, the trade name  6 is Xigris. And recently, we've tried to block, or  7 basically treat animals with low levels of activated  8 protein C with Xigris. The problem is a short half-  9 life. It's about 13 minutes, and NAPc2 is administered  10 by sub-cu injections, so you can use the squeeze  11 mechanism, and pull the animal up and inject.  12 And almost all the treatment studies that  13 we've done in Level 4 have been done with some kind of  14 an injection, and IV injection, a sub-cu injection.  15 This presented a real problem. With the 13 minute  16 half-life, you really needed to run an IV line. And  17 we were fortunate in that, because of the HIV field  18 and a lot of the work that's been done with the SHIV  19 monkeys, they had developed a tether system where they  20 insert a catheter, and they run the catheter down  21 right above the right atrium, and then the line is run  22 out through the back. The monkey is put into a jacket  23 so that it doesn't rip the catheter out.  24 And this is all fed through a flex cable,  25 and then on the outside, if you can see here, there's</p> <p style="text-align: right;">Page 113</p>

<p>1 actually a computer controlled pump system so that you  2 can continuously infuse the drug. Now, the biggest  3 problem that we had when we were developing this, or  4 trying to get it to work, we had to repeat the study  5 a couple of times, is the monkeys don't like to wear  6 these jackets. And you can see this is 30 days. You  7 have to adapt them, and again, it points out some  8 differences between the different species of non-human  9 primates.</p> <p>10 The rhesus macaques actually tend to be a  11 little bit smarter than the cynos or the greens and we  12 had to -- they were particularly tough to adapt to  13 these jackets. But anyway, the study that Lisa and I  14 did, we began -- we infected -- there was three  15 separate studies, and there was a cohort of 14 total  16 animals. There was 11 treated in three controls.  17 Controls got saline, the treated animals got th Eli  18 Lily's, drug Xigris, and we started treatment. We  19 infected the animals first with 1000 pfu of Zaire, and  20 the treatment was started probably about 60 to 90  21 minutes later. The drug has to go through the line.</p> <p>22 And the bottom line is, very similar to  23 the NAPc2 result, we had a group of animals that  24 didn't respond that died with the controls. We had a  25 group that responded significant delays in death, and</p> <p style="text-align: right;">Page 114</p>	<p>1 vaccine, the one thing that we notice that is  2 associated with survival, is the animals that survive  3 have lower levels of D-dimers. They're able to  4 maintain the protein C activity, and in certain  5 cytokines, especially IL-6 are maintained. And then  6 as Tony talked about in human cases, low viral load.  7 And this is a slide that shows these animals are all  8 plotted against historical controls, and I think  9 there's 23 historical control rhesus here, and you can  10 see where the cutoff is 4.2 log 10 pfu.</p> <p>11 Xigris is shown in blue and NAPc2 in red,  12 and then the VSV monkeys, but the bottom line is that,  13 at any point in the disease course, in a rhesus  14 macaque with ebola Zaire, if the viremia, serum  15 viremia, plasma viremia goes above about 4.2 logs, you  16 die. There's been a lot of discussion about vaccines,  17 and I'm running about a minute left here, so I'm going  18 to go through this quick.</p> <p>19 We have looked at aerosol models. The VSV  20 vaccine that Heinz developed protects, in a preventive  21 platform, against an aerosol challenge. So I'm going  22 to talk now briefly just about aerosol versus  23 injection. We had three control aerosol infected  24 monkeys with ebola Zaire, and two with Marburg. All  25 of these animals died. It took a little bit longer in</p> <p style="text-align: right;">Page 116</p>
<p>1 we had two animals that survived, and interestingly,  2 this is pretty much exactly -- the 19.4 percent effect  3 is pretty much exactly what this drug was licensed on  4 for use in humans.</p> <p>5 So again, I think this does show that  6 coagulation, and the ability to control the regulation  7 of coagulation has a dramatic effect on the disease  8 course. And Heinz talked about this, and I'm glad  9 because I'm running out of time, that he did, but the  10 other thing, probably one of the most amazing studies  11 that I've ever been involved with in my entire career  12 was using the VSV vaccine platform, as opposed to  13 exposure treatment, just like you would use for  14 rabies. And this is a study that Katie Diderio in our  15 lab published in the Lancet last year where, again,  16 you administer the vaccine as a treatment.</p> <p>17 Animals are infected with ebola or  18 Marburg. Twenty minutes later, they get the vaccine,  19 and the reason I'm showing you this is because I want  20 to try to draw some parallels with what correlates  21 with survival, but again, with the Marburg, 100  22 percent protection with that strategy, with ebola,  23 about 50 percent protection, but if we look at all of  24 these models, NAPc2 -- or treatments, I'm sorry,  25 NAPc2, Xigris, and then with the post-exposure VSV</p> <p style="text-align: right;">Page 115</p>	<p>1 the aerosol model to produce the lethal infection, all  2 other conditions being equal. And what was  3 interesting is we didn't really see a lot of pathology  4 that you might expect.</p> <p>5 This is the lung in a Marburg infected  6 monkey by IM versus aerosol. This is an animal that  7 had consolidation. This has nothing to do with this  8 study, just to show you what you would see if you had  9 a severe case of broncho pneumonia. So we didn't  10 really see this in this model. In fact, we saw  11 pathology that was very typical with the IM model,  12 lymphoid depletion, fibrin deposition, nice macular  13 rash, Marburg intense staining of the liver, macular  14 rash, again, lymphoid depletion.</p> <p>15 There's a little bit -- you see a little  16 bit more antigen in the lung, and then you see antigen  17 in some of the other tissues, epithelial cells in the  18 lip, nasal cavity and things like that. But it really  19 -- we didn't see any really significant difference in  20 the pathology between the aerosol and the  21 intramuscular. It's not really surprising, because we  22 did an oral conjunctival exposure a number of years  23 ago with Nancy Jackson at USAMRIID, and there really  24 wasn't any different pathology by that route, either.  25 And so I think I'm out of time there.</p> <p style="text-align: right;">Page 117</p>

<p>1           Somebody had asked earlier about a slide, 2 and I'd be happy to give this to you later. It just 3 shows the differences between the models, the macaques 4 with different clinical features of disease. Again, 5 the primary difference is the coagulation disorders 6 that we see with the macaque models, not quite as 7 prominent with the African greens, and so I think my 8 argument is pretty much that the rhesus macaque, 9 because of the time to death and a lot of the other 10 conditions, is probably the best model that we have 11 for human disease, but I was really fascinated. I 12 thought Tony's talk was fantastic because it was -- 13 there were so many parallels between the macaque 14 models, and what we now know, and what Tony has 15 started to show, and what Eric Larol is starting to 16 show with humans, and I'll take any questions, thank 17 you.</p> <p>18           (Applause)</p> <p>19           DR. JAHRLING: Surely there are questions 20 for Dr. Geisbert.</p> <p>21           DR. GEISBERT: Heinz.</p> <p>22           DR. FELDMANN: Going back to the old issue 23 about the controversial studies that Sharif showed, 24 and you with your monkeys in terms of the endothelial 25 cells, if I remember, someone was arguing maybe this</p> <p style="text-align: right;">Page 118</p>	<p>1 infected. But it's just not something you see until 2 the very end.</p> <p>3           PARTICIPANT: Tom, do you think there's 4 any differences in the ebola Zaire isolates, are there 5 some strange variations in terms of pathogenesis, 6 because in one vaccine study we did with Dr. Nabel's 7 group, we used a very low passage ebola Zaire `76. It 8 was a single E6 passage that we inoculated six pfu, 9 and we got a cynom to die in four days.</p> <p>10           DR. GEISBERT: I definitely think there 11 is, and that's why I was trying to make the point that 12 you have a -- it's very difficult when you start 13 comparing studies across different groups with 14 different virus isolates. Everything that I showed 15 you on that one table early was done in our group with 16 the exact same isolate, so the exact same Zaire 17 isolate for all the macaque studies. And I couldn't 18 agree with you more.</p> <p>19           We have a `76 Mayanga isolate that's 20 probably been passaged too many times, and the only 21 two macaques that I've ever known that survived ebola 22 Zaire were one that was on an oral contractival study 23 that survived that, and then a monkey that was on a 24 different study that actually survived an IM 25 injection. But what actually was with the Zaire `95,</p> <p style="text-align: right;">Page 120</p>
<p>1 is due to the late stage, like these are all post- 2 mortem studies that happened, but CDC people have to 3 correct me that, as far as I know, he found viral 4 antigen in endothelial cells in these patients, and so 5 on. Have you ever looked at animals that you found 6 dead, or that had already died, or whether you see 7 more in that, or is this clearly a difference between 8 the human and the non-human primates?</p> <p>9           DR. GEISBERT: We see -- there's a lot of 10 individual animal variability, first off. We do see 11 infected endothelial cells in the monkey model. It's 12 not that we don't see them, but it's really more the 13 terminal -- the end stage. So if an animal dies on 14 day 6, or whatever the terminal time point, in the 15 serial SAC studies, it was only at the very end that 16 we were finding infected endothelial cells, and I've 17 never noticed much of difference whether an animal is 18 euthanized versus whether an animal was, say, found 19 dead. If there was any difference in the distribution, 20 I've never seen it. It could be a difference between 21 the humans and the non-human primates, or it could 22 just be that you're looking at end stage, and you 23 really don't have the progression. Because I mean, if 24 you're looking at the end stage in the macaques, 25 you're going to say, yes, endothelial cells are</p> <p style="text-align: right;">Page 119</p>	<p>1 the Kikwit, we've never had a monkey survive that, a 2 rhesus or a cynom as a control animal. So I 3 completely agree with you. I think that that 4 certainly passage history, and certainly differences 5 between isolates, there could be differences. And 6 look at the outbreaks, I mean, there seems to be 7 differences in mortality rate with outbreak, and could 8 that be a reflection of the particular isolate in that 9 outbreak.</p> <p>10           PARTICIPANT: If I could offer up one 11 little observation that we have with some stock 12 viruses that we have, a spleen homogenate from a cyno, 13 compared to a tissue culture passage virus, if you 14 titrate those two stocks, and you titrate it on E6 15 cells, well the E6 stock is going to titrate out 16 higher, but then it will flip when you try infecting 17 on PBMCs from a cyno. So it seems that there's 18 perhaps a tropism, or an ability to grow inside 19 themselves or infect them is different.</p> <p>20           DR. GEISBERT: Yes, I mean, we see -- if 21 you put most of these viruses on a macrophage, you get 22 higher titers than you do on a Vero cell. And the 23 passage history of the Veros versus -- we do plaque 24 assays and Veros and E6s, and even the same sample, 25 you can get different titers depending on the passage</p> <p style="text-align: right;">Page 121</p>

1 history of the cells. It gets a little frustrating.  
2 PARTICIPANT: And it also may account for  
3 some of the problems we've had titrating human  
4 specimens, like from Kikwit, on E6 cells.  
5 DR. GEISBERT: Yes.  
6 DR. JAHRLING: One last question.  
7 PARTICIPANT: Tom, you showed a huge  
8 amount of pathology, of course, definitely a large  
9 amount of research data. I'm a little bit discouraged  
10 by the massive amount of pathology induced by this  
11 virus to develop a drug that can correct all this  
12 problem, but I'm truly encouraged by your paneer in  
13 post-exposure vaccination. The question is, actually,  
14 if you use the vaccine, for example, the VSV, actually  
15 there's a risk in my opinion. The VSV has to  
16 replicate to produce enough antigen to induce a  
17 response, while at the same time, the virus is  
18 actually replicating, as well, to see who will win the  
19 race.  
20 In your case actually the antigen -- I'm  
21 sorry, the vaccine does win the race, but my question  
22 is, have you looked at the, what's the time course in  
23 terms of the VSV vaccine to replicate, to generate  
24 enough antigen to induce the immune response and --  
25 DR. GEISBERT: Yes, the VSV vaccine

Page 122

1 replicates a lot faster than wild type virus. So you  
2 know, you can get 107 of the VSV vaccine in two days in  
3 cell culture, but you can't come close to that. So it  
4 definitely replicates a lot faster than the wild type  
5 virus. I did point out -- I don't know if Heinz  
6 pointed this out or not but, you know, we're trying to  
7 understand the mechanism of how that works. We know  
8 it's not an -- we know it's not non-specificity,  
9 because we had controls in there.  
10 We've had -- in the Marburg study, we've  
11 even used -- Katie has used Angola as a control for  
12 Musoke, and there is specificity. You need the  
13 specific vector. In other words, you need Musoke to  
14 protect against Musoke. You need Zaire to protect  
15 against Zaire. So it's not -- and we use non-specific  
16 vectors as controls in these studies, so it's not  
17 really that -- but your point is well-taken, and Heinz  
18 and I are trying to figure it out right now. I mean,  
19 it could be a race. It could be that it's just  
20 interfering, you know, the vector is infecting those  
21 cells before the virus can get there.  
22 PARTICIPANT: Tom, don't you think it's  
23 time to start combining some of these treatment  
24 strategies? I see the point that Mike brought up  
25 about the mouse model that a lot of treatment

Page 123

1 approaches that work there and don't work in the  
2 guinea pigs or the monkeys, that could be because of  
3 interferon. So I think that point is well-taken, but  
4 doesn't it, at the same time suggest that maybe there  
5 is a synergy between some of these treatments that  
6 work in mice and interferon, and we have the clinical  
7 precedence for that with reba virine and interferon  
8 for HCD. So why not starting combining some of these  
9 approaches, such as small model core, with an  
10 interferon, something that's immune stimulatory,  
11 something that's anti-viral?  
12 DR. GEISBERT: I couldn't agree more. I  
13 didn't have the time to present the data. Lisa's got  
14 some really nice data that she's presented at other  
15 meetings with interferon beta, and you can treat  
16 macaques with interferon beta, and while you don't get  
17 protection, you don't get any protection, you get a  
18 significant delay in death. So if you combine that  
19 with some kind of siRNA or something, would -- you  
20 know, and again, try to tip the balance in favor of  
21 the host. Get the viral load down less than 4.2 or  
22 whatever the critical threshold is, and we're working  
23 on things like that right now. We're looking at  
24 different strategies to combine maybe one of the  
25 anticoagulants with an anti-viral, and again, with the

Page 124

1 goal of lowering the load. Because I think, once --  
2 again, once a viral load, and Tony's data shows it and  
3 our data shows it, that you go over about four, four  
4 and a half logs, it's over.  
5 DR. JAHRLING: It was a very interesting  
6 presentation. It's been the fuel for a lot of ongoing  
7 discussion which, perhaps, we can do over lunch, but  
8 there is one presentation between us and lunch.  
9 Sorry, Doug. And I just would mention also that Dr.  
10 Ksaizek, and I think Sanchez also made note that  
11 respiratory infection is not the natural route of  
12 exposure, but certainly for concerns about  
13 bioterrorism and what have you, protection against  
14 aerosolized filoviruses is very much on the radar  
15 screen. So Dr. Reed, from USAMRIID, is going to talk  
16 about aerosolized filoviruses in three species of  
17 primates.  
18 DR. REED: All right, well, thanks to the  
19 organizers for inviting me to speak today. I'm going  
20 to talk about some work that we've been doing for the  
21 last couple of years. We don't have near the history  
22 that Peter, or Tom, or some of the people in this  
23 audience do, but we are doing our best to catch up.  
24 As Peter said, we are primarily concerned  
25 with the aerosol exposure. Why is a long and

Page 125

<p>1 complicated history. Aerosol dissemination is the  2 most effective way to disseminate a biological weapon  3 for a large scale attack. However, for filoviruses,  4 it was thought for a very long time that aerosol  5 transmission was not a concern during natural  6 outbreaks. However, there's been an accumulation of  7 data over the years. The ebola Reston outbreak, where  8 there was thought to be possible aerosol transmission  9 of the viruses, the infected animals that were in the  10 same room as other naive animals that have been  11 reported in a couple of instances, and there was  12 experimental evidence, reported by both USAMRIID and  13 the Russians, that showed that you could, in fact, get  14 aerosol infection of primates and guinea pigs.</p> <p>15 There have been allegations that the  16 former Soviet Union looked at both Marburg and ebola  17 as biological weapons with the potential for aerosol  18 dissemination. And then also a terrorist organization  19 was thought to have looked at these viruses as a  20 potential weapon. I think Tom and Mike have pretty  21 well covered what the rodent and primate models are  22 for these viruses, so I won't talk to anything about  23 that.</p> <p>24 Again, I said there's some experimental  25 evidence for aerosol infection with these viruses. A</p> <p style="text-align: right;">Page 126</p>	<p>1 about that today.</p> <p>2 The first part of it was to determine an  3 appropriate challenge dose. Historically, as Tom  4 mentioned, 1,000 pfu had been the traditional high  5 dose challenge for parenteral sub-cu or IM infection,  6 but the LD50s and LD99 have never been empirically  7 established for aerosol, and at least from what I've  8 been able to find in literature, for parenteral,  9 either, for animal models. It's known to be very low.  10 It's just never been formally established. The  11 pathology and the disease in these animal models,  12 then, compare that with what we know for the  13 parenteral infections, and as Tom has mentioned  14 already quite elegantly, this issue of what species do  15 you use, particularly when you get to non-human  16 primates. Do you use a cynon? Do you use a rhesus?  17 Do you use an African green? And we wanted to examine  18 whether the most commonly used model was or was not  19 necessarily the most relevant model of the human  20 disease, and in the case of both ebola Zaire and  21 Marburg Ci67, we have pathogenesis studies that have  22 been done in cynomolgus macaques for parenteral  23 infection that we can compare with quite nicely.</p> <p>24 And that's work that's been -- Tom just  25 talked about. And then also we wanted to evaluate the</p> <p style="text-align: right;">Page 128</p>
<p>1 number of studies that were published by the Russians  2 in the 1990s and early part of this century showing  3 that, with the Popp strain of Marburg or ebola Zaire,  4 you could produce lethal disease in guinea pigs or  5 non-human primates. The two caveats that I would  6 throw into that is that there are doses that they use.  7 They use a guinea pick LD50, which does not translate  8 easily to our tradition pfu measurements. And in  9 addition, they used the guinea pig adapted virus,  10 rather than wild type virus for most of their studies.  11 And as Tom has already said, USAMRIID scientists have  12 also looked at this using ebola Zaire and a rhesus  13 macaque model. There was also the oral conjunctival  14 infections that they looked at, but there's the  15 limited number of studies.</p> <p>16 So one of our goals is to develop the  17 animal models, and primarily for efficacy studies to  18 support licensure of these therapeutics or vaccines  19 under the animal rule. And in particular, because  20 we're a DOD agency, we're concerned about bio-defense.  21 We need to be able to show protection against an  22 aerosol exposure. And just to kind of go through the  23 objectives of how we've approached this, developing  24 both guinea pig and non-human primate models, we have  25 looked a little bit at mice, but I'm not going to talk</p> <p style="text-align: right;">Page 127</p>	<p>1 use of telemetry to monitor and record physiological  2 changes in these animals, and that's not been  3 previously done for filovirus infected animals. Why  4 ebola Zaire in `95? As it's already been eluded to,  5 it's a highly pathogenic virus, particularly in  6 humans. Prior to the Marburg outbreak in Angola, it's  7 probably the most virulent of the known filoviruses,  8 and with Mayanga 76 in there, as well. Highly  9 virulent in non-human primates. There is a prior  10 pathogenesis study, and there are prior vaccine  11 studies done for parenteral challenge. And one thing  12 that Tom eluded to just a few minutes ago about the  13 strain of the virus, the passage of the virus,  14 everything I'm talking about today, both for ebola and  15 a little bit of -- I'm going to show with Ci67, was  16 all done with the same isolate, the same passage that  17 Tom talked about for his viruses, and so we have some  18 nice comparability there.</p> <p>19 In terms of clinical observations and  20 pathology, we're not going to talk too much about  21 this, but what we saw clinically -- that's not good.  22 It didn't like that. Touch to resume. There we go.  23 All right, what we saw, and this is similar to what  24 Tom reported, both species of macaques developed a  25 notable petechial rash, similar to parenteral</p> <p style="text-align: right;">Page 129</p>

<p>1 inoculation. I would tend to say, from what Tom just 2 showed us, the pictures, the rash that we see for 3 aerosol exposure is not as severe as what we see with 4 the parenteral infection.</p> <p>5 The greens do not develop a significant 6 rash at all. If it's there at all, it's very light, 7 very faint. The clinical observations, these are the 8 observations from the technicians and the scientists 9 going into the rooms, we do tend to see more reports 10 of gastrointestinal discomfort, anorexia, with both 11 macaques species, and not with the greens. And then 12 in terms of the gross necropsy findings, which I'll 13 show you some of this, the lungs of the macaques and 14 the greens have a mild pulmonary congestion and edema, 15 whereas what we've seen with the rhesus macaques for 16 ebola was that they looked more like a parenteral 17 inoculation.</p> <p>18 The one comment I've seen from both 19 pathologists for both the ebola Zaire and the CI67 is 20 that, while it looks similar to what we see for 21 parenteral inoculation, there is a difference in -- 22 there is a notable difference in the dissemination of 23 the virus, particularly the infection of the 24 mediastinal lymph nodes. And a lot of this work is 25 still in progress.</p> <p style="text-align: right;">Page 130</p>	<p>1 succumbing to the infection. With African greens, 2 it's even more protracted. It goes out to day 8 or 3 day 9 on these animals at a higher dose of over 100, 4 usually around 1,000 pfu's. In this case, we actually 5 did see a notable temperature drop before the animal 6 succumbed to the infection. One of the issues Tom 7 mentioned about the end point, when do you decide to 8 euthanize an animal versus letting it continue? We 9 notice that with the African greens it was a lot more 10 difficult to tell when the animal was ready to be 11 euthanized.</p> <p>12 These animals would continue eating, show 13 no obvious signs of discomfort. They would have a 14 fever response. They would have other clinical signs. 15 Their white blood counts were different, but in terms 16 of walking in this room and looking at the animals, 17 you would be surprised to know that that animal was 18 sick. So in a lot of cases with the African greens, 19 even though we were trying to be diligent, we did have 20 several of these animals that died overnight because 21 we thought the animal had at least another day or two 22 in it, and we were surprised in the morning.</p> <p>23 This just shows you the temperature 24 profile. Now, this is the average for each group. 25 There's -- we had several groups that we did. We did</p> <p style="text-align: right;">Page 132</p>
<p>1 This just shows you the rash, and again, 2 you can see some differences there from what Tom 3 showed you. It's not quite as severe. However, we do 4 see it quite nicely in the rhesus macaques. We see it 5 also in the cynos, and this is just one of many 6 African greens in which we didn't see any rash at all.</p> <p>7 In terms of the fever response, and as I 8 told you, we used telemetry to monitor these animals. 9 We've got a lot of history working with alpha viruses 10 with telemetry showing the fever response, and being 11 able to quantify the fever response in addition to 12 just determining that there is, in fact, a fever. And 13 this is showing you a typical pattern for a cynomolgus 14 macaque infected with ebola Zaire. The black line 15 here is the predicted temperature based on what we 16 collected prior to exposure. And then the red values 17 here are the actual temperatures collected post- 18 exposure, and you can see this is a fairly typical 19 cyno exposed to ebola Zaire at a high dose, and this 20 animal died around day 6, day 7.</p> <p>21 When you then look at a rhesus macaque, 22 you can see that this is a little more protracted, 23 just like Tom reported for parenteral infection. 24 Again, you see quite nicely, there's, you know, a 25 fever that develops about two days prior to the animal</p> <p style="text-align: right;">Page 131</p>	<p>1 a high dose challenge with at least six animals, and 2 each species, and this just shows you the average 3 temperature daily after exposure, again using our 4 telemetry data. And you can see with the cyno in blue 5 here, it peaks around day 5, day 6, and then declines 6 rapidly. We had one animal that made it out to day 8. 7 The rest of them died on day 6 or day 7.</p> <p>8 With the African greens, there was a 9 fairly uniform fever, again, peaks about day 8, and 10 then by day 9, these animals are dead. And with the 11 rhesus macaques, these animals have a similar peak to 12 what we saw with the African greens, however, they 13 progress much faster after that fever peaks.</p> <p>14 This is a summary of the data we've 15 accumulated in the cynos for ebola Zaire. We first 16 did an LD50 study to actually try and determine the 17 LD50 for ebola Zaire in the cyno, and again, we went 18 with the cyno because that's the most commonly used 19 model at USAMRIID, and that was our thinking was that 20 that was the most likely to be used in a vaccine 21 study, and so you can see at very low doses, a dose of 22 two pfu, we had -- the animal goes out to about day 10 23 and dies. However, we had other animals in that same 24 range that survived infection. Our LD50, we estimate 25 is less than 10.</p> <p style="text-align: right;">Page 133</p>

1 And the primary reason we don't give a  
 2 hard and fast number is because it is a few -- it's  
 3 only five animals that we're talking about for that  
 4 determination. And in addition, you're talking about  
 5 the limits of the plaque assay and its ability to  
 6 determine exactly how many pfus. We just had an  
 7 elegant discussion about the E6 cells versus the Vero  
 8 cells, the passage number, all those kind of things  
 9 come into play. It's, I think, sufficient to say it  
 10 is indeed very low in terms of the LD50.

11 At the higher dose challenge, this is a  
 12 dose of around 300 pfus. You can see, most of these  
 13 animals, the mean time to death was 6.5 days, so right  
 14 in the same range as a parenteral infection. Fever  
 15 duration in terms of hours is about 80 hours, and the  
 16 severity is 120. This is the summation of all those  
 17 fever points. So everything we define is a  
 18 significant fever. And I'll show you how this  
 19 compares to the other species.

20 You can see maximum temperatures, really  
 21 not that significant. We did have a few animals that  
 22 were over 40 degrees centigrade, but most are in the  
 23 range of about 39. When you move onto the rhesus  
 24 macaques, in the rhesus, we did a dose response study,  
 25 rather than a traditional LD50. We did animals at 10,

Page 134

1 100 and 1,000 pfus. You can see we're reasonably  
 2 close. We had about 10 here, not quite as high as we  
 3 would have liked with those two animals, and then a  
 4 little over 1,000 for these.

5 And again, time to death is protracted at  
 6 the lower doses. This animal actually survived, had  
 7 no real significant fever. The others had fairly good  
 8 fevers. You can see the duration in terms of the  
 9 fever is the same even at the lower doses as it is  
 10 with the higher doses. However, the time to death is  
 11 much quicker with the higher dose. The mean time to  
 12 death was 7.3 days, so again in the same range as a  
 13 parenteral infection for a rhesus macaque.

14 Fever duration here was a little bit  
 15 shorter, 62 hours, but the severity was actually a  
 16 little worse, 135. And you can see there's a number  
 17 of animals here, five of these six animals. This  
 18 animal, the telemetry we had issues with, but these  
 19 five -- four of these other five animals all had  
 20 fevers over 40 degrees centigrade. And when you look  
 21 at the African greens, these -- again, these take  
 22 longer to die, even at the higher dose. At our dose  
 23 response study, you can see death was about day 9, day  
 24 10. It's not a whole lot faster at a higher dose.  
 25 It's day 8. The mean time to death was 8.3 days.

Page 135

1 Fever duration is the longest, 88 hours,  
 2 and the fever severity was the worst, 189. If you  
 3 look at serum viremia, and again, this is one of those  
 4 areas where we're still in the process of analyzing a  
 5 lot of these samples. We've collected, as you can  
 6 see, a large number of samples in the last two years.  
 7 But what we have done is day 5, 6 and 7 and 8 for  
 8 these animals, and you can see that the African greens  
 9 here are the green line, the cynos and the rhesus are  
 10 the blue and the red. The viremia in the African  
 11 green is actually significantly higher for this  
 12 aerosol exposure. Whether or not that will be  
 13 statistically significant is yet to be determined, but  
 14 so far it looks fairly high.

15 Changes in white blood cell count, this is  
 16 total white blood cells, not just lymphocytes, with  
 17 the rhesus and the cynos -- I mean, the rhesus and the  
 18 greens here, you see a significant elevation towards  
 19 the end, whereas with the -- this is a change from  
 20 baseline. The cynos, there's a slight elevation, and  
 21 then a decrease. If you break that out and to look at  
 22 the lymphocytes, similar to what Tom has reported, and  
 23 others with the cynos, you see a decline in lymphocyte  
 24 count over the time of exposure, and then a brief  
 25 surge right before death. With the greens and the

Page 136

1 rhesus, you see a slight drop, and then a significant  
 2 increase in the last few days.

3 Platelet counts drop, as we would expect.  
 4 There -- it's more significant with the cynos. For  
 5 whatever reason here, we had an increase compared to  
 6 baseline on day 2. There's no day 1 data for the  
 7 cynos because the Coulter counter decided to die on us  
 8 that day. So that's why there's no data there for  
 9 that point, but the rest of them you can see the  
 10 greens and the cynos trickle along, and then decline  
 11 after about day 4, whereas the cynos pretty much  
 12 decline after day 2, consistently.

13 We did also look at PT and APT times using  
 14 a veterinary coagulation analyzer. And what we saw  
 15 was that it's a little misleading. There are changes  
 16 here with both the cyno and the rhesus; however, the  
 17 greens, the differences in coagulation times were far  
 18 more severe, and we saw disruption of both pathways.  
 19 In terms of the pathogenesis, this is a picture from  
 20 the pathologist on the study, Don Nichols. And I  
 21 asked him for some nice slides that I could show you  
 22 guys, so again, I'm not a pathologist. I'll defer to  
 23 Tom or Tony who did the Marburg work that I'm going to  
 24 show you here in a minute, if you've got pathology  
 25 questions, but the notable here is the mediastinal

Page 137

1 edema in these African green monkeys.  
2 But in particular, Don noted this, and  
3 when I asked him what is the difference between these  
4 species when it comes to the infection, and it's this  
5 congestion and hemorrhage in the mucosa of the  
6 duodenum. It's found in the macaques. Tom showed  
7 some data there, it's not common. It's about 50  
8 percent or less of the macaques, see this, you see  
9 this. However, with what we saw in the aerosol, it  
10 was two of the six rhesus, one of the six cynos, but  
11 five of the six African greens at the high dose, all  
12 had this congestion. So there is clearly some  
13 differences in the pathogenesis between the three  
14 species.  
15 In terms of the virus isolation, this is  
16 another part that's still in progress. The greens and  
17 the rhesus are pretty much done. The cynos I would  
18 take a little bit with a grain of salt, because that's  
19 an N of 2 right now, so we still have a lot more  
20 animals to grind through to see what the virus is in  
21 these different tissues. You can see that there are  
22 some differences, in particular in the gonad in the  
23 African green. Why that would be, I don't know, but  
24 that's the major significant difference in these  
25 species in terms of total virus load in the different  
Page 138

1 tissues.  
2 To kind of wrap up then for the ebola  
3 Zaire work, it's obviously virulent in all three  
4 species, even at very low doses, because we did have  
5 that one rhesus survivor at 8 pfus, and because of the  
6 differences in times of death and such, we would say  
7 that the rhesus and maybe the African greens may be  
8 slightly more resistant to the infection than the  
9 cynos. The time course of the infection is extended  
10 at lower doses. For the higher doses, though, it is  
11 comparable to what we see with parenteral infection.  
12 There are differences between the species.  
13 The mean time to death to the African greens is  
14 significantly longer, and shortest in the cynos. It  
15 might be slightly accelerated in the rhesus macaques  
16 compared to parenteral, and I take that -- I say  
17 slightly, based on, you know, our N of 6. Clinical  
18 observations and signs, there are differences,  
19 particularly the rash and the -- we don't see it in  
20 the greens, and the fever duration and severity is  
21 worse in the greens than the other two species, and  
22 again, what I just showed you in terms of the gross  
23 necropsy and the changes in the coagulation pathways.  
24 Which species is more relevant to the  
25 humans, that's a question hopefully we can shed some  
Page 139

1 light on today. This is particularly true for aerosol  
2 exposure. As I told you, it's not something that  
3 occurs in natural outbreaks. There are very few  
4 reports of human beings infected by aerosol exposure  
5 to these viruses. So how well this compares with the  
6 human, we don't know. I am going to throw in a little  
7 bit of Marburg work, and this is courtesy of Tony  
8 Alvez, who's sitting down here in the audience. And  
9 so if you have any questions for this, I can deflect  
10 some of them to him. We have also repeated all of  
11 these studies now looking at cynos, rhesus and greens  
12 with Marburg in the Ci67 strain using the same  
13 isolate, the same passage that Tom used for his  
14 pathogenesis study. So we have that comparability.  
15 This is just showing you the pathology in  
16 the lungs, and in fact, if you do see some congestion  
17 in the lungs, although it's fairly mild in comparison  
18 to bronchial pneumonia. You do see viral antigen  
19 staining in the lungs. And this is just showing you  
20 the mediastinal lymph nodes, both the pathology, and  
21 then also the viral antigen quite nicely.  
22 And then I'm just going to wrap up. As I  
23 said, we've only been working on this now in terms of  
24 the non-human primate studies for about the last two  
25 years, but we have done a significant number of  
Page 140

1 animals. We have done a little bit of work with the  
2 ebola Sudan, just starting on the LD50 determination  
3 there. We've also done an LD50 for Musoke and found  
4 that, similar to Ci67 and ebola Zaire, is in the range  
5 of 10 pfus or less. And Marburg Ravn, we've not done  
6 any primates work yet, but we have done some guinea  
7 pigs, and Angola, we've done six cynos, and found that  
8 the time to death was comparable to what Tom reported  
9 for a high dose challenge.  
10 And then just kind of acknowledge, as Tom  
11 eluded, any primate studies require a huge effort.  
12 Not only the people in the center that I work for,  
13 Matt Lackemeyer, in particular has done a lot of the  
14 virus isolation in the viremia studies, and the  
15 coagulation that I showed you, but also Nicole.  
16 Aysegul, if I'd shown you any of the guinea pig work,  
17 all the necropsies were a combination of Aysegul and  
18 Matt. Justin Hartings, who is our physicist in our  
19 department, and helped a lot with the dose  
20 determinations.  
21 One of the things we've tried to refine  
22 with our doses in terms of aerosols, is to use a time  
23 calculated dose to insure that all of our animals  
24 receive a uniform dose rather than a scatter shot if  
25 we just did a simple 10-minute exposure. If you want,  
Page 141

1 I can talk a lot about how that can -- how aerosol  
2 exposures are done, but I focused on the animal models  
3 for this talk.  
4 And then Joan, who grew up the viruses  
5 that we used, and Tom, and also and his expertise,  
6 Lisa and her input, Gene, and Diane who helped provide  
7 many of the guinea pig viruses that we've used. Our  
8 Vet Med Division, Keith and Heather. In particular,  
9 I want to notice Sarah Norris, who does our  
10 statistics, and then our Pathology Division who did  
11 all the necropses(phonetic) of all those primates that  
12 I've shown you that we did, which is Don Nichols, Jeff  
13 Brubaker, and Tony Alvez. Thank you.  
14 (Applause)  
15 Any questions? Everybody is hungry for  
16 lunch.  
17 PARTICIPANT: Could you go back and put  
18 this slide up that showed the three temperature  
19 profiles?  
20 DR. REED: Yes, well, I think I can. I  
21 can do it on the computer here, but I don't know if we  
22 can get that. There we go. That's -- I mean, you  
23 really don't see much in terms, difference in terms of  
24 the fever onset. It's the duration of the fever and  
25 the severity that seems to be different between the

Page 142

1 three species. Cynos are much shorter than the  
2 others.  
3 PARTICIPANT: Can you make a comment about  
4 the aerosol, obviously, that's a data issue, not  
5 general public safety issue for the civilian work. I  
6 wonder, for the different species monkeys, you find  
7 some difference as you stated the last part of your  
8 talk. You said we don't have human aerosol data at  
9 all.  
10 DR. REED: Right.  
11 PARTICIPANT: But how we will proceed for  
12 drug development, and approve the issue with FDA if we  
13 don't have any data? Obviously, that's a whole issue  
14 for animal. And I wonder how can we proceed, you  
15 know, to select a model from the three species of  
16 monkey, even some time if you want to spread this  
17 problem more across other rodent or, you know, that's  
18 a big hurdle, I believe, for us at the DoD. Can you  
19 make, you know, guess what will happen with FDA?  
20 DR. REED: Yes, the approach we've taken  
21 is the comparison with the parenteral infection, and  
22 to cross that with the human data that is available to  
23 try and establish what correlations we can, and  
24 determine, you know, where we see differences between  
25 parenteral and aerosol infection in the macaques and

Page 143

1 the African greens, how do those things pertain to  
2 what has been reported in human cases.  
3 PARTICIPANT: I find your data very, very  
4 convincing. Actually, it clearly shows the virus can  
5 be transmitted from aerosol. I think, not only in  
6 bio-defense, in the bio-terrorism defense, I think in  
7 that scenario, somebody holding a can in a subway  
8 spraying a culture, putting some perfume in a subway -  
9 -  
10 DR. REED: Right.  
11 PARTICIPANT: -- what's the consequence,  
12 actually that will cause infection in urban area. I  
13 think this lack of aerosol transmission data in human,  
14 based on what I heard, has not been looked carefully.  
15 The health workers does wear full protection, wear  
16 mask.  
17 DR. REED: Right.  
18 PARTICIPANT: So actually, there is lack  
19 of data to show this can be transmittable, and  
20 actually it's not necessary -- it's proven not be able  
21 to transmit it through air, but this data is clearly  
22 is evidence that it can be transmitted through air.  
23 I'm glad, actually, Dr. Jahrling is here, and maybe he  
24 can tell me the quote. The ebola virus has the  
25 potential to go airborne. The airborne strain of

Page 144

1 ebola can circle around the world in 6 to 7 weeks.  
2 And that was disputed, but I think clearly your data  
3 show even the early times statement made by Dr.  
4 Jahrling, and actually have some question in --  
5 DR. REED: Yes, I mean, our data shows  
6 that aerosol infection is possible in the primate  
7 species and in guinea pigs. How that translates to  
8 humans, and outbreaks in humans in Africa, I don't  
9 know. You know, you have to get virus into the air.  
10 You have to assume survivability. We don't know any  
11 of that because we don't look at that kind of  
12 information. We're looking strictly at an animal in  
13 an experimental setting. Yes, Tom?  
14 PARTICIPANT: Doug, in your slides, like  
15 the one you have up here, what group of animals does  
16 that represent? In other words, you did --  
17 DR. REED: That's the high dose challenge.  
18 PARTICIPANT: That's how many animals was  
19 in --  
20 DR. REED: So that's six animals per  
21 group.  
22 PARTICIPANT: Six animals per group, okay.  
23 DR. REED: Right, yes, for each of those,  
24 that's six. That's average data for each day post-  
25 infection for those animals.

Page 145

1 DR. JAHRLING: Well, thank -- one more?  
 2 DR. KORTEPETER: Mark Kortepeter from  
 3 USAMRIID. Just, it's been mentioned a number of times  
 4 today about, certainly in the -- you know, when we  
 5 have these outbreaks for the filoviruses that  
 6 generally we don't see person-to-person spread in the  
 7 healthcare setting, or if we do, it's usually related  
 8 to some innoculum, you know, from a needle stick or  
 9 something like that.  
 10 DR. REED: Right.  
 11 DR. KORTEPETER: The question I'd have,  
 12 though, is we don't know how that first case occurs,  
 13 how that's infected from the bat in the cave or  
 14 wherever it's occurring. So my question would be, do  
 15 we -- is it possible it's occurring from bat waddo?  
 16 Is it possible it's coming from, you know, a bat  
 17 that's spraying a person in the face in the cave, or  
 18 are they actually coming themselves, and being  
 19 infected that way. So my question is, we don't know  
 20 in that setting, I don't think, or, you know, how it's  
 21 postulated, that that first case is infected that  
 22 eventually does get in the healthcare system, and then  
 23 you see, you know, a multiplication in the healthcare  
 24 setting.  
 25 So I wouldn't say that we don't -- that it

Page 146

1 doesn't necessarily happen, but I'd say that it's  
 2 certainly possible.  
 3 DR. REED: Yes, I'm just trying to think,  
 4 you know, the epidemiology has tended to rule it out,  
 5 but again, and we've never looked at the answer of,  
 6 you know, how survival was the virus in an aerosol  
 7 and, you know, because that would come into play and  
 8 you would, you know, your environment changes  
 9 depending on where you are, and that would also affect  
 10 that survival.  
 11 So whether or not it really occurs in a  
 12 natural outbreak, I don't know.  
 13 DR. JAHRLING: There are several issues  
 14 here. I mean, certainly the aerosol that could be put  
 15 up by bats in caves, and the possibility of that being  
 16 a natural route of infection, I think is very real,  
 17 hasn't been proved or disproved, but I think it's  
 18 plausible. I think it's also important, though, that  
 19 the secondary transmission rate is certainly a lot  
 20 lower than influenza or measles or any of the other --  
 21 small pox, any of the big VW threats. So all these  
 22 various scenarios that are being worked out and  
 23 countermeasures developed, and what have you, I think  
 24 need to take into account that the R0 is probably  
 25 going to be a whole lot less than one.

Page 147

1 Last question, then we're going to go to  
 2 lunch.  
 3 DR. CHRISTOPHER: George Christopher from  
 4 DTRA. One point about the aerosol transmission; even  
 5 though it may not occur, or not occur frequently in  
 6 the natural setting, we're interested in developing  
 7 and obtaining FDA licensure for therapeutics. So like  
 8 it or not, we're going to need to demonstrate efficacy  
 9 in an animal challenge model, most likely using  
 10 aerosol, to obtain our BW indication.  
 11 DR. REED: Right, and that's why -- that's  
 12 what's driven our work is that indication for the --  
 13 DR. JAHRLING: So I'd like to thank the  
 14 presenters for a very interesting session this  
 15 morning. We're breaking for lunch. My advice to you  
 16 is not to go outside the building, but to eat in the  
 17 wonderful cafeteria upstairs. We'll be back in an  
 18 hour.  
 19 (Whereupon at 12:22 p.m. a luncheon recess  
 20 was taken.)  
 21  
 22  
 23  
 24  
 25

Page 148

1 A-F-T-E-R-N-O-O-N S-E-S-S-I-O-N  
 2 1:31 p.m.  
 3 SESSION II. VACCINES: CORRELATES OF PROTECTION AND  
 4 RELEVANT FUNCTIONAL ASSAYS  
 5 DR. NABEL: First, let us get on. The  
 6 first speaker will be Alan Schmaljohn who's now at the  
 7 University of Maryland Medical School. The title of  
 8 his talk is "Filovirus vaccine design and rationale."  
 9 He's going to give us an overview of the past and  
 10 present vaccine candidates as well as strategies for  
 11 multivalent vaccines. Alan.  
 12 DR. SCHMALJOHN: Okay. I will save any  
 13 hot button issues for the middle of the talk so that  
 14 latecomers cannot feel terribly cheated here. As you  
 15 can see, I don't currently have a former association  
 16 with USAMRIID where I've been for just over 20 years.  
 17 Still I have a lot of great colleagues and unfinished  
 18 manuscripts and the like from there. But my current  
 19 professional anchor is a new appointment at the  
 20 Department of Microbiology and Immunology in the  
 21 Maryland Medical School in downtown Baltimore.  
 22 Let's see. I have two choices here.  
 23 Okay. I'm going to talk in general, some  
 24 generalities, some specific examples that lead us to  
 25 then a couple tables that we'll spend more time on

Page 149

<p>1 which are looking at some of the candidates. Now I've  2 made the assumption which looking around the room I  3 think is generally correct that most of you, maybe not  4 all of you, but most of you are very familiar with  5 this work and familiar with the literature. So I'm  6 not going to do a literature review. We've done that  7 kind of thing in publication and there are many good  8 reviews you can look at.</p> <p>9 If the overall goal of what we're looking  10 for is only safety and efficacy, the first thing that  11 makes that hard is that we're really talking about  12 that has to be so in humans and what else makes it  13 hard.</p> <p>14 This is just a teaching slide that I  15 sometimes use to emphasize that the outcome when we do  16 these vaccine experiments is dependent on so many  17 things and we're only trying to impact a single one of  18 them which is the acquired immunity portion and that  19 the outcome also in experimental settings goes to the  20 Lawrence butterfly effect of sensitive dependence on  21 initial conditions.</p> <p>22 So you are familiar with the general  23 structure of the virus. This gets to the question for  24 introduction in a general way of what components do we  25 use for a vaccine and do we know for sure that we're</p> <p style="text-align: right;">Page 150</p>	<p>1 look at six of the seven genes. We used an alpha  2 virus replicon of Venezuelan equine encephalitis  3 virus, replicon, a defective interfering vaccine,  4 replication defective vaccine, to look in mostly  5 strain 13 guinea pigs which is that lower line. The  6 highest number of pigs we used was in strain 13s and  7 we were looking at individual genes in this case of  8 which ones protect. This is just kind of at a quick  9 survey level and we have found just very good  10 protection with glycoprotein. We found some pretty  11 good protection with nucleoprotein, a little less with  12 VP-35.</p> <p>13 We checked in other strains of guinea pigs  14 which are a little more sensitive than strain 13s and  15 then a lot more sensitive in our limited experience  16 with strain 2 guinea pigs and Marburg virus. We did  17 not get protection in strain 2s with GP. We did not  18 even protect all the guinea pigs, I mean, with NP. We  19 didn't protect all of them, but GP was still the best.  20 We were kind in the middle of VP-35. So this is kind  21 of our first guide to say GP is looking like a strong  22 candidate.</p> <p>23 As you know, similar things have been done  24 with Ebola both in -- With Ebola, it's been mostly a  25 look at NP and GP as I recall. With Ebola in mice,</p> <p style="text-align: right;">Page 152</p>
<p>1 on the right track and all of us have probably been  2 queried by other virologists in other fields of  3 exactly that question why are you working with that  4 antigen, why not the homologue of what they're working  5 with.</p> <p>6 The general outline, again as you know,  7 but I wanted to emphasize for those of you who don't  8 know about the same gene order and then the numbers in  9 the middle are the percent identity in amino acid  10 sequence between Marburg and Ebola. So when we jump  11 ahead to that question of what are the possibilities  12 for a pantropic vaccine, a single vaccine that will  13 protect against both Marburg and Ebola and all strains  14 of Ebola and all isolates of Marburg, all variants, is  15 there any gene candidate within there that we would  16 want to look heavily at?</p> <p>17 You would normally expect that  18 glycoprotein to be one of the less well conserved, but  19 it's in the lower portion. But there's not high  20 conservation among the others either. There is within  21 each species but not across Marburg and Ebola. I  22 think those numbers probably refer to Musoke and  23 Zaire, Marburg Musoke and Ebola Zaire.</p> <p>24 So what we did early on and we'll extend  25 and probably hear from other speakers as well was to</p> <p style="text-align: right;">Page 151</p>	<p>1 they've looked at all the genes except the polymerase  2 L. I don't believe -- We never looked at it and  3 neither did anybody else. We're having hard enough  4 time getting it cloned and expressed and having the  5 tools define our expression.</p> <p>6 But with Ebola all of these genes can  7 protect mice depending on the strain of mice and the  8 degree to which you call it protection and the number  9 of mice protected. But that's mice and Ebola in  10 guinea pigs, it's GP is solidly protective. NP is  11 generally not.</p> <p>12 So when we went to monkeys, this is an old  13 experiment, but it illustrates several points. This  14 was our first one in Marburg Musoke and cynomolgus  15 macaques. The control animals get this high viremia,  16 108, and sometimes higher plaque-forming units/ml  17 coming up on Days 5 and 7. The NP-only group, two of  18 the three animals got viremic and overtly sick but  19 recovered and the third one died synchronous with  20 controls. Every vaccine, every animal that got a  21 glycoprotein containing vaccine with or without  22 nucleoprotein was solidly protected and our limit of  23 detection was 50 plaque-forming units/ml. So it's  24 pretty sensitive. It's hard to get down there with  25 PCR when you convert it back to per ml.</p> <p style="text-align: right;">Page 153</p>

<p>1 But this is kind of the general pattern  2 that we're looking for in so many other vaccines that  3 the others of you have reported. But you're looking  4 for no disease and no apparent viremia. Whether or  5 not that's sterile immunity depends on whether you can  6 find antibodies, for example, to VP-40 or something  7 like that. But it's below limited detection and it's  8 solid protection.</p> <p>9 When we consider next steps, this was kind  10 of a transitional thought we were having, what about  11 a couple of the more variable strains of Marburg  12 virus. We had started the Marburg Musoke and looking  13 then at Ravn and what's the variability. You see that  14 there's conservation on the N terminus and on the C  15 terminus and a lot of variability in the middle, a lot  16 of, at least, algorithm-predicted N-linked and O-  17 linked glycosylation sites and that is consistent with  18 what's been reported. I'm not citing everybody's  19 name. I'm thinking of Tony, but if I say that, I'll  20 make somebody else mad for being left out. So I won't  21 say Tony.</p> <p>22 (Laughter.)</p> <p>23 Looking at this in a slightly different  24 way, this is a comparison of Marburg strains among  25 themselves. These are, I think, five different,</p> <p style="text-align: right;">Page 154</p>	<p>1 So this is a slide that I had used over  2 the years in teaching just to see the approach is --  3 just demonstrate what some of the approaches are to  4 vaccine development. This is kind of the state-of-  5 the-art ten years ago and it has not moved  6 conceptually forward dramatically since then. We can  7 occasionally add bits and pieces, but basically where  8 we've moved is toward a lot greater proofs of concepts  9 on these various other kinds of replication defective  10 vectors. We've used the Replicon. There's the DNA,  11 the adeno, pox viruses were the hybrids and chimeras  12 of the VSV type and the live vector will come to you  13 as well.</p> <p>14 Again, these are published data just to  15 show that we looked and compared within our own  16 laboratory a number of approaches and the world has  17 compared an even higher number of approaches. So I'm  18 going to spend a little more time on this and leave  19 time for questions and new information and assertions.</p> <p>20 This is a range. You can either squint or  21 move forward, but I tried to get them to all fit on  22 here. This is arranged in approximate chronological  23 order of discovery and typical of scientists we, and  24 humans in general, sometimes get the misimpression  25 that newest is always best. It may be best, but</p> <p style="text-align: right;">Page 156</p>
<p>1 nonidentical isolates in a similarity plot in a moving  2 average and here are, I believe, it's the four Ebola  3 species is what I put onto this one. What you're  4 seeing is a high degree of conservation within Marburg  5 at both the GP-2 and the C terminus which contains the  6 putative fusion domain and in GP-1 which contains this  7 putative receptor binding domain and where the  8 variability occurs is also where all that  9 glycosylation is.</p> <p>10 Similar pattern with Ebola, but these are  11 different -- This is all one species with some  12 variance. These are different species. So there's  13 still the most variability here and the most here and  14 here. This would seemingly predict that maybe the  15 immune response you want is against these ends. But  16 that's been a little harder to fully demonstrate.  17 Those critical experiments are probably still coming  18 if we just look now.</p> <p>19 If we pool both the Marburg and the Ebola  20 viruses, take all the viruses that were considered  21 here and all of them that were considered here and  22 look at variability, still the highest conservation is  23 in these areas that are important in both structure  24 and entry and probably some of the same structure and  25 entry but also binding at a secondary step.</p> <p style="text-align: right;">Page 155</p>	<p>1 basically, and the killed vaccine and the live  2 attenuated are basically in the proof or concept  3 arena.</p> <p>4 So I've made comments here and these are  5 in a review article recently published. Maybe what I  6 want to focus on the most is what I'm calling  7 "principal concerns" and when I say "concerns" I don't  8 mean showstoppers. I don't mean that vaccine  9 shouldn't be pursued because of this concern. But  10 every vaccine approach has some concerns. Obviously,  11 on the killed vaccine, you're worried about the safety  12 of manufacture, the potency, the possibility of  13 exacerbation which Ignachev reported some evidence of  14 that long ago and we've seen similar trends I would  15 call them, not proofs of the possibility of disease  16 exacerbation which is something we need to keep in the  17 back of our minds given the old measles and RSV  18 experiences.</p> <p>19 Live vaccines, safety incomplete  20 attenuation or reversion would be dramatic. They  21 would be a showstopper except you can theoretically  22 mitigate or overcome those by a reverse genetics  23 approach. So that's not theoretically impossible, but  24 it would ordinarily be a big concern. Vaccinia-  25 vectored, vaccinia safety, the vector immunity and the</p> <p style="text-align: right;">Page 157</p>

<p>1 potency have caused us basically to back-burner that.  2 That vaccine, a vaccine for Ebola designed in that  3 way, did protect guinea pigs. But kind of the  4 concurrent literature developing at that time on other  5 vaccinia vectored vaccines in humans caused us to  6 table that.</p> <p>7 We did some early work with baculavirus.  8 My lab eventually deprioritized it, not because it  9 can't work but because the kind of work that I most  10 wanted to do did not involve adjuvant research and the  11 particulars of having to reconquer all the same  12 problems every time with every vaccine, it wasn't just  13 what I wanted to do. It's where the results led us.  14 We had a lot better success in guinea pigs and then in  15 monkeys with VEE replicon.</p> <p>16 And there we have terrific efficacy. We  17 can protect also with a single dose protecting a month  18 later. The vaccine tipping point as I'm calling it,  19 the minimal amount to protect, is about 108 infectious  20 units in monkeys that's in rhesus macaques.</p> <p>21 The concerns there are vector immunity  22 which you would not expect ordinarily to have the  23 first time, but on multiple uses of a VEE replicon  24 vaccine, vector immunity will be a concern and it has  25 to be looked at as to whether that needs to be</p> <p style="text-align: right;">Page 158</p>	<p>1 tell me about the monkey efficacy. I know there has  2 been some and the concerns to me are similar to  3 protein-based vaccines. Is it potent enough and does  4 it require an adjuvant and then what kind of  5 explorations does that require for human use?</p> <p>6 There are also the remarkably, efficacious  7 results with the VSV terrific in rodents and monkeys  8 with both Marburg and Ebola, single shot, rapid  9 immunity. You could add the manufacturer scale-up  10 which is very rapid. Those are all wonderful  11 characteristics of a vaccine. There is no overt  12 illness from the live vaccine itself and this is just  13 descriptive in the recombinant. The filovirus  14 glycoprotein replaces the VSV glycoprotein. So it's  15 a whole new virus.</p> <p>16 And the concerns about them that it's  17 still a live vaccine and some people, well not just  18 some people -- it's harder to get live vaccines  19 licensed newly than it is to keep using measles, mumps  20 and rubella. So we have a balance of safety and  21 potency. Not spoken about very much but I think  22 there's a significant environmental and release issue  23 that has to be considered in terms of global politics  24 and national politics and the like as the utility of  25 this and its use to be situational. And then most</p> <p style="text-align: right;">Page 160</p>
<p>1 engineered around or whether it's a showstopper.  2 Safety is a concern with everything really, but safety  3 at doses high enough to achieve potency because we  4 don't know what that's going to be in humans.</p> <p>5 The DNA has a lot of things going for it  6 and has protected monkeys especially against Marburg  7 virus. I don't think that DNA alone has been reported  8 to protect against Ebola virus in monkeys. It's been  9 explored for immunological priming. So for DNA alone,  10 the concern is potency.</p> <p>11 Defective adenovirus we had Drs. Nabel and  12 Sullivan here that have done a lot of work on this.  13 It's excellent in rodent and monkey efficacy at what  14 I call high doses because I'm used to working with the  15 replicon 1010 units. It was the first demonstration of  16 monkey efficacy with Ebola with a single shot, well,  17 monkey efficacy at all, and single shot in Ebola with  18 monkeys. Again, the concerns are what's going to be  19 the impact of vector immunity, what's the safety at  20 doses either this high or high enough to override  21 vector immunity and those have to be determined  22 experimentally.</p> <p>23 Virus-like particles, Simon Bavari and  24 Kelly Warfield have led work in that area. Good  25 rodent efficacy. You can come to the microphone and</p> <p style="text-align: right;">Page 159</p>	<p>1 recently, Alec Bouhkrief and company, the recombinant  2 paramyxovirus virus which again has shown efficacy in  3 guinea pigs and monkeys. It contains both the  4 parainfluenza and the Ebola glycoprotein. So it's a  5 different kind of live virus. It's a live vaccine and  6 it has the same general concerns as the VSV.</p> <p>7 Passing through and others will touch on  8 the topic of immunology, but basically do we know  9 enough about the roles of antibodies and how they  10 might act either in a nonneutralizing or neutralizing  11 capacity and the complex interactions with T cells to  12 really claim that we know what we're doing or that we  13 can rationally predict what the immune response is and  14 ought to be? Maybe Nancy will tell us that, yes,  15 we're there.</p> <p>16 I wanted to also say parenthetically and  17 with time running short, this is in review, these are  18 all the ways in which glycoprotein which looks like  19 the best candidate from the available evidence, all  20 the different ways in which it evades and fools the  21 immune system. One is the variation. Another is the  22 heavy glycosylation. There's the glycoprotein  23 shedding, the gene editing which is a feature of the  24 Ebola viruses. The potential structural issues of  25 those more conserved and vital domains at the N</p> <p style="text-align: right;">Page 161</p>

1 terminus and C terminus may be masked structurally by  
 2 the heavily glycosylated domains. We have the problem  
 3 that these viruses bind to a lot of different cell  
 4 types with low affinity and it may be hard to get  
 5 antibody to do classical neutralization on it. We got  
 6 the problem with the viruses themselves with trigger  
 7 cytokine release in cells so they can cause some  
 8 inflammation. The toxicity of the glycoprotein for  
 9 cells is both a production issue and potentially a  
 10 safety issue and the long familiar immunosuppressive  
 11 domain which we're still kind of waiting to see what  
 12 is that really all about.

13 This was just to remind that there is a  
 14 delicate balance of the innate immune system and the  
 15 inflammatory immune system getting out in front of the  
 16 virus because when it fails to get out in front of the  
 17 virus it often can make disease worse and we have to  
 18 be concerned and to what degree, if any, does partial  
 19 immunity have the capacity for harm?

20 Some definitions we won't got through.  
 21 So these were kind of my recommendations.  
 22 Humility, don't pretend we know more than we know.  
 23 Critical thinking, believe quality data, not someone  
 24 else's optimism or bias including my own. Prudence,  
 25 my recommendation is to define and manage the risks.

Page 162

1 These are all risk/benefit equations as to which  
 2 vaccine is going to take the lead ultimately. But I'm  
 3 not in favor of putting all the eggs in one basket  
 4 because we don't know enough yet.

5 The reality is that it requires public  
 6 funding of Phase I trials. There is not a commercial  
 7 market sufficient, and this is just a personal  
 8 philosophy. Government decision makers get in the  
 9 biggest trouble when they don't speak up and this was  
 10 just some of the reviews in Metaanalysis that you can  
 11 look at and maybe have seen some of them and that  
 12 leaves us a little less time than I wanted, but seven  
 13 minutes for people to tell me about their vaccines and  
 14 why I've understated its great value, etc.

15 (Laughter and applause.)  
 16 DR. NABEL: Thanks, Alan. We can open the  
 17 floor up for questions.  
 18 DR. SCHMALJOHN: I thought you were  
 19 mocking the microphone, Jevad. No.  
 20 PARTICIPANT: Alan, would you comment on  
 21 the lack of protection by the VEE replicon free Ebola  
 22 Zaire? It performs so well with the Marburg. It was  
 23 almost like it just had to work with the Ebola and  
 24 could you offer up any bits wisdom as to why it  
 25 didn't?

Page 163

1 DR. SCHMALJOHN: And the reason I didn't  
 2 show data on that, Gene or Bill, do you want to speak  
 3 to that directly? Gene or Mary Kate could, but it  
 4 think Gene has been doing his -- He's been in the suit  
 5 the most with these.

6 MR. OLINGER: So it really comes down to  
 7 two issues. One being is the vector has been enhanced  
 8 a little bit since those first experiments. Second is  
 9 the dose. The original dose is probably around 1 X  
 10 106 and at that dose, we're way below the threshold.  
 11 The threshold appears to be a little higher than that.  
 12 Once we've moved up to that threshold, we can actually  
 13 get protection within 28 days with one shot. So it's  
 14 similar or comparable to any of the vaccine platforms  
 15 that are out there. We haven't quite published all  
 16 this and we're moving towards that at the moment.  
 17 This is against IM challenge. We're working on the  
 18 aerosols at the moment.

19 DR. SCHMALJOHN: We actually had similar  
 20 experiments with Marburg, experience with Marburg when  
 21 we moved from Musoke to a Popp variant or we call it  
 22 Ci67 just because it's a different variant. At first  
 23 giving the monkeys giving cynos 107 infectious units,  
 24 they all died. When we get up to 108, either on  
 25 multiple shots or to our surprise or pleasant

Page 164

1 surprise, a single shot 30 days later, at 108 was  
 2 sufficient to protect them. Certainly, if we jump up  
 3 to high dose like 1010, they are solidly protected. So  
 4 in both cases, it came down to probably the amount of  
 5 antigen the animal sees in a bolus at one time which  
 6 is why it appeared early on to be less effective than  
 7 the adeno which was going in at a higher dose.

8 PARTICIPANT: Alan, could you comment on  
 9 the multivalency issue where there is a five, maybe  
 10 six, subtypes of filoviruses and how many of the  
 11 vaccine you can mix to still maintain the efficacy of  
 12 the vaccine?

13 DR. SCHMALJOHN: The number of strains and  
 14 species against which we want to protect is going to  
 15 be kind of medical and policy issue, certainly, Ebola  
 16 Zaire species, certainly Marburg species. The  
 17 question is one Marburg variant or maybe two.  
 18 Probably Ebola Zaire. So we're up to a minimum of  
 19 three, maybe four, if we do two Marburgs. The  
 20 question of can you mix them and not get what was  
 21 classically known as immunological interference or  
 22 antigenic competition? That is, do you get the same  
 23 response with the mixture to each of the four parts as  
 24 you get to individual parts when you do them  
 25 separately? Most of those definitive experiments have

Page 165

<p>1 not been done in monkeys and certainly not in humans.  2 So that's where there is opportunity for knowledge.  3 DR. NABEL: Alan, I'll make one comment  4 and ask one question. In terms of adeno to those of  5 us who work with adeno for vaccines and other areas,  6 1010 is actually not a high dose. It's an average dose  7 and we actually do have now over 900 person years of  8 safety experience that's actually going into an  9 efficacy trial for HIV probably later this month. So  10 I think that dose is probably okay. We still are  11 working on the question of pre-existing immunity and  12 hopefully we'll have that sorted out shortly.  13 My other question to you though or my  14 question to you is do you have any thoughts about in  15 these various different vaccine models what might be  16 a physiologically relevant challenge dose.  17 DR. SCHMALJOHN: Yeah, we are going to  18 come back to that in the panel discussion. But what  19 we have used and I think for very good reasons is 100  20 to 1,000 PFUs which is by LD-50 a high dose, by virus  21 particles pretty low and very realistic in terms of  22 either a fomite including needle stick and I guess I  23 would remind that these viruses are kind of outliers  24 from a lot of the biological threat agents is the  25 sense that they're just as deadly by a fomite</p> <p style="text-align: right;">Page 166</p>	<p>1 development and also for Marburg vaccine development  2 and basically, the way I'm going to run this talk is  3 to focus on some work that we did with the VEE  4 replicon and then to try to provide a summary or a  5 snapshot of the literature in terms of antibody and  6 CTL responses or T cell responses. At the end, that  7 is not inclusive again because of my time constraints  8 due to stolen files.  9 So what are the protective, adaptive  10 immune responses? Of course, those would be  11 antibodies and CTLs and we decided to look in the  12 mouse model because it's well characterized as far as  13 immunological reagents and the ability to assess the  14 different immune responses and acknowledging that the  15 model may be a little physiological different than the  16 non-human primate model and possibly the human model,  17 it still provided a method for assessing the  18 mechanisms of immunity.  19 Mike Bray gave a nice talk this morning  20 about this model and the mouse adaptative virus. I'm  21 not going to go into that in great detail except to  22 say that our challenge doses were in the 1,000 PFU  23 range.  24 So again, these studies were done using  25 the Venezuelan replicon expressing different Ebola</p> <p style="text-align: right;">Page 168</p>
<p>1 parenteral route as they are by aerosol. So, yes,  2 we're concerned about aerosol. We're also concerned  3 about other -- ought to be concerned that it also is  4 protective against other means of delivery. So that  5 dose being realistic, I guess I wouldn't want to have  6 any confidence in a vaccine that couldn't protect a  7 monkey against 1,000 PFUs.  8 DR. NABEL: Okay. We'll see if there are  9 any dissenters from that later on. Thank you.  10 The next speaker will be Mary Kay Hart.  11 She's going to talk to us about the role of antibodies  12 and cell-mediated immunity and conferring protection  13 against filoviruses.  14 DR. HART: Okay. Thank you. As with  15 Alan, I also want to acknowledge that this work was  16 performed at USAMRID when I was employed there and  17 about a year ago, I left to join DVC where we now work  18 on advanced development of vaccines. But because I  19 have moved on, I want to also acknowledge the help  20 that I had in putting this together by Jane Olander  21 and John Dye who stepped in with some electrons and  22 assistance after my computer was stolen in the last  23 few days.  24 As we've just heard, there are numerous  25 platforms that are being evaluated for Ebola vaccine</p> <p style="text-align: right;">Page 167</p>	<p>1 genes and the setup is shown here. Some of these  2 replicons were provided by Jon Smith and Curt Kamrud  3 from Alphavax and I'd like to acknowledge their  4 assistance with these studies as well.  5 So with the replicon, what happens is that  6 the structural proteins from the alpha virus are  7 replaced with the foreign protein of interest, in this  8 case, one of six Ebola virus proteins. We've actually  9 evaluated the T cell responses to the glycoprotein,  10 the nucleoprotein and four VPs. This is RNA is  11 transfected into the BHK cells and packaged into what  12 looks like a VEE virus. That particle goes into  13 animals and undergoes a single round of replication  14 and expresses the antigen of interest but does not  15 have additional rounds of replication.  16 So when we did our initial studies, the  17 references that support these studies are shown at the  18 bottom of each slide for acknowledging the personnel  19 who were involved in these studies. We tested the  20 different replicons expressing the protein shown on  21 the left and then looked in two mouse streams that  22 differed the MHC for protection and as you can see  23 here, we saw very good protection with all six  24 proteins in the BALB/C mice and with five of the six  25 proteins in C57BI/6 mice. I've highlighted here the</p> <p style="text-align: right;">Page 169</p>

<p>1 fact that we saw absolutely no protection in the 2 C57Bl/6 mice immunized with the VP24 replicon and 3 we'll come back to that in a moment when we get into 4 the CTL studies.</p> <p>5 We also took serum from these immunized 6 mice and passively transferred it into naive animals 7 and looked for protection and, as you can see here, we 8 only saw significant protection by antibodies that 9 were directed at the glycoprotein. We followed that 10 up in identifying monoclonal antibodies with 11 protective capacity. These are shown here. There 12 were five different epitopes that we've mapped that 13 are shown on the left in these groups.</p> <p>14 The specificities of these are shown in 15 the slides here. Three of those are linear and 16 restricted to the Zaire Ebola virus. Two of them are 17 conformational and crossreact with Ivory Coast and in 18 group four case with Sudan as well.</p> <p>19 As far as protection, we saw greater than 20 50 percent protection with all of these antibodies 21 transferred into mice prior to challenge and that 22 would be a day before challenge and we also evaluated 23 the capacity to protect when given as late as two or 24 three days after the mice were challenged and we saw 25 that with all of these groups that we could protect</p> <p style="text-align: right;">Page 170</p>	<p>1 The ICC assay is depicted here. We 2 basically were looking for gamma interferon and CD4 3 positive cells and we used the matrix approach with 4 peptides for each protein. What I'm showing here is 5 the VP24 protein. There were 14 pools of overlapping 6 peptides that marched down the sequence of that. I'm 7 sorry. Yes, 14 pools or 49 peptides. Each peptide 8 was put into two pools. So you can see here that the 9 first peptide is in pool one and pool eight and then 10 each of these pools were analyzed by ICC and looking 11 for ones that were positive shown here. You can go 12 back and deduce the peptides that may be the specific 13 epitopes that we were looking for or at least the ones 14 stimulating the responses and those are shown here in 15 green.</p> <p>16 So you can see that we had five 17 possibilities based on the positive pools and then 18 when we went back and tested those individually we saw 19 that four of those were actually positive. The fifth 20 one was not positive but there was a positive peptide 21 in each of the pools in which that peptide was 22 present.</p> <p>23 This slide indicates the ICC staining on 24 the day that we took the cells and tested them in 25 chromium release assay and then adoptively transferred</p> <p style="text-align: right;">Page 172</p>
<p>1 animals if the antibody was administered 24 hours 2 after challenge and in some cases when it was 3 administered as late as two days after challenge. But 4 if we went any further, we did not see a protective 5 efficacy.</p> <p>6 The isotypes are shown here. Most of 7 these protective antibodies are G2a which of course 8 has the ability to fix complement and so the secondary 9 characteristics of the antibody isotype may come into 10 play in protection although we did see protection with 11 the one, IgG1 antibody shown in group 3. I should 12 also point out that that particular monoclonal 13 antibody has a very high affinity.</p> <p>14 So moving into the CTL studies that we 15 did, this slide is just basically an overview of our 16 strategy and approach and pointing out that we were 17 looking primarily for CD8 positive T cells in this. 18 We've done some initial studies as well looking at CD4 19 studies, but I won't be presenting those here.</p> <p>20 Mice are vaccinated and their spleens 21 removed and restimulated in vitro with peptides for 22 seven days and then we put them into three different 23 assays that we were looking at, the ELISpot, ICC assay 24 for gamma interferon and then in functional chromium 25 51 release assay.</p> <p style="text-align: right;">Page 171</p>	<p>1 them into naive mice and challenged mice and as you 2 can see here, where we had good lytic activity for 3 three of these different peptides, the cells 4 transferred into mice also provided good protection 5 against subsequent challenge.</p> <p>6 What I'm showing here are the epitopes 7 that we defined for the glycoprotein and the 8 nucleoprotein. Also published are the other four 9 proteins that we mapped and what I'd like to emphasize 10 here is the comparison on the sensitivity of the three 11 assays that we used and their ability to correlate 12 with survival.</p> <p>13 So if we look at the colors, the yellow 14 boxes, we find that the ELISpot sometimes did not 15 detect protective epitopes. We found that it was very 16 good at predicting or identifying dominant responses 17 and less effective at picking up the more subtle 18 responses that we identified.</p> <p>19 The ICC assay was very good except that we 20 had one exception where it predicted protection and we 21 didn't actually see it when we transferred those cells 22 into mice and, interestingly enough, the chromium 23 release assay predicted that that epitope would not 24 protective and in all cases predicted protection with 25 lytic activity.</p> <p style="text-align: right;">Page 173</p>

<p>1 This is a summary of what we found as far 2 as protective immune mechanisms induced in mice by the 3 VRPs and, as you can see here, I've also noted that 4 others have detected CTLs within the glycoprotein as 5 well and some of that were different from the ones 6 that we identified and I'd like to take a moment to 7 indicate that it may be that the way the proteins are 8 expressed in vivo sometimes influences what is 9 detected in these assays and also point out that the 10 assays across the field are not yet standardized and 11 the reagents are not yet standardized.</p> <p>12 However, what we found here is that 13 antibody is protective in two mouse strains when the 14 antibody is directed at GP and we could identify CTL 15 responses with the number of epitopes shown here for 16 each of these proteins in the two mouse strains. As 17 I pointed out earlier, we saw no protection in the 18 C57Bl/6 mice immunized with the replicon expressing 19 VP24 and we could not identify despite intensive 20 efforts especially on the part of Gene Olinger to 21 identify CTL epitopes. We did not actually find any 22 and sera from those mice did not provide any 23 protection either.</p> <p>24 So what I'd like to do here is to provide 25 a snapshot that I took from a review article written</p> <p style="text-align: right;">Page 174</p>	<p>1 not always shown and again, here the gamma interferon 2 ELISpot and CTL responses that have been described.</p> <p>3 So the conclusions are that different 4 vaccine platforms are inducing antibody titers as 5 measured by ELISA in the three to five log range both 6 to Ebola virus and Marburg virus in different animal 7 models. T cells responses either measuring CTLs 8 directly or cytokine associated responses indicating 9 a Th1 response have been measured in animals 10 vaccinated with different platforms for Ebola and 11 Marburg. Antibodies and CTLs have been demonstrated 12 to provide protection from Ebola virus challenge in a 13 murine model and I think my opinion is that additional 14 studies evaluating nonhuman primate responses are 15 clearly warranted. And additional studies are 16 required to identify the correlates that will bridge 17 animal and human data for licensure under the Animal 18 Rule.</p> <p>19 These are the disclaimers on the use of 20 animals and especially the fact that these are my 21 opinions and not associated with the Army. And I'd 22 like to take a moment to acknowledge the people that 23 I worked with at USAMRIID on the data that I presented 24 here. Thank you. 25 (Applause.)</p> <p style="text-align: right;">Page 176</p>
<p>1 a few years ago of what has been described in the 2 literature. This is not a comprehensive list and I 3 apologize to those of you in the audience that I have 4 may missed in this list.</p> <p>5 What I would like to emphasize is that in 6 all the labs despite the platforms and despite the 7 assay variations that may exist between the labs, we 8 are seeing fairly strong ELISA titers induced in mice 9 and guinea pigs and macaques. For Marburg 10 glycoprotein, there are fewer reports of T cell assays 11 that have been used, although there are reports of 12 gamma interferon in the ELISpot and some proliferation 13 responses and again, this is just a continuation of 14 the list with Marburg looking at the ELISA titers that 15 have been described. Moving onto other proteins 16 within Marburg, there are some reports with ELISA 17 titers induced to the NP and VP35 also described.</p> <p>18 For Ebola glycoprotein, again looking at 19 the different platforms shown here, we see a strong 20 tendency for an antibody response and as detected in 21 ELISA, there are reports of CTLs being induced by 22 different platforms and proliferation in guinea pigs 23 as well.</p> <p>24 Again in macaques, we saw some 25 proliferation indices reported and CTLs reported but</p> <p style="text-align: right;">Page 175</p>	<p>1 DR. NABEL: Thank you, Mary Kate. Please 2 ask questions.</p> <p>3 PARTICIPANT: I have two questions. The 4 first question is some types of vaccines, mostly 5 frequently inactivated vaccines or subunit vaccines, 6 induced detectable by ELISA nonneutralizing antibody 7 response. In your opinion, what is correlation 8 between ELISA response and neutralizing response for 9 vectored vaccine against filoviruses and do you 10 believe ELISA validation of antibody response by ELISA 11 itself is sufficient? And the second question is how 12 would you comment on a recent study in which nonhuman 13 primates received high dose of neutralizing antibody 14 response but after the challenge failed during the 15 challenge completely. Thanks.</p> <p>16 DR. HART: Okay. I think the ELISA data 17 would have to be analyzed in terms of comparing the 18 protective efficacy and maybe identifying a threshold 19 of protection. My personal bias is not in favor of 20 using assays that aren't necessarily functional, but 21 we don't have a very good neutralizing antibody assay 22 for the filoviruses at least in vitro.</p> <p>23 So I think it's possible and I know that 24 at USAMRIID we were looking carefully at the titers 25 that were induced to see if we could find a threshold</p> <p style="text-align: right;">Page 177</p>

1 of protection. I think that's a little bit easier  
2 when the vaccine includes only the glycoprotein  
3 because then we haven't seen any protective antibodies  
4 for the other proteins and if we're doing an ELISA  
5 that has multiple proteins down in the well and we  
6 vaccinate with multiple proteins it may cloud the  
7 issue a bit.

8 With regard to passive transfer of  
9 nonhuman primates and antibodies, my personal feeling  
10 is that it will take a cocktail of antibodies and  
11 recognizing that one antibody or different polyclonal  
12 sera that are not necessarily well characterized have  
13 not provided protection doesn't mean that we can't get  
14 there and, with the antibodies that we have, there is  
15 a program underway with Gene and Jon sitting in the  
16 back to try to develop a cocktail that would provide  
17 better efficacy in nonhuman primates. So I think it's  
18 one of those things that the fact that the initial  
19 studies have failed like with the VEE replicon in  
20 nonhuman primates doesn't mean that there isn't any  
21 utility there, but that it needs to be further  
22 optimized and explored with regard to dosing and  
23 characterization.

24 PARTICIPANT: Mary Kate, you clearly have  
25 presented a huge amount of data versus antibody on CTL

Page 178

1 response and also clearly both antibody in CTL response  
2 play an important role in the immune protection. But  
3 in term how you are comparing the clinical trials and  
4 animal model, do you think we should actually --  
5 Because different vaccines have different strains.  
6 For example, protein tend to induce antibody response,  
7 not CTL response. Different technology, the ratio of  
8 antibody versus CTL response may be different. Should  
9 we actually establish a standard in animal models when  
10 we're comparing to -- When we're getting involved in  
11 clinical trial, what is a standard that would maybe  
12 show protection? For example, antibody seems three  
13 logs higher, but that also has to be in the presence  
14 of a CTL response. I would like to hear your comment.

15 DR. HART: I think it's a very difficult  
16 question. Looking forward to licensing a vaccine  
17 under the Animal Rule and having to develop those  
18 bridges and correlates and I expect that some of our  
19 colleagues will be getting this question a little bit  
20 later today and if not from some of you probably from  
21 me. But I think initially what we can do is to  
22 explore with the reagents and tools that we have  
23 available or that we can develop the relative  
24 frequency and specificity of the antibody responses  
25 and the CTL responses and look for the utility of

Page 179

1 different assays in predicting protection in those  
2 animal models. It is certainly a little more  
3 difficult to look at the T cell responses in nonhuman  
4 primates than in mice especially if you want to use  
5 chromium based assays, but that's also one of the  
6 reasons that we looked at different assays to see  
7 which ones would correlate the best and then tried to  
8 move those into nonhuman primates.

9 I think on the positive side the fact that  
10 most of the vaccines are using one or two proteins and  
11 they're only six or seven to look at all together  
12 within each virus is helpful in that it is possible  
13 and feasible to do the peptide analysis that we've  
14 shown here, whereas for other pathogens that would  
15 have 100 different proteins that would be much more  
16 daunting.

17 As far as correlating to human disease, I  
18 think that's the biggest challenge facing the  
19 filovirus vaccine development as far as licensure.  
20 There is some data that's coming forward on what  
21 happens in patients. But until we can actually get  
22 our hands on or our heads wrapped around what happens  
23 in terms of protection, in terms of antibodies and T  
24 cells responses, then it's going to be exceedingly  
25 difficult to bridge those responses from the animals

Page 180

1 and indicate that they're likely to protect in human.  
2 But again, I think I'll defer to our colleagues from  
3 the FDA.

4 PARTICIPANT: And also clearly the GP  
5 alone based on Dr. Nabel's map showed sufficient to  
6 provide protection against an Ebola challenge. But  
7 also they have problems. The glycoprotein tend to be  
8 very variable with a more conservative protein like NP  
9 protein. That may be better antigens for CTL  
10 response, but however it's also being criticized. It  
11 may or may not be necessary when including NP in a  
12 vaccine. But based on your data, it shows other  
13 conservative proteins. VP30s, VP40s show similar  
14 level of importance in terms of immune response. What  
15 would you comment and suggest as to what would be an  
16 ideal vaccine to provide broader protection? What  
17 would be the antigen you would suggest?

18 DR. HART: I think it depends on the goal  
19 of the program. If a program is to develop a  
20 pentavalent vaccine for Marburg and Ebola virus, then  
21 including multiple proteins from each of those viruses  
22 is going to make a very massive type vaccine and  
23 fairly daunting, I think, in terms of vectors that  
24 might be used to provide those antigens.

25 If one wants to develop a vaccine

Page 181

<p>1 specifically for Ebola Zaire, then one might include  2 the multiple proteins to ensure the best response  3 keeping in mind that as with some of the nonhuman  4 primates humans certainly are outbred in terms of  5 their expression of MHC class one alleles and that not  6 only within different ethnic groups, but across people  7 in general, the frequencies of those differ and so  8 finding enough CTL epitopes to induce a good response  9 in the majority of your vaccine recipients is better  10 achieved by including multiple proteins.</p> <p>11 DR. NABEL: Mary Kate, I have a quick  12 question and then I think what I'm going to suggest is  13 that we go onto the next talk because we're a little  14 ahead of time. I appreciate that.</p> <p>15 DR. HART: You're welcome.</p> <p>16 DR. NABEL: And then we'll take the break  17 after the next talk. The quick question is have you  18 looked at the adoptive transfer of your CTL clones in  19 this STAT knockout animals. In other words, is the  20 protection your seeing dependent on the interferon  21 response?</p> <p>22 DR. HART: We had not done that at the  23 time I left. Dick.</p> <p>24 PARTICIPANT: Yes, we haven't tried that  25 approach yet with the transfers. We have looked at</p> <p style="text-align: right;">Page 182</p>	<p>1 studies, the historical studies very briefly, but then  2 concentrate a lot more on the immune assessments.</p> <p>3 So the first generation vaccine contained  4 genes encoding the glycoprotein and the nucleoprotein.  5 These genes are expressed either in DNA vectors or  6 adenovirus vectors. Some of the human studies, the  7 first generation human studies, also use both of these  8 genes, but I'll point that out as I go along what the  9 different vaccines are.</p> <p>10 Of course, we targeted the glycoprotein as  11 a good target for neutralizing antibodies and the  12 nucleoprotein as was just discussed as potentially a  13 good target for a more consistent response and  14 cellular immunity.</p> <p>15 So it had been shown by several groups  16 that DNA vectors were very efficient at delivering  17 these genes and confirming protection in rodent models  18 and Gary Nabel's group was one of the first groups to  19 show this in mice and guinea pigs. Unfortunately, for  20 other pathogens and for Ebola as well, DNA vectors  21 alone did not transition very well into nonhuman  22 primates for conferring immune protection. So our  23 approach was to try to boost the response conferred  24 with DNA vectors with a boost using an adenovirus  25 vector. Adenovirus vectors yield very high levels of</p> <p style="text-align: right;">Page 184</p>
<p>1 the animals after transfer to see what presents and if  2 we transfer the CTLs, of course, they have antibody  3 responses after their convalescent and equally if we  4 give them antibody and then if we go look at them  5 later on, they have CTLs. So it's clearly both arms  6 are needed in order to protect them. Now what role  7 the interferon response is playing, I'm not sure.</p> <p>8 DR. HART: But we did also have some  9 studies that were ongoing in SCID mice just to tease  10 apart which ones were absolutely critical for  11 protection and which ones were keeping the virus in  12 check while other responses came up.</p> <p>13 DR. NABEL: Okay. Thank you. The next  14 talk will be by Nancy Sullivan on initial assessments  15 of correlates of protection for an Ebola vaccine.</p> <p>16 Okay. They're just loading. So bear with  17 us one minute. The good news for all this is that  18 we'll finish earlier this afternoon.</p> <p>19 DR. SULLIVAN: Okay. Thank you very much.  20 So Mary Kate's talk was a good lead-in now to discuss  21 what we do know about immune correlative protection.  22 We have worked with both CDC and USAMRID over the past  23 several years to try to gain a better understanding of  24 the immune responses that correlate with immune  25 protection. So I'd like to go over some of those</p> <p style="text-align: right;">Page 183</p>	<p>1 protein expression and they probably target dendritic  2 cells which is obviously favorable for antigen  3 presentation. And so I'll talk about two platforms  4 that the VRC uses. One is a DNA prime adenovirus  5 boost and then the other one is a single adenovirus  6 injection that just requires one shot.</p> <p>7 So just to summarize a lot of the  8 historical data that led up to these studies of immune  9 correlates, we've done a lot of work to show that we  10 can use a single immunization with an Ad vaccine and  11 provide uniform protection and the most effective  12 immunogen is Ebola GP. It's the outer glycoprotein.</p> <p>13 We targeted the Zaire and Sudan subtypes  14 of Ebola virus because those seem to cover most of the  15 epidemiological threats in the circulating strains,  16 again, GP from those subtypes, and one of the  17 important considerations, again, as Mary Kate alluded  18 to do you get, and I think Alan also talked about the  19 potential for diluting the immune response when you  20 start adding on different things. So what we were  21 able to show is that including GP didn't reduce  22 protective efficacy against Ebola Zaire. We were also  23 shown that we could eliminate NP without reducing  24 efficacy. So this simplifies the vaccine we're  25 focusing on the GPs. These are all published data.</p> <p style="text-align: right;">Page 185</p>

<p>1           Okay. So what is the way forward now to 2 move these vaccines out of the nonhuman primate model 3 into humans and, ultimately, licensure, and there is 4 a formal pathway now to do that and this is called the 5 FDA Animal Rule and it involves parallel development 6 in animal models and in human studies simply because 7 we cannot do efficacy studies in human subjects. So 8 the efficacy studies are conducted in animal models 9 early on to optimize the composition and the dose of 10 the vaccine, and then there's an iterative process to 11 show that these vaccines are safe in human subjects. 12           The next phase looks at immunogenicity of 13 these vaccines, and we need to demonstrate a immune 14 correlate of protection which I'll talk about in a 15 moment what that means, and then ultimately the animal 16 studies are bridged to the human studies through the 17 immune correlate. So we need to show in humans that 18 we can generate an immune response that's similar to 19 what is protective in the nonhuman primate model. 20           Okay. So the Animal Rule just in brief, 21 it's very long, but this highlights the essential 22 points is it's a mechanism for approving products 23 based on animal efficacy data. We can demonstrate the 24 effect in a single animal species as long as that 25 represents a sufficiently well characterized animal</p> <p style="text-align: right;">Page 186</p>	<p>1 a correlate of protection does not necessarily have to 2 be functional and let me just give you a little bit of 3 an example for how this differs from a mechanism of 4 protection which would be described by functional 5 antibody and cellular responses. 6           So, for example, B cells could be the 7 mechanism of protection. Antibodies could be 8 protective. But the correlate that you measure in 9 subjects may not be antibodies. It might be serum Th2 10 cytokines which are necessary to generate that 11 antibody response. Likewise, CD8+T cells could be the 12 mechanism of protection, but it may be that serum 13 antibodies are what we measure prechallenge that show 14 us that there's a potent immune response, and these 15 are a linked because CD4 cells are required to produce 16 both. So the immune correlate reflects the underlying 17 protective mechanisms, but it doesn't necessarily have 18 to be the same as the underlying protective mechanism. 19           Okay. So now moving to these biological 20 assessments, this is just a summary of our first study 21 that we did with Tony Sanchez and Pierre Rollin at CDC 22 with the DNA prime and adenovirus boost showing that 23 over a four injection study regimen we could generate 24 immune protection in all of the vaccinated subjects 25 and, of course, all of the control subjects succumbed</p> <p style="text-align: right;">Page 188</p>
<p>1 model, and it's got to be reasonably likely to provide 2 a clinical benefit in humans in order to go forward 3 through licensure. Now this sounds very subjective, 4 but the judgment itself is actually based upon 5 quantitative biological data and those data are what 6 we focus on for the immune correlates of protection. 7 So we're going to determine an immune response that 8 will predict protection from infection or disease. 9           This represents a new paradigm for vaccine 10 development because historically vaccines were 11 developed just by immunizing and then showing 12 protection in a study population. So what I'm just 13 showing you here is how the genetic vaccine works and 14 how the process that we're confronted with now differs 15 from that historical process. 16           So a genetic vaccine is injected directly 17 into the muscle. The muscle cells make the protein 18 that's picked up by antigen presenting cells and 19 delivered to cells of the adaptive immune response. 20 So when we think about correlates of immunity, we 21 think about this adaptive immune response and how to 22 best capture what those relevant responses are. 23           And I do want to take a moment. There has 24 been some discussion about functional antibodies and 25 functional assays and take a moment to point out that</p> <p style="text-align: right;">Page 187</p>	<p>1 to the disease, and the time course is essentially 2 what Tom described earlier. 3           And so our first approach at looking at 4 correlates of immunity was to say, okay, we know that 5 the DNA didn't protect. So how do these differ in the 6 immune responses that are generated when you have a 7 boost after the DNA prime? And what we observed was 8 that the cellular responses remained pretty consistent 9 throughout the immunization period either before or 10 after the adenovirus boost. But what differed 11 dramatically after the adenovirus boost was the 12 generation of very high antibody titers. So this gave 13 us the first inkling that antibody titers might be a 14 reasonable assessment of immune correlates of 15 protection. 16           Now this regimen is not amenable to doing 17 the iterative studies that I talked about in that 18 pathway and so for this reason and other reasons, we 19 wanted to develop an accelerated protocol, and what we 20 evaluated was the ability of just a single injection 21 of adenovirus to protect against infectious challenge 22 and that's just showing you this study that we did 23 with Tom Geisbert and Peter Jahrling and again all of 24 the vaccinated subjects, this is cynomolgus macaques, 25 vaccinated with a single shot of adenovirus and</p> <p style="text-align: right;">Page 189</p>

<p>1 challenged four weeks after immunization, all of the 2 vaccinated subjects were protected and the control 3 subjects died in the usual time course.</p> <p>4 So when we now look at immune correlates 5 in this model, we want to look at immune correlates 6 under conditions of infection break-throughs. So that 7 is not 100 percent protection or 100 percent lethality 8 and our approach to achieving that was to dose down 9 the vaccine to a point where we did see infection 10 break-through. So what I'm just showing you here is 11 a Kaplan-Meyer survival curve and what you can see is 12 our sort of gold standard vaccine which is the 13 adenovirus at a dose 1012 provides uniform protection 14 in all of the animals, and the control animals, of 15 course, die in about a week. We can dose down two 16 logs and still not have infection break-through, but 17 what we observed in this study was that the break- 18 through comes between a dose of 109 and 1010. So this 19 enables us to look at the difference between the 20 animals that didn't survive and the animals that did 21 survive in terms of their immune responses.</p> <p>22 The first thing we looked at was T cell 23 responses, and we've chosen a fairly sensitive assay 24 to measure these responses. It's an intercellular 25 cytokine assay that Mary Kate presented, I think, some</p> <p style="text-align: right;">Page 190</p>	<p>1 break-through range.</p> <p>2 However, that's looking at bulk T cell 3 responses and the question is is that really the 4 appropriate population to evaluate. Can we look at a 5 more relevant cell population and that would be 6 memory/effector cells and can we also describe these 7 responses a little bit better by looking at 8 functionality and a variety of cytokines?</p> <p>9 And so we've developed, Mario Roederer 10 actually at the VRC has developed a polychromatic flow 11 assay that uses, right now, we're using between 18 and 12 20 colors to better define the T cell responses both 13 in the CD4 and CD8 populations and the way we do this 14 is by eliminating dead and irrelevant cells here using 15 markers that gate out B cells and monocytes and other 16 things that might be nonspecifically secreting 17 cytokines. Then we can look at our lineage markers 18 and then further concentrate on the relevant memory 19 population here denoted by TM which is T memory cells.</p> <p>20 And our first approach at this showed us 21 that most of our responses were indeed, our antigen 22 specific responses were indeed, in this memory 23 population. And what we were able to achieve by 24 defining the assay better was now instead of looking 25 at responses, for example, for CD8 that are less than</p> <p style="text-align: right;">Page 192</p>
<p>1 data on but didn't go into the assay too much, and 2 what this allows you to do when a subject is immunized 3 and generates these T cell responses you can actually 4 measure antigen-specific T cell responses by the 5 amount of cytokines that are produced in these 6 different cell populations. And the way we do this 7 and look at antigen specificity is by stimulating 8 PBMCs with co-stimulatory antibodies and then the 9 specific antigen. In this case, it's overlapping 10 peptide span the open reading frame of GP.</p> <p>11 So then we just fix and permeabilize and 12 stain the cells and identify the different lymphocyte 13 subsets, either CD3, CD4, or CD8, and measure the 14 cytokine signals in those different cell populations.</p> <p>15 Looking at that experiment now where we 16 had infection break-through if we evaluate the CD4 and 17 CD8 responses by how much TNF alpha they produce, 18 there are a couple of things that we can see here. 19 One, the intercellular cytokine staining assay, the 20 readout, is often in the low percentages, single digit 21 percentages. Secondly, we can now look at that dose 22 range where we have break-through and ask are there 23 differences between survivors in yellow and fatalities 24 in red and, in fact, when we look at CD4 and CD8 25 responses we don't see dramatic differences in that</p> <p style="text-align: right;">Page 191</p>	<p>1 one percent we're looking at responses that are eight 2 percent which gives us better capability to discern 3 differences between different animals.</p> <p>4 The other thing that I just want to point 5 out is that when we look at a TNF response it's not 6 just a group of cells all producing TNF. That signal 7 comes from cells producing just TNF or some producing 8 also gamma or IL-2 or MIP-1 beta. It's a combination 9 of cell types that secrete these cytokines and that 10 defines the function of the cell.</p> <p>11 There are many possible combinations 12 depending on the number of cytokines that you 13 evaluate. But we have developed some methods for 14 interrogating these responses that allows us to assess 15 the response in a more manageable way, and that is 16 shown illustrated here where we can look at 17 functionality at this end of the spectrum. There are 18 polyfunctional secreting either four or three 19 cytokines and then as you go to this end of the 20 spectrum they're producing two or one cytokine.</p> <p>21 And this is just sort of an initial 22 assessment of the Ebola vaccine, but what you can see 23 straight away by looking at memory responses and 24 functionality, there are, in fact, differences between 25 nonsurvivors and survivors that begin to stand out now</p> <p style="text-align: right;">Page 193</p>

<p>1 that we couldn't assess by looking at just a single 2 cytokine in bulk CD8 cells. This is a promising start 3 to development of the methods for T cell analysis, and 4 I think it shows us that we lack resolution in some of 5 the older assays to discern these differences. But 6 this is provided now by looking at more complex T cell 7 phenotypes.</p> <p>8 The advanced T cell assays that evaluate 9 these functions are important in looking at functional 10 differences in the cellular responses. But the 11 complex assays and analysis that we currently have 12 present some challenges for universal application to 13 filovirus vaccine development. Not everyone can 14 measure 20 colors by flow cytometry and analyze the 15 data.</p> <p>16 So let me just move now to the antibody 17 responses that we measured in that break-through study 18 and here when we think about antibody responses it's 19 a little bit simpler than the T cell responses, but 20 functionally it can be complicated as well which 21 brings us back to the difference between looking at 22 simply an ELISA titer or looking at the function of 23 these antibodies. We, of course, elected to start 24 simply and get more complicated if we have to the way 25 we did with the T cell responses and in that break-</p> <p style="text-align: right;">Page 194</p>	<p>1 from these values because all nonhuman primate samples 2 have background, and it varies from monkey to monkey. 3 So we've made two changes to the assay. One is that 4 we calculate the titers a little bit differently and 5 we also make sure we subtract pre-immune values, and 6 the difference that this gives us now, if you look at 7 EC90 from background-subtracted values, is these 8 titers that would be the same by endpoint dilution, 9 now we have some resolution to discern differences in 10 those curves when they're different.</p> <p>11 Okay. So what we did was we went back, 12 the first study where we looked at break-through with 13 small groups of animals, three animals, and we wanted 14 to assure ourselves that this, in fact, was 15 generalizable before we moved ahead. And so what we 16 did was a cumulative assessment of lots of our 17 different vaccine studies, and this is a pretty 18 rigorous test because sometimes the vaccine had just 19 GP. Sometimes it had GP and NP, but the bottom line 20 is that the correlate held true when we looked at 21 historical samples. What I'm showing you here is the 22 mean ELISA titers again against GP in fatalities and 23 survivors, and there's a statistically significant 24 different in those titers.</p> <p>25 What you'll notice though is that there is</p> <p style="text-align: right;">Page 196</p>
<p>1 through study what we observed was that there was 2 indeed a correlate with ELISA IGG and immune 3 protection. This was not a great surprise to us 4 because we had several studies leading up to this that 5 suggested this might be the case.</p> <p>6 I just want to step back a minute and talk 7 about measuring ELISA titers because there is a 8 standard practice of measuring endpoint dilution 9 titers which is it's universal, it's simple, and 10 everyone can do it and compare results. The only 11 problem is endpoint titrations lack some precision. 12 So I'm just giving you an example here of two curves 13 from different animals that look pretty similar and if 14 you measure the endpoint titration, it's one to 5,000 15 for both animals.</p> <p>16 We've developed another method which 17 defines what we're calling the effective concentration 18 90 percent, the EC90, and the benefit of doing this 19 kind of calculation is that rather than relying on a 20 single point on the curve we integrate all of the 21 points on the curve which is important because this 22 area of the curve can be very noisy and that can 23 change your titer by a lot.</p> <p>24 We also have discovered that it's 25 absolutely essential to subtract pre-immune titers</p> <p style="text-align: right;">Page 195</p>	<p>1 a region of overlap in those different titers. So we 2 wanted to know what the predictive value of this 3 readout was, and it's actually not bad. We can 4 predict that below a titer of one to 500 there is no 5 survival. Above a titer of one to 3500 there is 100 6 percent survival. Again, this is with that dataset 7 that had all different vaccines in it.</p> <p>8 So our next step was to use our lead 9 vaccine candidate and immunize a number of animals and 10 do a similar assessment to what we've done here. I'm 11 just showing you the results of that study, this is a 12 Kaplan-Meyer curve, and showing that if we establish 13 an ELISA cutoff of one to 1400, we can predict 14 survival in 90 percent of the subjects. So the ELISA 15 titer depending on what degree of protection you want 16 to be assured of will vary. But the point is that you 17 can actually define the correlate in this way.</p> <p>18 Just to summarize the antibody analysis, 19 the endpoint titrations provide a uniform calculation, 20 but they're less precise than if you integrate all of 21 the values on the curve. Nonhuman primate sera 22 generate background signals, and it absolutely has to 23 be corrected before evaluating titers. And the EC90 24 titer is an immune correlate of protection in these 25 pilot studies where we evaluated, I think it was 30</p> <p style="text-align: right;">Page 197</p>

<p>1 animals.</p> <p>2 I just want to very quickly go through</p> <p>3 some of the human studies that we've done and show you</p> <p>4 how we're thinking about bridging these studies.</p> <p>5 The first study was using Ebola DNA. This</p> <p>6 was three injections of DNA and three different</p> <p>7 cohorts receiving two, four or eight milligrams of DNA</p> <p>8 and you can see the total number of subjects that were</p> <p>9 immunized. This was the first generation vaccine that</p> <p>10 contained both GP and NP. And these study subjects</p> <p>11 were evaluated for their ELISA responses and what's</p> <p>12 shown here is that we can measure very high titer</p> <p>13 ELISA responses in humans to both the Sudan, Gulu and</p> <p>14 the Zaire components in that vaccine. We also have</p> <p>15 antibody responses to NP, maybe not quite as high as</p> <p>16 GP, but the response rate is really quite good.</p> <p>17 The studies moving forward now are</p> <p>18 evaluating not only the DNA vaccine, but a single</p> <p>19 adenovirus injection or DNA priming followed by an</p> <p>20 adenovirus boost and you can see the details of the</p> <p>21 studies here using either 30 or 20 subjects in VRC 207</p> <p>22 and VRC 208 and this will be just the glycoprotein</p> <p>23 that's been defined now in the nonhuman primate</p> <p>24 studies is what we want to move forward with.</p> <p>25 So just to summarize, the preclinical</p> <p style="text-align: right;">Page 198</p>	<p>1 example of one of those alternative vectors that is</p> <p>2 currently under evaluation now, this is a chimeric</p> <p>3 vector between Ad5 which is the target for Ad5</p> <p>4 neutralizing antibodies and Ad35 which is resistant to</p> <p>5 those antibodies.</p> <p>6 It turns out that the antibody response is</p> <p>7 generated mostly to the hexon and indeed it's focused</p> <p>8 on the variable loops of the hexon. So what Dan did</p> <p>9 was swapped out the hexon variable loops of Ad5 which</p> <p>10 are neutralization sensitive with those of Ad35 which</p> <p>11 are resistant, and he did some mouse studies shown</p> <p>12 here using HIV genes and showed that in naive animals</p> <p>13 both the Ad5 and the HVR48 vectors performed quite</p> <p>14 well.</p> <p>15 When he looked at the performance of these</p> <p>16 vectors in the face of pre-existing immunity not</p> <p>17 unexpectedly the Ad5 vectors did not perform as well</p> <p>18 but the Ad48, the HVR48 vectors, performed very well</p> <p>19 even in the presence of neutralizing antibodies</p> <p>20 against Ad5. So this is now our lead candidate under</p> <p>21 evaluation now moving forward, and I think that, of</p> <p>22 course, we will continue to evaluate other alternative</p> <p>23 vectors as well because as Alan said, we're not going</p> <p>24 to put all our eggs in one basket. But it is</p> <p>25 promising, and we should know more about that within</p> <p style="text-align: right;">Page 200</p>
<p>1 development in the early human subject studies, the</p> <p>2 gene inserts include GP from Sudan and Zaire. ELISA</p> <p>3 IgG against GP is an immune correlate of protection</p> <p>4 and that's what we will develop for use in bridging to</p> <p>5 the human immunogenicity studies and the human</p> <p>6 clinical trials demonstrating safety and</p> <p>7 immunogenicity will provide the basis for licensure</p> <p>8 moving forward.</p> <p>9 So there's one area that we're still</p> <p>10 evaluating before we have our final vaccine candidate</p> <p>11 and that is does pre-existing immunity to Ad5 reduce</p> <p>12 vaccine efficacy. So there were some suggestions from</p> <p>13 studies that we've done and that others have done that</p> <p>14 indeed prior immunity to the vector can reduce the</p> <p>15 immune potency of the vaccine. Although studies of</p> <p>16 HIV infection suggest that Ad boosting in DNA prime</p> <p>17 subjects is really only marginally affected by prior</p> <p>18 immunity. In fact, the response rate was reduced by</p> <p>19 less than 10 percent.</p> <p>20 Despite that, since we are considering</p> <p>21 single adenovirus injections and we also want to have</p> <p>22 the best platform here, we're evaluating alternative</p> <p>23 adenovirus vectors that are resistant to Ad5 immunity</p> <p>24 and we're doing that in collaboration with Crucell and</p> <p>25 Dan Barouch at Harvard. And just to show you an</p> <p style="text-align: right;">Page 199</p>	<p>1 the next couple of months.</p> <p>2 Let me just acknowledge people who</p> <p>3 contributed to, first, the nonhuman primate studies.</p> <p>4 As I said, the early studies were done with Tony and</p> <p>5 Pierre and later studies with Pete, Tom and Joan. All</p> <p>6 of our adenovirus studies are done in collaboration</p> <p>7 with our biopharmaceutical partner, Crucell, most</p> <p>8 notably, Maria Pau and Isabella Versteeg. In my lab,</p> <p>9 there are several people who have performed the</p> <p>10 nonhuman primate studies and then, of course, the VRC</p> <p>11 collaborates quite closely on all of these studies and</p> <p>12 then just lastly, the clinical trials are a monumental</p> <p>13 effort by many, many people. I'd be happy to take</p> <p>14 questions.</p> <p>15 (Applause.)</p> <p>16 DR. NABEL: Please step forward to the</p> <p>17 mikes if you have questions.</p> <p>18 DR. SCHMALJOHN: Nancy, one of our</p> <p>19 unpublished aggravations went off that slide that I</p> <p>20 didn't talk about very much where we did in guinea</p> <p>21 pigs compared a number of vaccine strategies and</p> <p>22 within there we saw the DNA vaccine is very effective</p> <p>23 while inducing very low antibody titers; whereas, the</p> <p>24 baculavaccine in adjuvant gave very high antibody</p> <p>25 titers and some break-throughs and we also saw within</p> <p style="text-align: right;">Page 201</p>

<p>1 there that if we did DNA prime adeno boost, we had a 2 pretty substantial shift in isotypes, IgG isotypes, 3 which was easier in guinea pigs because they only have 4 two IgG isotypes.</p> <p>5 I guess parenthetically we used -- I would 6 be inclined to use -- always do the preimmune serum, 7 but also do a concurrent -- I background subtracted 8 irrelevant antigen on the same sample rather than 9 subtracting the prebleed. So that's another approach 10 especially if your antigen is a polyclonal activator 11 or your adjuvant is.</p> <p>12 But the point was, and then we went to 13 monkeys with the DNA only, the bacula only and the 14 prime boost and saw nothing of the same effect and we 15 didn't publish it not because we were embarrassed or 16 didn't want to but because proving the negative would 17 have taken a lot more monkeys. So I guess -- 18 wondering where you are on that. It appears you're 19 actually going to get those data out of humans before 20 any of us do out of monkeys in terms of does the DNA 21 only contrast with adeno only and is the prime boost 22 qualitatively different. You are comparing all three 23 arms?</p> <p>24 DR. SULLIVAN: Yes. So I will try to 25 touch on everything that you've raised, Alan. The</p> <p style="text-align: right;">Page 202</p>	<p>1 haven't evaluated that with the same precision that we 2 have with Ebola virus to identify the cutoff. Does 3 that answer your questions?</p> <p>4 DR. NABEL: I'll -- I think there was one 5 thing he was asking that maybe I can answer.</p> <p>6 DR. SULLIVAN: Okay.</p> <p>7 DR. NABEL: Which is I think you were also 8 asking with these platforms with DNA only versus adeno 9 versus prime boost whether we're seeing different 10 character immune responses as you go into humans and 11 the reason I'll answer is that most of the data there 12 is actually from HIV because we haven't done the prime 13 boost in Ebola yet. We've only done each one alone.</p> <p>14 But with HIV, it's very clear that both 15 the quantitative immune response as well as the 16 qualitative aspect of the immune response changes when 17 you boost a DNA prime and what we've learned, and it 18 is consistent between mouse and monkey and human, is 19 that when you DNA prime followed by an adeno boost you 20 actually with the DNA prime stimulate a broader 21 spectrum of CD4 responses so that you get more 22 diversity in the CD4 response. That in turn gives you 23 increased T cell help for the CD8s. So you get when 24 you then boost with adenovirus not increased diversity 25 of the CD8s but an increased magnitude of the same</p> <p style="text-align: right;">Page 204</p>
<p>1 first thing is the ability of rodent models to yield 2 an immune correlate that's the same as what we see in 3 primate models, and I think the answer there is no. 4 So the rodent models are extraordinarily useful for 5 sorting out immune mechanism for screening vaccine 6 candidates. So all of our initial screening is done 7 in rodent models.</p> <p>8 But what we were surprised by, and Tom and 9 Pete I'm sure will remember this very well, is one of 10 our earlier candidates where we deleted a portion of 11 the glycoprotein gene, tested that in a mouse model 12 and it yielded very high antibody titers. When we 13 moved it into nonhuman primates, it did not perform 14 well. So while the rodent model is useful for 15 screening, I don't think it's always going to 16 translate into the nonhuman primate for immune 17 assessments. I think we have to work with the model 18 that we're focused on and identify the immune 19 correlate in that model. It may also differ, Alan, 20 according to platform. So your baculavirus may have 21 a different immune correlate than the genetic vectors 22 that we're using.</p> <p>23 We have done this with Marburg using all 24 of our platforms, and we did generate antibody titers 25 with those platforms and immune protection. We</p> <p style="text-align: right;">Page 203</p>	<p>1 CD8s that you would otherwise generate.</p> <p>2 You also generate much enhanced antibody 3 responses. This now we have also seen in humans with 4 antibodies to HIV and we would presume that would 5 translate as well into Ebola. So thus far, we are 6 seeing good parallels and I think that's very good.</p> <p>7 PARTICIPANT: (Off microphone) With HIV 8 glycoprotein or --</p> <p>9 DR. NABEL: That's against envelope. Next 10 question.</p> <p>11 PARTICIPANT: I have a question regarding 12 to the pre-existing immunity against adenoviral 13 vectors. I think clearly it's a very important issue 14 and often we get criticized when anybody used an 15 adenovirus is suggesting the pre-existing immunity 16 making the virus basically incapable to induce any 17 immune response. We often quote Dr. Nabel's statement 18 in the HIV vaccine. Even in the presence of immunity, 19 a vaccine does induce a quite significant immune 20 response against the virus.</p> <p>21 We also have some data to show actually 22 using high dose in animal models we do not see the 23 pre-existing immunity has been a problem, but it turns 24 out you have to meet two conditions. One is to use 25 minimum effective dose so reduction, we'll be able to</p> <p style="text-align: right;">Page 205</p>

1 see in the vaccine.  
2 But, secondly, the repeat vaccination has  
3 to be in the period within two to three months which  
4 is the peak of immune response. In the effort we did  
5 in the serotype rotation tried to avoid the pre-  
6 existing immunity using different viruses in large  
7 number of monkeys. Actually if you revaccinate or  
8 vaccinate with different serotypes, after three months  
9 period, the benefit is minimum. So the opposite is  
10 true. You use the same vector as long as you avoid  
11 the two to three months acute immune response. You  
12 can repeat vaccinate as we've seen shown -- and also  
13 in non-human primates.  
14 So my question is can you actually  
15 disclose some of your clinical trial data? Do you see  
16 the differences because that ultimately becomes  
17 important and also they're suggesting that if you use  
18 inter-nasally or orally pre-existing immunity may not  
19 be a problem.  
20 DR. SULLIVAN: Yes. So the next studies  
21 that I talked about for the human trials do indeed  
22 stratify by preimmunity and your points, sir, are very  
23 good. It may not be an issue and we may not see as  
24 much of a problem as we anticipate seeing. That said  
25 we're being highly conservative in just making sure

Page 206

1 that we move forward with other vectors in the event  
2 that Ad5 immunity is an issue.  
3 PARTICIPANT: And I would also like to  
4 make a comment. Some of the pre-existing immunity is  
5 related to the pox virus. Actually, the differences  
6 between, you all know, the adenoviral vector and pox  
7 virus vector is the adenoviral vector only is  
8 expressed in the gene of interest so in -- de novo.  
9 So therefore, immune response is against the antigen  
10 you're presenting.  
11 DR. SULLIVAN: Sure. Yes.  
12 PARTICIPANT: In pox virus, there are over  
13 roughly 100 proteins made at the same time as the  
14 antigen is being made, the antigen of interest. So  
15 there is antigen dilution, not necessarily pre-  
16 existing immunity.  
17 DR. SULLIVAN: Yes.  
18 PARTICIPANT: I just had a technical  
19 question. In your ELISAs, you said you subtract the  
20 background values from your preimmune sera. Is that  
21 for the absorbency?  
22 DR. SULLIVAN: We use our preimmune sera  
23 as background on an individual basis. So rather than  
24 just subtracting an assay background, we subtract  
25 background for every single subject and we perform

Page 207

1 that assay three times.  
2 PARTICIPANT: Is that all on the same  
3 plate, too?  
4 DR. SULLIVAN: I'm sorry.  
5 PARTICIPANT: Is that all on the same  
6 plate?  
7 DR. SULLIVAN: It's internally controlled.  
8 It's all on the same plate. Yes. All very important  
9 points.  
10 PARTICIPANT: I was asking because we see  
11 similar issues with looking at ELISAs for alpha virus  
12 vaccines.  
13 DR. SULLIVAN: Yes. Very important  
14 points.  
15 PARTICIPANT: (Off microphone) Is that  
16 four parameter --  
17 DR. SULLIVAN: I think it's quadratic, but  
18 I would have to check.  
19 PARTICIPANT: Thanks Dr. Sullivan. One,  
20 first of all, is good refinement on the ELISA. That's  
21 something we've been fighting with for years here and  
22 that's a good step forward. The one thing I'll  
23 caution though is what we've seen with several vaccine  
24 platforms is that we actually have monkeys that  
25 actually have higher titers, a log and a log and a

Page 208

1 half higher, than monkeys that do not survive  
2 challenge and animals that do survive challenge. So  
3 it may not always hold true with another vector  
4 system.  
5 The other issue is the cellular. It's  
6 really an impressive system you have set up. One of  
7 the things we've kind of noticed in our kind of more  
8 basic ICCs is that we see transient responses and we  
9 don't really see the epitope-specific response that we  
10 see after challenge and that's consistent with the  
11 literature. But it's kind of confusing, too, because  
12 you get good responses across the board, but they  
13 don't seem to correlate to the after challenge and  
14 what you see is an epitope-specific response that  
15 actually is likely a lytic response that's part of the  
16 protection. How do you propose of getting to that  
17 point with your assay?  
18 DR. SULLIVAN: Yes. So that gets back to  
19 the difference between correlate and mechanism. And  
20 that's defined in empirically. So we have to  
21 interrogate these T cell responses very carefully over  
22 multiple animals under conditions of infection break-  
23 through to find the parameter that is the correlate  
24 that's going to predict what happens post challenge.  
25 So it's really important to separate mechanism from

Page 209

1 correlate.  
2 PARTICIPANT: I agree.  
3 DR. NABEL: Okay. Since we're all in  
4 agreement, a good time for a break and we'll convene  
5 in a half hour. Thanks.  
6 (Whereupon, at 2:59 p.m., the above-  
7 entitled matter recessed and reconvened at 3:32 p.m.  
8 the same day.)  
9 DR. NABEL: We'll get on with the last  
10 presentations. The next discussions will really be  
11 involving revolving around regulatory issues and so  
12 the next talk will be on regulatory perspectives on  
13 the use of animal models to study vaccines for  
14 filovirus infections by Mark Abdy from CBER, FDA.  
15 DR. ABDY: Hi everyone. Before -- I  
16 usually don't look at any notes, but I want to hit --  
17 There are about six quick points that I want to sort  
18 of bring up real quick. The first is that I certainly  
19 don't consider myself an expert and I don't think too  
20 many people within CBER consider themselves experts on  
21 the filoviruses, and essentially I really appreciate  
22 the opportunity to be here to learn from you folks who  
23 are doing a lot of this work. Certainly, I'm talking  
24 about -- everything I'm talking about is from the  
25 perspective of the Office of Vaccines here. So I

Page 210

1 appreciate having that opportunity to learn because we  
2 do need to have a dialogue between each other to sort  
3 of help us move forward.  
4 The second thing is -- what I'm going to  
5 present in this talk today is certainly a set of  
6 questions. I mean you're dealing with the animal  
7 rule. It sits in the CFR and there's, I don't know,  
8 ten paragraphs to it, and I've given this talk 30  
9 something times. There's only so many ways you can  
10 spin ten paragraphs. So it's the same language. I'm  
11 not going to present data to you. But basically what  
12 I'm trying to do is to show you what sort of questions  
13 that come up in our minds that we want to see sponsors  
14 address as they move forward, and I think that's going  
15 to sit up nicely for what the panel is going to  
16 discuss in the next session.  
17 The other thing that I've sort of noticed  
18 today is as speakers have set up here I've heard a lot  
19 of "I don't know," "Maybe," "Promising start." That  
20 tells me something. There's a long way to go still.  
21 And then the other thing to be aware of is  
22 I'm not going to stand here today and tell you "We  
23 have these models," "These assays we want you to use."  
24 Again, we're here to learn and try to develop stuff so  
25 that we can move forward hopefully together.

Page 211

1 And then finally, I'm not going to address  
2 any individual regulatory issue. If you have an IND  
3 or something like that and you have a specific  
4 question, the appropriate way to do that is to address  
5 it through your pre-IND or IND mechanism. I just  
6 don't want to get into those issues today.  
7 Okay. Let's move forward. So essentially  
8 what I'm going to do here is I'm going to cover some  
9 background information on the Rule, what type of data  
10 are we looking for and issues to consider when  
11 developing models.  
12 The reason the Rule was needed is because  
13 human efficacy studies were not feasible or ethical  
14 and essentially it's either epidemiology precludes a  
15 field trial or it's just not ethical to challenge  
16 people with these agents.  
17 Very quickly, it came about around the  
18 early 1990s and since many of you have heard this talk  
19 already, it was essentially the final Rule was issued  
20 in May of 2002, and it's published in two different  
21 places within the Code of Federal Regulations. For  
22 biologicals, it's in Section 601 and, for drugs, it's  
23 in Section 314, the same language. But essentially,  
24 what it does is it allows the FDA to approve a product  
25 for which human safety data has been established and

Page 212

1 for which the Animal Rule requirements are met, and  
2 these would be based on adequate and well controlled  
3 animal models, animal studies and result of which  
4 establishes that this product has a reasonable  
5 likelihood to provide a clinical benefit in humans.  
6 There are four essential pillars to the  
7 Animal Rule, four requirements that you need to think  
8 about as you develop these disease models and  
9 essentially efficacy models as well, and I'm going to  
10 go through them here and I had tried to put sort of a  
11 spin onto the filovirus field with some of these  
12 questions to think about.  
13 But the first is there is a reasonably  
14 well understood pathophysiological mechanism of the  
15 toxicity of the substance and its prevention or  
16 substantial reduction by the product. What that means  
17 is do we understand the pathogenesis or pathology of  
18 filoviruses reasonably well. Do we understand the  
19 differences between Ebola and Marburg viruses  
20 reasonably well and between the strains of each virus,  
21 i.e., Reston versus Zaire? And then in the vaccine  
22 world, do we understand how the vaccine works?  
23 But the second pillar that you need to --  
24 it's a criteria that you need to meet is that the  
25 effect must be demonstrated in more than one animal

Page 213

<p>1 species expected to react with a response predictive 2 for humans, and then you can see I have it in a 3 smaller font, unless the effect is demonstrated in a 4 single animal species that represents a sufficiently 5 well characterized animal model.</p> <p>6 This is not the "Two Animal Rule" that 7 many people refer to it. In fact, in some cases for 8 some products you are probably looking at using more 9 than one -- using more than two species to move toward 10 approval. It's really handled on a case-by-case 11 basis. I will be honest with you. I don't foresee in 12 the vaccine world at the present time us using one 13 species toward approval. You're probably looking at 14 two species minimum.</p> <p>15 But which animal models, which species, 16 which strains, are most relevant? Hopefully, the 17 panel can sort of start to bring that to the surface 18 at the next session. And also after this morning's 19 talk, from what country of origin is important. The 20 vaccine perspective, does the immune response in 21 animals resemble that that we see in humans?</p> <p>22 The third criteria is that the animal 23 study endpoint is clearly related to a desired benefit 24 in humans, generally, the enhancement of survival of 25 prevention of major morbidity. Putting it in language</p> <p style="text-align: right;">Page 214</p>	<p>1 II and III sections of the IND work, and the reason I 2 put that up there is while we do not talk about Animal 3 Rule studies as a Phase I study or a Phase II study or 4 a Phase III study and it would be inappropriate to do 5 that, it does make sense that you start this model 6 development off at some stage and work it somewhat 7 parallel to your clinical development so that in your 8 pre-IND phase I study you need to be thinking about 9 which models, do some early proof concept, early 10 immunogenicity work and gradually you work your way 11 through your Phase III when you have a definitive or 12 pivotal efficacy study designed according to GLP. 13 You're using your final formulation. You're bridging 14 your animal and human immunogenicity data. You have 15 a prospective statistical plan, and you're using 16 validated assays. So that's your goal.</p> <p>17 Essentially, what I do tell people to 18 think about is they say "Well, what do we need for a 19 pivotal study in animals," think about it with the 20 same sort of robustness and detail that you would if 21 you were designing a Phase III clinical study in 22 humans. It's that kind of detail we're after.</p> <p>23 But this is key particularly with the 24 Animal Rule because we are learning together on this. 25 Meet with the FDA on a regular basis to discuss your</p> <p style="text-align: right;">Page 216</p>
<p>1 that we understand, does the disease induced in 2 animals resemble that seen in humans? Something 3 that's not an issue for filoviruses but for other 4 agents that people are thinking about using the Animal 5 Rule for. The way I interpret this and this is my 6 personal opinion is when you see major morbidity that 7 means your animal model must develop major morbidity. 8 Just because an animal can get infected by something 9 and clear it, that's not sufficient to meet this 10 criteria. Again, not an issue for filoviruses.</p> <p>11 And the fourth and final criteria is that 12 data or information on the kinetics or 13 pharmacodynamics of the product or other relevant data 14 or information in animals and humans allows for the 15 selection of an effective dose in humans. From a 16 vaccine perspective, what components of the immune 17 response are important for protection and how can they 18 best be measured? And also you need to be able to 19 make that bridge which we've heard about already today 20 from the immune response in animals to humans.</p> <p>21 Very quickly, you don't give a 22 presentation from the FDA without having something 23 like this up on the screen. But essentially, this is 24 your route to licensure, and I use this sort of to 25 concentrate you on the Phase II/Phase III, I mean, I,</p> <p style="text-align: right;">Page 215</p>	<p>1 findings and your future studies. I don't like 2 finding out -- I mean it's not like. It's somewhat 3 unnecessary from my point of view that you find out 4 that people have done a whole bunch of animal studies. 5 A whole bunch of animals have lost their lives. And 6 the data doesn't quite fit what we want. It's much 7 better to come up front and say, "This is what we're 8 thinking of doing, what do you guys think" and work 9 through it together, and that has certainly worked 10 well for other products that we're working on.</p> <p>11 Just a point of clarification because this 12 has come up in some of the previous talks I've given, 13 preclinical pharm/tox animal studies, these have 14 little or nothing to do with the Animal Rule studies 15 and should be conducted prior to entry into a Phase I 16 study. So basically what I'm saying is these 17 pharm/tox studies are safety studies and they've pre 18 Phase I. They're what you do to get you into a Phase 19 I study. Your Animal Rule studies are efficacy 20 studies and they're post Phase I. There are sponsors 21 that have missed that point, and you don't, 22 incidentally, have to use that same species as well. 23 You use the species that are appropriate.</p> <p>24 So how do we select animal models? I've 25 sort of come up with these four bullet points here,</p> <p style="text-align: right;">Page 217</p>

1 but basically the selection of species is done on a  
2 case-by-case basis, we consult with outside experts,  
3 we look at the literature and then we also listen to  
4 recommendations made at scientific meetings and the  
5 three that have been -- well, the two so far that have  
6 been very useful to us in vaccines and in certainly we  
7 hope this one will be are an anthrax meeting in 2002  
8 and a plague meeting in 2004. It's good to get all,  
9 as many of the experts together, hear what they're  
10 saying and sort of hear what sort of rises to the top  
11 as far as choices go. But then the sponsor will still  
12 need to justify their choice of the model to the FDA  
13 with data.

14 Some issues to be considered when  
15 developing an animal disease model, basically what you  
16 need to be able to do is you need to be able to  
17 describe the clinical characteristics of human disease  
18 first and that needs to be the symptoms, incubation  
19 period, progression and pathology, and then describe  
20 if you can the outcome of untreated human cases. Does  
21 the animal model mimic the human findings? How do  
22 different exposure routes impact the disease in these  
23 animals? And if what you are testing or developing in  
24 the animal model is different from the human exposure  
25 route, you must justify why your animal study was

Page 218

1 conducted that way. Make the assumption that if it's  
2 going to be a certain way in humans you should be  
3 doing it the same way in animals.

4 Some other points. To what degree does  
5 the animal data compare with the human data? Are  
6 there any clinical biomarkers that indicate a pending  
7 onset of severe disease or death? Perhaps more  
8 important for a therapeutic than it is for a  
9 prophylactic but certainly something to be thinking  
10 about and we've heard some of that information today  
11 and then how reproducible is this animal model?

12 In addition, you start thinking about an  
13 efficacy model. How does the timing of the  
14 intervention in the animal studies compare to what  
15 happens in the clinical setting? Certainly, in the  
16 vaccine world, you can think of two scenarios and  
17 they've sort of been raised today. But there's the  
18 idea that you vaccinate and then challenge some time  
19 later as a prophylactic and then there's this post  
20 exposure data. We've heard about it with the  
21 fasciculus dermatitis vaccine today. Not criticizing  
22 this work because it's great to know that's there.  
23 But from a regulatory point of view, I want to see  
24 some data that's beyond 30 minutes after challenge  
25 because that's the reality and certainly we've been

Page 219

1 through that with some other agents as well. Not  
2 criticizing it, it's just you have some great work to  
3 start with, but you sort of need to know where your  
4 goal is going. Think of what the indication is going  
5 to be for your product and sometimes design your  
6 studies. So go backwards and then design your studies  
7 for that indication.

8 Are there concerns about immune responses  
9 between the different species and humans? How will  
10 this data be bridged to humans and then is there is a  
11 correlate of protection and if this correlate can be  
12 obtained, will you have protection?

13 This is a blank table on purpose because  
14 I don't have enough information that wouldn't fit on  
15 a slide. But this is sort of how I've suggested to  
16 sponsors to approach us when they're suggesting this  
17 is a model we want to look at and justify it. And  
18 what you need to do is develop a detailed table where  
19 you take your species and in the case of filoviruses,  
20 let's go with humans, monkeys and rodents and then  
21 under each category -- sorry. So under each species  
22 or monkey, list each species of interest that you're  
23 interested in or strain and sort of list them in a  
24 table and then start filling in this table with  
25 clinical disease. So what clinical markers would you

Page 220

1 find? Immunology, which assays, what data do you have  
2 that sort of you've done them as a comparison between  
3 the two and then put pathology as well, both clinical  
4 pathology and gross and histopathology. So obviously,  
5 you can't fit it all in this table, but that's a  
6 fairly detailed table when you start to think about  
7 it. But when you do that, you start to realize where  
8 your gaps are, and those gaps are places that probably  
9 need to be filled and sort of will help you in your  
10 mission for research.

11 Appropriate facilities, again you need to  
12 have select agent requirements. This is sort of  
13 preaching to the choir here. Small and large animal  
14 model capability and experienced staff, some  
15 validation experience, BSL-3 and BSL-4 capability,  
16 particularly in this case BSL-4 and, of course,  
17 there's only a handful of facilities in the U.S. that  
18 can conduct aerosol studies at this level. So the  
19 queue is long.

20 GLP, very few facilities in the U.S. can  
21 conduct an infectious agent aerosol challenge in  
22 accordance with GLP regulations. I think it may be  
23 three. But a brief note on GLP, if you read the  
24 Animal Rule in the Federal Register, not in the CFR,  
25 but in the Federal Register, it will say that all

Page 221

<p>1 studies must be conducted in accordance with pre-  2 existing requirements under Good Laboratory Practices  3 and the Animal Welfare Act.</p> <p>4 At the present time and I've been saying  5 this for four plus years now, that is being amended in  6 the CFR under Section 58 which is where the GLP regs  7 are to reflect that. It is stuck much higher up than  8 my level in the FDA, that language getting amended  9 into the CFR 58.</p> <p>10 But because of this requirement and  11 knowing what the facilities are like in this country,  12 CBER for some time now has basically said that we  13 expect GLP facilities to be used and a GLP study to be  14 used for definitive or pivotal animal studies and for  15 any study which you will use or you want to describe  16 in the label or the package insert so that you don't  17 have to do GLP studies for pilot studies. CDER has a  18 slightly different interpretation I believe, but I'll  19 let them address that.</p> <p>20 Assays and immunology, a lot of work needs  21 to be done to develop these assays and validate them.  22 You will need to validate assays for both animal and  23 human assays before you do your pivotal study, and the  24 goal should be a validated functional assay or one  25 that has been correlated to a functional assay. And</p> <p style="text-align: right;">Page 222</p>	<p>1 a prospective statistical plan in place.</p> <p>2 Some potential misunderstandings. If you  3 can get your product licensed using any other route,  4 you must use this alternative. Perhaps not essential  5 for filoviruses, but there are some sponsors out there  6 that have sort of misinterpreted what that means.</p> <p>7 Safety must still be demonstrated in your  8 human subjects. So you still have to do your Phase I,  9 Phase II and Phase III studies. This rule, the Animal  10 Rule, is not an accelerated or fast track approval.  11 You would have to apply for those and get that  12 separately, if I've worded that terminology correctly,  13 but they're not the same thing.</p> <p>14 And certainly this point, I think, for  15 those of us that have been working in this arena for  16 five plus years now, this rule is not a shortcut to  17 approval and, in fact, I'm willing to say will take  18 longer because this is a tremendous amount of work to  19 do, and something that's worth remembering is that the  20 purpose of the Animal Rule is to develop a product for  21 humans, not for animals. Just because you can get it  22 to work in monkeys or mice doesn't mean that we will  23 accept that. You need to be able to show to us that  24 there's a reasonable likelihood that it will work in  25 human beings and that may mean that you have to work</p> <p style="text-align: right;">Page 224</p>
<p>1 certainly if you think I've said something that you  2 don't think is possible or you have a way around that,  3 put something together with a lot of data, show it to  4 us and let's talk about it.</p> <p>5 By the time a definitive animal study is  6 conducted, you should be able to predict the outcome  7 of the negative controls when infected with the  8 predetermined route, dose and strain of the infectious  9 agent. Preparation and administration of the  10 infectious agent should be consistent with earlier  11 studies that led the design of that definitive study  12 and by that what I mean and I think I've heard this  13 sort of today is don't just -- Tom Geisbert, I think  14 he used the same virus, the same challenge dose the  15 entire time for a whole set of studies. That's what  16 we're after so that you can make better comparisons.  17 The less variables you have in a set of studies to  18 evaluate the better.</p> <p>19 And then when possible use validated  20 assays to monitor the response and bridge the data to  21 animals. Certainly, nonvalidated assays will be  22 useful and we are moving forward in a number of  23 products where we are getting nonvalidated assay data  24 that is very helpful as well. So don't just ignore  25 it. Certainly, be working on that as well. And have</p> <p style="text-align: right;">Page 223</p>	<p>1 with different titers in your vaccine to get immune  2 responses that are somewhat comparable. But just  3 don't assume that if you give an adult human dose to  4 a monkey and they're protected we're going to be okay  5 with that. You need to be thinking about the fact  6 that you have to try and get it to the human being  7 point of view.</p> <p>8 Route of exposure is important. We've  9 certainly talked about that some today. We certainly  10 expect that it should mimic what is expected during an  11 attack and outbreak. So again, if whatever the  12 indication of your product is, that's what you should  13 be thinking of as your exposure route. Certainly,  14 other exposure routes are helpful, but I guess the one  15 that's going to come up in discussion is aerosol.</p> <p>16 If your indication is going to be we want  17 to protect so and so against an aerosol infection or  18 an aerosol challenge, then you will need to do aerosol  19 data. You can certainly use the other data to help  20 you, but at some stage we will need to see that. I  21 have a hard time believing that there is not  22 significant differences in pathology between the two.  23 We just haven't learnt enough about it. I have heard  24 too many "mays," "possibly" and that sort of stuff  25 today.</p> <p style="text-align: right;">Page 225</p>

<p>1 I think it's a safe bet that you need to 2 be looking at your route of exposure that you want to 3 put in your package insert. Certainly, if you don't 4 think that's correct, you're more than welcome to put 5 your argument together and submit it to us. 6 And I've heard this done today as well. 7 I mean, actually what's been interesting sitting in 8 this meeting is that stuff that five years ago we 9 didn't hear these terms and listen to these studies. 10 These studies have already been done in some of this 11 work, but a life history study is very useful because 12 you'll learn more about the disease in each species 13 and at the time line as well. But you will probably 14 not have everything in one animal model and thus, I'm 15 getting back to that point that you're probably 16 looking at at least two species. 17 And then, as I said, I think making a very 18 detailed table and then looking at where the gaps are 19 and trying to fill those gaps will be useful. 20 It is certainly a rule that is new to both 21 us and industry and collaboration between the two of 22 us is essential and, certainly from my point of view, 23 I think that's happening. I believe we've been pretty 24 receptive to talking to sponsors about issues. If 25 they've had a protocol they wanted reviewed, talk to</p> <p style="text-align: right;">Page 226</p>	<p>1 talk 30 times, but I'm still awake and it's a sleeping 2 vervet monkey" as we call it from South Africa. 3 Thank you and any questions. 4 (Applause.) 5 DR. NABEL: Questions? 6 PARTICIPANT: Yes, I have a question. I 7 appreciate the comments about the VSV post exposure in 8 30 minutes and we certainly want to walk that out and 9 see how far you can take it out. 10 DR. ABDY: Great. 11 PARTICIPANT: One of the concerns that a 12 lot of us in this business have is lab accidents and 13 it's kind of something that we don't talk about very 14 much. But with all these new labs being built, it's 15 reality and even with the best folks. Thirty minutes 16 is realistic to treat a lab exposure and so very 17 selfishly, I think myself and, I don't know where 18 Heinz is, would like to see that platform advanced for 19 that purpose and for that indication. 20 Let's say that it doesn't work 24 or 48 21 hours later, but let's say it works at two or three 22 hours. How would you address that? Is there an 23 approach that you would use and do you still see value 24 or utility to that? 25 DR. ABDY: Certainly, I think we need to</p> <p style="text-align: right;">Page 228</p>
<p>1 us. 2 Early and frequent communication with us 3 always works best. Expect interactions with your 4 product as you move forward with an advisory 5 committee. In some cases, it may be prior to an 6 animal efficacy trial for concurrence with our 7 concepts or certainly following the Agency's BLA 8 review. 9 Just a few acknowledgments here. My 10 immediate supervisor is Dave Green who is a 11 toxicologist. Tim Nelle is our primary reviewer 12 within the Division of Vaccines as is Erik Henschal on 13 some files. Dale Slavin is also fairly involved with 14 Animal Rule work within the division. Carolyn Wilson 15 is not in our office. She is in the Office of 16 Cellular and Gene Therapy, and then Barbara Styr is 17 in the Office of Antivirals in CDER, and I thank them 18 for looking at the talk and certainly we had offline 19 communications about this meeting. 20 And as my final slide for those of you 21 that, as I said, I've given this talk close to 30 22 times and there are a couple of individuals who have 23 probably heard this talk 30 times and one of them is 24 Ed Nuzum and while I'm not going to put it in here, it 25 probably should read "I'm Ed Nuzum. I've heard this</p> <p style="text-align: right;">Page 227</p>	<p>1 see -- I mean, I hate to use this answer from the FDA 2 because we seem to use it so much. But a lot of our 3 thoughts are data driven. But I would need to see, in 4 something like this, I would like to see a timeline of 5 in detail this animal gets infected with this dose, 6 with this agent, this route and at this time it starts 7 to show signs of severe disease. 8 Now we don't want to see it at that 9 timeline for a post exposure vaccine. That then 10 becomes therapeutic. But I haven't seen any data yet, 11 and that's the sort of data that needs to be generated 12 to say that we feel certain that during this time 13 period is when we want to use a post exposure route 14 and then we would like to push it because we think 15 that's probably reality in the human world. 16 But, of course, you've just presented a 17 scenario where you're in a lab in very difficult 18 circumstances to work with. I think you would have to 19 put that into a submission and say these are our 20 reasons why that we feel we have to do it this way. 21 Be honest with us though. If you have options, let us 22 know because certainly we'll do some digging around. 23 Don't take the easy route. 24 I mean this is hard work and looking at 25 the data today, there's a tremendous amount of work.</p> <p style="text-align: right;">Page 229</p>

1 I've never worked in a BSL-4. I've done some animal  
 2 work and it's a lot of work you guys have done and  
 3 certainly we recognize that, but this is not an easy  
 4 way. There is not an easy option. So lay out what  
 5 you want to do and tell us why and use data to back it  
 6 up.  
 7 PARTICIPANT: Thanks.  
 8 DR. NABEL: Tom, I have to say though that  
 9 just listening to your comment, why wouldn't you take  
 10 the vaccine ahead of time instead of waiting to be  
 11 stuck -- I think the point is though that there is  
 12 room for licensure for a needle stick exposure and I  
 13 think that's really the point that --  
 14 DR. ABDY: I think if a sponsor wants to  
 15 pursue that as sort of that indication, then that  
 16 absolutely is an appropriate way to be going.  
 17 DR. NABEL: Alan.  
 18 DR. SCHMALJOHN: Yes, and I just wanted to  
 19 kind of follow up on that conundrum. Certainly, it's  
 20 estimated that the most catastrophic potential for  
 21 these viruses is in an aerosol release, and it's still  
 22 a very significant biological threat in a parenteral  
 23 exposure. But if you take the effort to license for  
 24 aerosol exposure, does that mean that Tom Ksiazek and  
 25 Heinz Feldmann, etc., going to an outbreak and

Page 230

1 vaccinating themselves and vaccinating others because  
 2 their exposure is almost certainly parenteral, is that  
 3 an off-license use?  
 4 DR. ABDY: I don't - we have not had to  
 5 address that issue yet. Again, my own personal  
 6 feeling is if you're going to use it for a route that  
 7 has not been shown to be approved for the Animal Rule,  
 8 then it would not be approved. You would need to show  
 9 the route that you want to use is the route that's  
 10 going to be approved. But again, that's my personal  
 11 opinion. We have not had to address that. Certainly,  
 12 we've talked about it in the plague workshop where  
 13 people asked -- if you get licensed for pneumonic  
 14 plague will it work for bubonic and we've said you  
 15 need to show us bubonic data.  
 16 DR. SCHMALJOHN: Just a final follow-up  
 17 then, people should be attentive to this is absolutely  
 18 in no way like plague or anthrax or smallpox.  
 19 DR. ABDY: I agree. No, absolutely. I'm  
 20 just showing you -- giving you some background in the  
 21 way we've been thinking on that though.  
 22 DR. NABEL: Yes, I just want to also add  
 23 to what Alan just said which is that even in the event  
 24 of an aerosol attack, I know some of the modeling  
 25 that's been done that really among the biggest

Page 231

1 concerns are the contacts of the people who have  
 2 gotten it by aerosol delivery. So whether it's Tom  
 3 going to Africa or whether it's secondary contacts in  
 4 the setting of an outbreak, the actual droplet  
 5 mediated transmission is one that I think we shouldn't  
 6 make a second class indication. I think it's right up  
 7 there.  
 8 PARTICIPANT: You mentioned starting early  
 9 talking to FDA.  
 10 DR. ABDY: Yes.  
 11 PARTICIPANT: And I was wondering how  
 12 early is --  
 13 DR. ABDY: Early.  
 14 PARTICIPANT: -- what you're talking about  
 15 and also what is the logistics of that? Is that like  
 16 a type B meeting you have to apply for and takes two  
 17 months to set it up or is it very informal?  
 18 DR. ABDY: Certainly, for those people who  
 19 are just thinking about it and have never communicated  
 20 with us, we have such things as pre-IND meetings and,  
 21 in fact, we have such things as pre-pre-IND meetings  
 22 and once you're in, like we have some agents now  
 23 moving forward with Animal Rule approval that are in  
 24 Phase I/Phase II type studies. We certainly have as  
 25 an amendment to the IND asked folks to please submit

Page 232

1 your protocols to us so that we can review them. In  
 2 some cases, it's been just a small group of us that  
 3 say this looks fine, but sometimes we want to get our  
 4 statisticians involved and that means that the whole  
 5 review team has to come in. So you can use it as an  
 6 amendment to an IND.  
 7 The other route that has happened and this  
 8 is more the approach that NIH has taken is to use the  
 9 master file where they've developed an animal model,  
 10 and if it hasn't involved an individual sponsor's  
 11 product, that data is going into a master file that  
 12 others can use, and those are certainly approaches  
 13 that are working well. I mean, we have some ways to  
 14 go still but we've come a long way.  
 15 DR. NABEL: Let's just take these two  
 16 questions and then we're going to have a panel  
 17 discussion. So this won't be the end of it, and I do  
 18 want to let the panel get on.  
 19 PARTICIPANT: Yes, we've seen data on some  
 20 rodent models and on a couple of different nonhuman  
 21 primate. If the nonhuman primate, as they appear to  
 22 be, are far superior in terms of predictive to humans,  
 23 could you use two different nonhuman primate species  
 24 to satisfy your two different species or multiple  
 25 species requirement?

Page 233

<p>1 DR. ABDY: So you all heard that question.  2 Absolutely. The Animal Rule does not tell you what  3 species you have to use, which ones. So if you think  4 you have two nonhuman primate models that you need to  5 move forward, that could be done. I still think  6 because of numbers in some issues, you may need some  7 rodent data. But certainly, you don't have to use  8 monkeys and, in this case, I think you're probably  9 going to have to, but in some other products you --  10 well, everything I've looked at, there probably is a  11 monkey needed.</p> <p>12 PARTICIPANT: If immune enhancement  13 happens to be a component of human pathogenesis in a  14 particular disease, does that have to be displayed in  15 the pivotal animal model as well?</p> <p>16 DR. ABDY: If not displayed, it needs to  17 be addressed and in some sort of -- nothing's going to  18 be perfect in these models. Otherwise, we'd have  19 human beings. So you're not going to have everything,  20 but certainly, that's an issue that we would have to  21 talk about. But I think it would have to be  22 addressed. There is certainly another product I can  23 think about where that is a concern and they're going  24 to have to address it.</p> <p>25 DR. NABEL: Okay. Thank you, Mark. After  Page 234</p>	<p>1 and I wanted to remind everybody that there is going  2 to be a filovirus symposium in Libreville in Gabon  3 March 27 to 29, 2008. Eric Leroy and Jean-Paul  4 Gonzalez are the local organizing committee and the  5 website is here. If you just google Filovirus  6 Symposium 2008 you'll find it.</p> <p>7 I guess this really gets into the question  8 now and question one was which animal model and  9 looking at the symptoms, and this is a slide, one of  10 the last slides of my talk, looking at the summary of  11 the clinical features for filovirus infections with  12 the different animal models, mouse, guinea pig and the  13 three species of nonhuman primates and how that  14 compares to human disease.</p> <p>15 And a couple of points I guess that I  16 wanted to make and kind of open this up for  17 discussion, again I think that the data that we have  18 regarding the nonhuman primates and the humans shows  19 that the coagulation disorders and, in particular, the  20 fibrin deposition, very prominent features in the  21 macaque models and in the humans, not quite as  22 prominent in the African greens or in the guinea pigs  23 or the rodents, and I think that this is very  24 important. I think that the data that we have that  25 Lisa and I have done with the coagulation of  Page 236</p>
<p>1 30 times, I think you have it down. So I will hand  2 over the chair position to Ed Nuzum now who will  3 proceed with the panel discussion on vaccine  4 development and if the members of the panel could come  5 up to the table, I think we can get started.</p> <p>6 DR. NUZUM: Okay. So I think the panel,  7 myself and the panel, will take the table up here, and  8 the general approach is going to be we have four  9 questions that are going to be up and each panel  10 member is going to address one of them and then the  11 other panels members are free to address each question  12 and we'll take questions from the audience on each  13 question and, if there's time, I have some summary  14 notes and comments that I'll go over as well.</p> <p>15 So the first question is going to deal  16 with species strains and Tom Geisbert is going to talk  17 about that.</p> <p>18 DR GEISBERT: Okay. A real quick  19 announcement. Heinz was going to put this in this  20 morning and I got here late. Heinz and I just wanted  21 to inform everybody. This is kind of a follow-up  22 meeting. Heinz and I and Yoshi Kawaoka started a  23 series of filovirus meetings in Winnipeg. Actually,  24 there's a series that the last one Gary Nabel had one  25 before and Heinz had one in Winnipeg and just -- Heinz  Page 235</p>	<p>1 inhibitors particularly NPC-2 and Xigris clearly show  2 that coagulopathy plays a huge role in disease and  3 that you can mitigate the effects of the disease by  4 targeting that cascade.</p> <p>5 So I would argue very strongly that any  6 animal model that would be developed for filoviruses  7 would really have to show that, not saying that  8 there's not utility in guinea pigs or mice; there  9 certainly is, but I think that's one of the big  10 things. I think if you look at viremia, the average  11 viremia in the mouse is 108, 109, and that's from one  12 of Mike Bray's papers. If you look at the macaques,  13 106, 107, and the only human paper that was published  14 back in the '70s it looks like about six and a half  15 logs. So that's very consistent. And I think the  16 target cells, of course, are consistent among all of  17 the species.</p> <p>18 We didn't really talk about the cytokine-  19 chemokine response, but mostly, the same profiles are  20 seen in the macaques. Interestingly, the rhesis IL-10  21 is up and it's also up in humans, not really so much  22 with the cynos.</p> <p>23 But that's kind of in summary of what  24 we've seen so far and I guess, Ed, if you want to --  25 I don't know how you want to do this if you want to  Page 237</p>

1 open it up for discussion or if the other panel  
 2 members want to take a crack at that.

3 DR. SCHMALJOHN: Tom, I guess I'd like to  
 4 leave a little wiggle room in terms of often when you  
 5 say this happens in filovirus infection in monkeys, it  
 6 is more often your vast Ebola Zaire experience in  
 7 terms of the coagulopathy varies. We see coagulopathy  
 8 with Marburg and we haven't looked at it in all  
 9 species of monkeys and all strains of Marburg, but  
 10 often it's a consequence of liver damage, not the same  
 11 kind that you see with Ebola Zaire and cynos and in  
 12 rhesus.

13 Then the other column is the degree of  
 14 consistency of those observations in humans. It's,  
 15 yes, true that they occur in humans, but it's not  
 16 necessarily true that it's even a majority of cases.

17 DR. GEISBERT: I think that came up in  
 18 Tony's talk this morning, too, is there is going to be  
 19 variability and with these new outbreaks, hopefully we  
 20 get more information on human disease. And so I think  
 21 all we can base it on at this point is what Erik has  
 22 gotten and what Tony has gotten from some of these  
 23 models and it does show that it's consistent at least  
 24 for Ebola Zaire and Ebola Sudan. I mean, Tony's work  
 25 was in Gulu, and I didn't have time to show all the

Page 238

1 data, but it's very consistent between Sudan and  
 2 Zaire.

3 Marburg, you're correct. The liver is  
 4 more involved. Some of the coagulation defects are  
 5 probably because hepatocytes are not functioning and  
 6 making certain coagulation factors and things like  
 7 that. But we do see almost everything else. For  
 8 Angola, for example, we've looked at, Lisa and I have  
 9 looked at that pretty closely and you do have a lot --  
 10 you get D-dimers. You get fibrin, not quite as to the  
 11 same. It's more of a temporal thing and you see it  
 12 more towards the end; whereas Zaire comes up earlier.

13 So I think you're correct in the sense  
 14 that we need more human data to marry up with the  
 15 monkey data. But I think for at least Zaire and Sudan  
 16 and probably Angola and maybe Ci67 that we have a  
 17 pretty good dataset for nonhuman primates.

18 DR. SCHMALJOHN: Yes, I'd love to hear  
 19 from anybody who has real experience with a human  
 20 disease lately and can add onto what's published.

21 PARTICIPANT: We've got experience with  
 22 the disease but we aren't collecting the data you're  
 23 talking about. That's part of the problem.

24 DR. GEISBERT: I mean, I think if you look  
 25 at what Tony showed this morning clearly D-dimers and

Page 239

1 then also what was interesting was nitrate and we see  
 2 the same thing in macaques and I think that that plays  
 3 a significant role in some of the vascular dysfunction  
 4 that we see during filovirus infection. So I think  
 5 while we certainly don't have the data yet, I think  
 6 that the work that Tony did is fantastic and I think  
 7 that that's exactly the kind of studies that need to  
 8 be done in the future to solidify this. But I think  
 9 that from what we have so far, it certainly looks like  
 10 the macaques are very consistent with what Tony and  
 11 Erik have shown and the rodents, I think, there's  
 12 clearly a lot of issues there.

13 DR. NUZUM: I just want to ask cynos  
 14 versus rhesus. According to your table, they look  
 15 very similar. Would you pick on over the other?

16 DR. GEISBERT: We pretty much use them  
 17 interchangeably. I mean, if you want to lower the bar  
 18 a little bit, I think it's probably easier to protect  
 19 the rhesus and I think that if you look at the disease  
 20 course, all things being equal and again it was  
 21 mentioned, I mean, the studies that Lisa and I have  
 22 done have used the same exact challenge virus, exact  
 23 same dose, everything is identical. So if you look at  
 24 large numbers, we have 20 some historical control  
 25 rhesus with the Ebola Zaire Kikwit 95 isolate and 30

Page 240

1 something cynos and there is about a two day  
 2 difference in disease course with the rhesus being  
 3 longer and I guess you could probably argue that the  
 4 rhesus may be a little bit more like human if you look  
 5 at the disease course. But I personally think they  
 6 can be used interchangeably.

7 My personal bias and again this is a  
 8 personal bias is that the rhesus is probably a little  
 9 bit more like human than cyno. But I think they're  
 10 again very interchangeable. I think the green is  
 11 definitely the outlier and again I think when you look  
 12 at between studies and some of the variability, one of  
 13 the slides I showed this morning, we're using a number  
 14 of different subspecies of cynos and we really haven't  
 15 dissected that out and with the rhesus, we've only,  
 16 Lisa and I have pretty much exclusively used Chinese  
 17 rhesus. So there is very strong consistency. In  
 18 Nancy Sullivan's studies and Gary Nabel's studies with  
 19 the exception of one study with Philippine cynos,  
 20 they've all been Vietnamese and with the studies that  
 21 we've done with Heinz, they've all been Indonesian for  
 22 the VSVs. So there is at least consistency in those  
 23 two vaccine studies.

24 But, you know, whether some of these  
 25 biomarkers as Tony called this morning would be

Page 241

<p>1 identical in a Mauritius cyno versus a Vietnamese 2 cyno, I don't know. I think that we also need to take 3 that into consideration. But again, to answer your 4 question, rhesus or cyno, I think either one. 5 PARTICIPANT: I would like to ask a 6 question of the panel as well as maybe Mark from FDA. 7 Clearly, actually, Tom, you showed extensive data 8 pathology. The monkeys appear to have similar disease 9 or same disease as humans although as you indicated 10 there is not enough clinical human samples to do the 11 similar study. 12 I was wondering if monkey can be 13 identified because in general they get the same 14 disease. They are not necessarily a model. They get 15 the same disease as human. They have the same 16 behavior of the infection. If we find an animal 17 model, I couldn't think of any better animal model 18 than the monkeys as filovirus infection. 19 So, for example, donkeys, you can infect 20 high dose. They get a viremia. They don't get the 21 disease. But can we actually just use this as the 22 example, that monkeys, regardless of which subspecies 23 can be used as the animal model to meet the Animal 24 Model Rule or do we have to look into the specific 25 disease pathology, coagulation, to match with human</p> <p style="text-align: right;">Page 242</p>	<p>1 excellent and they're going to generate the kind of 2 data that FDA will need to see to make those 3 decisions. I just think it's a little early to answer 4 a litany of questions like that. 5 PARTICIPANT: Would it be safe to say at 6 least at the beginning if you have a Marburg vaccine 7 you have to test every subtype? If you have a Ebola 8 vaccine, you have to test every subtype to show 9 protection before you can apply for the FDA approval. 10 DR. ABDY: So the question is what 11 challenge virus should you be using, etc. Certainly, 12 you need to choose one that causes disease 13 consistently, causes severe morbidity and mortality 14 and is one that the people that know what's going on 15 there in the epidemiology of these diseases thinks we 16 could be exposed to. Certainly, we don't expect for 17 approval up front to have done it against a number of 18 different subtypes. That would be a tremendous amount 19 of work. 20 We have said that you may as you get post 21 approval perhaps and one thing I did not mention in my 22 talk that's worth remembering, I didn't talk about all 23 the post marketing studies that need to be done 24 because we have a long way to go with filoviruses. 25 But there is a whole set of requirements there as well</p> <p style="text-align: right;">Page 244</p>
<p>1 which is very hard to get the data? And that's my 2 question. 3 Secondly, can we use -- what actually is 4 the FDA thinking of which strain of the filovirus as 5 a model? Can we use, for example, Zaire as a model 6 if we protect Zaire using the same technology, for 7 example, antibodies expressing Sudan GP or Marburg GP? 8 Can that be accepted as the model so you don't have to 9 do every subtype in the challenge model? 10 There is an example, but however, there is 11 also concern that filovirus does behave differently, 12 different strain, subspecies behave specifically. For 13 example, flu, you don't have to test every strain of 14 flu as long as the vaccines work. So what's the 15 consensus and that's my question. 16 DR. NUZUM: I think the questions you're 17 asking require a lot of data and a lot of evaluation 18 and a lot of natural history studies and until 19 sponsors come to FDA with their product, their 20 indication, their data, their justification, rationale 21 for their product development plan, I don't think 22 there's specific answers for that. 23 I think where we're at now is we have a 24 lot of good preliminary data. The natural history 25 studies that Doug Reed presented, I think, are</p> <p style="text-align: right;">Page 243</p>	<p>1 that follow in the Animal Rule. 2 But you very likely, to get back to your 3 question, may have to do a couple of pilot type 4 studies with a different strain just to see if it 5 protects. But certainly the label is going to reflect 6 what you tested it against. That's what's going to 7 say. 8 DR. SCHMALJOHN: If I could get back a 9 minute to Ed's question on rhesus versus cyno, I think 10 Tom and I agree in a general sense in a bias toward 11 rhesus, we started with cynos and then moved toward 12 rhesus both for availability but also more importantly 13 for reagent match. A lot more the reagents that are 14 so well characterized for humans are known for their 15 characteristics in rhesus and maybe Nancy could talk 16 about the 20 parameter in rhesus versus cyno. 17 DR. SULLIVAN: Yes. So we've developed 18 panels that work in both rhesus and cyno and they're, 19 for the most part, I would say interchangeable for the 20 immune reagents. 21 PARTICIPANT: This afternoon we're 22 focusing on vaccine development and I guess tomorrow 23 morning we'll be looking at therapeutics. So I'm 24 wondering since these are two different targets, two 25 different goals, to what extent the clinical features</p> <p style="text-align: right;">Page 245</p>

1 of disease are relevant for vaccine development or is  
2 what you're trying to do is simply prevent the disease  
3 altogether and why shouldn't we be looking more immune  
4 responses and comparing those among animal models in  
5 choosing an appropriate model?  
6 DR. NUZUM: Yes, but you're getting ahead  
7 of us. I mean, we have questions tomorrow that  
8 address that. After we go through today on vaccines,  
9 we're going to talk -- I think there's an opportunity  
10 to talk about differences relevant to therapeutics  
11 tomorrow and obviously, the big differences are  
12 difference in immune response, kinetics for vaccines  
13 and pharmacokinetics of drugs for therapeutics or  
14 antibodies, whatever.  
15 But we're running out of time. Let's do  
16 real quick comments. We have three more questions to  
17 get through and some of them we've already gotten  
18 into. So let's do these quick.  
19 PARTICIPANT: Yes. So just a couple of  
20 very quick questions. In terms of challenge dose that  
21 you would use in a nonhuman primate model, what dose  
22 would you use using correlates to human exposure?  
23 Just refer 10 PFUs, 1,000 PFUs, what would you --  
24 DR. SULLIVAN: Part of that depends on the  
25 route. So I mean, Tony told you that he challenges IP  
Page 246

1 with 6 PFU and if you think about an IP challenge  
2 that's full of dendritic cells and that are going to  
3 be early targets of infection, six PFU is uniformly  
4 lethal. Whereas, Tom talked about 1,000 PFU in an IM  
5 challenge. The LD-50s haven't been done for both  
6 routes to really understand how those compare.  
7 DR. GEISBERT: And to answer Tony's  
8 question from this morning, that could be some of the  
9 differences too. An IP challenge may be why your  
10 monkeys died quicker than IM. So certainly, routes  
11 could matter in addition to dose and a whole lot of  
12 the other variables. But I think 1,000 has pretty  
13 much been the standard that we've figured would  
14 replicate an accidental needle stick whether it's in  
15 a lab or whether it's in Africa.  
16 PARTICIPANT: And just one more question.  
17 You know this may be more relevant to tomorrow's  
18 discussion, but at what point post exposure would you  
19 expect for a therapeutic treatment to basically mimic  
20 the stage of the disease in terms of human infection  
21 and when one might treat per se?  
22 DR. GEISBERT: I'm not quite sure I  
23 understand the question exactly.  
24 PARTICIPANT: Well, we just had the point  
25 that one half hour after infection might not be  
Page 247

1 appropriate for therapeutic.  
2 DR. GEISBERT: I think -- I mean, Tony's  
3 right behind you. Maybe he's seen human cases. He  
4 can maybe answer this better than I. But I think that  
5 when -- You know, I can tell you in the macaque models  
6 the disease goes pretty quick and I think when you  
7 start seeing symptoms and you have a full blown rash  
8 and you have 104 or 105 viremia or more, I don't think  
9 any treatment is going to work if you wait that long.  
10 So I think it's something in the early stage. I don't  
11 know. Do you want to comment on that, Tony?  
12 DR. SANCHEZ: Yes. I very much believe  
13 that you're in a horse race once you get to that  
14 point. If you reach that level of infection, I think  
15 trying to treat with a therapeutic or a vaccine is not  
16 going to work.  
17 PARTICIPANT: And when do you human  
18 patients come in would be one question and where are  
19 they usually? They're not except in rare exceptions  
20 where we have known exposure like Dr. Matthew in Gulu  
21 for instance. They're coming in when they come in and  
22 generally they have very high viremia.  
23 DR. ABDY: Tom, this is a question more or  
24 less to get it on the record. But you very quickly  
25 said the African green you didn't think was a suitable  
Page 248

1 model. Certainly, I see your point on the rash. But  
2 could you please tell me or tell us other reasons why  
3 you don't think the African green is an appropriate  
4 model?  
5 DR. GEISBERT: That's really the -- I  
6 didn't say -- I don't want to say there's no -- that  
7 African green has no utility because I don't -- Maybe  
8 that came across a little different than I wanted it  
9 to. Compared to the macaques, I do think that the  
10 coagulopathy, the presence of the rash, there is  
11 fibrin in the greens. But from what we've seen with  
12 Zaire in our lab, it's nothing close to what we see  
13 with the macaques.  
14 And again as Alan pointed out we don't  
15 really know yet with humans. We have a handful of  
16 cases. But it does appear from what we have looked at  
17 for humans that it's more consistent with the  
18 macaques. So I think if your choice is a rhesus or a  
19 cyno versus a green, I think handsdown it's a rhesus  
20 or a cyno.  
21 PARTICIPANT: I have a question but it's  
22 more a regulatory question but also in general which  
23 is not necessarily only applies to the filoviruses.  
24 I understand that FDA is very concerned about safety  
25 and safety comes before everything else and that's  
Page 249

1 totally understandable. But my question is is FDA  
2 also sometimes concerned about raising the bar so high  
3 that would result in depriving the public from a  
4 potential product that could be very useful and I  
5 think that could be the case where the animal model is  
6 more sensitive than human.  
7 I don't know if it is necessarily the case  
8 for filoviruses. But if you just look at the numbers,  
9 filoviruses in most nonhuman primate models are 100  
10 percent lethal; whereas, in humans, there's been  
11 rarely an outbreak with 100 percent fatality.  
12 Now I just want to tie this specifically  
13 to a situation we were just talking about and that is  
14 the route of exposure. If 6 PFU in IP kills but  
15 doesn't kill IM and now the FDA forces you to show  
16 that the all possible exposure routes and the vaccine  
17 fails or to some degree fails, not completely fails,  
18 like instead of having 100 percent protection, you  
19 have 50 percent protection at some exposure routes,  
20 does that justify to really stop the development of  
21 that vaccine?  
22 DR. NUZUM: But FDA isn't going to force  
23 anyone to show all. I mean, this is my opinion I  
24 guess. I'm not FDA, but they're not going to force  
25 anybody to do anything. They're going to want you to  
Page 250

1 provide data for what you want the vaccine for, what's  
2 your indication, and this whole business of 30 minutes  
3 post challenge, that's a whole separate discussion in  
4 itself. You're talking post event, post exposure,  
5 therapeutic, pre exposure, post event, post exposure,  
6 which are two different things in my mind, and  
7 therapeutic, and your models will have to address  
8 whatever you're going to put on the label, and FDA is  
9 not going to say what you want. The sponsor is going  
10 to come with their product.  
11 PARTICIPANT: But does the route of  
12 exposure go on the label?  
13 DR. NUZUM: Yes. The indication is part  
14 of the label and 30 minutes, to me, 30 minutes post  
15 challenge --  
16 PARTICIPANT: I understand that for the  
17 therapeutic. But I'm talking about vaccine. I mean  
18 when you vaccinate somebody, you don't even know the  
19 route of exposure.  
20 DR. NUZUM: No, if you say --  
21 PARTICIPANT: Is it a legal issue?  
22 DR. NUZUM: If you want your label to say  
23 it's being labeled for aerosol exposure, you have to  
24 have that on the label as an indication for that and  
25 you have to have data to show that.  
Page 251

1 PARTICIPANT: And if you don't identify  
2 the route of exposure or can you not identify the  
3 route of exposure?  
4 DR. NUZUM: I think -- well, Mark would  
5 say I think you have to specify.  
6 DR. ABDY: Yes. The bar is very difficult  
7 for an Animal rule approval. With Animal Rule  
8 approvals, we're already not having the ideal  
9 situation. We're already at some stage going to have  
10 a lot of very good scientific data but there's going  
11 to be a leap of faith that this is going to work in a  
12 human being. Therefore, the bar has to be high and I  
13 can -- certainly, the colleagues that I talk to in my  
14 office, if a product is going to be tested, if it's  
15 going to be used for an aerosol bioterrorism attack  
16 with Ebola, we're going to want to see data that says  
17 that if this vaccine is going to be given, we want to  
18 see data that it's going to work, not just -- we  
19 definitely -- that's when you talk to us up front and  
20 you find out what your route of exposure is and where  
21 you need to be going. It's talking to us up front  
22 early.  
23 DR. NUZUM: Tony, did you have --  
24 DR. SANCHEZ: Yes, getting back to the  
25 plaque-forming unit discussion, we really need to  
Page 252

1 define exactly what that means because I have virus  
2 stocks of Ebola that have come out of cynos and  
3 spleens and I can't get those to plaque. But they  
4 have infectious capability and having said that, what  
5 is the -- how does that equate to infectious units,  
6 plaque-forming units? I think that's a very fuzzy  
7 area.  
8 DR. GEISBERT: There's a lot of witchcraft  
9 too in the plaque assays to be honest with you. I  
10 mean, the history of the cells, the passage history,  
11 some passages don't work. Some do. I think that's  
12 part of it. The other thing we know that the plaque  
13 to particle ratio is not one to one. I mean we've  
14 done EM counts before. I can tell you, you know, Mike  
15 Bray's mouse adapted seed, it's 30 particles per  
16 plaque. In our monkeys, Zaire 95 seed it's about 27  
17 particles per plaque.  
18 DR. SANCHEZ: Yes, we did an LD50 with the  
19 guinea pig adapted Ebola Zaire, and the LD50 turned  
20 out to be 0.01 plaque-forming unit.  
21 DR. SULLIVAN: Tony, that's correct. The  
22 only way to measure infectivity is LD50. Plaque-  
23 forming unit will give you an internal comparison  
24 stock to stock so you get an idea of what your stocks  
25 look like, but LD50 is what you need to determine  
Page 253

1 infectivity.

2 PARTICIPANT: One more question regarding

3 the challenge dose, there were a number of challenge

4 studies and the doses however were ranging from 6 PFU

5 to, as far as I remember, 7,000 PFU in the case of

6 Alan's vaccine and all these doses, of course, are

7 uniform lethal infection, and Nancy has shown that

8 very nice correlates of protection for the dose of

9 1,000 PFU, and the question regarding the accidental

10 exposure to viruses remains open. Of course, in the

11 case of stick of hand with the virus, the realistic

12 dose may be 103 or higher infectious doses.

13 However, in the case of transmission

14 through mucosal surfaces taking into consideration the

15 limited stability of the virus in the environment, the

16 challenge dose may be in real outbreak may be perhaps

17 1 PFU and 1 infectious in humans and -- of course a

18 lethal infection.

19 The question is do you think it's worth to

20 look at a correlate of protection against various

21 doses and say 103, 102 or 10 infectious units and maybe

22 the difference is not huge in difference of antibody

23 response which requires protection against these

24 different doses maybe this difference in antibody

25 response would not be huge, would be taking rapid

Page 254

1 development of infection. But do you think this needs

2 to be directly addressed?

3 DR. SULLIVAN: So you're absolutely right

4 that the infectious dose, a lower infectious dose, may

5 elicit a smaller antibody response and likewise fewer

6 antibodies may be necessary to neutralize an

7 infectious dose that's smaller. But what we're

8 looking at is pre-challenge immune correlates. All of

9 these studies will be done in humans pre-challenge.

10 So the immune correlate has to bridge to an immune

11 response that's measured pre-challenge.

12 DR. NUZUM: And I think challenge dose,

13 it's another one of these unanswered issues that,

14 again, will be developed as the product matures and

15 there's conversation with FDA. I mean for purposes of

16 this meeting I think if people have good ideas and

17 rationale for what a challenge dose should be, you're

18 welcome to send it to any of us in the organizing

19 group, anybody on the panel, after the meeting and

20 that will be taken into consideration.

21 But to some extent some of these things

22 are probably going to go back into smaller working

23 groups or with or without sponsors and it will evolve

24 as we get more data and it comes out. But anyone is

25 welcome to provide their input.

Page 255

1 Let's go ahead and move on to Question 2

2 or we'll run out of time and Alan Schmaljohn is going

3 to address this.

4 DR. SCHMALJOHN: Just running through

5 quickly comments that came to me, in terms of the

6 strain, I would begin with the supposition that most

7 of us investigators have already argued about these

8 questions so we can be guided in part by what's been

9 done. Look at the public literature which was what

10 Mark was saying as well, that is to say, in terms of

11 what exact strain USAMRIID for Zaire, we've used all

12 the monkey challenges have all been with a single pass

13 of a single pool. But before we decide that that's

14 the gold standard or that the one at CDC is the gold

15 standard or somebody else's, we have to also say well,

16 we have enough to share with the world of that

17 passage, that seed, whatever. They all kill monkeys.

18 Okay. Point two, standardization will

19 provide results that are more consistent and

20 comparable among laboratories to minimize the

21 possibility of artifacts. That is catastrophic

22 contamination with another agent in there or drifts in

23 virulence that happened upon passage that lead to

24 variability.

25 Three, however, choice of strain in terms

Page 256

1 of relevance, that is in terms of the threat such as

2 it is or the outbreak, the new outbreak, is what our

3 favorite Secretary of Defense calls "a known unknown."

4 The threat from nature or "evildoers" is

5 unpredictable. What we don't know is whether any

6 strain is uniquely potent in terms of evoking broad

7 immunity. That is going back to the construction of

8 the vaccine. What strains should be in the vaccine?

9 An example would be among the Marburg viruses you have

10 these related clades that are different. It could be

11 fortuitous that one of them is the one that induces

12 the broadest immunity to all the Marburg viruses, but

13 we don't know that yet. So we can't pick that one.

14 The other is the transition to the rodent

15 experiments. Without exception thus far in the

16 question mark is if anybody can rebut that and that's

17 fine. This is just what I think I know. The

18 filoviruses in human cases are practically uniformly

19 lethal for nonhuman primates but lethality in rodents,

20 viruses have to be adapted by repeated passage and

21 selection.

22 The special case of rodent adapted

23 viruses, the genotypic associations are not consistent

24 and predictable. There's not a single -- there are

25 many lesions that will cause loss in virulence. We're

Page 257

<p>1 still discovering what causes the increase in 2 adaptation of virulence because it's not the same in 3 every rodent at that adapted virus.</p> <p>4 Where I know of it, the guinea pig adapted 5 viruses are generally not virulent for mice. There is 6 very few data, data that Mike Bray published in three 7 rhesus, I believe, on whether rodent adaptation 8 diminishes virulence for nonhuman primates, and this 9 just hasn't been a priority use of nonhuman primates.</p> <p>10 Another difference is that most of the 11 rodent adapted viruses have been subjected to 12 threefold plaque purification. This isn't necessarily 13 a bad thing, but it's different from the typical 14 nonhuman primate challenge seeds which we have tended 15 and I presume you have also, Tony, stated a fairly low 16 passage, as low a passage as we could, and still the 17 heterogenous swarm of viruses is what we've used for 18 nonhuman primate challenge. Even the one or two 19 passages in Vero cells may shift the population. 20 Maybe that's another reason I favor using a slightly 21 higher challenge dose to make sure that the drift 22 isn't influencing too much of what you observe.</p> <p>23 Let's see. Not all the viruses have been 24 adapted to successfully cause lethal disease in 25 rodents, though the number is piling up. Speak to Page 258</p>	<p>1 tissues and cell culture to 108 per ml and higher. 2 (B) This moderate dose results in less variability in 3 time to death. Maybe the variability is a good thing. 4 But if you go to very low dose, you tend to get a 5 longer spread of time to death.</p> <p>6 (C) By reducing the challenge dose in the 7 low range in the 1 to 10, I believe it's unhelpful 8 both in terms of relevance. But as a practical 9 experimentalist, I like to measure, go back and 10 measure, the same day or freeze the sample and measure 11 another day exactly how much did that diluted sample 12 contain. I know that the original seed contained 2 x 13 108. When I diluted it down, did it still contain 5 14 PFUs? I can't measure 5 PFU -- well, actually you 15 can. It's just harder. So you can get down there and 16 measure it.</p> <p>17 And just also for practical terms in just 18 doing animal experiments over the years, I have 19 avoided where I can a very low dose because it makes 20 it hard to tell between whether the immunity prevented 21 all disease, symptoms and viremia or whether in the 22 vagaries of virology and Poisson distribution, the 23 animal was just missed. That's not going to happen at 24 100 or 1,000 PFU challenge.</p> <p>25 So those are my logics on that. I guess Page 260</p>
<p>1 Kelly Warfield among them. Guinea pigs typically have 2 been easier than mice, and that's just visuals.</p> <p>3 This was just to talk about how different 4 the viruses are and how many viruses we would pick. 5 If you look within a species Ebolas have been pretty 6 closely related. With the Marburgs, they're more 7 heterogenous. Marburgs consist of a single species 8 with more variation.</p> <p>9 I wanted to just get my two cents worth 10 again on dose. One is just again what has been done 11 and what's worked. But those studies mostly used 100 12 to 1,000 PFUs which is at least that many LD50. I 13 appreciate Tony's point that we too went from 14 marvelously consistent plaquing on Vero E6 cells to 15 something changed in the cells and we go from -- we 16 have to hit them at a particular point to get the 17 exact same number. That doesn't mean we can 18 necessarily substitute that with PCR because then 19 we're measuring genomes and we don't know whether 20 we're measuring infectivity. We can measure LD50, but 21 that only applies in monkeys. So it's hard to do a 22 lot of such things.</p> <p>23 The logic. This is relevant for a full 24 miter needle stick and easily achievable in aerosol. 25 That's not a high dose when these things grow in Page 259</p>	<p>1 that's all.</p> <p>2 DR. NUZUM: Alan, if I interpreted that, 3 pretty much your comments are related to feasibility 4 and what makes sense from an animal model point of 5 view. As far as getting back to relevance to humans, 6 lacking what known human challenge doses are, it's 7 hard. I don't think we can really address that at 8 this point.</p> <p>9 DR. SCHMALJOHN: No data. Right?</p> <p>10 PARTICIPANT: Thinking about reference 11 strains and stuff like that, has anyone looked at 12 using reverse genetics to sort of standardize the 13 strain and sort of creating a reference pool of virus 14 to sort of use the stock?</p> <p>15 DR. SCHMALJOHN: That's entirely feasible 16 and doable, but it begets a whole range of other 17 problems as to whether it represents what you want it 18 to represent and how faithful really is even the 19 reverse genetics in a 19 kilobase genome and how many 20 errors did you introduce. So as practical 21 virologists, we've been happy with things at a lower 22 passage. But that is the extreme of consistency. So 23 those are somewhere between science and philosophy.</p> <p>24 DR. GEISBERT: We have put Yoshi Kawaoka's 25 clone derived Zaire 76 virus into four rhesus monkeys Page 261</p>

<p>1 and it killed all four of them, and the disease was 2 similar to wild type.</p> <p>3 PARTICIPANT: One comment with regards to 4 dose and I think you guys are very close, during 5 Nancy's talk, I think ultimately when you come up with 6 a vaccine since we don't know the relevant dose we're 7 probably going to have to do breakthrough and find out 8 exactly how protective a vaccine is against how high 9 a dose and I think ultimately that's the only data 10 that we're going to have.</p> <p>11 DR. SCHMALJOHN: We have not done that and 12 I don't know whether any of the rest -- we have not 13 hit a challenge dose where we break through by 14 whatever miscalculation in the first experiments. We 15 challenged with nearly 10,000 PFUs and they were fully 16 protected, and we have not ramped up. It's just been 17 a matter of prioritizing which experiments to do in 18 what order. Has anybody else tried to override in 19 challenge dose?</p> <p>20 DR. GEISBERT: We made a mistake on one of 21 the VSVs and I think hit about 5,000 and we saw no 22 breakthrough either.</p> <p>23 DR. NUZUM: That's 5,000 PFUs.</p> <p>24 DR. GEISBERT: PFU.</p> <p>25 DR. NUZUM: IM.</p> <p style="text-align: right;">Page 262</p>	<p>1 quicker as far as -- they're all 100 percent lethal 2 and we know that, but it's a quicker time to death and 3 we don't know why.</p> <p>4 But I think that for vaccine purposes I 5 think you would have to address Angola based on what 6 we've seen in the macaques and based on, you know, for 7 years we thought Marburg was maybe around 50 percent, 8 20 to 50 percent, and then all of a sudden with the 9 Durba outbreak and with Angola, I mean, I guess you 10 could argue the numbers. But the published number is 11 90 percent.</p> <p>12 PARTICIPANT: I mean it's sort of 13 paradoxical but that virus is actually probably more 14 similar to the original Marburg strains than anything 15 else which had a 25 percent mortality as I recall. So 16 I don't know why it's more lethal. I guess nobody has 17 really run down all these strains. I mean, we 18 probably have the most extensive collection because 19 generally we get sent initial specimens and we 20 certainly attempt virus isolations from those, but one 21 of our goals is not to try them all on monkeys.</p> <p>22 DR. GEISBERT: We only tried the one you 23 gave us, Tom.</p> <p>24 DR. NUZUM: Let's go on to Question 3, the 25 challenge.</p> <p style="text-align: right;">Page 264</p>
<p>1 DR. GEISBERT: Yes.</p> <p>2 DR. NUZUM: And Fred has a good point. In 3 our anthrax studies, one of the things we'll do at 4 some point is challenge at a very high dose, much 5 higher than our routine studies, just simply to ask 6 the question is it effective and no one said it has to 7 be effective at high doses. You can just do the study 8 and you have the information and right here, we hear 9 that they may be effective at very high doses. So a 10 lot of this becomes academic if that's the case.</p> <p>11 One thing I have heard today mentioned as 12 far as strains a lot are Ebola Zaire and Marburg Ci67, 13 I think maybe those are used the most, perhaps the 14 best characterized, and that's one of the things we'll 15 look at is availability, pedigree, the current 16 characterization before deciding on which strains to 17 go forward with and standardize on and those are all 18 things we would look at.</p> <p>19 DR. GEISBERT: One of the things I think 20 we need to think about with Marburg is Angola and 21 we've started to pretty much move over to Angola based 22 on the disease course and the data that CDC and Heinz 23 Feldmann got in humans and it clearly looks like the 24 Angola strain was more pathogenic in humans and we've 25 clearly shown that in our macaque models. It is</p> <p style="text-align: right;">Page 263</p>	<p>1 PARTICIPANT: Can I say something?</p> <p>2 DR. NUZUM: Okay.</p> <p>3 PARTICIPANT: I have a comment on the 4 dose. At least for the mouse model, you have to be a 5 little bit careful in going up with the dose. When we 6 give that virus like IV higher doses or IP, mice start 7 to survive the challenge. They get all sick.</p> <p>8 So if you do a vaccine experiment and look 9 for complete protection, you're not going to see that. 10 But if you look at that paper by Hideki Ebihara in 11 PLoS Pathogens, some of the recombinants that he made 12 in order to try to find out which mutation is 13 important for that adaptation, some of these have a 14 very, very narrow range where they are very viral and 15 then kill the mice. But if you up to one or the other 16 side, they don't kill at all anymore. This is mouse. 17 We see a little bit similar issue with guinea pig, but 18 not to that extent. I have no idea about nonhuman 19 primates because whatever we have done is using 1,000 20 as Tom and many others do. But just to keep that in 21 mind that that can happen.</p> <p>22 DR. SCHMALJOHN: I just want to say that's 23 a good point and I was wondering also out of Doug 24 what's a practical upper limit of what dose you could 25 use in aerosol if Doug Reed is still here.</p> <p style="text-align: right;">Page 265</p>

1 DR. GEISBERT: I don't know. We gave 105  
2 oral and conjunctival in that study in rhesus and it  
3 killed them.  
4 DR. SCHMALJOHN: Yes. It's harder to get  
5 that kind of inhaled dose in an aerosol and it would  
6 rapidly consume the seeds we have because you're going  
7 concentrated stuff 10 mls in order to get enough  
8 exposure in the air. So there's an upper limit to  
9 what we would --  
10 DR. GEISBERT: Louise is here. Maybe --  
11 DR. PITT: Yes. I was just going to  
12 comment because it would use a huge amount of virus to  
13 do the challenges, but technically we can go logs  
14 higher than 103.  
15 DR. SCHMALJOHN: How many logs? It's an  
16 eight log prep let's say.  
17 DR. PITT: 105 at least.  
18 DR. NUZUM: Okay. So let's go to Question  
19 three. Tom. This is on challenge route and this has  
20 been kicked around a lot today already. So maybe it  
21 won't take long.  
22 DR. KSIAZEK: Doug, could you read the  
23 exact question I guess? I didn't transcribe it.  
24 DR. NUZUM: What challenge routes should  
25 be used if the purpose is to develop a vaccine to

Page 266

1 protect against a bioterrorist attack?  
2 DR. KSIAZEK: We tend to deal more with  
3 natural disease that occurs in outbreaks, but clearly  
4 for biodefense depending on whose scenario you're  
5 following I think aerosol attack is what seems to be  
6 more commonly coming up. I guess if you're from the  
7 Food and Drug Administration dealing with food borne  
8 diseases I've also heard that scenario. But we  
9 haven't really thought a lot about it at CDC I guess.  
10 So the attack would just be assumed to be  
11 an aerosol challenge for individuals. Although as  
12 somebody has pointed out, I think that infectious  
13 control practices in the U.S. are pretty effective but  
14 there would undoubtedly be some secondary cases in  
15 individuals that were exposed in the initial attack.  
16 So I mean I guess for a biodefense vaccine the route  
17 that you would want to protect against would be  
18 aerosol challenge.  
19 DR. NUZUM: And I think a practical matter  
20 here is and we heard from DOD this morning ultimately  
21 things like this are going to come down to who the  
22 sponsor is and the funding agency. They're going to  
23 more or less dictate what they want and then the  
24 product development path will be geared towards that.  
25 So clearly, I mean, my summary from what

Page 267

1 I've heard today on this is epidemiologically aerosol  
2 transmission is not a factor. But for biodefense, we  
3 will need to have aerosol models. I think -- I don't  
4 know. Does anyone else on the panel have any other  
5 comments on that?  
6 DR. GEISBERT: I think we have to think  
7 differently after 9/11 a little bit. I mean, a lot of  
8 the aerosol and I understand the whole issue with  
9 biodefense -- USAMRIID so long. A lot of that was  
10 based on the former Soviet program when we all know  
11 that there was a program to develop bomblets and  
12 dispense an aerosol on a civilian population. I think  
13 after 9/11 and you think about folks flying airplanes  
14 into buildings, we start thinking about other routes  
15 and we look at what happened with the anthrax letters  
16 and five letters shut down the postal service and it's  
17 fear. It's bioterrorism too and it's not just  
18 necessarily just an aerosol, but what if somebody --  
19 This is a pretty stable virus actually, and what if  
20 somebody threw it on a salad bar or something like  
21 that. So you're talking oral possibly and we know  
22 it's a highly infectious by oral, very lethal. So I  
23 don't know that you can just solely say aerosol as far  
24 as bioterrorism. My two cents.  
25 DR. KSIAZEK: Again, our sort of take on

Page 268

1 natural routes of infection it's more likely to be  
2 some sort of mucosal exposure, but probably not  
3 aerosol via either small droplet from direct proximity  
4 to people shedding fairly large quantities of virus or  
5 contamination of your hands and then auto-inoculation.  
6 These seem like the epidemiology at least fits the  
7 experience that we've had in the field in terms of  
8 taking preventive measures which seem to be effective.  
9 And clearly, I would acknowledge that  
10 aerosol infection is a fairly efficient means if you  
11 have the means and the wherewithal to create the  
12 aerosol in a laboratory environment. We've seen a lot  
13 of data today that suggests how efficient that is and  
14 that the dose required is not particularly high.  
15 I think one thing that I get a little bit  
16 animated about when you go to a lot of these  
17 biodefense or bioterrorism sessions is I don't think  
18 a lot of people realize how difficult it is to make a  
19 prep and get it up that's going to go up efficiently.  
20 And I think there's a real art to that that certain  
21 people certainly can do, and I think state sponsored  
22 programs can manage that. I kind of question myself  
23 whether a bunch of guys with rags on their head are  
24 going to manage it or not.  
25 I mean the aerosol challenge I think is a

Page 269

<p>1 little bit more perhaps challenging. I mean there are 2 examples with other viruses, Venezuelan Equine 3 Encephalitis and Rift Valley Fever where vaccines that 4 have been demonstrated particularly in rodent models 5 to be effective against parenteral challenge had not 6 been effective when aerosol challenge was used as the 7 means of trying to demonstrate efficacy against that 8 route.</p> <p>9 So the bar is probably a little bit higher 10 in terms of being able to do that. But I guess I 11 would also raise the issue of whether you're really 12 trying to attempt to develop a mucosal immunity that's 13 effective against, I guess, the aerosol route and at 14 that same time you probably accomplish a decent 15 protection against the other mucosal routes as well. 16 I mean, I don't know so much about this.</p> <p>17 DR. GEISBERT: Make a point on that. 18 Heinz is here as well. We've done two different 19 studies looking at this for Ebola and RSV vaccine is 20 a single injection completely protects monkeys against 21 an aerosol challenge with Ebola. Also it completely 22 protects monkeys against an aerosol challenge with 23 Marburg and in Winnipeg, Heinz actually immunized 24 monkeys orally and inter-nasally and completely 25 protected against IM.</p> <p style="text-align: right;">Page 270</p>	<p>1 principally goes systemic with a lot of liver damage 2 in the case of Marburg. It's still --</p> <p>3 So I don't think it's a higher bar either 4 way. Either one would suit, and I would ask you, Tom, 5 does it still hold that when patients present with 6 disease and the route of exposure turns out to be 7 needle stick, this is humans, that the mortality rate, 8 let's see, in 86 was 100 percent. Is it still 9 hovering at 100 percent with needle stick?</p> <p>10 DR. KSIAZEK: I think the only data was 11 from that 76.</p> <p>12 DR. SCHMALJOHN: Seventy-six, I'm sorry.</p> <p>13 DR. KSIAZEK: There was a lot of needle 14 stick in that outbreak. I think HIV came along and 15 kind of changed the equation even in Africa in terms 16 of needle reuse to some extent or the policy would be 17 use your own needle again.</p> <p>18 PARTICIPANT: I just have a couple of 19 comments. I was fortunate enough to be the 20 pathologist assigned to Doug Reed's study. So I 21 posted probably about 20 of these nonhuman primates 22 that have been infected by aerosol spray with Marburg 23 and he in his presentation earlier was showing that 24 the doses ranged anywhere between 10 to 100 to 1,000 25 PFUs and grossly what I did see in the lungs is</p> <p style="text-align: right;">Page 272</p>
<p>1 DR. KSIAZEK: Right.</p> <p>2 DR. GEISBERT: So it doesn't seem to be 3 quite as important. In fact, our data was slightly 4 different than Doug's and again, there's just a lot of 5 different variables. But in our model that Lisa and 6 I have used it actually looked like it was easier to 7 protect against aerosol than intramuscular everything 8 being equal. But because you have -- our monkeys died 9 a little bit later when they were challenged by 10 aerosol.</p> <p>11 DR. KSIAZEK: I think the experience with 12 VEE and Rift was the use of chilled vaccines which 13 were pretty effective I guess in developing a humoral 14 response. But there are some questions about whether 15 there was really a good cellular response in those 16 animals.</p> <p>17 DR. SCHMALJOHN: I guess I would want to -- 18 - the viruses that are more -- the viruses and 19 bacteria that are more lethal and difficult to protect 20 against by aerosol are those that either cause kind of 21 a unique lung pathology or the ones like these that 22 cause an encephalitis and get straight to the brain 23 tract. Now Marburg was noted to cause encephalitis 24 late in some patients and I suppose this is typical, 25 but the virus -- it's principally a viremia and</p> <p style="text-align: right;">Page 271</p>	<p>1 depending, and this is not dose dependent, some at 10 2 as well as some at 1,000 PFUs did have medial stinal 3 edema, pulmonary congestion as well as large tracheal- 4 bronchial lymph nodes as well as the classic liver 5 that Tom had showed that you typically see with 6 Marburg.</p> <p>7 One thing that I did notice and I'm glad 8 you brought this up with the Marburg Angola, we did 9 see or I have seen histologically as well as both with 10 PTH and with ourfibrin immunohistochemical stain 11 increased fibrin deposition in a number of tissues 12 which is a little bit different than what we have 13 seen, I guess, with the IM or IP exposure with 14 Marburg. I just wanted to put that out.</p> <p>15 DR. KSIAZEK: Yes. I think your point is 16 that endstage disease is not dissimilar or perhaps 17 with a few differences. Has anybody actually done any 18 serial sacks with aerosol infection to see how it 19 progresses? I know you've done the same with 20 parenteral inoculation.</p> <p>21 I mean you get moderate clinical 22 parameters and it doesn't appear to be all that 23 dissimilar.</p> <p>24 PARTICIPANT: I have one other question. 25 Sorry. Is it on? Dr. Sanchez had put up earlier that</p> <p style="text-align: right;">Page 273</p>

1 with the Ebola infections in humans you do see an  
 2 increase in nitric oxide. You had increase in  
 3 nitrate. Is that --  
 4 DR. GEISBERT: We had talked about that  
 5 earlier. Yes, we see the same thing in the macaques.  
 6 PARTICIPANT: So on your slide it had an  
 7 increase in nitrates, but he had nitric oxide. That's  
 8 all I was just asking. I don't know if it was one and  
 9 the same.  
 10 DR. GEISBERT: Nitrates, what we're  
 11 measuring in vivo.  
 12 DR. KSIAZEK: Then there are besides I  
 13 think what we've heard talking about again from a  
 14 biodefense route of exposure certainly other exposures  
 15 that are more allied with natural routes of infection.  
 16 One of the sort of interesting things is again this  
 17 business with the skin and certainly conjunctival and  
 18 oral routes have been done in, I think, rodents as well  
 19 as in primates and they appear to be quite efficient.  
 20 So these are all routes that we have to be concerned  
 21 with. It just depends on what you're targeting these  
 22 vaccines for.  
 23 One of the other questions, I mean, that's  
 24 come up a number of times in sort of reviews we've  
 25 done I guess for intelligence sort of guys is does

Page 274

1 infection occur through intact skin and I don't really  
 2 have an answer. I don't know if anybody has done any  
 3 particular studies along that route. We don't believe  
 4 it probably is. We don't make an effort to garb  
 5 ourselves in such a way that some casual splash onto  
 6 your face or the back of your neck or areas like that  
 7 would necessarily be guarded against. When we go into  
 8 the field, we certainly try and protect our  
 9 respiratory tree when we're doing dissections and that  
 10 sort of thing.  
 11 I think one of the other issues is hand  
 12 washing and the degree that people in sort of rough  
 13 settings have microabrasions on particularly their  
 14 hands. It probably all contributes to a more  
 15 parenteral exposure perhaps in circumstances where  
 16 aerosol might be a role. But I think these are  
 17 probably more common in what does happen.  
 18 I think I already raised this issue. I  
 19 wonder if mucosal and aerosol exposure and sort of  
 20 being more or less amenable to the same sort of  
 21 vaccine stimulation to begin with.  
 22 DR. NUZUM: I think the route again is a  
 23 function of the product development path and probably  
 24 this whole vaccine development effort are so complex  
 25 anyway you need to identify your goal, determine the

Page 275

1 indication you need, design studies to get there and  
 2 get FDA buy-in. Then if there are other routes that  
 3 you need to protect against or whatever that you may  
 4 do supplemental studies or bridging studies to expand  
 5 the label indication. Mainly, we just have to  
 6 determine our goals and focus the resources so we can  
 7 get to where we want to get to.  
 8 So speaking of that, Question 4 is on  
 9 correlates. Nancy, would you like to address that?  
 10 DR. SULLIVAN: You know, we've reached a  
 11 late hour and in the interest of time, I don't think  
 12 we need to see any more slides. What I would like to  
 13 do is just raise a couple of summary points that I  
 14 think are important and probably haven't been resolved  
 15 between the scientific community and the FDA.  
 16 One of those is, you know, development of  
 17 an immune correlate is empirical, and it depends on  
 18 your system, your vaccine or your therapeutic and what  
 19 you're testing. So I think basically you just have to  
 20 provide data. We have to generate data that shows  
 21 what the correlate is.  
 22 What I'd like to caution against is  
 23 getting preoccupied with functional assays. When one  
 24 speaks of a neutralizing antibody assay being  
 25 functional, that presumes that this in vitro assay is

Page 276

1 representing the in vivo situation and I think most of  
 2 us who have worked with viruses of many types and  
 3 neutralization assays realize that that's not the case  
 4 at all. Indeed you have to titrate the virus  
 5 precisely to even see neutralization in vivo, and in  
 6 addition, there are multiple ways that antibodies can  
 7 function in vivo.  
 8 It's not just the traditional  
 9 neutralization where you preincubate virus with  
 10 antibody and see if it enters cells. There's ADCC.  
 11 In vivo you have cytokine involvement and T cell help.  
 12 So I really hope that we can move away from thinking  
 13 that in vitro assays are somehow representing a  
 14 functional endpoint.  
 15 DR. NUZUM: Any other comments from the  
 16 panel?  
 17 DR. SCHMALJOHN: I'll hold people a second  
 18 longer. I agree. She speaks my mind. It's important  
 19 to continue the research on what the mechanisms are  
 20 because they'll help us improve the vaccine. But in  
 21 terms -- an example, a smallpox where for 200 years  
 22 the best correlate of immunity was the vaccination  
 23 scar and so we need something that correlates well.  
 24 But we also need to understand what's going on.  
 25 DR. SULLIVAN: Yes.

Page 277

1 PARTICIPANT: I will make a couple of  
2 comments because we're doing this for anthrax and  
3 again we're struggling with smallpox and a variety of  
4 other pathogens. I think the correlative data I've  
5 seen with the ELISA today are really encouraging and  
6 I would definitely say to pursue that. I think the  
7 level of comfort and again, Mark talks about how high  
8 the bar has to be for the Animal Rule, the level of  
9 comfort is always going to be the functional antibody  
10 or any kind of mechanistic assay that you can come up  
11 with. I'm seeing some really nice T cell data, some  
12 chromium release assays that look very, very  
13 promising.

14 And so one of the things to keep in mind  
15 is if you can come up with some mechanistic correlate  
16 or perhaps even a surrogate even if it's impractical  
17 you can use that to set the basis of your efficacy  
18 data and then actually do your bridging possibly with  
19 a different assay, doing subsets of the more difficult  
20 assays and then doing larger studies with some of the  
21 more simple assays as in the ELISA.

22 DR. SULLIVAN: Yes. So I just want to  
23 follow up on what Allen said and indeed understanding  
24 mechanism can be very helpful in defining immune  
25 correlates. But I still -- if you refer to a very

Page 278

1 nice chromium release assay, in my view that assay has  
2 almost nothing to do with what's going on in vivo. So  
3 you're looking at an in vitro lytic function that  
4 probably almost never occurs in vivo. I think to  
5 ascribe the word functionality to that assay is just  
6 a misnomer.

7 DR. NUZUM: Any other comments? I would  
8 comment on this, but I think I'd better not because  
9 I'm not sure I'd say the right thing and, again, this  
10 is going to come down to the data and conversation  
11 between the sponsor and FDA. I mean that's where the  
12 ultimate decision is made. But as has been said  
13 today, you make a case for the goal you're trying to  
14 achieve and the justification and it's really between  
15 the sponsor and the FDA at the end of the day.

16 Are there any other comments on this  
17 issue? Okay.

18 Then let me just summarize very quickly.  
19 I think one thing that I've heard today, if I can find  
20 it. The main point I wanted to make I think is when  
21 talking about -- when I've talked about the Animal  
22 Rule, I've talked about the importance of getting  
23 human data on the disease to help design the animal  
24 studies. What I've heard today though we have a lot  
25 more data in animals than in humans and to some extent

Page 279

1 maybe we need to be really looking at the data in the  
2 animals to help design field studies or whatever so  
3 that when there is, when we do have cases, the few  
4 cases we do have of human disease, we can really look  
5 at that disease in humans and try and find, kind of go  
6 the other direction, try and find endpoints and  
7 correlates in humans that we're seeing in animals. So  
8 it's a little different approach in that regard.

9 And one thing I thought I heard Tony say,  
10 did you not say there was delayed antibody response in  
11 humans?

12 DR. SANCHEZ: I did.

13 DR. NUZUM: Right. So if that's happening  
14 in humans and we're using an ELISA antibody assay as  
15 a critical correlate, I think that's a good example of  
16 the kind of things we have to be aware of. I think  
17 the animal data we have is very good, and we just need  
18 to make the most of that. So maybe we need some kind  
19 of approach or evaluation of all this data so when  
20 there is a natural outbreak in human disease that the  
21 medical personnel and the field investigators really  
22 start to look for the right endpoints and try to see  
23 if the endpoints we're looking at in animals really  
24 are relevant.

25 I think that was my main point. I think

Page 280

1 we had really good discussion on the species. I think  
2 the strains given what's known about strains,  
3 challenge strains and so forth, the individual people  
4 working with these viruses can go forward on that and,  
5 of course, we welcome any input from anyone on, like  
6 I say, strains, dose or anything else.

7 Are there any other questions or comments  
8 from the audience? Okay. Then I want to thank  
9 everyone for attending. It's been a long day. I  
10 think it's been a very good day. I especially want to  
11 thank the speakers and the panel members, and we'll  
12 see most of you tomorrow.

13 (Whereupon, at 5:12 p.m., the above-  
14 entitled matter was concluded.)

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Page 281

<b>A</b>				
<b>abbreviations</b> 11:5 11:17	<b>accidents</b> 228:12	<b>acute</b> 57:1 58:23 59:24 88:24 89:5 206:11	<b>adenoviral</b> 205:12 207:6,7	144:5,13 145:6 147:6,14 148:4,10
<b>abdomen</b> 98:10	<b>account</b> 122:2 147:24	<b>Ad</b> 185:10 199:16	<b>adenovirus</b> 159:11 184:6,24,25 185:4 185:5 188:22 189:10,11,21,25 190:13 198:19,20 199:21,23 201:6 204:24 205:15	167:1,2 221:18,21 225:15,17,18,18 230:21,24 231:24 232:2 251:23 252:15 259:24 265:25 266:5 267:5,11,18 268:1 268:3,8,12,18,23 269:3,10,12,25 270:6,13,21,22 271:7,10,20 272:22 273:18 275:16,19
<b>Abdy</b> 1:13 210:14 210:15 228:10,25 230:14 231:4,19 232:10,13,18 234:1,16 244:10 248:23 252:6	<b>accumulate</b> 35:9	<b>adapt</b> 73:17 88:25 89:7,11 114:7,12	<b>adequate</b> 7:9 39:24 40:19 213:2	277:5,11,18 268:1 268:3,8,12,18,23 269:3,10,12,25 270:6,13,21,22 271:7,10,20 272:22 273:18 275:16,19
<b>ability</b> 33:7 43:14 54:20 86:25 87:5 115:6 121:18 134:5 168:13 171:8 173:11 189:20 203:1	<b>accumulated</b> 35:22 37:8 133:15	<b>adaptation</b> 14:22 14:23 56:20 69:2 70:6,20 73:20 258:2,7 265:13	<b>adequately</b> 7:14	<b>aerosolized</b> 2:13 125:14,16
<b>able</b> 29:18,21 36:21 43:4 57:21 60:20 60:23 61:20 64:12 67:24 70:10,13 71:19 72:12 73:10 79:6,20 80:25 87:12 89:20 112:17 116:3 127:21 128:8 131:11 144:20 185:21 192:23 205:25 215:18 218:16,16 223:6 224:23 270:10	<b>accumulating</b> 65:2	<b>adaptative</b> 168:20	<b>adhere</b> 107:23	<b>aerosols</b> 50:18 52:7 141:22 164:18
<b>above-entitled</b> 67:12	<b>accumulation</b> 126:6	<b>adapted</b> 73:8 74:9 74:21 77:6 86:11 86:22 87:9 93:4 127:9 253:15,19 257:20,22 258:3,4 258:11,24	<b>Adjourn</b> 2:25	<b>Affairs</b> 3:5,25
<b>absolutely</b> 170:1 183:10 195:25 197:22 230:16 231:17,19 234:2 255:3	<b>achievable</b> 28:15 259:24	<b>adapting</b> 72:24 89:2	<b>adjuvant</b> 89:24 158:10 160:4 201:24 202:11	<b>affect</b> 147:9
<b>absorbency</b> 207:21	<b>achieve</b> 159:3 192:23 279:14	<b>adaptive</b> 80:18,22 81:6 168:9 187:19 187:21	<b>administer</b> 115:16	<b>affinity</b> 162:4 171:13
<b>abundant</b> 29:14	<b>achieved</b> 16:16 65:11 182:10	<b>Adby</b> 49:7,7	<b>administered</b> 113:9 171:1,3	<b>afforded</b> 40:4 43:2 44:20
<b>academic</b> 263:10	<b>achieving</b> 190:8	<b>ADCC</b> 277:10	<b>administration</b> 1:3 223:9 267:7	<b>Africa</b> 35:1 38:13 42:13 44:2 45:2 65:8 92:20 145:8 228:2 232:3 247:15 272:15
<b>accelerated</b> 55:4 139:15 189:19 224:10	<b>acid</b> 151:9	<b>add</b> 8:24 45:23 156:7 160:9 231:22 239:20	<b>adoptive</b> 182:18	<b>African</b> 45:18 92:24 95:12 98:2 98:5 100:8 118:7 128:17 131:6 132:1,9,18 133:8 133:12 135:21 136:8,10 138:1,11 138:23 139:7,13 144:1 236:22 248:25 249:3,7
<b>accept</b> 224:23	<b>acids</b> 22:11	<b>added</b> 36:12 64:11	<b>adult</b> 69:11 73:6 225:3	<b>afternoon</b> 183:18 245:21
<b>accepted</b> 74:2 243:8	<b>acknowledge</b> 46:3 141:10 167:15,19 169:3 176:22 201:2 269:9	<b>addicted</b> 86:16	<b>advanced</b> 8:2,8 22:1 31:2 32:12 34:14 167:18 194:8 228:18	<b>Agarose</b> 58:5
<b>accepting</b> 9:2	<b>acknowledging</b> 168:14 169:18	<b>adding</b> 6:4 185:20	<b>advantage</b> 33:8 70:11	<b>age</b> 69:6
<b>access</b> 30:7 94:17	<b>acknowledgments</b> 227:9	<b>addition</b> 7:9 127:9 131:11 134:4 219:12 247:11 277:6	<b>adversaries</b> 32:9	<b>agency</b> 1:2 127:20 267:22
<b>accidental</b> 91:15 247:14 254:9	<b>acquire</b> 32:23 33:18	<b>address</b> 5:14 6:20 10:14 19:15,17 50:6 101:6 211:14 212:1,4 222:19 228:22 231:5,11 234:24 235:10,11 246:8 251:7 256:3 261:7 264:5 276:9	<b>advice</b> 148:15	<b>Agency's</b> 227:7
	<b>acquisition</b> 8:4	<b>addressed</b> 234:17 234:22 255:2	<b>Ad35</b> 200:4,10	<b>agenda</b> 2:2 3:18,19 3:20
	<b>act</b> 14:15 161:10 222:3	<b>addressing</b> 18:17	<b>Ad48</b> 200:18	
	<b>activate</b> 28:4	<b>adeno</b> 156:11 165:7 166:4,5 202:1,21 204:8,19	<b>Ad5</b> 199:11,23 200:3,3,9,13,17 200:20 207:2	
	<b>activated</b> 60:15 113:5,7		<b>aerosol</b> 50:10,12 51:16,18,21,24,25 52:6 116:19,21,22 116:23 117:1,6,20 125:25 126:1,4,8 126:14,17,25 127:22 128:7 130:3 136:12 138:9 140:1,4 142:1 143:4,8,25	

<b>agent</b> 221:12,21 223:9,10 229:6 256:22	97:20 182:5 <b>Allen</b> 278:23 <b>ALLERGY</b> 1:1	194:14 <b>analyzed</b> 172:10 177:17	217:4,13,14,19,24 218:15,21,24,25 219:5,11,14	223:21 224:21 271:16 279:25 280:2,7,23
<b>agents</b> 32:6 33:21 166:24 212:16 215:4 220:1 232:22	<b>allied</b> 274:15 <b>allow</b> 23:25 89:10 <b>allowed</b> 73:15 <b>allows</b> 82:12 191:2 193:14 212:24 215:14	<b>analyzer</b> 137:14 <b>analyzing</b> 136:4 <b>anchor</b> 22:22 149:19	221:13,24 222:3 222:14,22 223:5 224:9,20 226:14 227:6,14 229:5 230:1 231:7 232:23 233:9	<b>animated</b> 269:16 <b>anniversary</b> 4:15 5:15 <b>announcement</b> 235:19
<b>aggravations</b> 201:19	<b>allude</b> 26:24 <b>alluded</b> 15:3 23:1 185:17	<b>anchoring</b> 22:18 <b>Andromeda</b> 34:17 <b>Angola</b> 12:5 34:25 37:15 38:22 100:14,20,20 104:2,3 106:10,11 123:11 129:6 141:7 239:8,16 263:20,21,24 264:5,9 273:8	234:2,15 236:8,12 237:6 242:16,17 242:23,23 245:1 246:4 250:5 252:7 252:7 260:18,23 261:4 278:8 279:21,23 280:17	<b>annual</b> 6:5 <b>anorexia</b> 130:10 <b>answer</b> 65:17 90:10 147:5 203:3 204:3 204:5,11 229:1 242:3 244:3 247:7 248:4 275:2
<b>ago</b> 15:4,18 68:6 76:9 88:13 92:14 95:15 96:15 104:11 107:7 111:17 117:23 129:12 156:5 157:14 167:17 175:1 226:8	<b>Alphavax</b> 169:3 <b>alpha/beta</b> 71:24 85:13 <b>ALT</b> 62:7 78:15 106:23 <b>alter</b> 88:17 <b>alternative</b> 199:22 200:1,22 224:4	<b>animal</b> 1:4 2:5,11 2:22 3:13 6:23 7:2,11,17,18,20 8:9,11,12,16,18 8:18,20 9:9 26:19 28:10 40:8 45:6 51:18,20 55:2 56:20 57:20 63:6 63:10 64:6,19,23 65:14 67:17 68:11 68:18 69:13 70:22 74:4 76:22 82:23 84:7 87:22 91:17 101:22 102:1,4,11 113:11 117:6 119:10,13,17,18 121:2 127:17,19 128:9,11 131:20 131:25 132:5,8,10 132:17,21 133:6 133:22 135:6,18 142:2 143:14 145:12 148:9 153:20 165:5 176:6,17,17 179:4 179:9,17 180:2 186:5,6,8,15,20 186:23,24,25 205:22 210:13 211:6 213:1,3,3,7 213:25 214:4,5,6 214:15,22 215:4,7 215:8 216:2,14,24	<b>animals</b> 5:25 27:6 27:11,18,20 51:16 65:23 70:1,3,5,19 71:10 72:16 74:22 75:8 85:24 87:14 89:6 91:4,5 97:7 97:20 100:21 101:2,15,15 104:22 107:11,20 111:24 112:2,4,14 113:7 114:16,17 114:19,23 115:1 115:17 116:2,7,25 119:5 126:9,10 129:2,3 131:8 132:3,12,16,20 133:1,10,11,23 134:3,13,21,25 135:3,17,17,19 136:8 138:20 141:1,23 145:15 145:18,20,22,25 153:15,18 169:13 170:6 171:1 176:9 176:20 180:25 182:19 183:1 190:14,14,20,20 193:3 195:13,15 196:13,13 197:9 198:1 200:12 209:2,22 214:21 215:2,14,20 216:19 217:5 218:23 219:3	<b>answers</b> 243:22 <b>antagonist</b> 14:4,19 90:2,5 <b>antagonistic</b> 56:17 <b>antagonists</b> 30:8 85:9 91:21 <b>anthrax</b> 218:7 231:18 263:3 268:15 278:2 <b>anti</b> 16:8 25:7 <b>antibodies</b> 2:18 16:16,18,20,24 57:3 59:25 83:20 84:22,23,23 154:6 161:9 167:11 168:11 170:8,10 170:20 171:7 176:11 178:3,9,10 178:14 180:23 184:11 187:24 188:7,9,13 191:8 194:23 200:4,5,19 205:4 243:7 246:14 255:6 277:6 <b>antibody</b> 26:14 47:18,25 48:1,13 59:12,15,17,22 60:7 84:19 162:5 168:5 171:1,9,11 171:13 174:13,14 175:20 176:4 177:6,10,13,21 178:11,25 179:6,8 179:12,24 183:2,4
<b>agreements</b> 210:4 <b>ahead</b> 11:20 151:11 182:14 196:15 230:10 246:6 256:1	<b>Alvez</b> 140:8 142:13 <b>amazing</b> 115:10 <b>amenable</b> 189:16 275:20 <b>amended</b> 222:5,8 <b>amendment</b> 232:25 233:6 <b>American</b> 104:18 <b>amino</b> 22:11 151:9 <b>amount</b> 7:10 44:17 51:1 55:15 56:7 58:16 61:6 88:21 122:8,9,10 158:19 165:4 178:25 191:5 224:18 229:25 244:18 266:12 <b>amounts</b> 50:24 66:13 105:16 <b>Amylase</b> 62:17 <b>analogues</b> 17:5 <b>analysis</b> 20:16 23:9 180:13 194:3,11 197:18 <b>analyze</b> 61:20	<b>air</b> 144:21,22 145:9 266:8 <b>airborne</b> 144:25,25 <b>airplanes</b> 268:13 <b>Alan</b> 1:22 149:6,11 163:16,20 165:8 166:3 167:15 185:18 200:23 202:25 203:19 230:17 231:23 249:14 256:2 261:2 <b>Alan's</b> 254:6 <b>Albert</b> 86:11 <b>albumin</b> 62:19 107:25 108:7 <b>Alec</b> 161:1 <b>ALEV</b> 93:1 <b>algorithm-predic...</b> 154:16 <b>allegations</b> 126:15 <b>allele</b> 60:24 <b>alleles</b> 60:22 97:18		

188:5,11 189:12 189:13 194:16,18 197:18 198:15 200:6 201:23,24 203:12,24 205:2 254:22,24 255:5 276:24 277:10 278:9 280:10,14 <b>anticipate</b> 206:24 <b>anticipated</b> 4:24 5:20 <b>anticipating</b> 5:21 <b>anticoagulants</b> 124:25 <b>antigen</b> 49:25 57:14 58:12,14,16 59:3,5,10,14,18 61:5,7,9 77:6 80:10 105:15 117:16,16 119:4 122:16,20,24 140:18,21 151:4 165:5 169:14 181:17 185:2 187:18 191:7,9 192:21 202:8,10 207:9,14,14,15 <b>antigenic</b> 165:22 <b>antigens</b> 59:16 107:15,18 181:9 181:24 <b>antigen-specific</b> 191:4 <b>antiprotease</b> 22:9 <b>antisense</b> 17:6 <b>antisera</b> 60:6 <b>antiviral</b> 17:17 20:24 28:3 <b>antivirals</b> 17:16,23 17:23 28:9 30:10 30:11 227:17 <b>antivirus</b> 16:20 <b>anti-coagulant</b> 112:16 <b>anti-interferon</b> 83:19 <b>anti-viral</b> 124:11 124:25 <b>anti-virals</b> 30:5 <b>anybody</b> 153:3	179:1 205:14 239:19 250:25 255:19 257:16 262:18 273:17 275:2 <b>anymore</b> 265:16 <b>anyway</b> 29:1 78:1 101:7 111:25 114:13 275:25 <b>apart</b> 183:10 <b>apologize</b> 53:2,7 56:21 175:3 <b>apoptosis</b> 61:9,12 61:14 76:3,6,11 77:20 78:11 80:16 82:20 83:7 106:5 <b>apoptotic</b> 26:2 <b>apparent</b> 154:4 <b>apparently</b> 72:17 74:19 80:17 <b>appear</b> 59:25 75:20 233:21 242:8 249:16 273:22 274:19 <b>appearance</b> 34:17 59:2 61:10 <b>appeared</b> 165:6 <b>appearing</b> 75:9 <b>appears</b> 11:7 41:2 53:10 77:13 78:7 164:11 202:18 <b>applause</b> 28:23 46:8 63:25 86:19 118:18 142:14 163:15 176:25 201:15 228:4 <b>applicable</b> 9:3 <b>application</b> 194:12 <b>applications</b> 8:13 <b>applied</b> 8:6 <b>applies</b> 249:23 259:21 <b>apply</b> 224:11 232:16 244:9 <b>appointment</b> 149:19 <b>appreciate</b> 9:24 182:14 210:21 211:1 228:7 259:13	<b>approach</b> 21:25 28:14 143:20 156:2 157:10,23 171:16 172:3 182:25 184:23 189:3 190:8 192:20 202:9 220:16 228:23 233:8 235:8 280:8 280:19 <b>approached</b> 7:1 127:23 <b>approaches</b> 17:3 124:1,9 156:3,16 156:17 233:12 <b>approaching</b> 6:8 <b>appropriate</b> 7:17 7:17 128:3 192:4 212:4 217:23 221:11 230:16 246:5 248:1 249:3 <b>approval</b> 7:11 214:10,13 224:10 224:17 232:23 244:9,17,21 252:7 <b>approvals</b> 252:8 <b>approve</b> 143:12 212:24 <b>approved</b> 231:7,8 231:10 <b>approving</b> 186:22 <b>approximate</b> 156:22 <b>April</b> 6:13 <b>APT</b> 137:13 <b>area</b> 15:16,23 30:1 36:11 37:17 40:14 43:16 44:23,25 79:11 88:19 90:16 95:1 98:10 107:15 144:12 159:24 195:22 199:9 253:7 <b>areas</b> 40:3 52:5 54:19 59:3 136:4 155:23 166:5 275:6 <b>arena</b> 157:3 224:15 <b>argue</b> 237:5 241:3 264:10	<b>argued</b> 256:7 <b>arguing</b> 118:25 <b>argument</b> 118:8 226:5 <b>arms</b> 183:5 202:23 <b>Army</b> 176:21 <b>arranged</b> 156:22 <b>art</b> 269:20 <b>article</b> 84:13 157:5 174:25 <b>artifacts</b> 256:21 <b>ascribe</b> 279:5 <b>aside</b> 15:21 <b>asked</b> 118:1 137:21 138:3 231:13 232:25 <b>asking</b> 93:2 204:5,8 208:10 243:17 274:8 <b>aspect</b> 57:4 204:16 <b>aspects</b> 30:15 107:6 <b>assay</b> 134:5 171:23 171:25 172:1,25 173:19,23 175:7 177:21 190:23,25 191:1,19 192:11 192:24 196:3 207:24 208:1 209:17 222:24,25 223:23 276:24,25 278:10,19 279:1,1 279:5 280:14 <b>assays</b> 2:16 47:25 57:6,13,14 58:1,3 59:14 65:19,21 121:24 149:4 171:23 173:11 174:9,10 175:10 177:20 180:1,5,6 187:25 194:5,8,11 211:23 216:16 221:1 222:20,21 222:22,23 223:20 223:21 253:9 276:23 277:3,13 278:12,20,21 <b>assemble</b> 106:2 <b>assembly</b> 17:12 <b>assertions</b> 156:19 <b>assess</b> 168:13	193:14 194:1 <b>assessing</b> 58:10 168:17 <b>assessment</b> 50:11 189:14 193:22 196:16 197:10 <b>assessments</b> 2:20 183:14 184:2 188:20 203:17 <b>assigned</b> 272:20 <b>assist</b> 60:2 <b>assistance</b> 167:22 169:4 <b>assistant</b> 89:24 <b>associated</b> 13:10,21 14:18 36:2 38:7 40:14 41:20 44:13 45:5 55:18 56:9 57:24 58:12,14 60:24,25 61:14 66:12 116:2 176:8 176:21 <b>association</b> 112:21 149:15 <b>associations</b> 257:23 <b>assortment</b> 63:4 <b>assume</b> 145:10 225:3 <b>assumed</b> 267:10 <b>assuming</b> 47:1 <b>assumption</b> 7:5 150:2 219:1 <b>assure</b> 196:14 <b>assured</b> 197:16 <b>AST</b> 62:7,7,8 78:15 78:16 106:23,24 <b>asymptomatic</b> 48:9 <b>atrium</b> 113:21 <b>attachment</b> 16:14 <b>attack</b> 126:3 225:11 231:24 252:15 267:1,5,10 267:15 <b>attained</b> 68:3 <b>attempt</b> 31:11 51:10 79:19 264:20 270:12 <b>attempting</b> 33:12 <b>attempts</b> 23:5 43:18 49:25
--	---	--	--	--

<b>attend</b> 5:2	13:8 26:25 27:17	21:10 32:15,20	<b>behalf</b> 3:11	147:21 157:24
<b>attendance</b> 9:15	36:3 38:6 40:21	45:22,25 59:14	<b>behave</b> 243:11,12	237:9 246:11
<b>attending</b> 281:9	50:5 57:22 61:19	90:18 131:15	<b>behavior</b> 242:16	<b>biggest</b> 114:2 163:9
<b>attention</b> 36:17	67:10 73:4,16	139:17 144:14	<b>beings</b> 140:4	180:18 231:25
<b>attentive</b> 231:17	78:24 82:3 86:10	172:17 180:5	224:25 234:19	<b>bilirubin</b> 62:13
<b>attenuated</b> 19:3,17	88:17 96:3,24	181:5,12 186:23	<b>believe</b> 23:19,22	<b>Bill</b> 164:2
19:18 21:8,16,19	98:13 107:1 111:8	187:4 213:2	38:11 48:3 49:13	<b>bind</b> 15:11 29:19
86:24 157:2	111:17 113:22	263:21 264:5,6	50:21 52:12 62:8	59:15 106:1 162:3
<b>attenuation</b> 21:13	118:22 142:17	268:10	79:2 81:18 88:24	<b>binding</b> 15:2,5,10
23:17 157:20	148:17 153:25	<b>baseline</b> 136:20	111:23 143:18	15:18 29:22 60:7
<b>atypical</b> 61:11	157:17 166:18	137:6	153:2 155:2	155:7,25
<b>audience</b> 23:4 64:4	170:3 172:12,18	<b>basic</b> 8:6 44:19	162:23 177:10	<b>bio</b> 30:5 32:8
73:14 125:23	178:16 194:21	79:17 209:8	222:18 226:23	<b>biodefense</b> 3:5,25
140:8 175:3	195:6 196:11	<b>basically</b> 19:22	248:12 258:7	267:4,16 268:2,9
235:12 281:8	209:18 226:15	20:1 22:21 46:23	260:7 275:3	269:17 274:14
<b>Auditorium</b> 1:9	230:5 237:14	46:24 47:3,6 54:8	<b>believing</b> 225:21	<b>biological</b> 6:10
<b>Australian</b> 35:2	245:2,8 252:24	79:18 113:7 156:7	<b>beneficial</b> 112:13	54:6 126:2,17
<b>authorities</b> 36:18	255:22 257:7	157:1,2 158:1	<b>benefit</b> 187:2	166:24 187:5
<b>autocrine</b> 90:20	260:9 261:5 275:6	161:8 168:2	195:18 206:9	188:19 230:22
<b>auto-inoculation</b>	<b>background</b> 196:2	171:15 172:2	213:5 214:23	<b>biologicals</b> 212:22
269:5	197:22 202:7	205:16 211:11	<b>Bernard</b> 42:22	<b>biomarker</b> 54:2
<b>availability</b> 71:19	207:20,23,24,25	217:16 218:1,15	<b>best</b> 7:14 9:12	59:22
95:14 245:12	212:9 231:20	222:12 247:19	33:14 101:7	<b>biomarkers</b> 2:9
263:15	<b>background-subt...</b>	276:19	118:10 125:23	53:20 55:21 58:20
<b>available</b> 68:19	196:7	<b>basics</b> 67:20	152:19 156:25,25	63:4 64:5 66:17
143:22 161:19	<b>backwards</b> 39:6	<b>basis</b> 6:5 40:5 85:7	161:19 180:7	219:6 241:25
179:23	220:6	199:7 207:23	182:2 187:22	<b>biomedical</b> 96:1
<b>avenues</b> 31:6	<b>back-burner</b> 158:1	214:11 216:25	199:22 215:18	<b>biopharmaceutical</b>
<b>average</b> 132:24	<b>bacteria</b> 271:19	218:2 278:17	227:3 228:15	201:7
133:2 145:24	<b>bacula</b> 202:13	<b>basket</b> 163:3	263:14 277:22	<b>BioShield</b> 8:4
155:2 166:6	<b>baculavaccine</b>	200:24	<b>bet</b> 226:1	<b>bioterrorism</b>
237:10	201:24	<b>bat</b> 38:12,12 46:25	<b>beta</b> 85:11 124:15	125:13 252:15
<b>avoid</b> 8:23 206:5,10	<b>baculavirus</b> 158:7	47:4,17,24 146:13	124:16 193:8	268:17,24 269:17
<b>avoided</b> 260:19	203:20	146:15,16	<b>Bethesda</b> 1:11	<b>bioterrorist</b> 267:1
<b>awake</b> 228:1	<b>bad</b> 62:5 80:12	<b>bats</b> 42:8 45:7	<b>better</b> 6:3 55:12	<b>bio-defense</b> 30:6
<b>aware</b> 4:23 9:15	98:5 197:3 258:13	46:22 47:12 48:2	57:15 79:6 96:2,3	127:20 144:6
77:3 79:13 211:21	<b>Baka</b> 19:22	69:15 89:6 91:18	104:12,13 158:14	<b>bio-hazards</b> 9:3
280:16	<b>balance</b> 124:20	147:15	178:17 181:9	<b>bio-terrorism</b>
<b>awhile</b> 70:23	160:20 162:14	<b>Bavari</b> 159:23	182:9 183:23	11:23 144:6
<b>Aysegul</b> 141:16,17	<b>BALB/C</b> 169:24	<b>beacons</b> 58:9	192:7,12,24 193:2	<b>birth</b> 69:8
<b>A-F-T-E-R-N-O-...</b>	<b>Baltimore</b> 149:21	<b>bear</b> 183:16	217:7 223:16,18	<b>bit</b> 4:17 5:10 8:16
149:1	<b>bang</b> 5:16	<b>becoming</b> 33:8	242:17 248:4	10:6,9 19:13
<b>a.m</b> 1:11 3:2 67:15	<b>bar</b> 240:17 250:2	87:14	279:8	24:18 26:7 28:1
	252:6,12 268:20	<b>beets</b> 93:5,5	<b>beyond</b> 40:1	37:6,6,19 38:15
	270:9 272:3 278:8	<b>beg</b> 64:21	219:24	39:1 41:21 42:5
<b>B</b>	<b>Barbara</b> 227:16	<b>began</b> 5:16 114:14	<b>BHK</b> 169:11	43:2 44:21 48:14
<b>B</b> 188:6 192:15	<b>BARDA</b> 8:2	<b>begets</b> 261:16	<b>bias</b> 162:24 177:19	48:17,21 55:8
232:16 260:2	<b>Barouch</b> 199:25	<b>beginning</b> 5:13	241:7,8 245:10	59:6 66:10 68:13
<b>baboons</b> 92:25 93:3	<b>base</b> 38:2 238:21	38:14 98:24	<b>big</b> 22:5 24:10	79:5 83:8 88:23
93:4,8,9	<b>based</b> 16:12 18:22	103:21 244:6	56:24 143:18	92:17 95:8 99:17
<b>back</b> 9:4 12:4 13:2				

100:7 102:19	<b>boniface</b> 100:13	194:17 196:12	<b>bulls</b> 73:9	187:22
105:11,24 106:7	<b>book</b> 35:20,21	<b>break-throughs</b>	<b>BUN</b> 62:18 78:20	<b>carcass</b> 57:21
106:24 110:20,21	<b>boost</b> 184:23,24	190:6 201:25	<b>bunch</b> 217:4,5	<b>cardiovascular</b>
114:11 116:25	185:5 188:22	<b>bridge</b> 176:16	269:23	62:3
117:15,16 122:9	189:7,10,11	180:25 215:19	<b>business</b> 228:12	<b>care</b> 34:15 36:15
127:25 129:15	198:20 202:1,14	223:20 255:10	251:2 274:17	39:16 40:1,17
135:14 138:18	202:21 204:9,13	<b>bridged</b> 186:16	<b>butterfly</b> 150:20	41:10 42:9,9
140:7 141:1 164:8	204:17,19,24	220:10	<b>button</b> 149:13	43:25 44:2 48:25
178:1,7 179:19	<b>boosting</b> 199:16	<b>bridges</b> 179:18	<b>buy-in</b> 276:2	52:13 63:15 85:15
188:2 192:7	<b>border</b> 35:12	<b>bridging</b> 23:23	<b>BW</b> 148:10	101:22
194:19 196:4	<b>bornaviruses</b> 16:10	198:4 199:4	<b>B7</b> 60:24	<b>career</b> 115:11
240:18 241:4,9	<b>borne</b> 267:7	216:13 276:4		<b>careful</b> 8:11 265:5
265:5,17 268:7	<b>Botswana</b> 46:25	278:18	<b>C</b>	<b>carefully</b> 144:14
269:15 270:1,9	<b>Botswanapol's</b>	<b>brief</b> 31:17 136:24	<b>C</b> 22:11 56:10	177:24 209:21
271:9 273:12	69:14	186:20 221:23	112:18,19,22	<b>Carolyn</b> 227:14
<b>bits</b> 156:7 163:24	<b>bottom</b> 15:12 23:3	<b>briefly</b> 17:19 26:25	113:5,8 116:4	<b>carried</b> 37:1 90:3
<b>BLA</b> 227:7	41:12 78:23 86:7	46:21 85:7 116:22	154:14 155:5	<b>carries</b> 90:3
<b>black</b> 49:16 131:14	111:9 114:22	184:1	162:1 260:6	<b>carry</b> 43:17
<b>Blackberries</b> 53:9	116:12 169:18	<b>bring</b> 19:13 25:5	<b>cable</b> 113:24	<b>cartoon</b> 110:22
<b>blank</b> 220:13	196:19	34:3 210:18	<b>cadavers</b> 51:5	<b>cascade</b> 237:4
<b>bleed</b> 81:19	<b>Bouhkrief</b> 161:1	214:17	<b>cafeteria</b> 148:17	<b>case</b> 5:20 7:4 11:25
<b>bleeding</b> 51:1	<b>bounced</b> 46:20	<b>brings</b> 19:23 45:13	<b>calcium</b> 62:19,20	12:5 18:1,2,14,19
81:14,22 109:2,11	<b>bound</b> 111:2	194:21	<b>calculate</b> 196:4	19:16 25:22 26:11
<b>bleeds</b> 54:24	<b>box</b> 59:2	<b>briskly</b> 85:2	<b>calculated</b> 141:23	27:11,19 38:16
<b>block</b> 25:11 94:20	<b>boxes</b> 173:14	<b>broad</b> 257:6	<b>calculation</b> 195:19	40:10 42:16 43:25
111:12 113:6	<b>boy</b> 36:3	<b>broader</b> 6:7 67:6	197:19	59:16 64:6 80:4
<b>blocked</b> 26:14,17	<b>Bradford</b> 76:3	181:16 204:20	<b>call</b> 39:23 153:8	89:21 91:4 94:23
112:12	<b>brain</b> 271:22	<b>broadest</b> 257:12	157:15 159:14	94:25 102:2 117:9
<b>blocking</b> 14:5,20	<b>branch</b> 31:16 32:8	<b>bronchial</b> 140:18	164:21 228:2	122:20 128:20
<b>blocks</b> 26:14	38:5 41:5 46:4	273:4	<b>called</b> 13:14 36:8	132:4 146:12,21
111:14,15	<b>Bray</b> 1:13 4:11	<b>broncho</b> 117:9	37:16 70:6 95:15	152:7 169:8
<b>blood</b> 29:14 43:10	53:8 67:16 86:20	<b>brought</b> 36:17	111:13 113:5	170:18 191:9
43:15 50:8 51:1	87:3,7,24 88:2	94:11 123:24	186:4 241:25	195:5 220:19
55:8,16 59:3,18	90:9 109:10	273:8	<b>calling</b> 157:6	221:16 234:8
61:7,9,16 65:4	168:19 258:6	<b>Brubaker</b> 142:13	158:18 195:17	250:5,7 254:5,11
75:7,9 78:5,14	<b>Bray's</b> 237:12	<b>BSL</b> 21:24	<b>calls</b> 257:3	254:13 257:22
82:19 108:21	253:15	<b>BSL-3</b> 221:15	<b>Canada</b> 1:16 9:21	263:10 272:2
111:8 132:15	<b>break</b> 10:11 33:24	<b>BSL-4</b> 221:15,16	<b>candidate</b> 151:15	277:3 279:13
136:15,16	67:9 136:21	230:1	152:22 161:19	<b>cases</b> 4:24 27:6
<b>blot</b> 48:21 58:1	182:16 190:17	<b>bubonic</b> 231:14,15	197:9 199:10	34:15 36:10 37:25
<b>blown</b> 248:7	194:25 209:22	<b>budding</b> 14:18 16:5	200:20	38:2 39:13,25
<b>blue</b> 35:21 116:11	210:4 262:13	111:1	<b>candidates</b> 149:10	40:15 50:9 53:10
133:4 136:10	<b>breaking</b> 148:15	<b>building</b> 44:11	150:1 203:6,10	53:14 58:17 60:16
<b>boar</b> 97:14	<b>breakthrough</b>	148:16	<b>capability</b> 193:2	61:23,25 62:14
<b>board</b> 209:12	262:7,22	<b>buildings</b> 268:14	221:14,15 253:4	67:5 80:17,17
<b>Bob</b> 47:20	<b>breakup</b> 77:19	<b>build-up</b> 51:23	<b>capable</b> 89:3,18	81:4 94:19 98:18
<b>bodies</b> 77:25	<b>break-off</b> 111:2	<b>built</b> 97:13 101:10	<b>capacity</b> 161:11	103:3 107:5 108:1
<b>body</b> 52:14 60:11	<b>break-through</b>	228:14	162:19 170:11,23	108:22 109:16
<b>bolus</b> 165:5	190:10,16 191:16	<b>bulk</b> 192:2 194:2	<b>capsid</b> 12:11	112:25 113:1
<b>bomblets</b> 268:11	191:22 192:1	<b>bullet</b> 217:25	<b>capture</b> 57:14	116:6 132:18

144:2 165:4 171:2 173:24 214:7 218:20 227:5 233:2 238:16 248:3 249:16 257:18 267:14 280:3,4	<b>CD4</b> 171:18 172:2 188:15 191:13,16 191:24 192:13 204:21,22 <b>CD8</b> 60:14,15 171:17 191:13,17 191:24 192:13,25 194:2 <b>CD8s</b> 204:23,25 205:1 <b>CD8+T</b> 188:11 <b>cell</b> 19:4,6 22:18 25:22 26:10,16 78:5 90:25 121:22 123:3 136:15 162:3 168:6 169:9 175:10 180:3 190:22 191:3,4,6 191:14 192:2,5,12 193:9,10 194:3,6 194:8,19,25 204:23 209:21 260:1 277:11 278:11 <b>cells</b> 18:6 19:9 21:6 23:17,18 24:21 25:9,23 26:1 27:24 28:4 60:11 60:13,15 75:19,21 75:24 77:8,15,15 77:19,24 78:7 80:11,13,23 81:5 81:9 82:14,19 83:3 105:20,21 106:20 107:10,14 107:21 110:9 111:3 117:17 118:25 119:4,11 119:16,25 121:15 122:1,4 123:21 134:7,8 136:16 161:11 162:7,9 169:11 171:17 172:3,24 173:3,21 176:7 180:24 185:2 187:17,18 187:19 188:6,11 188:15 191:12 192:6,14,15,19 193:6,7 194:2	237:16 247:2 253:10 258:19 259:14,15 277:10 <b>cellular</b> 60:9 97:23 184:14 188:5 189:8 194:10 209:5 227:16 271:15 <b>cell-mediated</b> 2:18 167:12 <b>center</b> 1:10 39:18 40:23 141:12 <b>centered</b> 43:1 <b>Centers</b> 1:3 31:14 <b>centigrade</b> 134:22 135:20 <b>central</b> 13:6,7 42:13 45:2 72:14 75:20 <b>cents</b> 259:9 268:24 <b>century</b> 127:2 <b>cerebral</b> 86:12 <b>certain</b> 8:15 116:4 219:2 229:12 239:6 269:20 <b>certainly</b> 5:12 7:5 31:10 48:19 52:7 53:15 81:21 98:19 110:10 121:4,4 125:12 146:4 147:2,14,19 165:2 165:15,16 166:1 180:2 182:4 210:18,23 211:5 217:9 218:6 219:9 219:15,25 223:1 223:21,25 224:14 225:9,9,13,19 226:3,20,22 227:7 227:18 228:8,25 229:22 230:3,19 231:2,11 232:18 232:24 233:12 234:7,20,22 237:9 240:5,9 244:11,16 245:5 247:10 249:1 252:13 264:20 269:21 274:14,17 275:8 <b>CFR</b> 211:7 221:24	222:6,9 <b>chain</b> 37:1 <b>chains</b> 36:22 37:4 <b>chair</b> 235:2 <b>Chairperson</b> 10:19 <b>challenge</b> 65:5 103:13 104:7 112:3 116:21 128:3,5 129:11 133:1 134:11 141:9 145:17 148:9 164:17 166:16 168:22 170:21,22 171:2,3 173:5 176:12 177:14,15 180:18 181:6 189:21 209:2,2,10,13,24 212:15 219:18,24 221:21 223:14 225:18 240:22 243:9 244:11 246:20 247:1,5,9 251:3,15 254:3,3 254:16 255:12,17 258:14,18,21 260:6,24 261:6 262:13,19 263:4 264:25 265:7 266:19,24 267:11 267:18 269:25 270:5,6,21,22 281:3 <b>challenged</b> 27:7,7 170:24 173:1 190:1 262:15 271:9 <b>challenges</b> 103:18 194:12 246:25 256:12 266:13 <b>challenging</b> 270:1 <b>change</b> 10:14 11:19 73:6 136:19 195:23 <b>changed</b> 23:24 259:15 272:15 <b>changes</b> 23:24 77:18 78:5 83:7 129:2 136:15 137:15 139:23	147:8 196:3 204:16 <b>character</b> 204:10 <b>characteristic</b> 54:4 54:16 77:20 <b>characteristics</b> 55:1 86:23 160:11 171:9 218:17 245:15 <b>characterization</b> 178:23 263:16 <b>characterized</b> 28:12 55:23 56:6 168:12 178:12 186:25 214:5 245:14 263:14 <b>charge</b> 67:21 <b>charges</b> 95:17 <b>charging</b> 95:16 <b>cheated</b> 149:14 <b>check</b> 3:20 57:9 183:12 208:18 <b>checked</b> 152:13 <b>chemical</b> 6:10 <b>chemistries</b> 61:17 61:19 78:14 <b>chemokine</b> 237:19 <b>chemokines</b> 81:25 87:16 <b>chemokine/cytok...</b> 87:3 <b>Chen</b> 1:14 3:3,3 <b>chest</b> 98:10 <b>chilled</b> 271:12 <b>chimeras</b> 156:11 <b>chimeric</b> 200:2 <b>chimpanzee</b> 42:17 <b>chimps</b> 42:19 <b>Chinese</b> 95:23 96:5 96:21 97:19 241:16 <b>choice</b> 18:7 218:12 249:18 256:25 <b>choices</b> 149:22 218:11 <b>choir</b> 221:13 <b>choose</b> 68:19 244:12 <b>choosing</b> 246:5 <b>chosen</b> 190:23
---	--	--	---	--

<b>Chow</b> 64:2,2	179:1 181:4 183:5	239:4,6 242:25	10:3 13:2,8 25:20	272:19 277:15
<b>Christopher</b> 148:3	205:13 214:23	<b>coagulopathy</b> 26:4	27:17 30:22 38:6	278:2 279:7,16
148:3	237:1 239:25	26:20 76:24 79:4	50:5 62:9 66:4	281:7
<b>chromium</b> 171:24	240:12 242:7	108:24 112:10	68:9 79:1 81:11	<b>commercial</b> 163:6
172:25 173:22	263:23,25 267:3	237:2 238:7,7	101:23 107:1	<b>committee</b> 101:22
180:5 278:12	267:25 269:9	249:10	123:3 134:9 147:7	227:5 236:4
279:1	<b>cleavage</b> 15:20	<b>Coast</b> 56:2 67:4	156:12 159:25	<b>common</b> 38:8
<b>chromosome</b> 14:12	22:21 23:11,14	170:17	166:18 170:3	50:16 138:7
<b>chronic</b> 69:16	24:1,13	<b>coat</b> 97:11	171:9 211:13	275:17
<b>chronological</b>	<b>cleave</b> 22:8 23:15	<b>cocktail</b> 178:10,16	217:7,12,25	<b>commonly</b> 44:7
156:22	<b>cleaved</b> 22:16	<b>Code</b> 212:21	225:15 233:5,14	128:18 133:18
<b>circle</b> 145:1	<b>clinging</b> 89:5	<b>cofactor</b> 14:3	235:4 243:19	267:6
<b>circulating</b> 185:15	<b>clinical</b> 2:7 35:19	<b>coffee</b> 10:11	248:18,21 251:10	<b>communicated</b>
<b>circulation</b> 109:25	35:22 43:6 53:19	<b>coffin</b> 64:14	253:2 262:5	232:19
<b>circumstances</b>	55:7 61:19 118:4	<b>cohort</b> 99:25	267:21 274:24	<b>communication</b>
229:18 275:15	124:6 129:19	103:16 114:15	278:10,15 279:10	227:2
<b>citing</b> 154:18	130:7 132:14	<b>cohorts</b> 198:7	<b>comes</b> 9:21 11:4	<b>communications</b>
<b>civilian</b> 143:5	139:17 179:3,11	<b>collaborates</b>	19:6 26:7 32:10	227:19
268:12	187:2 199:6	201:11	66:22 82:3 110:6	<b>community</b> 37:4
<b>Ci67</b> 105:3 128:21	201:12 206:15	<b>collaboration</b>	138:4 164:6	40:17 46:20
129:15 130:19	213:5 216:7,21	199:24 201:6	190:18 193:7	276:15
140:12 141:4	218:17 219:6,15	226:21	239:12 249:25	<b>companies</b> 99:6
164:22 239:16	220:25,25 221:3	<b>collaborators</b> 46:5	255:24	<b>company</b> 113:3
263:12	236:11 242:10	<b>colleagues</b> 4:25	<b>comfort</b> 278:7,9	161:1
<b>clades</b> 257:10	245:25 273:21	73:15 149:17	<b>comfortable</b> 54:24	<b>comparability</b>
<b>claim</b> 161:12	<b>clinically</b> 129:21	179:19 181:2	<b>coming</b> 4:4,9 11:24	129:18 140:14
<b>clarification</b>	<b>clone</b> 20:15,17,19	252:13	12:5,19 13:11,18	<b>comparable</b> 23:21
217:11	261:25	<b>collected</b> 35:14	26:25 36:3 53:16	139:11 141:8
<b>class</b> 182:5 232:6	<b>cloned</b> 153:4	48:3 55:1 131:16	67:23 74:2 79:22	164:14 225:2
<b>classic</b> 72:25 109:5	<b>clones</b> 182:18	131:17 136:5	100:15 146:16,18	256:20
273:4	<b>close</b> 8:22 24:8 55:5	<b>collecting</b> 239:22	153:17 155:17	<b>comparatively</b>
<b>classical</b> 30:11	123:3 135:2	<b>collection</b> 43:6	180:20 248:21	68:13
162:5	227:21 249:12	264:18	267:6	<b>compare</b> 19:3 24:5
<b>classically</b> 165:21	262:4	<b>collections</b> 47:14	<b>commemorating</b>	67:24 68:11 99:9
<b>CLAUSEN</b> 46:9,16	<b>closed</b> 42:3	<b>colobus</b> 49:12,17	63:14	99:18 101:12
<b>clean</b> 57:11	<b>closely</b> 77:17	<b>color</b> 105:16	<b>comment</b> 10:22	102:14 128:12,23
<b>cleaned</b> 38:1	201:11 239:9	<b>colors</b> 173:13	29:4,12 47:4	195:10 219:5,14
<b>cleaner</b> 57:13	259:6	192:12 194:14	66:16 88:22 89:13	247:6
<b>clear</b> 21:13 38:3	<b>closer</b> 110:19	<b>column</b> 238:13	130:18 143:3	<b>compared</b> 24:11
73:8 204:14 215:9	<b>closest</b> 55:2	<b>combination</b> 17:17	163:20 165:8	61:23 121:13
<b>clearance</b> 60:1,10	<b>clotting</b> 108:20	72:5 85:14 141:17	166:3 177:12	137:5 139:16
<b>cleared</b> 59:18	<b>cloud</b> 178:6	193:8	179:14 181:15	156:15,17 201:21
<b>clearly</b> 7:4 21:11	<b>coags</b> 78:22	<b>combinations</b>	207:4 230:9	249:9
25:14 51:17 72:13	<b>coagulation</b> 94:10	193:11	248:11 262:3	<b>compares</b> 134:19
72:19 83:17 85:14	95:7 108:21	<b>combine</b> 90:9	265:3 266:12	140:5 236:14
95:5,9 108:8	109:13 110:24	124:18,24	279:8	<b>comparing</b> 120:13
109:12 112:10,11	111:8 112:16	<b>combined</b> 28:14	<b>comments</b> 9:17,18	177:17 179:3,10
119:7 138:12	115:6,7 118:5	<b>combining</b> 123:23	10:21 157:4 228:7	202:22 246:4
144:4,21 145:2	137:14,17 139:23	124:8	235:14 246:16	<b>comparison</b> 2:7
176:15 178:24	141:15 236:19,25	<b>come</b> 5:19 7:12	256:5 261:3 268:5	53:19 140:17

143:21 154:24 173:10 221:2 253:23 <b>comparisons</b> 55:9 223:16 <b>competent</b> 19:3,14 <b>competition</b> 165:22 <b>complaints</b> 10:20 <b>complement</b> 171:8 <b>complete</b> 25:1 265:9 <b>completed</b> 23:10 <b>completely</b> 26:14 121:3 177:15 250:17 270:20,21 270:24 <b>complex</b> 13:21,23 14:8 15:2 20:2 161:11 194:6,11 275:24 <b>complicated</b> 7:22 25:19 88:23 126:1 194:20,24 <b>component</b> 61:10 64:12 95:9 234:13 <b>components</b> 150:24 198:14 215:16 <b>composition</b> 186:9 <b>compounds</b> 61:21 62:25 <b>comprehensive</b> 175:2 <b>computer</b> 114:1 142:21 167:22 <b>concentrate</b> 184:2 192:18 215:25 <b>concentrated</b> 266:7 <b>concentration</b> 78:21 195:17 <b>concept</b> 157:2 216:9 <b>concepts</b> 156:8 227:7 <b>conceptually</b> 156:6 <b>concern</b> 11:22,23 89:9,11 126:5 157:9,24 158:24 159:2,10 234:23 243:11 <b>concerned</b> 9:17	125:24 127:20 162:18 167:2,2,3 249:24 250:2 274:20 <b>concerns</b> 11:24 125:12 157:7,7,10 158:21 159:18 160:2,16 161:6 220:8 228:11 232:1 <b>concluded</b> 24:12,19 281:14 <b>conclusion</b> 28:8 <b>conclusions</b> 63:3 176:3 <b>concomitantly</b> 25:10 <b>concurrence</b> 227:6 <b>concurrent</b> 158:4 202:7 <b>conditions</b> 21:24 50:3 65:3 117:2 118:10 150:21 190:6 205:24 209:22 <b>conduct</b> 221:18,21 <b>conducted</b> 186:8 217:15 219:1 222:1 223:6 <b>Conference</b> 1:10 <b>conferred</b> 184:23 <b>conferring</b> 2:19 167:12 184:22 <b>confidence</b> 167:6 <b>confirmation</b> 4:21 18:16 19:11 <b>confirming</b> 184:17 <b>conflict</b> 33:5 <b>conflicts</b> 33:10 <b>conformational</b> 170:17 <b>confronted</b> 187:14 <b>confuse</b> 8:11 <b>confused</b> 48:21 <b>confusing</b> 48:14 209:11 <b>congestion</b> 109:8 109:13 130:14 138:5,12 140:16 273:3	<b>Congo</b> 4:22 5:2 33:13 42:13 43:24 45:3 <b>conjugate</b> 47:24 <b>conjunction</b> 47:11 <b>conjunctival</b> 117:22 127:13 266:2 274:17 <b>consensus</b> 243:15 <b>consequence</b> 26:3 144:11 238:10 <b>consequences</b> 66:12 <b>conservation</b> 151:20 154:14 155:4,22 <b>conservative</b> 181:8 181:13 206:25 <b>conserved</b> 12:17 151:18 161:25 <b>consider</b> 34:13 52:15 65:16 154:9 210:19,20 212:10 <b>considerable</b> 6:20 <b>considerably</b> 79:3 <b>consideration</b> 21:9 242:3 254:14 255:20 <b>considerations</b> 185:17 <b>considered</b> 155:20 155:21 160:23 218:14 <b>considering</b> 199:20 <b>consist</b> 259:7 <b>consistency</b> 238:14 241:17,22 261:22 <b>consistent</b> 49:9 154:17 184:13 189:8 204:18 209:10 223:10 237:15,16 238:23 239:1 240:10 249:17 256:19 257:23 259:14 <b>consistently</b> 137:12 244:13 <b>consists</b> 13:7 <b>consolidation</b> 117:7	<b>constant</b> 108:3 <b>constraints</b> 168:7 <b>construction</b> 257:7 <b>consult</b> 218:2 <b>consume</b> 266:6 <b>contact</b> 52:4 103:3 <b>contacts</b> 232:1,3 <b>contain</b> 260:12,13 <b>contained</b> 184:3 198:10 260:12 <b>containing</b> 51:24 153:21 <b>containment</b> 20:18 32:6 <b>contains</b> 155:5,6 161:3 <b>contaminated</b> 44:12 <b>contamination</b> 256:22 269:5 <b>CONTENTS</b> 2:1 <b>contest</b> 81:2 <b>context</b> 5:5 6:7 <b>continuation</b> 175:13 <b>continue</b> 6:19 73:24 86:17 99:10 132:8,12 200:22 277:19 <b>continued</b> 70:4 <b>continuing</b> 65:5 112:16 <b>continuously</b> 52:22 114:2 <b>contractual</b> 120:22 <b>contrast</b> 202:21 <b>contributed</b> 201:3 <b>contributes</b> 275:14 <b>contributing</b> 110:3 <b>contributions</b> 9:2 <b>control</b> 1:3 28:19 31:14 33:4 34:14 39:17,21 40:19 99:25 115:6 116:9 116:23 121:2 123:11 153:15 188:25 190:2,14 240:24 267:13 <b>controlled</b> 114:1 208:7 213:2	<b>controls</b> 114:16,17 114:24 116:8 123:9,16 153:20 223:7 <b>controversial</b> 15:16 101:6 118:23 <b>controversy</b> 51:3 <b>conundrum</b> 230:19 <b>convalescence</b> 43:12 <b>convalescent</b> 183:3 <b>convene</b> 210:4 <b>conventional</b> 58:5 <b>conversation</b> 255:15 279:10 <b>conversations</b> 3:9 <b>convert</b> 153:25 <b>convincing</b> 144:4 <b>cooper</b> 106:20 <b>copy</b> 3:18,19 20:7 <b>core</b> 13:6,7 124:9 <b>correct</b> 65:25 85:6 119:3 122:11 150:3 226:4 239:3 239:13 253:21 <b>corrected</b> 197:23 <b>correctly</b> 224:12 <b>correlate</b> 64:5,10 173:11 180:7 183:24 186:14,17 188:1,8,16 195:2 196:20 197:17,24 199:3 203:2,19,21 209:13,19,23 210:1 220:11,11 254:20 255:10 276:17,21 277:22 278:15 280:15 <b>correlated</b> 222:25 <b>correlates</b> 2:15,20 101:24 115:20 149:3 176:16 179:18 183:15 185:9 187:6,20 189:4,14 190:4,5 246:22 254:8 255:8 276:9 277:23 278:25 280:7 <b>correlating</b> 180:17
---	--	--	---	---

<b>correlation</b> 177:7	189:1 190:3,15	<b>CTLs</b> 168:11 174:4	237:22 238:11	126:7 133:4,14
<b>correlations</b> 143:23	194:23 200:22	175:21,25 176:7	240:13 241:1,14	137:6,8 138:7
<b>correlative</b> 183:21	201:10 221:16	176:11 183:2,5	241:19 245:11	143:4,8,13,22
278:4	229:16 237:16	<b>culture</b> 21:6 22:18	253:2	144:3,13,19,21
<b>correspond</b> 78:11	240:20 241:2,5	24:18 26:2 34:10	<b>cytokine</b> 61:2 87:8	145:2,5,24 156:14
<b>corresponds</b> 58:24	254:6,10,17	121:13 123:3	88:14 110:12	162:23 164:2
<b>cost</b> 95:14	263:22 281:5	144:8 260:1	162:7 176:8	176:17,23 177:16
<b>costly</b> 95:21	<b>courtesy</b> 140:7	<b>cumulative</b> 196:16	190:25 191:14,19	178:25 180:20
<b>Cote</b> 11:16	<b>cover</b> 185:14 212:8	<b>curious</b> 48:7	193:20 194:2	181:12 185:8,25
<b>cotton</b> 40:15	<b>covered</b> 126:21	<b>current</b> 45:13	237:18 277:11	186:23 187:5,5
<b>coughing</b> 51:23	<b>covers</b> 7:23 37:10	149:18 263:15	<b>cytokines</b> 61:2	191:1 194:15
<b>Coulter</b> 137:7	<b>co-factor</b> 30:17	<b>currently</b> 11:6	79:10 80:7 81:25	202:19 204:11
<b>count</b> 33:21,23	<b>co-receptors</b> 15:15	21:25 149:15	83:11,12 87:17	205:21 206:15
41:15 136:15,24	<b>co-stimulatory</b>	194:11 200:2	110:13 116:5	211:11 212:9,25
<b>counted</b> 53:14	191:8	<b>Curt</b> 169:2	188:10 191:5	215:12,13 216:14
<b>counter</b> 137:7	<b>CP</b> 56:12	<b>curve</b> 190:11	192:8,17 193:9,12	217:6 218:13
<b>countermeasures</b>	<b>crack</b> 238:2	195:20,21,22	193:19	219:5,5,20,24
147:23	<b>create</b> 18:25 51:24	197:12,21	<b>cytometry</b> 58:2	220:10 221:1
<b>counter-measures</b>	52:8 269:11	<b>curves</b> 195:12	194:14	223:3,20,23
6:17	<b>created</b> 32:4 50:18	196:10	<b>cytopathic</b> 24:17	225:19,19 229:3
<b>counting</b> 39:13	52:6 86:12	<b>cutoff</b> 116:10	56:9	229:10,11,25
<b>country</b> 5:7 34:12	<b>creating</b> 261:13	197:13 204:2	<b>cytosol</b> 16:5,9	230:5 231:15
37:16 214:19	<b>creatinine</b> 62:18	<b>cycle</b> 15:8 16:9	<b>cytotoxic</b> 60:11	233:11,19 234:7
222:11	<b>creation</b> 32:7	17:15	<b>C57Bl/6</b> 169:25	236:17,24 239:1
<b>counts</b> 78:6 82:19	<b>creatures</b> 38:9	<b>cyno</b> 100:5 121:12	170:2 174:18	239:14,15,22
102:7 132:15	<b>criteria</b> 213:24	121:17 131:19		240:5 242:7 243:1
137:3 253:14	214:22 215:10,11	133:4,17,18	<b>D</b>	243:17,20,24
<b>couple</b> 10:16 15:21	<b>critical</b> 7:10 34:15	137:16 241:9	<b>D</b> 61:24 65:24	244:2 251:1,25
31:22 32:17,22	44:2 60:10 124:22	242:1,2,4 245:9	66:11	252:10,16,18
34:22 41:3 47:15	155:17 162:23	245:16,18 249:19	<b>daily</b> 40:5 133:3	255:24 258:6,6
52:23 61:21 64:18	183:10 280:15	249:20	<b>Dale</b> 227:13	261:9 262:9
80:20 84:16 96:15	<b>criticized</b> 181:10	<b>cynom</b> 120:9	<b>damage</b> 62:4	263:22 269:13
103:20 114:5	205:14	<b>cynomolgus</b> 97:18	238:10 272:1	271:3 272:10
125:21 126:11	<b>criticizing</b> 219:21	97:22 103:15	<b>damaged</b> 108:9	276:20,20 278:4
149:25 154:11	220:2	104:20 110:9	<b>Dan</b> 199:25 200:8	278:11,18 279:10
191:18 201:1	<b>cross</b> 64:21 143:22	128:22 131:13	<b>dangerous</b> 54:22	279:23,25 280:1
227:22 233:20	<b>crossreact</b> 170:17	153:14 189:24	<b>Danish</b> 36:3	280:17,19
236:15 245:3	<b>cross-linked</b>	<b>cynon</b> 121:2 128:16	<b>darker</b> 97:11 98:9	<b>databases</b> 37:24
246:19 272:18	111:10	<b>cynos</b> 92:22 95:12	<b>data</b> 7:7,8,9,10,18	<b>dataset</b> 197:6
276:13 278:1	<b>Crucell</b> 199:24	96:13,17,18,25	7:20 11:14 13:11	239:17
<b>course</b> 2:7 5:18	201:7	97:1 98:1 99:1,4	13:17 21:9 25:4	<b>date</b> 49:19 79:5
16:14 20:23 34:2	<b>Crumrine</b> 1:15 9:7	99:11,12,13,25	26:10 27:4 38:1	94:19
34:5 35:3 54:17	9:10 29:2 31:7	100:3,12 102:15	42:10 48:11 64:7	<b>daunting</b> 180:16
56:18 78:10,12	52:24	114:11 131:5	65:2,10 66:4 67:1	181:23
80:9 90:19 100:22	<b>crux</b> 5:23 7:16	133:15 136:9,17	68:6 75:5 79:11	<b>Dave</b> 227:10
103:14 115:8	<b>CTL</b> 168:6 170:4	136:20,23 137:4,7	79:12 83:9 86:1	<b>day</b> 17:7 73:3,3
116:13 122:8,22	171:14 174:14,21	137:10,11 138:10	87:8,18 90:17	75:7,7,10,10,12
139:9 168:10	176:2 178:25	138:17 139:9,14	94:13 95:7 99:18	77:5,10,13 87:13
171:7 183:2	179:1,7,8,14,25	140:11 141:7	110:12 122:9	95:20 96:22
184:10 188:25	181:9 182:8,18	143:1 164:23	124:13,14 125:2,3	100:21 102:9,9

104:4,6,24,24	<b>decade</b> 17:20 31:2	<b>degree</b> 39:21 40:3	57:4 98:17,17	<b>detail</b> 168:21
105:8,13,17	<b>decent</b> 270:14	48:25 49:4 56:6	102:17 121:25	216:20,22 229:5
107:11,12,20	<b>decide</b> 101:20	106:22 153:8	147:9 153:7	<b>detailed</b> 220:18
108:15,16 109:7	132:7 256:13	155:4 162:18	193:12 197:15	221:6 226:18
109:18 112:19	<b>decided</b> 137:7	197:15 219:4	267:4 273:1	<b>details</b> 43:10 56:14
119:14 131:20,20	168:11	238:13 250:17	<b>depends</b> 154:5	70:23 198:20
132:2,3,21 133:5	<b>deciding</b> 263:16	275:12	181:18 246:24	<b>detect</b> 58:17,19
133:5,6,7,7,9,10	<b>decision</b> 163:8	<b>degrees</b> 98:19	274:21 276:17	87:13 173:15
133:22 135:23,23	279:12	109:1 134:22	<b>depicted</b> 172:1	<b>detectable</b> 177:6
135:25 136:7	<b>decisions</b> 244:3	135:20	<b>depletion</b> 117:12	<b>detected</b> 174:4,9
137:6,6,8,11,12	<b>declarations</b> 5:7	<b>delay</b> 3:23 67:16	117:14	175:20
145:24 170:22	<b>decline</b> 78:10	111:24 124:18	<b>deposited</b> 109:20	<b>detecting</b> 61:9
172:24 210:8	136:23 137:10,12	<b>delayed</b> 59:1	<b>deposition</b> 76:18	<b>detection</b> 58:6,7
241:1 260:10,11	<b>declines</b> 133:5	280:10	76:20 77:2 82:5,7	153:23 154:7
279:15 281:9,10	<b>decrease</b> 56:12	<b>delays</b> 114:25	94:3,5,10 117:12	<b>determination</b>
<b>days</b> 59:12 64:18	58:25 136:21	<b>deleted</b> 203:10	236:20 273:11	134:4 141:2
71:2,3,13 72:1,1	<b>decreased</b> 60:17	<b>deletes</b> 56:11	<b>deposits</b> 94:7 95:1	<b>determinations</b>
72:18 94:6 99:6	<b>deduce</b> 172:12	<b>delicate</b> 162:14	107:22 110:14	141:20
100:3,19,19	<b>defective</b> 152:3,4	<b>delivered</b> 187:19	<b>deprioritized</b> 158:8	<b>determine</b> 83:5
102:15,15,16	156:9 159:11	<b>delivering</b> 184:16	<b>depriving</b> 250:3	128:2 133:16
103:2,5,17,17,20	<b>defects</b> 239:4	<b>delivery</b> 167:4	<b>derive</b> 49:24	134:6 143:24
104:23 114:6	<b>defense</b> 1:2 3:12	232:2	<b>derived</b> 46:1	187:7 253:25
120:9 123:2	144:6 257:3	<b>delta</b> 22:10,10	261:25	275:25 276:6
131:25 134:13	<b>defer</b> 90:13 137:22	<b>demonstrate</b> 148:8	<b>dermatitis</b> 219:21	<b>determined</b> 136:13
135:12,25 137:2	181:2	155:16 156:3	<b>describe</b> 17:8 20:8	159:21
153:17 164:13	<b>deferon</b> 90:6	186:13,23 270:7	22:14 49:17 69:3	<b>determining</b>
165:1 167:23	<b>define</b> 23:5 134:17	<b>demonstrated</b>	192:6 218:17,19	131:12
170:24 171:3,22	153:5 162:25	39:20 52:10	222:15	<b>Detrick</b> 4:8 40:21
<b>de</b> 207:8	192:12 197:17	176:11 213:25	<b>described</b> 15:10,12	<b>develop</b> 8:18 17:16
<b>dead</b> 42:17 119:6	253:1	214:3 224:7 270:4	37:2 41:1 85:19	30:4 59:17 98:8
119:19 133:10	<b>defined</b> 173:7	<b>demonstrating</b>	175:1,15,17 176:2	104:12,12 122:11
192:14	198:23 209:20	199:6	188:4 189:2	127:16 130:5
<b>deadly</b> 166:25	<b>defines</b> 97:18	<b>demonstration</b>	<b>describing</b> 21:1	178:16 179:17,23
<b>deal</b> 32:2,5,5	193:10 195:17	83:21 159:15	76:9 78:17	181:19,25 189:19
235:15 267:2	<b>defining</b> 192:24	<b>dendritic</b> 25:23	<b>descriptive</b> 160:13	199:4 211:24
<b>dealing</b> 6:15,22 9:1	278:24	28:4 75:21 77:15	<b>design</b> 2:17 105:4	213:8 215:7
84:6 211:6 267:7	<b>definitely</b> 18:16	80:11,13,23 83:3	149:8 220:5,6	220:18 222:21
<b>death</b> 59:11 73:20	23:18 30:12 94:9	105:21 185:1	223:11 276:1	224:20 266:25
73:21 75:13,25	120:10 122:8	247:2	279:23 280:2	268:11 270:12
81:23,23 83:1	123:4 241:11	<b>dengue</b> 86:10,11,13	<b>designation</b> 11:5	<b>developed</b> 17:21
100:2,18 101:25	252:19 278:6	86:14	<b>designations</b> 13:15	18:22 27:14 28:12
102:14 103:10,12	<b>definition</b> 54:4	<b>denoted</b> 192:19	<b>designed</b> 158:2	30:19,21 34:12
104:4 111:24	<b>definitions</b> 162:20	<b>department</b> 3:12	216:12	68:22 70:5 71:13
114:25 118:9	<b>definitive</b> 165:25	141:19 149:20	<b>designing</b> 216:21	102:5 110:23
124:18 134:13	216:11 222:14	<b>dependence</b> 150:20	<b>desired</b> 214:23	113:19 116:20
135:5,10,12,23,25	223:5,11	<b>dependent</b> 15:20	<b>despite</b> 174:19	129:24 147:23
136:25 139:6,13	<b>deflect</b> 140:9	18:13 25:14	175:6,6 199:20	187:11 192:9,10
141:8 219:7 260:3	<b>degeneration</b> 106:9	150:16 182:20	<b>destroyed</b> 77:16	193:13 195:16
260:5 264:2	<b>degradation</b>	273:1	80:14	233:9 237:6
<b>deaths</b> 53:11	108:19	<b>depending</b> 12:6,23	<b>destruction</b> 77:14	245:17 255:14

<b>developing</b> 103:21 104:16 114:3 127:23 148:6 158:4 212:11 218:15,23 271:13	164:24 190:3 247:10 271:8 <b>dies</b> 119:13 133:23 <b>Dieter</b> 20:8 <b>differ</b> 182:7 189:5 203:19 <b>differed</b> 169:22 189:10 <b>difference</b> 14:13 22:6 24:11,23 76:15 77:2 94:2 100:14 117:19 118:5 119:7,17,19 119:20 130:21,22 138:3,24 142:23 143:7 190:19 194:21 196:6 209:19 241:2 246:12 254:22,22 254:24 258:10 <b>differences</b> 12:14 12:15 13:1 33:12 38:21 66:21 93:12 94:9 107:2 110:8 110:18 114:8 118:3 120:4 121:4 121:5,7 131:2 137:17 138:13,22 139:6,12,18 143:24 191:23,25 193:3,24 194:5,10 196:9 206:16 207:5 213:19 225:22 246:10,11 247:9 273:17 <b>different</b> 8:9 25:21 25:23,25 26:9 33:10,10 48:6,10 66:21 75:19 93:16 93:16 96:17,23 97:7,12,13,22 98:22 99:18,19 100:9 101:12 102:13 103:25 106:11 111:20 114:8 117:24 118:4 120:13,14 120:24 121:19,25 124:24 132:15 138:21,25 142:25	143:6 154:23,25 155:11,12 161:5 161:20 162:3 164:22 166:15 168:14,15,25 169:20 170:12 171:22 173:3 174:5 175:19,22 176:3,6,10 178:11 179:5,5,7,8 180:1 180:6,15 182:6 184:9 185:20 191:6,12,14 193:3 195:13 196:10,17 196:24 197:1,7 198:6 202:22 203:21 204:9 206:6,8 212:20 218:22,24 220:9 222:18 225:1 233:20,23,24 236:12 241:14 243:12 244:18 245:4,24,25 249:8 251:6 254:24 257:10 258:13 259:3 270:18 271:4,5 273:12 278:19 280:8 <b>differently</b> 196:4 243:11 268:7 <b>differs</b> 187:14 188:3 <b>difficult</b> 32:2 43:17 48:15 61:8 65:2 70:21 71:4 90:15 98:9 101:9,25 120:12 132:10 179:15 180:3,25 229:17 252:6 269:18 271:19 278:19 <b>difficulties</b> 65:3 <b>diffusion</b> 16:25 23:22 <b>digging</b> 229:22 <b>digit</b> 191:20 <b>diligent</b> 132:19 <b>diluted</b> 260:11,13 <b>diluting</b> 185:19	<b>dilution</b> 195:8 196:8 207:15 <b>dimers</b> 61:25 65:25 66:12 <b>diminishes</b> 258:8 <b>direct</b> 50:22 52:4 82:14 269:3 <b>directed</b> 170:9 174:14 <b>direction</b> 280:6 <b>directly</b> 29:22 77:16 164:3 176:8 187:16 255:2 <b>Director</b> 3:24 <b>dirty</b> 50:7 <b>discern</b> 193:2 194:5 196:9 <b>disclaimers</b> 176:19 <b>disclose</b> 206:15 <b>disclosure</b> 8:25 <b>discomfort</b> 130:10 132:13 <b>discouraged</b> 122:9 <b>discovered</b> 31:23 42:16 195:24 <b>discovering</b> 258:1 <b>discovery</b> 156:23 <b>discrepancies</b> 93:13 <b>discuss</b> 183:20 211:16 216:25 <b>discussed</b> 7:14 31:4 184:12 <b>discussion</b> 2:24 5:23 7:19 16:17 20:20 45:22 90:13 116:16 125:7 134:7 166:18 187:24 225:15 233:17 235:3 236:17 238:1 247:18 251:3 252:25 281:1 <b>discussions</b> 3:21 7:1 64:18 210:10 <b>disease</b> 1:3 5:10 24:11 31:14,17 32:10,15 43:11 48:24 49:6,20 50:13,23 54:10,15	54:17 55:1,6,13 55:19 56:24 57:4 58:15,24 59:23 61:4,8 64:13 67:20 68:12,14,23 69:2,11 70:5 71:14 74:12,13,17 75:1 78:19 79:17 79:24 81:10 86:9 95:10 100:22 102:22 103:14 104:15 107:6 115:7 116:13 118:4,11 127:4 128:11,20 154:4 157:15 162:17 180:17 187:8 189:1 213:8 215:1 218:15,17,22 219:7 220:25 226:12 229:7 234:14 236:14 237:2,3 238:20 239:20,22 240:19 241:2,5 242:8,9 242:14,15,21,25 244:12 246:1,2 247:20 248:6 258:24 260:21 262:1 263:22 267:3 272:6 273:16 279:23 280:4,5,20 <b>diseases</b> 1:1 3:7 5:19 6:2 31:19 32:5 33:7 109:9 244:15 267:8 <b>disfusion</b> 23:24 <b>disorder</b> 109:14 <b>disorders</b> 94:10 109:13 118:5 236:19 <b>dispense</b> 268:12 <b>displaced</b> 58:7 <b>displayed</b> 234:14 234:16 <b>disproved</b> 147:17 <b>dispute</b> 16:18 <b>disputed</b> 145:2 <b>disregulation</b> 62:2
--	---	--	---	--

<b>disrupt</b> 62:3	101:18 125:20,23	159:20 168:22	230:14,17,18	<b>drop</b> 103:18,24
<b>disrupted</b> 62:13	161:12 164:4	254:4,6,12,21,24	231:4,16,19,22	104:1,5 108:6
<b>disruption</b> 137:18	178:4 189:16	261:6 263:7,9	232:10,13,18	112:21 132:5
<b>dissected</b> 241:15	195:18 199:24	265:6 272:24	233:15 234:1,16	137:1,3
<b>dissections</b> 275:9	210:23 217:8	<b>dosing</b> 178:22	234:25 235:6,18	<b>droplet</b> 52:16
<b>disseminate</b> 82:13	219:3 260:18	<b>Doug</b> 1:20 125:9	238:3,17 239:18	232:4 269:3
126:2	275:9 278:2,19,20	145:14 243:25	239:24 240:13,16	<b>dropped</b> 71:6
<b>disseminated</b> 26:3	<b>domain</b> 15:17	265:23,25 266:22	243:16 244:10	102:8,9
<b>disseminates</b> 80:4	23:20,25 155:6,7	272:20	245:8,17 246:6,24	<b>drops</b> 107:25
<b>dissemination</b>	162:11	<b>Doug's</b> 271:4	247:7,22 248:2,12	112:19
40:16 90:22 126:1	<b>domains</b> 30:13	<b>downtown</b> 149:21	248:20,23 249:5	<b>Drs</b> 159:11
126:18 130:22	161:25 162:2	<b>Dr</b> 3:3,24 4:2 9:10	250:22 251:13,20	<b>drug</b> 1:3 3:4 54:10
<b>dissenters</b> 167:9	<b>dominant</b> 173:16	9:22 10:1 28:24	251:22 252:4,6,23	84:11,15 85:25
<b>dissimilar</b> 273:16	<b>Don</b> 137:20 138:2	29:2,11,20 30:9	252:24 253:8,18	99:6 111:13
273:23	142:12	31:7,7,8,10 46:13	253:21 255:3,12	112:11 113:3
<b>distinct</b> 28:14	<b>Dong</b> 51:14,14	46:17,21 47:8	256:4 261:2,9,15	114:2,18,21 115:3
<b>distress</b> 62:16	<b>donkeys</b> 242:19	48:12 49:7,16	261:24 262:11,20	122:11 143:12
<b>distributed</b> 73:11	<b>donor</b> 19:5	50:11 51:14 52:2	262:23,24,25	267:7
<b>distribution</b> 119:19	<b>dose</b> 27:7 72:17	52:24 53:2,23	263:1,2,19 264:22	<b>drugs</b> 66:20 83:24
260:22	82:24 89:22	56:8 64:1,1,2,8,17	264:24 265:2,22	84:6 86:2 89:25
<b>disulfide</b> 23:23	103:13,22 104:7	65:12,18,24 66:7	266:1,4,10,11,15	212:22 246:13
<b>Divalshkoff</b> 22:14	128:3,5 131:19	66:22 67:8,16	266:17,18,22,24	<b>DTRA</b> 3:12 64:2
<b>diversity</b> 37:11	132:3 133:1,21	86:20,20 87:3,7	267:2,19 268:6,25	148:4
204:22,24	134:11,12,24	87:24 88:2 90:9	270:17 271:1,2,11	<b>due</b> 23:19 61:11
<b>division</b> 3:5 142:8	135:11,22,22,24	92:1,4,6 118:19	271:17 272:10,12	62:20 119:1 168:8
142:10 227:12,14	138:11 141:9,19	118:20,21,22	272:13 273:15,25	<b>duodenum</b> 138:6
<b>DNA</b> 156:10 159:5	141:23,24 145:17	119:9 120:6,10	274:4,10,12	<b>duration</b> 134:15
159:7,9 184:5,16	158:17 164:9,9,10	121:20 122:5,6,25	275:22 276:10	135:8,14 136:1
184:20,24 185:4	165:3,7 166:6,6	124:12 125:5,9,15	277:15,17,25	139:20 142:24
188:22 189:5,7	166:10,16,20	125:18 142:20	278:22 279:7	<b>Durb</b> 47:1
198:5,6,7,18,19	167:5 177:13	143:10,20 144:10	280:12,13	<b>Durba</b> 36:8 264:9
199:16 201:22	186:9 190:8,13,15	144:17,23 145:3,5	<b>drabs</b> 94:18	<b>Durba-Watsa</b> 12:4
202:1,13,20 204:8	190:18 191:21	145:17,20,23	<b>draft</b> 3:18	36:8
204:17,19,20	205:22,25 215:15	146:1,2,10,11	<b>dramatic</b> 24:6 95:5	<b>DVC</b> 1:17 167:17
<b>DNP</b> 21:4	223:8,14 225:3	147:3,13 148:3,11	100:15 102:7	<b>DVM</b> 1:13,18
<b>doable</b> 261:16	229:5 240:23	148:13 149:5,12	105:13 106:21	<b>dwel</b> 32:8
<b>documented</b> 40:12	242:20 246:20,21	163:16,18 164:1	110:15,17 115:7	<b>dwellings</b> 50:16
41:11 102:23	247:11 254:3,8,12	164:19 165:13	157:20 191:25	<b>Dye</b> 167:21
103:3	254:16 255:4,4,7	166:3,17 167:8,14	<b>dramatically</b> 156:6	<b>dying</b> 55:11 71:14
<b>documenting</b> 69:15	255:12,17 258:21	177:1,16 179:15	189:11	81:9,14 91:5
84:13	259:10,25 260:2,4	181:5,18 182:11	<b>drastically</b> 73:22	107:11
<b>documents</b> 35:21	260:6,19 262:4,6	182:15,16,22	<b>draw</b> 115:20	<b>dysfunction</b> 240:3
<b>DoD</b> 64:2 127:20	262:9,13,19 263:4	183:8,13,19	<b>DRC</b> 45:20 53:11	<b>d'Ivoire</b> 11:16
143:18 267:20	265:4,5,24 266:5	201:16,18 202:24	53:17 55:19 67:5	<b>D-dimer</b> 64:10
<b>doing</b> 29:21 47:10	269:14 273:1	204:4,6,7 205:9	<b>dreamed</b> 68:7	<b>D-dimers</b> 62:3 66:2
47:25 68:10 72:20	281:6	205:17 206:20	<b>dribs</b> 94:17	66:4 76:23 108:11
73:1 74:1 79:2	<b>doses</b> 103:9 127:6	207:11,17,22	<b>drift</b> 258:21	108:17,18 116:3
80:9 81:14 87:22	133:21 135:6,9,10	208:4,7,13,17,19	<b>drifts</b> 256:22	239:10,25
90:14 91:21 93:15	139:4,10,10	209:18 210:3,9,15	<b>driven</b> 16:6 148:12	
96:15,21 99:5	141:22 159:3,14	228:5,10,25 230:8	229:3	

<b>e</b> 11:18	47:10,13 53:14	197:23	<b>effort</b> 43:5,8 63:21	194:22 195:2,7
<b>earlier</b> 6:11 26:12	56:1 57:15 58:18	<b>Ed</b> 1:20 50:4	141:11 201:13	196:22 197:13,14
72:5 75:17 107:25	60:12,15,18,20	227:24,25 235:2	206:4 230:23	198:11,13 199:2
110:20 118:1	61:13,15 62:10	237:24	275:4,24	208:20 278:5,21
174:17 183:18	66:18,23 68:23	<b>edema</b> 130:14	<b>efforts</b> 6:18 63:14	280:14
189:2 203:10	69:14 70:16 71:9	138:1 273:3	84:20 174:20	<b>ELISAs</b> 207:19
223:10 239:12	71:24 72:24 73:8	<b>editing</b> 22:13 24:15	<b>effusion</b> 15:19	208:11
272:23 273:25	74:15 77:7,23	24:16,19 25:3	<b>eggs</b> 163:3 200:24	<b>ELISA-based</b>
274:5	89:1 92:10,19	161:23	<b>Egyptian</b> 47:17	57:14
<b>early</b> 8:7 39:15	95:4,5 98:7 100:4	<b>Ed's</b> 245:9	<b>egypticus</b> 47:17	<b>ELISpot</b> 171:23
53:5 55:5 56:23	100:6 102:4 103:9	<b>effect</b> 16:21 24:6	<b>EID</b> 76:9	173:14 175:12
58:5 66:2,5 69:4	104:22 105:1,7,12	25:11 56:9,13,17	<b>eight</b> 27:11 102:15	176:2
69:13,18 78:6	105:22 106:6,13	81:25 84:5 90:21	103:17 172:9	<b>Elke</b> 19:21
84:17 98:4,13	106:17,21,24	93:25 112:13	193:1 198:7	<b>else's</b> 162:24
99:6 109:17 110:9	107:10 108:1,13	115:2,7 150:20	266:16	256:15
111:12 120:15	109:8,10 110:1,8	186:24 202:14	<b>either</b> 8:4 10:11	<b>eluded</b> 94:1 129:4
127:2 145:3	110:16,17,20,22	213:25 214:3	13:14 23:10 58:8	129:12 141:11
151:24 158:7	111:4 112:1,18	<b>effective</b> 28:13,15	58:11 80:13 96:20	<b>EM</b> 107:19 253:14
165:6 186:9 199:1	115:17,22 116:14	72:22 80:18 81:6	112:5 117:24	<b>emanated</b> 36:10
201:4 212:18	116:24 120:4,7,21	89:2 104:16 126:2	128:9 151:20	<b>embarrassed</b>
216:9,9 227:2	126:7,16 127:3,12	165:6 173:17	156:20 159:20	202:15
232:8,12,13 244:3	128:20 129:4,14	185:11 195:17	161:10 164:24	<b>emergency</b> 31:15
247:3 248:10	130:16,19 131:14	201:22 205:25	166:22 174:23	<b>emerging</b> 6:2
252:22	131:19 133:15,17	215:15 263:6,7,9	176:7 184:5 189:9	<b>emphasis</b> 65:6
<b>easier</b> 67:18 178:1	139:2 141:2,4	267:13 269:8	191:13 193:18	<b>emphasize</b> 150:15
202:3 240:18	144:24 145:1	270:5,6,13 271:13	198:21 212:14	151:7 173:9 175:5
259:2 271:6	151:10,13,14,21	<b>effects</b> 24:4 25:23	242:4 262:22	<b>empirical</b> 276:17
<b>easily</b> 58:2 65:11	151:23 152:24,24	82:15,20 84:10	269:3 271:20	<b>empirically</b> 128:6
65:20 84:21 127:8	152:25 153:6,9	237:3	272:3,4	209:20
259:24	155:2,10,19 158:2	<b>efficacious</b> 160:6	<b>elaborate</b> 87:1	<b>employed</b> 167:16
<b>easy</b> 57:8,10 58:19	159:8,16,17 160:8	<b>efficacy</b> 7:3,18,20	<b>elected</b> 194:23	<b>employee</b> 92:12
85:5 86:1 88:4	161:4,24 163:21	8:11,19,20 16:18	<b>electrons</b> 167:21	<b>enables</b> 190:19
97:14 98:12	163:23 165:15,18	64:23 84:14	<b>electrophoresis</b>	<b>encapsidation</b>
229:23 230:3,4	167:25 168:25	127:17 148:8	58:6	13:24
<b>eat</b> 93:4,5 148:16	169:8 170:16	150:10 158:16	<b>elegant</b> 134:7	<b>encapsulated</b> 16:2
<b>eating</b> 132:12	175:18 176:6,10	159:13,16,17,25	<b>elegantly</b> 128:14	<b>encephalitis</b> 86:14
<b>Ebihara</b> 265:10	176:12 181:6,20	160:1 161:2	<b>elevated</b> 62:8,17	152:2 270:3
<b>ebola</b> 2:21 4:21	182:1 183:15	165:11 166:9	78:18 108:22	271:22,23
11:3,14,15,16,16	184:20 185:12,14	171:5 177:18	<b>elevation</b> 136:18,20	<b>encoded</b> 85:9
11:23 12:6,23	185:22 193:22	178:17 185:22,24	<b>elevations</b> 79:9	<b>encoding</b> 184:4
13:3 14:8 18:24	198:5 204:2,13	186:7,8,23 199:12	<b>Eli</b> 113:4 114:17	<b>encouraged</b> 122:12
20:9,11,23 21:1,2	205:5 213:19	212:13 213:9	<b>elicit</b> 255:5	<b>encouraging</b> 278:5
21:11,24 22:6,6	238:6,11,24,24	216:12 217:19	<b>eliminate</b> 7:6	<b>encyto</b> 77:11
22:13,22 26:22,23	240:25 244:7	219:13 227:6	185:23	<b>ended</b> 73:5
27:6,10,11,25	252:16 253:2,19	270:7 278:17	<b>eliminating</b> 81:5	<b>endocytosis</b> 15:9
28:2,5 30:18	263:12 270:19,21	<b>efficiency</b> 47:25	192:14	<b>endoprotease</b>
31:24 33:16 37:2	274:1	<b>efficient</b> 52:16	<b>ELISA</b> 57:15 59:13	22:17
37:5,12 39:1,15	<b>Ebolas</b> 259:5	60:10 184:16	175:8,14,16,21	<b>endosomal</b> 15:19
40:21 41:1,7,7	<b>echoing</b> 54:12	269:10,13 274:19	176:5 177:6,8,10	<b>endothelial</b> 25:9
43:22 44:12 47:5	<b>EC90</b> 195:18 196:7	<b>efficiently</b> 269:19	177:10,16 178:4	107:9,13,21

118:24 119:4,11 119:16,25 <b>endothelium</b> 107:17,18,19,24 108:3,5 109:21 <b>endpoint</b> 195:8,11 195:14 196:8 197:19 214:23 277:14 <b>endpoints</b> 280:6,22 280:23 <b>ends</b> 12:12 155:15 <b>endstage</b> 273:16 <b>engineer</b> 35:11 <b>engineered</b> 159:1 <b>England</b> 40:9 <b>enhance</b> 60:8 89:23 <b>enhanced</b> 164:7 205:2 <b>enhancement</b> 214:24 234:12 <b>enhancing</b> 21:22 61:4 <b>enjoy</b> 10:9 <b>ensure</b> 182:2 <b>enter</b> 7:15 15:8 <b>enters</b> 277:10 <b>entertain</b> 63:24 <b>entire</b> 37:10 115:11 223:15 <b>entirely</b> 38:3 261:15 <b>entitled</b> 210:7 281:14 <b>entry</b> 17:25 18:11 18:12,15 19:11 60:8 63:7 155:24 155:25 217:15 <b>envelop</b> 13:6 <b>envelope</b> 205:9 <b>environment</b> 52:8 70:9 147:8 254:15 269:12 <b>environmental</b> 160:22 <b>enzymes</b> 55:8 102:8 106:22 <b>epidemiologic</b> 36:21 <b>epidemiological</b>	185:15 <b>epidemiologically</b> 268:1 <b>epidemiology</b> 2:7 52:2 53:19 147:4 212:14 244:15 269:6 <b>episode</b> 41:23 44:11 <b>episodes</b> 55:12,14 <b>epithelial</b> 117:17 <b>epitope</b> 173:23 <b>epitopes</b> 170:12 172:13 173:6,15 174:15,21 182:8 <b>epitope-specific</b> 209:9,14 <b>equal</b> 100:6 117:2 240:20 271:8 <b>equally</b> 183:3 <b>equate</b> 253:5 <b>equation</b> 64:25 272:15 <b>equations</b> 163:1 <b>equine</b> 152:2 270:2 <b>equivalent</b> 12:12 14:3,16 <b>ER</b> 16:3 <b>Eric</b> 38:9 47:11 118:15 236:3 <b>Erik</b> 227:12 238:21 240:11 <b>errors</b> 261:20 <b>especially</b> 116:5 159:6 174:20 176:20 180:4 202:10 281:10 <b>essential</b> 186:21 195:25 213:6 224:4 226:22 <b>essentially</b> 37:9 49:22 52:18 189:1 210:21 212:7,14 212:19,23 213:9 215:23 216:17 <b>establish</b> 143:23 179:9 197:12 <b>established</b> 20:15 28:10 128:7,10 212:25	<b>establishes</b> 213:4 <b>establishment</b> 28:3 65:8 <b>estimate</b> 133:24 <b>estimated</b> 230:20 <b>ethical</b> 212:13,15 <b>ethically</b> 7:4 <b>ethics</b> 9:1 <b>ethnic</b> 182:6 <b>etiology</b> 31:24 <b>Europe</b> 36:3 <b>euthanize</b> 132:8 <b>euthanized</b> 119:18 132:11 <b>evades</b> 161:20 <b>evaluate</b> 128:25 191:16 192:4 193:13 194:8 200:22 223:18 <b>evaluated</b> 54:5 167:25 169:9 170:22 189:20 197:25 198:11 204:1 <b>evaluating</b> 176:14 197:23 198:18 199:10,22 <b>evaluation</b> 7:3 200:2,21 243:17 280:19 <b>evasion</b> 87:15 <b>event</b> 4:12 15:24 23:22 24:20 207:1 231:23 251:4,5 <b>events</b> 6:9 17:9 25:21,25 36:23 54:15 63:7 <b>eventually</b> 36:18 42:3 72:8 89:11 146:22 158:8 <b>everybody</b> 67:10 77:3 82:9 86:14 142:15 235:21 236:1 <b>everybody's</b> 154:18 <b>evidence</b> 38:13 51:25 126:12,25 144:22 157:13 161:19 <b>evildoers</b> 257:4	<b>evoking</b> 257:6 <b>evolutionary</b> 24:20 <b>evolve</b> 89:8 255:23 <b>evolved</b> 8:1 <b>exacerbation</b> 157:13,16 <b>exact</b> 43:10 100:1 120:16,16 240:22 240:22 256:11 259:17 266:23 <b>exactly</b> 54:2 103:23 115:2,3 134:6 151:3 240:7 247:23 253:1 260:11 262:8 <b>examine</b> 128:17 <b>examined</b> 71:7 <b>example</b> 17:13,25 19:9,25 20:22 23:11 24:5 122:14 154:6 179:6,12 188:3,6 192:25 195:12 200:1 239:8 242:19,22 243:5,7,10,13 257:9 277:21 280:15 <b>examples</b> 84:16 149:24 270:2 <b>exceeding</b> 12:6 <b>exceedingly</b> 180:24 <b>excellent</b> 68:2 159:13 244:1 <b>exception</b> 16:10 47:7 173:20 241:19 257:15 <b>exceptions</b> 32:25 248:19 <b>excess</b> 42:18 <b>exchanged</b> 3:8 <b>excluded</b> 38:2 <b>excluding</b> 45:19 <b>exclusively</b> 103:7 241:16 <b>exist</b> 16:19 175:7 <b>existing</b> 206:6 207:16 222:2 <b>expand</b> 276:4 <b>expect</b> 63:1 76:5,22 81:13 82:25 83:2	83:6 108:6 117:4 137:3 151:17 158:22 179:18 222:13 225:10 227:3 244:16 247:19 <b>expected</b> 50:17 214:1 225:10 <b>expects</b> 53:4 <b>experience</b> 45:22 57:8 59:7 152:15 164:20 166:8 221:15 238:6 239:19,21 269:7 271:11 <b>experienced</b> 221:14 <b>experiences</b> 32:21 157:18 <b>experiment</b> 153:13 191:15 265:8 <b>experimental</b> 126:12,24 145:13 150:19 <b>experimentalist</b> 260:9 <b>experimentally</b> 159:22 <b>experiments</b> 24:3 29:22,23 65:14 150:16 155:17 164:8,20 165:25 257:15 260:18 262:14,17 <b>expert</b> 210:19 <b>expertise</b> 142:5 <b>experts</b> 210:20 218:2,9 <b>explain</b> 92:17 <b>explained</b> 52:20 <b>explains</b> 91:20 <b>explanation</b> 84:25 <b>explorations</b> 160:5 <b>explore</b> 86:2 179:22 <b>explored</b> 159:9 178:22 <b>exploring</b> 85:17 <b>export</b> 41:20 <b>exported/imported</b> 43:24
--	--	---	--	---

<b>exposed</b> 131:19 244:16 267:15	<b>extraordinarily</b> 203:4	<b>factory</b> 40:15	250:11	134:14,17,18
<b>exposure</b> 27:5 45:5 48:7,25 49:5 50:7 50:22 52:20 61:5 102:20 103:3 115:13 117:22 125:12,25 127:22 130:3 131:16,18 133:3 136:12,24 140:2,4 141:25 218:22,24 219:20 225:8,13,14 226:2 228:7,16 229:9,13 230:12,23,24 231:2 246:22 247:18 248:20 250:14,16,19 251:4,5,5,12,19 251:23 252:2,3,20 254:10 266:8 269:2 272:6 273:13 274:14 275:15,19	<b>extra-vascular</b> 107:15	<b>failed</b> 177:14 178:19	<b>fatty</b> 106:8	135:7,9,14 136:1 136:2 139:20 142:24,24 270:3
<b>exposures</b> 142:2 274:14	<b>extreme</b> 261:22	<b>fails</b> 162:16 250:17 250:17,17	<b>favor</b> 54:23 124:20 163:3 177:19 258:20	<b>fevers</b> 135:8,20
<b>express</b> 18:6 22:15	<b>extremely</b> 53:8 79:21 93:17 107:2	<b>failure</b> 62:5	<b>favorable</b> 185:2	<b>fewer</b> 175:10 255:5
<b>expressed</b> 21:3,4 61:3 153:4 174:8 184:5 207:8	<b>extrinsic</b> 111:7	<b>faint</b> 130:7	<b>favorite</b> 257:3	<b>fibrilytic</b> 108:21
<b>expresses</b> 169:14	<b>eye</b> 73:9 97:11	<b>fair</b> 44:17,18	<b>FDA</b> 1:13 3:12 8:21 54:3 64:22 143:12 143:19 148:7 181:3 186:5 210:14 212:24 215:22 216:25 218:12 222:8 229:1 232:9 242:6 243:4,19 244:2,9 249:24 250:1,15 250:22,24 251:8 255:15 276:2,15 279:11,15	<b>fibrin</b> 76:17,20 77:2 82:5,7 94:3,5 94:10 95:1,3 107:22 108:19 109:17,20 110:14 111:10,11 117:12 236:20 239:10 249:11 273:11
<b>expressing</b> 20:23 21:1 27:10 168:25 169:20 174:18 243:7	<b>e-mail</b> 3:8	<b>fairly</b> 36:12 38:2 50:14,24 69:4 131:18 133:9 135:7 136:14 140:17 175:8 181:23 190:23 221:6 227:13 258:15 269:4,10	<b>FDA's</b> 6:24	<b>field</b> 10:17,23 28:18 31:1,12 46:1,6 57:20 65:3 65:8 68:2 93:15 94:16 96:1 113:17 174:10 212:15 213:11 269:7 275:8 280:2,21
<b>expression</b> 13:2 21:7 22:4 60:17 85:5 153:5 182:5 185:1	<b>e-mailed</b> 3:18 4:10	<b>faith</b> 252:11	<b>fear</b> 268:17	<b>fields</b> 151:2
<b>extend</b> 104:1 151:24	<b>E6</b> 120:8 121:14,15 122:4 134:7 259:14	<b>faithful</b> 261:18	<b>feasibility</b> 261:3	<b>fifth</b> 172:19
<b>extended</b> 59:5 139:9	<b>E6s</b> 121:24	<b>familiar</b> 6:14 150:4 150:5,22 162:10	<b>feasible</b> 7:4 180:13 212:13 261:15	<b>fighting</b> 208:21
<b>extensive</b> 81:17 242:7 264:18	<hr/> <b>F</b> <hr/>	<b>family</b> 11:2	<b>feature</b> 76:7 161:23 <b>features</b> 91:25 118:4 236:11,20 245:25	<b>figure</b> 29:5,10 78:25 93:21 123:18
<b>extent</b> 26:7 50:2 106:14 108:9 245:25 255:21 265:18 272:16 279:25	<b>face</b> 98:9 146:17 200:16 275:6	<b>fantastic</b> 88:21 94:20 101:23 118:12 240:6	<b>fed</b> 113:24	<b>figured</b> 247:13
	<b>faced</b> 6:1	<b>far</b> 11:3 20:10 32:1 35:13 45:15 48:22 67:1 68:21 83:9 97:8 119:3 136:14 137:17 168:12 170:19 174:1 180:17,19 205:5 218:5,11 228:9 233:22 237:24 240:9 254:5 257:15 261:5 263:12 264:1 268:23	<b>Federal</b> 212:21 221:24,25	<b>figures</b> 37:22
	<b>facial</b> 97:12	<b>fatal</b> 38:17 58:12 60:16,18 61:7,14 61:23 79:24	<b>feeds</b> 111:8	<b>filamentous</b> 13:5
	<b>facilitate</b> 65:9	<b>fatalities</b> 11:25 191:23 196:22	<b>feel</b> 149:14 229:12 229:20	<b>file</b> 233:9,11
	<b>facilities</b> 30:21 39:16 40:18 41:20 63:17 221:11,17 221:20 222:11,13	<b>fatality</b> 12:5 61:1	<b>feeling</b> 87:20 178:9 231:6	<b>files</b> 168:8 227:13
	<b>facility</b> 41:9 42:2,6 42:9,9		<b>Feldmann</b> 1:15 9:23 10:1 28:24 29:11,20 30:9 31:7 46:21 65:12 65:13,24 97:4 118:22 230:25 263:23	<b>fill</b> 226:19
	<b>facing</b> 180:18		<b>fever</b> 42:15 58:21 58:22 67:18 68:8 71:15 79:15 81:12 109:11 131:7,10 131:11,12,25 132:14 133:9,13	<b>filled</b> 221:9
	<b>fact</b> 4:13,17,20 5:6 5:9 6:6 8:19 39:13 64:10 66:8 68:18 73:21 76:16 79:6 81:20 83:10 84:13 89:6 117:10 126:13 131:12 140:16 170:1 176:20 178:18 180:9 191:24 193:24 196:14 199:18 214:7 224:17 225:5 232:21 271:3			<b>filling</b> 220:24
	<b>factor</b> 56:19 80:8 82:21 107:16 109:24 110:2,3 111:1,5,6,7,9,15 111:15 112:12,12 268:2			<b>filoviridae</b> 11:2
	<b>factors</b> 30:18 239:6			<b>filoviral</b> 67:18 68:8 71:15 79:14 80:12 81:12 84:1 88:8
				<b>filovirus</b> 1:4 2:4,8,9 2:11,12,17,23 3:13 5:11 7:5 9:9 12:9 31:1 53:21 54:17 55:6,22,24 57:25 59:1 60:5 63:4,7 68:2 69:9 71:7 72:7,17 78:5 79:4 81:7 84:9

91:23 92:5,23	<b>fine</b> 11:4 233:3	<b>flood</b> 4:8	<b>formation</b> 14:17	193:18 198:7
93:15 129:3 149:8	257:17	<b>floor</b> 163:17	<b>former</b> 126:16	208:16 213:6,7
160:13 180:19	<b>finger</b> 33:23	<b>flow</b> 58:1 192:10	149:15 268:10	217:25 222:5
194:13 210:14	<b>finish</b> 63:11 183:18	194:14	<b>forming</b> 27:8	235:8 261:25
213:11 235:23	<b>finished</b> 29:24	<b>flowery</b> 9:12	253:23	262:1
236:2,5,11 238:5	<b>first</b> 9:5,21 10:2,6	<b>flu</b> 5:22 8:5 243:13	<b>formulants</b> 57:19	<b>fourth</b> 42:16
240:4 242:18	10:13 12:4 15:3	243:14	<b>formulation</b> 216:13	215:11
243:4,11	16:15 19:22 20:8	<b>fluid</b> 51:22	<b>Fort</b> 4:8 40:21	<b>frame</b> 104:25 105:1
<b>filoviruses</b> 2:6,13	20:22 21:1 22:14	<b>fluorescence</b> 19:25	<b>forth</b> 41:17 42:1	191:10
2:19 6:15 10:5,8	29:21 30:4 34:1,2	<b>fly</b> 5:2	50:9 281:3	<b>frank</b> 109:15
14:1 15:8,11	34:23 39:3 40:13	<b>flying</b> 268:13	<b>fortuitous</b> 257:11	<b>Fred</b> 263:2
18:18,20 31:19	42:13 48:15 51:22	<b>focal</b> 78:2	<b>fortunate</b> 4:25	<b>free</b> 163:21 235:11
33:16 57:7 58:3	65:16,17 68:15	<b>focus</b> 93:10 95:11	28:13 32:13 94:22	<b>freeze</b> 260:10
68:20 71:23 76:14	69:25 71:8 74:23	157:6 168:3 187:6	113:17 272:19	<b>French</b> 35:11
79:20 83:15 85:10	76:19 88:23 89:12	276:6	<b>fortunately</b> 97:2	<b>frequencies</b> 182:7
85:14,21,23 91:14	92:6 98:9 105:8	<b>focused</b> 31:16 47:6	<b>forward</b> 3:10 4:4	<b>frequency</b> 179:24
91:16 96:10 98:4	112:1 114:19	56:7 96:2 142:2	6:19 30:25 156:6	<b>frequent</b> 227:2
100:10 125:14,16	119:10 128:2	200:7 203:18	156:21 179:16	<b>frequented</b> 42:8
126:3 129:7 146:5	133:15 146:12,21	<b>focuses</b> 6:21	180:20 186:1	<b>frequently</b> 148:5
165:10 167:13	149:5,6 150:10	<b>focusing</b> 185:25	187:2 198:17,24	177:5
177:9,22 210:21	152:21 153:14	245:22	199:8 200:21	<b>FRET</b> 58:7
213:18 215:3,10	158:23 159:15	<b>folks</b> 35:20 210:22	201:16 207:1	<b>front</b> 3:19 97:14
220:19 224:5	164:8,22 172:9	228:15 232:25	208:22 211:3,14	162:15,16 217:7
237:6 244:24	177:4 184:3,7,18	268:13	211:25 212:7	244:17 252:19,21
249:23 250:8,9	188:20 189:3,13	<b>follow</b> 57:5 230:19	223:22 227:4	<b>Frontieres</b> 43:4
257:18	190:22 192:20	245:1 278:23	232:23 234:5	<b>fruit</b> 38:12 42:8
<b>final</b> 7:16 199:10	196:12 198:5,9	<b>followed</b> 6:12	263:17 281:4	46:25 89:6
212:19 215:11	201:3 203:1	34:19 170:9	<b>found</b> 24:15 26:21	<b>frustrating</b> 93:17
216:13 227:20	208:20 210:18	198:19 204:19	36:14,23 38:12	122:1
231:16	213:13 218:18	<b>following</b> 59:23	45:6 47:16 48:1	<b>fuel</b> 125:6
<b>finally</b> 5:13 31:23	235:15 262:14	64:16 227:7 267:5	51:6 54:4 58:11	<b>full</b> 8:25 14:11 16:2
94:12 212:1	<b>fit</b> 156:21 217:6	<b>followup</b> 44:19	60:22 61:22 68:22	20:5,14 52:14
<b>find</b> 27:19 37:1,11	220:14 221:5	<b>follow-up</b> 15:22	69:22 70:4 71:9	59:15 144:15
48:14,18,19 49:3	<b>fits</b> 269:6	51:15 231:16	71:21 72:4 73:18	247:2 248:7
51:10,12 76:11,17	<b>five</b> 27:19 67:9 72:1	235:21	94:2 97:20 111:5	259:23
77:1,1,18 94:6	73:3 92:9 99:15	<b>fomite</b> 166:22,25	119:3,5,18 138:6	<b>fully</b> 12:25 14:1
128:8 143:6 144:3	100:4 134:3	<b>font</b> 214:3	141:3,7 152:9,10	20:15 30:15
154:6 173:14	135:17,19,19	<b>food</b> 1:3 93:4 267:7	173:15 174:1,12	155:16 262:15
174:21 177:25	138:11 154:25	267:7	<b>founders</b> 46:3	<b>function</b> 13:12,17
209:23 217:3	165:9 169:24	<b>fools</b> 161:20	<b>four</b> 11:14 13:7,19	13:23 14:9 15:10
221:1 236:6	170:12 172:16	<b>force</b> 250:22,24	17:19 20:1 22:22	15:15 23:7,8 25:3
242:16 252:20	176:5 224:16	<b>forces</b> 44:24 250:15	24:7 27:11 41:1	25:8,13 29:8,13
262:7 265:12	226:8 268:16	<b>forefront</b> 5:19	47:20 70:4 71:2	82:1,21 193:10
279:19 280:5,6	<b>fix</b> 57:21 171:8	<b>foreign</b> 169:7	71:14 72:1 101:15	194:22 275:23
<b>finding</b> 38:11 76:1	191:11	<b>foresee</b> 214:11	104:24 120:9	277:7 279:3
119:16 182:8	<b>fixed</b> 57:19	<b>forest</b> 42:18 45:6	125:3,3 135:19	<b>functional</b> 2:16
217:2	<b>flanking</b> 19:24	<b>forgot</b> 58:21	155:2 165:19,23	30:13 62:15 149:4
<b>findings</b> 56:18 72:3	<b>flashing</b> 33:2	<b>form</b> 5:22 89:9	169:10 170:18	171:24 177:20
72:6 130:12 217:1	<b>flex</b> 113:24	<b>formal</b> 186:4	172:19 173:8	187:24,25 188:2,4
218:21	<b>flip</b> 121:16	<b>formally</b> 128:10	188:23 190:1	194:9 222:24,25

276:23,25 277:14 278:9 <b>functionality</b> 192:8 193:17,24 279:5 <b>functionally</b> 194:20 <b>functioning</b> 108:9 239:5 <b>functions</b> 23:1,5 194:9 <b>funding</b> 163:6 267:22 <b>funny</b> 52:19 <b>furin</b> 22:8,16,16 23:13 24:3,12 <b>furin-like</b> 22:9 <b>further</b> 3:23 11:11 18:21 19:13 20:4 21:12 27:22 31:5 61:21 85:25 171:4 178:21 192:18 <b>fuse</b> 23:25 <b>fusion</b> 15:6,6,21 23:20 155:6 <b>future</b> 16:22 25:17 65:11 97:25 217:1 240:8 <b>fuzzy</b> 253:6	<b>gate</b> 192:15 <b>geared</b> 267:24 <b>Geisbert</b> 1:16 38:20 51:17 55:4 64:11 68:4 76:4 84:12 90:13 92:4 92:6 118:20,21 119:9 120:10 121:20 122:5,25 124:12 189:23 223:13 235:16,18 238:17 239:24 240:16 247:7,22 248:2 249:5 253:8 261:24 262:20,24 263:1,19 264:22 266:1,10 268:6 270:17 271:2 274:4,10 <b>gene</b> 12:10,15,16 12:20 19:25 22:4 22:7 30:3 100:24 142:6 151:8,15 161:23 164:2,3,4 174:20 178:15 199:2 203:11 207:8 227:16 <b>genera</b> 11:2 <b>general</b> 36:13 143:5 149:23 150:22,24 151:6 154:1 156:24 161:6 182:7 235:8 242:13 245:10 249:22 <b>generalities</b> 149:24 <b>generalizeable</b> 196:15 <b>generally</b> 32:1 37:4 146:6 150:3 153:11 214:24 248:22 258:5 264:19 <b>generate</b> 80:22 122:23 186:18 188:10,23 197:22 203:24 205:1,2 244:1 276:20 <b>generated</b> 23:13 25:4 189:6 200:7	229:11 <b>generates</b> 23:14 191:3 <b>generating</b> 7:19 18:5 24:22 <b>generation</b> 20:19 69:25 184:3,7 189:12 198:9 <b>generations</b> 70:12 <b>genes</b> 18:5 152:1,7 153:1,6 169:1 184:4,5,8,17 200:12 <b>genetic</b> 13:19 18:24 20:5 23:12 37:9 37:11 187:13,16 203:21 <b>genetics</b> 19:20 58:4 85:22 88:16 157:22 261:12,19 <b>genome</b> 12:10 13:7 13:24 14:10 16:2 18:4,8,24 19:23 20:10 30:14 96:7 261:19 <b>genomes</b> 259:19 <b>genomic</b> 12:18 19:2 20:7 <b>genotype</b> 86:23 <b>genotypic</b> 257:23 <b>GenPhar</b> 51:14 <b>genus</b> 11:10 <b>geographic</b> 97:21 <b>George</b> 148:3 <b>Germany</b> 34:7 <b>getting</b> 6:3 9:25 65:7 68:17 153:4 162:15 179:10,19 209:16 222:8 223:23 226:15 246:6 252:24 261:5 276:23 279:22 <b>GFB</b> 21:3 <b>GFP</b> 21:1 <b>girlfriend</b> 35:2,4 <b>give</b> 3:25 5:5 31:17 39:4 75:4 83:23 83:24 84:24 104:15 118:2	134:1 149:9 183:4 188:2 215:21 225:3 253:23 265:6 <b>given</b> 10:5 42:10 43:10 50:3 57:11 76:18 157:17 170:23 211:8 217:12 227:21 252:17 281:2 <b>gives</b> 57:23 193:2 196:6 204:22 <b>giving</b> 4:6 35:17 36:5,15 41:10 44:4 84:16 89:22 164:23,23 195:12 231:20 <b>glad</b> 9:22 94:12 115:8 144:23 273:7 <b>glands</b> 51:6 <b>global</b> 160:23 <b>GLP</b> 216:12 221:20 221:22,23 222:6 222:13,13,17 <b>glucose</b> 62:12 <b>glycoprotein</b> 12:13 13:3,13 15:5 16:6 17:10 18:4,6,8,25 19:6 22:5,8,16,24 151:18 152:10 153:21 160:14,14 161:4,18,22 162:8 169:9 170:9 173:7 174:4 175:10,18 178:2 181:7 184:10 198:22 203:11 205:8 <b>glycoproteins</b> 16:3 22:23 <b>glycosylated</b> 162:2 <b>glycosylation</b> 154:17 155:9 161:22 <b>go</b> 7:19 8:7 11:20 16:3,4,15 17:12 17:19,22 20:4 21:8 22:24 24:17 30:4 44:6 53:18 54:20 55:23 57:21	75:5 77:10 88:17 99:9 101:5 105:18 114:21 116:18 125:3 127:22 129:22 142:17,22 144:25 148:1,16 168:21 172:11 182:13 183:4,25 184:8 187:2 191:1 193:19 198:2 204:10 211:20 213:10 218:11 220:6,20 233:14 235:14 244:24 246:8 251:12 255:22 256:1 259:15 260:4,9 263:17 264:24 266:13,18 269:16 269:19 275:7 280:5 281:4 <b>goal</b> 33:5 125:1 150:9 181:18 216:16 220:4 222:24 275:25 279:13 <b>goals</b> 127:16 245:25 264:21 276:6 <b>goes</b> 8:3,9 35:24 73:20 76:21 116:15 132:2 133:22 150:19 169:12 248:6 272:1 <b>going</b> 4:22 8:16 9:6 9:6 26:21 31:11 32:18 33:22,24 34:3 54:12 63:20 63:21 64:24 67:17 68:10,15 70:23 75:4,5 76:19 78:21,22 80:1,24 81:8 82:1 90:12 92:13,17 93:10 95:11 96:13 99:17 99:22 101:5,6 102:16 105:1 107:23 108:25 116:17,21 118:22
<b>G</b>				
<b>Gabon</b> 38:10 42:12 43:23 44:1 45:3 47:11 55:14 60:15 236:2 <b>Gabonese</b> 45:18 <b>gain</b> 183:23 <b>Galveston</b> 73:12 <b>gamma</b> 60:18 85:13 171:24 172:2 175:12 176:1 193:8 <b>gaps</b> 221:8,8 226:18,19 <b>garb</b> 275:4 <b>Gary</b> 1:19 15:3 56:8 64:2 184:18 235:24 241:18 <b>gastroduodenal</b> 109:6 <b>gastrointestinal</b> 130:10				

119:25 121:15 125:15,19 127:25 129:15,20 130:9 137:23 140:6,22 147:25 148:1,8 149:9,23 150:6 156:18 159:4,5,18 163:2 165:7,14 166:8,17 167:11 168:2,21 180:24 181:22 182:12 187:7 200:23 202:19 203:15 209:24 211:4,11 211:14,15,22 212:1,8,8 213:9 219:2 220:4,4 225:4,15,16 227:24 230:16,25 231:6,10 232:3 233:11,16 234:9 234:17,19,23 235:8,9,10,15,16 235:19 236:1 238:18 244:1,14 245:5,6 246:9 247:2 248:9,16 250:22,24,25 251:8,9,9 252:9 252:10,11,14,15 252:16,17,18,21 255:22 256:2 257:7 260:23 262:7,10 265:5,9 266:6,11 267:21 267:22 269:19,24 277:24 278:9 279:2,10 <b>gold</b> 36:10 190:12 256:14,14 <b>Golgi</b> 16:3 <b>gonad</b> 138:22 <b>Gonzalez</b> 236:4 <b>good</b> 10:1 21:17 32:17 41:8 42:3 53:8,24 59:22 64:3,5 67:4 68:21 71:17 77:17 79:15 82:23 86:17 92:6 94:11 129:21	135:7 150:7 152:9 152:11 159:24 166:19 169:23 173:2,4,16,19 177:21 182:8 183:17,20 184:11 184:13 198:16 205:6,6 206:23 208:20,22 209:12 210:4 218:8 222:2 239:17 243:24 252:10 255:16 260:3 263:2 265:23 271:15 280:15,17 281:1 281:10 <b>goodies</b> 67:11 <b>google</b> 236:5 <b>gorillas</b> 42:19 51:19 <b>gotten</b> 232:2 238:22,22 246:17 <b>government</b> 37:23 63:14 163:8 <b>GP</b> 13:14 23:15 27:10,10 56:6 152:17,19,21,25 153:10 174:14 181:4 185:12,16 185:21 191:10 196:19,19,22 198:10,16 199:2,3 243:7,7 <b>GPs</b> 185:25 <b>GP-1</b> 155:6 <b>GP-2</b> 155:5 <b>GP1</b> 22:17,19 23:15 56:9 <b>GP12</b> 13:14 22:22 <b>GP2</b> 22:17,17 23:15,21 56:21 <b>Grabon</b> 94:23 <b>gradually</b> 6:4 216:10 <b>grain</b> 138:18 <b>gram</b> 106:17 <b>grand</b> 36:5 <b>granulocytes</b> 78:8 <b>grayer</b> 97:11 <b>great</b> 3:15 42:8	43:5 62:15 64:20 81:3 107:4 149:17 163:14 168:21 195:3 219:22 220:2 228:10 <b>greater</b> 156:8 170:19 <b>greatest</b> 56:7 <b>greatly</b> 39:25 <b>green</b> 19:25 95:16 98:5,16 128:17 136:9,11 138:1,23 172:15 227:10 241:10 248:25 249:3,7,19 <b>greens</b> 92:24 93:11 95:12 98:2,23 100:8 114:11 118:7 130:5,11,14 131:6 132:1,9,18 133:8,12 135:21 136:8,18,25 137:10,17 138:11 138:16 139:7,13 139:20,21 140:11 144:1 236:22 249:11 <b>grew</b> 142:4 <b>grind</b> 138:20 <b>gross</b> 130:12 139:22 221:4 <b>grossly</b> 272:25 <b>ground</b> 6:25 <b>group</b> 10:19 11:13 15:3 20:8,25 23:13 34:16 38:10 56:8 68:5 79:2 96:4 99:17 101:16 102:16 104:10 112:2,4 114:23,25 120:7,15 132:24 145:15,21,22 153:17 170:18 171:11 184:18 193:6 233:2 255:19 <b>groups</b> 33:10 39:12 99:18 100:2 101:12,14 120:13 132:25 170:13,25	182:6 184:15,18 196:13 255:23 <b>grow</b> 23:16 73:10 121:18 259:25 <b>grows</b> 23:17 <b>guarded</b> 275:7 <b>guess</b> 4:7 34:19,23 47:1 50:2 143:19 166:22 167:5 202:5,17 225:14 236:7,15 237:24 238:3 241:3 245:22 250:24 260:25 264:9,16 266:23 267:6,9,16 270:10,13 271:13 271:17 273:13 274:25 <b>guide</b> 152:21 <b>guided</b> 256:8 <b>guinea</b> 14:24 64:9 67:24 68:25 69:3 69:4,21,23 70:15 70:18,21,25 72:21 73:2 74:9 75:8,22 76:5,13,25 78:6 78:17 79:5,8,10 80:17 83:8 84:3 84:22 94:7 124:2 126:14 127:4,7,9 127:24 141:6,16 142:7 145:7 152:5 152:13,16,18 153:10 158:3,14 161:3 175:9,22 184:19 201:20 202:3 236:12,22 237:8 253:19 258:4 259:1 265:17 <b>Gulu</b> 44:16 51:9 55:14 59:6,16 100:11 198:13 238:25 248:20 <b>guts</b> 74:14 <b>guys</b> 65:1 93:2 137:22 217:8 230:2 262:4 269:23 274:25 <b>gycloprotein</b> 184:4	185:12 <b>gycosylation</b> 17:11 <b>G2a</b> 171:7 <hr/> <b>H</b> <hr/> <b>habit</b> 5:6 <b>half</b> 44:12 53:5 98:18 108:24 113:8 125:4 209:1 210:5 237:14 247:25 <b>half-life</b> 113:16 <b>hallmarks</b> 19:19 <b>hamadryad</b> 92:25 <b>hamsters</b> 69:19 <b>hand</b> 31:1 103:6 235:1 254:11 275:11 <b>handful</b> 221:17 249:15 <b>handfuls</b> 94:18 <b>handled</b> 214:10 <b>handling</b> 57:19 <b>hands</b> 47:12 180:22 269:5 275:14 <b>handsdown</b> 249:19 <b>Hans</b> 20:7 <b>happen</b> 81:1,2 95:6 109:1 143:19 147:1 260:23 265:21 275:17 <b>happened</b> 42:7 111:25 119:2 233:7 256:23 268:15 <b>happening</b> 27:23 70:7 72:11 78:25 88:6 90:24 110:24 226:23 280:13 <b>happens</b> 69:12 72:15 76:6 86:6 88:8 91:23 94:14 105:6 109:5,18,18 110:19 169:5 180:21,22 209:24 219:15 234:13 238:5 <b>happy</b> 28:12 118:2 201:13 261:21 <b>hard</b> 58:17 59:8
---	---	---	---	--

77:1,25 134:2 150:11,13 153:3 153:24 162:4 225:21 229:24 243:1 259:21 260:20 261:7 <b>harder</b> 48:17 155:16 160:18 260:15 266:4 <b>harm</b> 162:19 <b>Hart</b> 1:17 167:10 167:14 177:16 179:15 181:18 182:15,22 183:8 <b>Hartings</b> 141:18 <b>Harvard</b> 199:25 <b>harvest</b> 34:9 <b>hasten</b> 45:23 <b>hate</b> 229:1 <b>Hawkins-Reed</b> 53:16 <b>HCD</b> 124:8 <b>head</b> 269:23 <b>heads</b> 180:22 <b>health</b> 1:11 11:22 144:15 <b>healthcare</b> 146:7 146:22,23 <b>healthy</b> 70:3 102:11 <b>hear</b> 17:7 55:4 151:25 179:14 218:9,10 226:9 239:18 263:8 <b>heard</b> 30:23 47:2 67:20 68:24 81:16 144:14 167:24 211:18 212:18 215:19 219:10,20 223:12 225:23 226:6 227:23,25 234:1 263:11 267:8,20 268:1 274:13 279:19,24 280:9 <b>heart</b> 62:9 <b>Heather</b> 142:8 <b>heavily</b> 151:16 162:2 <b>heavy</b> 97:14 161:22	<b>Heinz</b> 1:15 9:22 29:25 31:11 38:19 46:18 53:25 54:13 56:4,16 60:5 65:12 67:19 79:15 84:17 85:19 97:4 115:8 116:20 118:21 123:5,17 228:18 230:25 235:19,20,22,25 235:25 241:21 263:22 270:18,23 <b>Heinz's</b> 53:7 75:17 <b>held</b> 196:20 <b>help</b> 167:19 204:23 211:3 221:9 225:19 277:11,20 279:23 280:2 <b>helped</b> 141:19 142:6 <b>helpful</b> 17:22 20:24 180:12 223:24 225:14 278:24 <b>helpless</b> 84:8 <b>hemoconcentrati...</b> 78:19 <b>Hemoglobin</b> 78:20 <b>hemorrhage</b> 81:12 81:12,17 109:15 138:5 <b>hemorrhagic</b> 67:18 68:8 71:15 79:15 81:21 109:10 <b>Henschal</b> 227:12 <b>Hensley</b> 68:5 88:12 92:15 <b>hepatic</b> 78:3 <b>hepatocytes</b> 75:23 81:8 106:20 239:5 <b>herring</b> 49:18 <b>heterogenous</b> 258:17 259:7 <b>hexon</b> 200:7,8,9 <b>HHS</b> 6:11 8:1 <b>Hi</b> 210:15 <b>hide</b> 49:13,22 <b>Hideki</b> 265:10 <b>high</b> 12:1 20:18 27:7 30:20 32:6 44:23 48:18,25	62:6 75:13 76:23 79:22 82:25 91:6 102:8 103:9 105:10 128:4 131:19 133:1 135:2 136:14 138:11 141:9 145:17 151:19 153:15 155:4 159:3,14,20,20 165:3 166:6,20 171:13 177:13 184:25 189:12 198:12,15 201:24 203:12 205:22 242:20 248:22 250:2 252:12 259:25 262:8 263:4,7,9 269:14 278:7 <b>higher</b> 60:12 61:24 61:25 66:9 78:16 89:22 106:14 121:16,22 132:3 134:11 135:10,11 135:22,24 136:11 139:10 153:16 156:17 164:11 165:7 179:13 208:25 209:1 222:7 254:12 258:21 260:1 263:5 265:6 266:14 270:9 272:3 <b>highest</b> 38:23 59:4 152:6 155:22 <b>highlight</b> 6:6,13 7:24 31:25 <b>highlighted</b> 8:8 169:25 <b>highlights</b> 5:6 7:25 186:21 <b>highly</b> 12:17 21:15 25:19 26:5 62:16 73:25 83:15 129:5 129:8 206:25 268:22 <b>histo</b> 94:20 <b>histochemistry</b>	50:1 <b>histologically</b> 273:9 <b>histopathology</b> 75:15 221:4 <b>historical</b> 33:17 116:8,9 184:1 185:8 187:15 196:21 240:24 <b>historically</b> 34:2,24 92:22 94:13 100:16 128:3 187:10 <b>history</b> 100:24 101:3 121:4,23 122:1 125:21 126:1 131:9 226:11 243:18,24 253:10,10 <b>hit</b> 210:16 259:16 262:13,21 <b>hits</b> 47:15 <b>HIV</b> 96:1 113:17 166:9 199:16 200:12 204:12,14 205:4,7,18 272:14 <b>HLA-B</b> 60:19,22 <b>hold</b> 59:20 209:3 272:5 277:17 <b>holding</b> 59:18 144:7 <b>holes</b> 77:13 <b>homeostasis</b> 62:2 <b>homogenate</b> 70:2 73:4 121:12 <b>homologue</b> 151:4 <b>honest</b> 10:20 214:11 229:21 253:9 <b>hope</b> 5:13 10:8 21:14 25:4 218:7 277:12 <b>hopefully</b> 17:16 55:19 67:4 139:25 166:12 211:25 214:16 238:19 <b>horse</b> 248:13 <b>hospital</b> 35:16 44:18 <b>hospitalized</b> 36:4 <b>hospitals</b> 43:1	<b>host</b> 2:9 14:22 25:13,13,16 29:7 29:9 53:20 55:21 58:20 63:4,8 70:14 81:3 89:2,5 89:12 91:14,20 124:21 <b>hot</b> 109:12 149:13 <b>Hotchkiss</b> 76:8 <b>hour</b> 148:18 210:5 247:25 276:11 <b>hours</b> 4:20 53:6 112:4 134:15,15 135:15 136:1 171:1 228:21,22 <b>House</b> 9:1 <b>hovering</b> 272:9 <b>huge</b> 108:12 122:7 141:11 178:25 237:2 254:22,25 266:12 <b>Huggins</b> 71:9 98:13 <b>human</b> 2:9 7:3,7 32:10,15 33:15 38:23 41:19 51:22 53:20 54:14 55:5 55:5 56:13,15 58:25 63:5 64:6 66:3,8 68:25 69:10 72:16 78:4 79:23 88:14 91:16 94:13,15 95:10 96:5 98:18 102:22 107:5 108:1,25 112:25 116:6 118:11 119:8 122:3 128:19 140:4,6 143:8,22 144:2,13 160:5 168:16 176:17 180:17 181:1 184:6,7 186:6,7 186:11,16 198:3 199:1,5,5 204:18 206:21 212:13,25 216:14 218:17,20 218:21,24 219:5 222:23 224:8,25 225:3,6 229:15 234:13,19 236:14
--	--	--	--	--



<b>increase</b> 25:10 26:13 33:6 62:6 137:2,5 258:1 274:2,2,7	49:3,4,9,11 50:19 50:22 52:4 227:22 267:11,15	70:20 71:1,7 72:13,17 74:10,20 74:22 76:12 80:14 80:15 83:2,17,21 84:2,7 85:2 88:8 91:6,16 92:5,11 100:8 103:11 105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	254:1 259:20	120:25 185:6 188:23 189:20 198:19 270:20
<b>increased</b> 47:24 60:14 65:6 78:20 78:20 83:5 106:23 204:23,24,25 273:11	<b>Indonesian</b> 96:21 97:5,6 241:21	80:15 83:2,17,21 84:2,7 85:2 88:8 91:6,16 92:5,11 100:8 103:11 105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>infects</b> 67:3 75:23 75:24 110:1	<b>injections</b> 102:24 102:25 103:1 113:10 198:6 199:21
<b>increases</b> 51:1 81:10 108:16 110:12	<b>induce</b> 61:6 83:24 93:23 122:16,24 179:6 182:8 205:16,19	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>inflammation</b> 162:8	<b>injury</b> 82:14 107:9
<b>incredible</b> 68:3 105:16	<b>induced</b> 122:10 174:2 175:8,17,21 177:6,25 215:1	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>inflammatory</b> 25:8 25:24 26:6 79:8 80:8 82:18 162:15	<b>inkling</b> 189:13
<b>incubation</b> 103:2,4 103:11 218:18	<b>induces</b> 257:11	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>influences</b> 174:8	<b>innate</b> 162:14
<b>incumbent</b> 63:18 63:22	<b>inducing</b> 176:4 201:23	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>influencing</b> 258:22	<b>innoculum</b> 146:8
<b>IND</b> 212:2,5 216:1 232:25 233:6	<b>induction</b> 14:5 87:11	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>influenza</b> 147:20	<b>inoculated</b> 84:8 120:8
<b>index</b> 40:15	<b>industry</b> 226:21	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>inform</b> 235:21	<b>inoculation</b> 74:13 86:13 130:1,17,21 273:20
<b>Indian</b> 95:23	<b>ineffective</b> 72:20	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>informal</b> 232:17	<b>input</b> 142:6 255:25 281:5
<b>Indians</b> 96:2	<b>infect</b> 23:18 27:24 51:18 85:22 89:3 121:19 242:19	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>information</b> 9:20 18:24 19:2 30:2 35:19 45:24 53:15 55:7,15,20 64:8 66:22,23 88:22 145:12 156:19 212:9 215:12,14 219:10 220:14 238:20 263:8	<b>inscriptional</b> 12:16
<b>indicate</b> 59:3 174:7 181:1 219:6	<b>infected</b> 25:13 34:9 35:4,15 36:25 41:9 44:2 46:11 52:21 60:21 62:14 62:16 75:8,21 76:14 78:4 79:4 81:9,24 82:14,16 87:9 90:25 91:4 92:20 102:4 105:25 107:13 111:4 112:1 114:14,19 115:17 116:23 117:5 119:11,16 120:1 126:9 129:3 131:14 140:4 146:13,19,21 215:8 223:7 229:5 272:22	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>inform</b> 235:21	<b>insert</b> 113:20 222:16 226:3
<b>indicated</b> 242:9	<b>infected</b> 25:13 34:9 35:4,15 36:25 41:9 44:2 46:11 52:21 60:21 62:14 62:16 75:8,21 76:14 78:4 79:4 81:9,24 82:14,16 87:9 90:25 91:4 92:20 102:4 105:25 107:13 111:4 112:1 114:14,19 115:17 116:23 117:5 119:11,16 120:1 126:9 129:3 131:14 140:4 146:13,19,21 215:8 223:7 229:5 272:22	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>informal</b> 232:17	<b>inserts</b> 199:2
<b>indicates</b> 172:23	<b>infected</b> 25:13 34:9 35:4,15 36:25 41:9 44:2 46:11 52:21 60:21 62:14 62:16 75:8,21 76:14 78:4 79:4 81:9,24 82:14,16 87:9 90:25 91:4 92:20 102:4 105:25 107:13 111:4 112:1 114:14,19 115:17 116:23 117:5 119:11,16 120:1 126:9 129:3 131:14 140:4 146:13,19,21 215:8 223:7 229:5 272:22	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>information</b> 9:20 18:24 19:2 30:2 35:19 45:24 53:15 55:7,15,20 64:8 66:22,23 88:22 145:12 156:19 212:9 215:12,14 219:10 220:14 238:20 263:8	<b>inside</b> 121:18
<b>indicating</b> 176:8	<b>infected</b> 25:13 34:9 35:4,15 36:25 41:9 44:2 46:11 52:21 60:21 62:14 62:16 75:8,21 76:14 78:4 79:4 81:9,24 82:14,16 87:9 90:25 91:4 92:20 102:4 105:25 107:13 111:4 112:1 114:14,19 115:17 116:23 117:5 119:11,16 120:1 126:9 129:3 131:14 140:4 146:13,19,21 215:8 223:7 229:5 272:22	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>inform</b> 235:21	<b>insight</b> 104:16
<b>indication</b> 48:23 57:24 148:10,12 220:4,7 225:12,16 228:19 230:15 232:6 243:20 251:2,13,24 276:1 276:5	<b>infected</b> 25:13 34:9 35:4,15 36:25 41:9 44:2 46:11 52:21 60:21 62:14 62:16 75:8,21 76:14 78:4 79:4 81:9,24 82:14,16 87:9 90:25 91:4 92:20 102:4 105:25 107:13 111:4 112:1 114:14,19 115:17 116:23 117:5 119:11,16 120:1 126:9 129:3 131:14 140:4 146:13,19,21 215:8 223:7 229:5 272:22	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>inform</b> 235:21	<b>insights</b> 55:12
<b>indicator</b> 54:5	<b>infected</b> 25:13 34:9 35:4,15 36:25 41:9 44:2 46:11 52:21 60:21 62:14 62:16 75:8,21 76:14 78:4 79:4 81:9,24 82:14,16 87:9 90:25 91:4 92:20 102:4 105:25 107:13 111:4 112:1 114:14,19 115:17 116:23 117:5 119:11,16 120:1 126:9 129:3 131:14 140:4 146:13,19,21 215:8 223:7 229:5 272:22	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>inform</b> 235:21	<b>instance</b> 35:7,18 47:18 52:12 93:9 248:21
<b>indices</b> 175:25	<b>infected</b> 25:13 34:9 35:4,15 36:25 41:9 44:2 46:11 52:21 60:21 62:14 62:16 75:8,21 76:14 78:4 79:4 81:9,24 82:14,16 87:9 90:25 91:4 92:20 102:4 105:25 107:13 111:4 112:1 114:14,19 115:17 116:23 117:5 119:11,16 120:1 126:9 129:3 131:14 140:4 146:13,19,21 215:8 223:7 229:5 272:22	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>inform</b> 235:21	<b>instances</b> 39:14 126:11
<b>indirect</b> 82:15	<b>infected</b> 25:13 34:9 35:4,15 36:25 41:9 44:2 46:11 52:21 60:21 62:14 62:16 75:8,21 76:14 78:4 79:4 81:9,24 82:14,16 87:9 90:25 91:4 92:20 102:4 105:25 107:13 111:4 112:1 114:14,19 115:17 116:23 117:5 119:11,16 120:1 126:9 129:3 131:14 140:4 146:13,19,21 215:8 223:7 229:5 272:22	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>inform</b> 235:21	<b>Institut</b> 42:22
<b>individual</b> 35:15 37:3,10 42:20 45:18 52:19 53:17 119:10 152:7 165:24 207:23 212:2 233:10 281:3	<b>infected</b> 25:13 34:9 35:4,15 36:25 41:9 44:2 46:11 52:21 60:21 62:14 62:16 75:8,21 76:14 78:4 79:4 81:9,24 82:14,16 87:9 90:25 91:4 92:20 102:4 105:25 107:13 111:4 112:1 114:14,19 115:17 116:23 117:5 119:11,16 120:1 126:9 129:3 131:14 140:4 146:13,19,21 215:8 223:7 229:5 272:22	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>inform</b> 235:21	<b>INSTITUTE</b> 1:1
<b>individually</b> 45:4 172:18	<b>infected</b> 25:13 34:9 35:4,15 36:25 41:9 44:2 46:11 52:21 60:21 62:14 62:16 75:8,21 76:14 78:4 79:4 81:9,24 82:14,16 87:9 90:25 91:4 92:20 102:4 105:25 107:13 111:4 112:1 114:14,19 115:17 116:23 117:5 119:11,16 120:1 126:9 129:3 131:14 140:4 146:13,19,21 215:8 223:7 229:5 272:22	105:2,19 107:20 108:4 109:21 117:1 125:11 126:14,25 128:5 128:23 130:4,23 131:23 132:1,6 133:24 134:14 135:13 138:4 139:8,9,11 143:21 143:25 144:12 145:6,25 147:16 187:8 190:6,9,16 191:16 199:16 209:22 225:17 238:5 240:4 242:16,18 247:3 247:20,25 248:14 254:7,18 255:1 269:1,10 273:18 274:15 275:1	<b>inform</b> 235:21	<b>Institutes</b> 1:10
<b>individuals</b> 32:22 34:9,16 35:5 36:15 43:10 48:19	<b>infected</b> 25:13 34:9 35:4,15 36:25 41:9 44:2 46:11 52:21 60:21 62:14 62:16 75:8,21 76:14 78:4 79:4 81:9,24 82:14,			

190:24 191:19 <b>interchangeable</b> 97:23 241:10 245:19 <b>interchangeably</b> 240:17 241:6 <b>interest</b> 8:25 169:7 169:14 207:8,14 220:22 276:11 <b>interested</b> 74:4 104:11 148:6 220:23 <b>interesting</b> 4:10 36:20 37:8,12 76:15 92:2 93:1 109:16 117:3 125:5 148:14 226:7 240:1 274:16 <b>interestingly</b> 78:16 105:24 115:1 173:22 237:20 <b>interfere</b> 16:12 17:16 <b>interference</b> 165:21 <b>interferes</b> 85:11 <b>interfering</b> 14:21 16:14 17:5,13 123:20 152:3 <b>interferon</b> 14:4,6 14:19,20,21 16:25 17:1 56:19 60:18 71:22,24 72:10,12 72:14,19,22 80:2 80:2 82:12 83:16 83:22,23,24,25 84:2 85:3,9,11,12 85:13,15,18 87:10 87:11,13 89:15,18 89:20,22,23 90:2 90:4,5,11,14,16 90:17,18,19,21 91:2,4,7,8,9,19,21 93:23 124:3,6,7 124:10,15,16 171:24 172:2 175:12 176:1 182:20 183:7 <b>internal</b> 253:23	<b>internally</b> 208:7 <b>interpret</b> 101:9 215:5 <b>interpretation</b> 222:18 <b>interpreted</b> 261:2 <b>interrogate</b> 209:21 <b>interrogating</b> 193:14 <b>intervention</b> 28:10 54:8 219:14 <b>interventions</b> 104:16 <b>inter-nasally</b> 206:18 270:24 <b>Inter-vascular</b> 26:20 <b>intra</b> 74:12,25 86:12 <b>intramuscular</b> 74:23 99:21,23 102:20 117:21 271:7 <b>intravenous</b> 74:13 <b>intravenously</b> 74:25 <b>intrinsic</b> 111:9 <b>introduce</b> 3:24 9:5 10:8 17:8 31:8,12 261:20 <b>introducing</b> 31:20 37:3 <b>introduction</b> 2:3,6 10:5 31:17 52:20 53:24 79:16 150:24 <b>introductions</b> 9:13 <b>intubating</b> 44:3 <b>investigate</b> 28:9 <b>investigated</b> 39:7 44:16 <b>investigation</b> 32:11 32:16 38:6 39:10 45:2 47:10 48:2 <b>investigations</b> 32:13 40:7 46:1,6 <b>investigator</b> 42:17 <b>investigators</b> 256:7 280:21 <b>invitation</b> 10:3	<b>inviting</b> 92:7 125:19 <b>involve</b> 158:10 <b>involved</b> 12:24 13:24 14:19,23 15:1,17,20 16:7 31:14 38:16 40:25 41:24 44:3 47:23 50:2,8 105:12 106:8,12 110:22 115:11 169:19 179:10 227:13 233:4,10 239:4 <b>involvement</b> 277:11 <b>involves</b> 63:12 186:5 <b>involving</b> 14:5 35:10 40:16 210:11 <b>IP</b> 246:25 247:1,9 250:14 265:6 273:13 <b>irrelevant</b> 91:16 192:14 202:8 <b>Isabella</b> 201:8 <b>isolate</b> 54:21 69:10 69:24 74:19 100:13,17,20 120:16,17,19 121:8 129:16 140:13 240:25 <b>isolated</b> 88:14 <b>isolates</b> 36:22 56:3 101:1 105:4 120:4 120:14 121:5 151:14 155:1 <b>isolation</b> 49:24 138:15 141:14 <b>isolations</b> 264:20 <b>isotype</b> 171:9 <b>isotypes</b> 171:6 202:2,2,4 <b>issue</b> 5:3,13,23 18:17 29:13 41:17 46:22 64:21 93:4 118:22 128:14 143:4,5,12,13 160:22 162:9,10 165:9,15 178:7	205:13 206:23 207:2 209:5 212:2 215:3,10 231:5 234:20 251:21 265:17 268:8 270:11 275:18 279:17 <b>issued</b> 42:1 63:14 212:19 <b>issues</b> 18:20 19:12 19:17 49:18 132:6 135:18 147:13 149:13 161:24 164:7 208:11 210:11 212:6,10 218:14 226:24 234:6 240:12 255:13 275:11 <b>Italy</b> 41:25 42:1 <b>ITEM</b> 2:2 <b>iterative</b> 186:10 189:17 <b>IV</b> 113:14,16 265:6 <b>Ivory</b> 56:2 67:4 170:17 <b>i.e</b> 213:21 <b>I/Phase</b> 232:24	101:23 107:4 <b>Johannesburg</b> 35:1 43:25 <b>John</b> 24:9 51:14 71:9,18 96:21 98:12 167:21 <b>Johnson</b> 100:24 <b>join</b> 167:17 <b>joined</b> 104:10 <b>Jon</b> 169:2 178:15 <b>Journal</b> 97:17 104:19 <b>judgment</b> 187:4 <b>jump</b> 151:10 165:2 <b>junction</b> 109:6 <b>jurkat</b> 19:6,8 <b>justification</b> 243:20 279:14 <b>justify</b> 218:12,25 220:17 250:20 <b>Justin</b> 141:18
<hr/> <b>K</b> <hr/>				
<b>Kamrud</b> 169:2 <b>Kaplan-Meyer</b> 190:11 197:12 <b>Karen</b> 53:16 <b>karyorexis</b> 77:19 <b>Kate</b> 1:17 164:3 177:1 178:24 182:11 185:17 190:25 <b>Kate's</b> 183:20 <b>Katie</b> 115:14 123:11 <b>Kawaoka</b> 235:22 <b>Kawaoka's</b> 261:24 <b>Kay</b> 167:10 <b>keep</b> 10:23 21:17 25:3 27:21 73:4 95:20 99:12 103:8 157:16 160:19 265:20 278:14 <b>keeping</b> 182:3 183:11 <b>Keith</b> 142:8 <b>Kelly</b> 73:14 74:6 159:24 259:1 <b>Kelly's</b> 79:2 <b>Kenya</b> 35:12				
<hr/> <b>J</b> <hr/>				
<b>Jack</b> 18:23 <b>jacket</b> 113:22 <b>jackets</b> 114:6,13 <b>Jackson</b> 117:23 <b>Jahrling</b> 1:17 4:6 9:11 41:4 53:1,2 64:1,17 67:8 68:5 86:20 92:1,15,25 118:19 122:6 125:5 144:23 145:4 146:1 147:13 148:13 189:23 <b>Jane</b> 167:20 <b>Jean-Paul</b> 236:3 <b>Jeff</b> 142:12 <b>Jevad</b> 163:19 <b>JIV</b> 100:16 <b>Joan</b> 96:14 142:4 201:5 <b>job</b> 10:18 41:8				

<b>kicked</b> 266:20	<b>know</b> 5:8 9:10 10:8 10:10 15:15,17 16:17,19 20:10 21:20 23:4 25:15 28:24 30:3,5,7,11 30:13,25 32:10,12 32:20 33:15 46:23 46:25 49:11,21 50:1,5 53:3,3,8 64:4,19 65:6,20 68:7,12,13 69:8 71:16 75:6 77:12 78:23 79:15,16 81:17 82:6,21 83:9 84:18,24 86:15 87:17 88:3 88:5,12 90:16,19 91:1,12,15,24 94:15 99:4 100:23 101:3,10,18 104:15,23 105:13 105:18 106:2 108:3 118:14 119:3 123:2,5,6,7 123:8,20 124:20 128:12 131:24 132:17 138:23 139:17 140:6 142:21 143:15,17 143:19,24 145:9,9 145:10 146:4,8,12 146:16,19,20,23 147:4,6,7,8,12 150:25 151:6,8 152:23 159:4 160:1 161:8,12 162:22,22 163:4 177:23 183:21 189:4 197:2 200:25 207:6 211:7,19 219:22 220:3 228:17 229:22 231:24 237:25 241:24 242:2 244:14 247:17 248:5,11 249:15 250:7 251:18 253:12,14 257:5,13,17 258:4 259:19 260:12	262:6,12 264:2,3 264:6,16 266:1 268:4,10,21,23 270:16 273:19 274:8 275:2 276:10,16 <b>knowing</b> 222:11 <b>knowledge</b> 31:2 33:6,19 166:2 <b>known</b> 11:2 31:12 94:14 120:21 128:9 129:7 165:21 245:14 248:20 257:3 261:6 281:2 <b>Koboka's</b> 23:13 <b>Kortepeter</b> 146:2,2 146:11 <b>Ksaizek</b> 1:18 100:13 125:10 <b>Ksiazek</b> 31:9,10 46:13,17 47:8 48:12 49:16 50:11 52:2 94:11 230:24 266:22 267:2 268:25 271:1,11 272:10,13 273:15 274:12 <b>Kurilla</b> 1:19 3:24 4:2	269:12 <b>labs</b> 17:21 20:13 101:10 175:6,7 228:14 <b>lack</b> 18:3 23:15 25:12 40:18,18 79:12 144:13,18 163:21 194:4 195:11 <b>Lackemeyer</b> 141:13 <b>lacking</b> 30:2 261:6 <b>Lake</b> 11:9 <b>Lancet</b> 111:16 115:15 <b>landslide</b> 97:8 <b>language</b> 211:10 212:23 214:25 222:8 <b>large</b> 33:21 38:2 42:25 50:24 55:15 56:6 73:10 108:16 122:8 126:3 136:6 206:6 221:13 240:24 269:4 273:3 <b>largely</b> 32:5,20 33:3 39:12 68:4 70:13 <b>larger</b> 278:20 <b>largest</b> 37:14 <b>Larol</b> 118:15 <b>lastly</b> 201:12 <b>late</b> 8:3 53:3 54:16 60:1 67:9 87:11 119:1 170:23 171:3 235:20 271:24 276:11 <b>latecomers</b> 149:14 <b>lately</b> 239:20 <b>Laughter</b> 154:22 163:15 <b>Lawrence</b> 150:20 <b>lay</b> 230:4 <b>LDH</b> 78:18 <b>LD-50</b> 166:20 <b>LD-50s</b> 247:5 <b>LD50</b> 101:19 127:7 133:16,17,24 134:10,25 141:2,3	253:18,19,22,25 259:12,20 <b>LD50s</b> 128:6 <b>LD99</b> 128:6 <b>lead</b> 39:17 149:24 163:2 197:8 200:20 256:23 <b>leader</b> 19:24 <b>leading</b> 54:15 195:4 <b>leads</b> 26:11 62:23 82:24 <b>lead-in</b> 183:20 <b>leak</b> 78:22 <b>leakage</b> 26:1 <b>leap</b> 252:11 <b>learn</b> 210:22 211:1 211:24 226:12 <b>learned</b> 12:1 204:17 <b>learning</b> 216:24 <b>learnt</b> 225:23 <b>leave</b> 16:16 23:8 156:18 238:4 <b>leaves</b> 163:12 <b>leaving</b> 5:1 <b>led</b> 37:20 50:19 69:2 158:13 159:24 185:8 223:11 <b>left</b> 19:15 44:1 108:13 116:17 154:20 167:17 169:21 170:13 182:23 <b>leg</b> 41:12 <b>legal</b> 251:21 <b>Legino</b> 42:22 <b>legs</b> 102:7 <b>length</b> 14:11 16:2 20:14 <b>Leroy</b> 47:11 236:3 <b>Leroy's</b> 38:10 <b>lesion</b> 109:5 <b>lesions</b> 257:25 <b>lesser</b> 106:22 <b>lesson</b> 70:18 <b>lethal</b> 70:5,25 73:6 75:14 83:20 89:19 100:7 104:21
		<b>L</b>		
	<b>L</b> 153:2			
<b>lab</b> 18:23 24:2 40:8 45:19 46:11 55:17 73:13 95:20 101:22 115:15 158:8 201:8 228:12,16 229:17 247:15 249:12 <b>label</b> 222:16 245:5 251:8,12,14,22,24 276:5 <b>labeled</b> 251:23 <b>laboratories</b> 56:14 256:20 <b>laboratory</b> 1:16 28:19 32:13,23 52:8 66:25 101:11 156:16 222:2				

112:7 117:1 127:4 247:4 250:10 254:7,18 257:19 258:24 264:1,16 268:22 271:19 <b>lethality</b> 38:22 190:7 257:19 <b>letters</b> 268:15,16 <b>letting</b> 132:8 <b>let's</b> 5:4 149:22 212:7 220:20 223:4 228:20,21 233:15 246:15,18 256:1 258:23 264:24 266:16,18 272:8 <b>Leukopenia</b> 61:10 <b>level</b> 27:15 52:16 61:24 75:11 76:23 90:23,25 91:6 95:20 113:13 152:9 181:14 221:18 222:8 248:14 278:7,8 <b>levels</b> 27:14 58:14 62:6 90:18 106:23 108:23 112:19,23 113:1,7 116:3 184:25 <b>Libreville</b> 236:2 <b>license</b> 230:23 <b>licensed</b> 115:3 160:19 224:3 231:13 <b>licensing</b> 179:16 <b>licensure</b> 127:18 148:7 176:17 180:19 186:3 187:3 199:7 215:24 230:12 <b>life</b> 15:8 69:13 113:9 226:11 <b>light</b> 43:21 98:11 107:17 130:6 140:1 <b>liked</b> 135:3 <b>likelihood</b> 213:5 224:24 <b>likewise</b> 188:11 255:5	<b>Lily</b> 113:4 <b>Lily's</b> 114:18 <b>limit</b> 30:7 153:22 265:24 266:8 <b>limitations</b> 32:18 <b>limited</b> 24:4 32:21 33:9 45:11 55:6 78:24 87:12 127:15 152:15 154:7 254:15 <b>limits</b> 33:7 113:1 134:5 <b>line</b> 15:13 23:3 78:23 86:7 111:9 113:16,21 114:21 114:22 116:12 131:14 136:9 152:5 196:19 226:13 <b>lineage</b> 192:17 <b>linear</b> 170:15 <b>linked</b> 60:19 154:17 188:15 <b>lip</b> 102:6 117:18 <b>Lisa</b> 68:4 88:12 92:15 95:18 99:14 104:10,11 105:2 107:15 110:23 112:17 114:13 142:6 236:25 239:8 240:21 241:16 271:5 <b>Lisa's</b> 104:19 124:13 <b>list</b> 6:5 13:16 64:5 175:2,4,14 220:22 220:23 <b>listed</b> 3:21 55:13 <b>listen</b> 218:3 226:9 <b>listening</b> 230:9 <b>litany</b> 244:4 <b>literature</b> 15:16 16:13 66:11 98:21 101:8,9 128:8 150:5,6 158:4 168:5 175:2 209:11 218:3 256:9 <b>little</b> 4:5 7:21 10:6 10:9 18:21 19:13	26:7 28:1 37:5,6 37:19 38:15 39:1 41:21 42:5 43:2 44:21 47:9 48:14 48:17,21 53:25 55:7 59:2,6 66:10 66:23 67:1 68:13 76:17 79:5,11 87:10 88:23 92:17 94:14 95:8 97:6 97:12 99:17 100:7 102:19 105:11,24 106:7,24 110:19 110:20,21 114:11 116:25 117:15,15 121:11 122:1,9 127:25 129:15 131:22 135:4,14 135:16 137:15 138:18 140:6 141:1 152:11,14 155:16 156:18 163:12 164:8,11 168:15 178:1 179:19 180:2 182:13 188:2 192:7 194:19 196:4 217:14 238:4 240:18 241:4,8 244:3 249:8 265:5,17 268:7 269:15 270:1,9 271:9 273:12 280:8 <b>live</b> 10:25 18:16 19:17 156:12 157:1,19 160:12 160:17,18 161:5,5 <b>liver</b> 55:8 62:8,9,12 62:14 70:1 75:23 77:23 102:8 105:10,11 106:7 106:12,22 110:21 117:13 238:10 239:3 272:1 273:4 <b>livers</b> 73:3 <b>lives</b> 217:5 <b>living</b> 55:11 <b>load</b> 58:9 116:6 124:21 125:1,2	138:25 <b>loading</b> 183:16 <b>loads</b> 58:12 <b>local</b> 90:22 236:4 <b>locally</b> 90:20 <b>location</b> 13:12 23:20 36:16 <b>locations</b> 33:1 <b>lock</b> 24:8 <b>locks</b> 24:7 <b>locus</b> 60:22 <b>log</b> 116:10 176:5 208:25,25 266:16 <b>logic</b> 259:23 <b>logical</b> 58:15 <b>logics</b> 260:25 <b>logistical</b> 43:6 <b>logistically</b> 32:2 <b>logistics</b> 44:18 232:15 <b>logs</b> 103:24 116:15 125:4 179:13 190:16 237:15 266:13,15 <b>long</b> 15:18 29:1 34:20 36:12 37:17 38:15 58:3 59:21 66:14 94:16 109:25 125:25 126:4 157:14 162:10 186:21,24 206:10 211:20 221:19 233:14 243:14 244:24 248:9 266:21 268:9 281:9 <b>longer</b> 17:21 37:6 39:1 100:7 116:25 135:22 139:14 224:18 241:3 260:5 277:18 <b>longest</b> 136:1 <b>longstanding</b> 5:12 <b>long-term</b> 46:4 <b>look</b> 4:4 6:1,14 12:14 17:2,8,14 17:22 19:22 20:3 27:16 31:6 33:17 34:1 44:19 50:15 54:2 55:21 57:22	60:20,21 61:20 65:14,15,21 66:1 67:5 75:1 76:10 76:17,25 77:1,17 88:18 94:6,22 97:7,9 98:10,21 101:7,8 102:13 105:8 108:13 110:6 115:23 121:6 131:21 135:20 136:3,21 137:13 145:11 150:8 151:16 152:1,4,25 155:18 155:22 163:11 168:11 173:13 179:25 180:3,11 183:4 190:4,5,19 191:7,21,24 192:4 192:17 193:5,16 195:13 196:6 210:16 218:3 220:17 237:10,12 239:24 240:14,19 240:23 241:4,11 242:24 250:8 253:25 254:20 256:9 259:5 263:15,18 265:8 265:10 268:15 278:12 280:4,22 <b>looked</b> 10:6 15:23 24:14 29:12 43:13 49:25 60:4 64:15 96:24 97:3 116:19 119:5 122:22 126:16,19 127:12 127:14,25 130:16 144:14 147:5 153:1,2 156:15 158:25 169:21 170:7 180:6 182:18,25 190:22 196:12,20 200:15 234:10 238:8 239:8,9 249:16 261:11 271:6 <b>looking</b> 3:10 12:4,7 15:7 17:25 30:8 54:8 55:8 57:5,16
---	---	--	---	---

61:22,24 71:14 77:4,12 79:3,14 91:13,15,22 95:22 97:25 119:22,24 124:23 132:16 140:11 145:12 150:1,2,9 152:7 152:21 154:2,3,12 154:23 171:17,18 171:23 172:2,10 172:13 175:14,18 177:24 179:16 189:3 191:15 192:2,7,24 193:1 193:23 194:1,6,9 194:21,22 208:11 212:10 214:8,13 226:2,16,18 227:18 229:24 236:9,10 245:23 246:3 255:8 270:19 279:3 280:1,23	110:6 111:3,10 113:18 116:16 117:3 118:9 119:9 123:1,4,25 125:6 130:24 131:9 132:9,18 135:24 136:5 138:19 141:13,19 142:1 147:19,25 149:17 152:15 154:15,15 156:8 158:14 159:5,12 162:3 166:24 184:2 185:7,9 195:23 202:17 210:23 211:18 222:20 223:3 228:12 229:2 230:2 239:9 240:12 243:17,17 243:18,24 245:13 247:11 252:10 253:8 259:22 263:10,12 266:20 267:9 268:7,9 269:12,16,18 271:4 272:1,13 279:24	<b>lungs</b> 51:23 130:13 140:16,17,19 272:25 <b>luxury</b> 57:23 68:1 <b>lymph</b> 77:8 105:9 107:14 130:24 140:20 273:4 <b>lymphocyte</b> 76:6 76:11 78:11 83:7 106:1 136:23 191:12 <b>lymphocytes</b> 61:11 76:1 77:9,15,18 78:9 80:11,16 82:20 105:23,24 106:3 136:16,22 <b>lymphocytosis</b> 77:19 <b>lymphoid</b> 117:12 117:14 <b>lymphopenia</b> 105:23 <b>lympocytes</b> 80:24 110:11 <b>lytic</b> 173:2,25 209:15 279:3	133:11 134:24 138:6,8 139:15 143:25 153:15 158:20 175:9,24 189:24 237:12,20 240:2,10 249:9,13 249:18 264:6 274:5 <b>macrophages</b> 110:1 <b>macrophage</b> 79:24 82:11 88:3,10 105:9 121:21 <b>macrophages</b> 26:17 75:16,17,20 77:7,14 79:21 80:5,6 81:24 82:16 83:2 87:1 87:21,25 88:4,14 88:18 105:21 110:25 <b>macular</b> 98:8 102:5 117:12,13 <b>mad</b> 21:14 154:20 <b>magazine</b> 40:22 <b>magnitude</b> 204:25 <b>main</b> 1:9 13:1,23 25:21 94:2 279:20 280:25 <b>maintain</b> 24:20 116:4 165:11 <b>maintained</b> 116:5 <b>maintenance</b> 29:7 29:9 91:14,20 <b>major</b> 11:24 26:15 53:15 80:3,10 82:11 84:5 107:5 109:22 138:24 214:25 215:6,7 <b>majority</b> 80:16 81:3 182:9 238:16 <b>makers</b> 163:8 <b>making</b> 4:7 65:1 91:9,10,10 205:16 206:25 226:17 239:6 <b>manage</b> 5:4 162:25 269:22,24 <b>manageable</b> 193:15 <b>manner</b> 39:6 <b>manufacture</b>	157:12 <b>manufacturer</b> 160:9 <b>manufacturing</b> 34:8 41:24 <b>manuscript</b> 74:2 <b>manuscripts</b> 149:18 <b>map</b> 40:21 181:5 <b>mapped</b> 170:12 173:9 <b>Marburg</b> 11:2,10 11:10,24 12:2,22 13:4 14:9 18:24 20:9,10 22:6,24 27:6,10,18 28:2,5 33:16 34:1,6,21 35:6,14,20 37:4 39:2 45:8,18 46:23 47:5,9,15 49:21 56:3 57:16 58:18 61:13 66:18 67:2 69:1,10,22 71:5,23 72:5 73:2 73:18 74:19 92:10 92:20 94:23 95:4 95:5 98:3,7 100:14,25 104:2,3 104:8,20 105:1,4 105:5,11,15,22 106:6,8,10,16,21 106:24 108:14 110:5,5,8,14,17 110:19,22 111:4 115:18,21 116:24 117:5,13 123:10 126:16 127:3 128:21 129:6 137:23 140:7,12 141:5 151:10,13 151:14,21,23 152:16 153:14 154:11,12,24 155:4,19 159:6 160:8 163:22 164:20,20 165:16 165:17 168:1 175:9,14,16 176:6 176:11 181:20 203:23 213:19
<b>looks</b> 130:20 136:14 161:18 169:12 186:12 233:3 237:14 240:9 263:23 <b>loops</b> 200:8,9 <b>lose</b> 21:6 <b>losing</b> 80:15 <b>loss</b> 82:19 105:22 110:10 257:25 <b>lost</b> 37:18 80:23 217:5 <b>lot</b> 4:7,17 5:8 13:11 16:17 20:20 24:3 25:2 48:23 51:24 54:12 62:8 64:8 64:19 67:18,20 76:16,20 77:24 81:7,8,14 83:7 85:15 87:8,18 88:21 90:13 92:16 93:15,21 94:3,5 95:4,13 96:9 99:5 99:6,8,11 100:16 101:10,21 102:1 102:17 106:8,19 107:23 109:8,9	<b>lots</b> 83:3 196:16 <b>Louise</b> 266:10 <b>love</b> 239:18 <b>low</b> 11:25 12:3 101:1 113:1,7 116:6 120:7 128:9 133:21 134:10 139:4 162:4 166:21 191:20 201:23 258:15,16 260:4,7,19 <b>lower</b> 58:14 116:3 135:6,9 139:10 147:20 151:19 152:5 240:17 255:4 261:21 <b>lowering</b> 125:1 <b>lucky</b> 80:24 <b>lunch</b> 74:7 125:7,8 142:16 148:2,15 <b>luncheon</b> 148:19 <b>lung</b> 117:5,16 271:21	<b>M</b> <b>macaque</b> 95:17,19 96:7,10 102:3,21 103:1 104:3,21 106:4 108:2,13,15 108:17 109:3,16 110:7,9 111:18 116:14 118:6,8,13 120:17 127:13 131:14,21 135:13 236:21 248:5 263:25 <b>macaques</b> 27:9 41:18 92:22 93:5 93:10 94:6 95:22 96:12 97:19,22 98:1,20 101:17 103:7 111:19 114:10 118:3 119:24 120:21 124:16 128:22 129:24 130:11,13 130:15 131:4	<b>maintain</b> 24:20 116:4 165:11 <b>maintained</b> 116:5 <b>maintenance</b> 29:7 29:9 91:14,20 <b>major</b> 11:24 26:15 53:15 80:3,10 82:11 84:5 107:5 109:22 138:24 214:25 215:6,7 <b>majority</b> 80:16 81:3 182:9 238:16 <b>makers</b> 163:8 <b>making</b> 4:7 65:1 91:9,10,10 205:16 206:25 226:17 239:6 <b>manage</b> 5:4 162:25 269:22,24 <b>manageable</b> 193:15 <b>manner</b> 39:6 <b>manufacture</b>	

238:8,9 239:3 243:7 244:6 257:9 257:12 263:12,20 264:7,14 270:23 271:23 272:2,22 273:6,8,14 <b>Marburgs</b> 165:19 259:6,7 <b>March</b> 236:3 <b>marched</b> 172:6 <b>marginal</b> 77:8,12 94:5 95:1 <b>marginally</b> 199:17 <b>margins</b> 53:9 <b>Maria</b> 201:8 <b>Mario</b> 192:9 <b>mark</b> 1:13 146:2 210:14 234:25 242:6 252:4 256:10 257:16 278:7 <b>marked</b> 34:21 <b>marker</b> 20:23 65:16 66:6 <b>markers</b> 62:5 65:21 192:15,17 220:25 <b>market</b> 163:7 <b>marketing</b> 244:23 <b>marry</b> 239:14 <b>Marsocci</b> 100:17 <b>MARTIN</b> 1:15 <b>Marty</b> 9:6,7 <b>marvelously</b> 259:14 <b>Mary</b> 1:17 164:3 167:10 177:1 178:24 182:11 183:20 185:17 190:25 <b>Maryland</b> 1:11,22 149:7,21 <b>mask</b> 144:16 <b>masked</b> 162:1 <b>massive</b> 58:17 61:7 66:13 77:14 106:4 122:10 181:22 <b>master</b> 233:9,11 <b>match</b> 242:25 245:13	<b>materials</b> 54:21 <b>matrix</b> 12:13 13:9 14:16 172:3 <b>Matt</b> 141:13,18 <b>matter</b> 67:13 72:18 104:9 210:7 247:11 262:17 267:19 281:14 <b>Matthew</b> 248:20 <b>mature</b> 106:2 <b>matures</b> 255:14 <b>Mauritius</b> 96:21 97:9,10,19 242:1 <b>maximum</b> 134:20 <b>Mayanga</b> 120:19 129:8 <b>mays</b> 225:24 <b>MCC</b> 97:20 <b>MCP-1</b> 79:9 110:13 <b>mean</b> 30:23,23 46:19 47:8 49:16 50:7 59:11 68:1,2 87:24 88:13 100:2 100:18 102:14 103:1,4,10,17 104:4 119:23 121:6,20 123:18 134:13 135:11,25 136:17 139:13 142:22 145:5 147:14 152:18 157:8,8 178:13,20 196:22 211:6 215:25 217:2 223:12 224:22,25 226:7 229:1,24 230:24 233:13 238:24 239:24 240:17,21 246:7 246:25 248:2 250:23 251:17 253:10,13 255:15 259:17 264:9,12 264:17 267:16,25 268:7 269:25 270:1,16 273:21 274:23 279:11 <b>meaning</b> 25:16 <b>meaningful</b> 65:5	<b>means</b> 52:3 91:7 167:4 186:15 213:16 215:7 224:6 233:4 253:1 269:10,11 270:7 <b>meant</b> 54:2 <b>measles</b> 45:12 147:20 157:17 160:19 <b>measure</b> 28:20 54:9 83:5 91:2,3 188:8,13 190:24 191:4,13 194:14 195:14 198:12 253:22 259:20 260:9,10,10,14,16 <b>measured</b> 54:5 63:5 176:5,9 194:17 215:18 255:11 <b>measurements</b> 127:8 <b>measures</b> 269:8 <b>measuring</b> 176:7 195:7,8 259:19,20 274:11 <b>mechanically</b> 52:8 <b>mechanism</b> 80:10 89:22 90:5 109:23 113:11 123:7 186:22 188:3,7,12 188:18 203:5 209:19,25 212:5 213:14 278:24 <b>mechanisms</b> 8:4 28:7,15 168:18 174:2 188:17 277:19 <b>mechanistic</b> 278:10 278:15 <b>Med</b> 142:8 <b>Medecines</b> 43:4 <b>medial</b> 273:2 <b>mediastinal</b> 130:24 137:25 140:20 <b>mediate</b> 15:10 <b>mediated</b> 15:9 232:5 <b>mediates</b> 15:6 <b>mediators</b> 26:11	82:16 83:4 <b>medical</b> 1:24 36:15 39:16 40:1,4,17 43:25 48:25 52:13 52:18 149:7,21 165:15 280:21 <b>medium</b> 27:14 45:4 <b>medulla</b> 109:19 <b>meet</b> 205:24 213:24 215:9 216:25 242:23 <b>meeting</b> 3:9,10,11 3:15,17 4:5 9:14 9:16,16,19 31:4 31:20 218:7,8 226:8 227:19 232:16 235:22 255:16,19 <b>meetings</b> 124:15 218:4 232:20,21 235:23 <b>MEK</b> 26:17 <b>melt</b> 81:19 <b>member</b> 235:10 <b>members</b> 38:5 46:4 235:4,11 238:2 281:11 <b>membrane</b> 13:9,10 14:18 16:6 22:18 111:2,3 <b>membranes</b> 111:5 <b>memory</b> 192:18,19 192:22 193:23 <b>memory/effector</b> 192:6 <b>mention</b> 26:25 80:1 125:9 244:21 <b>mentioned</b> 16:8 49:10 69:22 74:8 82:17 84:17 85:8 85:20 87:16 91:2 100:14 128:4,13 132:7 146:3 232:8 240:21 263:11 <b>met</b> 1:9 213:1 <b>Metaanalysis</b> 163:10 <b>metalloprotease</b> 22:20 <b>method</b> 58:6	168:17 195:16 <b>methods</b> 193:13 194:3 <b>MHC</b> 97:18 169:22 182:5 <b>mice</b> 21:10 67:24 69:6,7 71:4,7,10 71:20,22 72:6,21 72:24 73:1,3,4,6 73:17,19,24 74:1 74:10,18 75:8,22 76:1,13 77:1 78:6 78:16,23 79:1,4,8 80:17 83:8,10,14 83:22,23 84:21 85:5,18,19 86:3,5 86:13 87:8 89:1 89:17,18 91:2 94:8 124:6 127:25 152:25 153:7,7,9 153:9 169:24,25 170:2,6,21,24 171:20 173:1,1,4 173:22 174:2,18 174:22 175:8 180:4 183:9 184:19 224:22 237:8 258:5 259:2 265:6,15 <b>Michael</b> 3:24 <b>Mick</b> 22:14 <b>microabrasions</b> 275:13 <b>Microbiology</b> 1:15 3:6,6 149:20 <b>microphone</b> 53:1 159:25 163:19 205:7 208:15 <b>microphones</b> 29:3 <b>microscopically</b> 77:4 <b>microscopy</b> 76:17 <b>micro-circulation</b> 62:4 <b>micro-particles</b> 111:2 <b>middle</b> 149:13 151:9 152:20 154:15 <b>Mike</b> 1:13,19 4:11
---	--	---	--	---

4:19 53:8 88:20 92:1,16 93:12,22 94:1 105:19,23 106:22 108:12 109:9 111:13 123:24 126:20 168:19 237:12 253:14 258:6 <b>mikes</b> 76:20 201:17 <b>mild</b> 58:15 69:2 98:15 130:14 140:17 <b>mildly</b> 69:23 <b>milligrams</b> 198:7 <b>mimic</b> 18:10,12 21:23 218:21 225:10 247:19 <b>mind</b> 10:23 21:17 25:5 27:21 80:21 89:16 103:8 182:3 251:6 265:21 277:18 278:14 <b>minds</b> 157:17 211:13 <b>mine</b> 36:10,25 38:7 82:10 <b>miner</b> 36:24 <b>miners</b> 38:16 <b>mines</b> 38:8 50:9 <b>minigenome</b> 19:21 20:16 <b>minigenomes</b> 21:23 <b>minimal</b> 158:19 <b>minimize</b> 256:20 <b>minimum</b> 165:18 205:25 206:9 214:14 <b>minus</b> 22:22 59:8 <b>minute</b> 80:1 113:15 116:17 137:24 183:17 195:6 245:9 <b>minutes</b> 27:9,22 57:9 67:9 112:3 113:9 114:21 115:18 129:12 163:13 219:24 228:8,15 251:2,14 251:14 <b>MIP-1</b> 193:8	<b>miscalculation</b> 262:14 <b>misimpression</b> 156:24 <b>misinterpreted</b> 224:6 <b>misleading</b> 137:15 <b>misnomer</b> 279:6 <b>missed</b> 65:13 175:4 217:21 260:23 <b>missing</b> 53:7 <b>mission</b> 40:11 44:18 221:10 <b>mistake</b> 262:20 <b>misunderstandin...</b> 224:2 <b>misuse</b> 20:21 <b>miter</b> 259:24 <b>mitigate</b> 157:22 237:3 <b>mix</b> 165:11,20 <b>mixed</b> 45:11 70:8 <b>mixture</b> 165:23 <b>ml</b> 153:25 260:1 <b>mls</b> 266:7 <b>mobilization</b> 78:8 <b>mocking</b> 163:19 <b>modalities</b> 16:22 <b>modality</b> 27:5 <b>mode</b> 51:19 <b>model</b> 1:4 2:12 3:13 7:18 8:9,16 8:18 14:24,25 25:1,19,21 26:12 26:19 32:17 45:25 55:3 64:6 69:4 70:22 71:17 77:3 82:23 86:6,11,15 87:19,22 89:1,17 92:5 98:6,24 100:6 102:21 103:1,13,21 104:21 105:15 110:9 111:18 112:7,18 117:1,10 117:11 118:10 119:11 123:25 124:9 127:13 128:18,19 133:19 143:15 148:9	168:12,15,16,16 168:20 176:13 179:4 186:2,19 187:1 190:5 203:11,14,17,19 214:5 215:7 216:5 218:12,15,21,24 219:11,13 220:17 221:14 226:14 233:9 234:15 236:8 237:6 242:14,17,17,23 242:24 243:5,5,8 243:9 246:5,21 249:1,4 250:5 261:4 265:4 271:5 <b>modeled</b> 96:2 <b>modeling</b> 30:12 231:24 <b>models</b> 2:5,11,23 5:25 8:12,12,19 9:9 14:24 28:11 32:14,19 40:8 51:18 55:2 56:20 57:21 63:6,10 64:16,20 67:17,23 68:11,16,17,18,21 69:13,20 74:4 75:14 81:1 82:4 83:13,25 85:17 86:8,16,17 92:10 92:18 93:13 96:3 104:12 105:14,20 106:5 107:6 108:2 108:13,15,17 109:4,16 110:7,21 112:11 115:24 116:19 118:3,6,14 126:21 127:17,24 128:9,11 142:2 166:15 176:7 179:9 180:2 184:17 186:6,8 203:1,3,4,7 205:22 210:13 211:23 212:11 213:3,8,9 214:15 216:9 217:24 233:20 234:4,18 236:12,21 238:23	246:4 248:5 250:9 251:7 263:25 268:3 270:4 <b>moderate</b> 260:2 273:21 <b>modifications</b> 85:21 <b>molecular</b> 58:9 108:7 <b>molecule</b> 56:10 <b>moment</b> 4:5 30:24 42:11 92:12 100:23 164:16,18 170:3 174:6 176:22 186:15 187:23,25 <b>monitor</b> 57:3 58:22 129:1 131:8 223:20 <b>monkey</b> 49:12,22 50:2 66:9 92:21 98:11 113:22 117:6 119:11 120:23 121:1 143:16 159:13,16 159:17 160:1 167:7 196:2,2 204:18 220:22 225:4 228:2 234:11 239:15 242:12 256:12 <b>monkeys</b> 35:13 67:3 95:8 96:22 98:5 100:1,1,21 103:14,15,16 112:1 113:19 114:5 116:12,24 118:24 124:2 138:1 143:6 153:12 158:15,20 159:6,8,18 160:7 161:3 164:23 166:1 202:13,17 202:20 206:7 208:24 209:1 220:20 224:22 234:8 238:5,9 242:8,18,22 247:10 253:16 256:17 259:21	261:25 264:21 270:20,22,24 271:8 <b>monoclonal</b> 57:3 84:23 170:10 171:12 <b>monocytes</b> 26:17 105:21 110:1,25 192:15 <b>mononegavirales</b> 11:1 12:9 16:11 <b>month</b> 3:7 13:18 158:17 166:9 <b>months</b> 31:23 52:23 76:9 201:1 206:3,8,11 232:17 <b>monumental</b> 201:12 <b>morbidity</b> 214:25 215:6,7 244:13 <b>morning</b> 10:1,4 53:4,6 81:11 92:6 95:7 132:22 148:15 168:19 235:20 238:18 239:25 241:13,25 245:23 247:8 267:20 <b>morning's</b> 214:18 <b>mortalities</b> 44:7 <b>mortality</b> 35:6,17 36:5 37:25 38:17 38:19,23 42:19 44:4 48:6 100:2 100:12 102:25 103:4,10 121:7 244:13 264:15 272:7 <b>mortem</b> 119:2 <b>Moshkoff</b> 22:20 <b>motif</b> 56:22 <b>mounted</b> 36:19 <b>mouse</b> 14:24 69:11 71:21,25 72:9 73:8 74:8,15,21 77:6,6 83:19,25 85:23 86:11,22 87:9 123:25 168:12,20 169:21 174:13,16 200:11
--	--	---	--	---

203:11 204:18 236:12 237:11 253:15 265:4,16 <b>mouse-adapted</b> 77:23 <b>move</b> 52:25 61:16 92:3 103:19 104:5 134:23 156:21 180:8 186:2 194:16 198:24 207:1 211:3,14,25 212:7 214:9 227:4 234:5 256:1 263:21 277:12 <b>moved</b> 156:5,8 164:12,21 167:19 196:15 203:13 245:11 <b>moving</b> 53:18 54:14 58:20 155:1 164:16 171:14 175:15 188:19 198:17 199:8 200:21 223:22 232:23 <b>MPH</b> 1:13 <b>MSF</b> 43:4 <b>mucin</b> 56:9 <b>mucosa</b> 138:5 <b>mucosal</b> 50:25 254:14 269:2 270:12,15 275:19 <b>multiple</b> 158:23 164:25 178:5,6 181:21 182:2,10 209:22 233:24 277:6 <b>multiplication</b> 146:23 <b>multivalency</b> 165:9 <b>multivalent</b> 149:11 <b>multi-focal</b> 78:2 <b>mumps</b> 160:19 <b>murine</b> 89:17 90:6 176:13 <b>muscle</b> 187:17,17 <b>muscles</b> 62:10 <b>Musoke</b> 106:12 123:12,13,14 141:3 151:22,23	153:14 154:12 164:21 <b>mutant</b> 23:14 <b>mutation</b> 265:12 <b>mysterious</b> 66:14 <b>mystery</b> 42:5 <b>M.D</b> 1:13,15,19,19 <hr/> <b>N</b> <b>N</b> 52:14 138:19 139:17 154:14 161:25 <b>Nabel</b> 1:19 149:5 159:11 163:16 166:3 167:8 177:1 182:11,16 183:13 201:16 204:4,7 205:9 210:3,9 228:5 230:8,17 231:22 233:15 234:25 235:24 <b>Nabel's</b> 15:3 56:8 120:6 181:5 184:18 205:17 241:18 <b>nail</b> 64:14 <b>Nairobi</b> 35:16 36:5 <b>naive</b> 126:10 170:6 173:1 200:12 <b>name</b> 3:3 34:5,6 39:4 81:13 113:5 154:19 <b>names</b> 6:4 <b>Nancy</b> 1:25 96:16 96:24 117:23 161:14 183:14 201:18 241:18 245:15 254:7 276:9 <b>Nancy's</b> 96:22 97:1 262:5 <b>nap</b> 10:12 <b>NAPc2</b> 112:3 113:9 114:23 115:24,25 116:11 <b>narrow</b> 265:14 <b>nasal</b> 117:18 <b>Natcher</b> 1:10 <b>national</b> 1:1,10,15 160:24	<b>natural</b> 50:13 70:7 112:15 125:11 126:5 140:3 147:12,16 148:6 243:18,24 267:3 269:1 274:15 280:20 <b>nature</b> 34:18 50:17 89:4 91:17 257:4 <b>near</b> 125:21 <b>nearly</b> 262:15 <b>necessarily</b> 128:19 147:1 177:20 178:12 188:1,17 207:15 238:16 242:14 249:23 250:7 258:12 259:18 268:18 275:7 <b>necessary</b> 144:20 181:11 188:10 255:6 <b>neck</b> 275:6 <b>necomotic</b> 102:5 <b>necropses(phone...</b> 142:11 <b>necropsies</b> 141:17 <b>necropsy</b> 130:12 139:23 <b>necrosis</b> 75:23,25 78:3 81:8 83:2 <b>necrotic</b> 77:24 <b>need</b> 6:20 7:6 8:10 8:17 18:16 19:11 19:16 20:1 22:2 29:6 39:21 92:3 97:25 112:9 123:12,13,14 127:21 147:24 148:8 157:16 186:13,17 211:2 213:7,23,24 215:18 216:8,18 218:12,16,16 220:3,18 221:9,11 222:22 224:23 225:5,18,20 226:1 228:25 229:3 231:8,15 234:4,6 239:14 240:7	242:2 244:2,12,23 252:21,25 253:25 263:20 268:3 275:25 276:1,3,12 277:23,24 280:1 280:17,18 <b>needed</b> 81:5 113:16 183:6 212:12 234:11 <b>needle</b> 102:23,25 146:8 166:22 230:12 247:14 259:24 272:7,9,13 272:16,17 <b>needles</b> 50:8 102:24 103:9 <b>needn't</b> 52:13 <b>needs</b> 7:13 15:19 15:23 20:18 22:13 28:14 31:4 54:21 158:25 178:21 218:18 222:20 229:11 234:16 255:1 <b>negative</b> 12:10,18 47:13 69:20 202:16 223:7 <b>neglected</b> 37:19 <b>neither</b> 153:3 <b>Nelle</b> 227:11 <b>nematode</b> 111:14 <b>NEPA</b> 108:4 <b>neutralization</b> 60:6 162:5 200:10 277:3,5,9 <b>neutralize</b> 255:6 <b>neutralizes</b> 26:15 <b>neutralizing</b> 16:16 16:18,20,20 84:19 161:10 177:8,13 177:21 184:11 200:4,19 276:24 <b>neutrophils</b> 78:9 <b>never</b> 11:7 69:21 74:14 81:15 94:8 98:14,16 119:17 119:20 121:1 128:6,10 147:5 153:2 230:1 232:19 279:4	<b>new</b> 10:16 11:20 13:11,17 31:6 55:19 63:17 70:14 79:22 89:2,8 101:10 149:19 156:19 160:15 187:9 226:20 228:14 238:19 257:2 <b>Newberger</b> 19:21 <b>newborn</b> 73:1 <b>newest</b> 156:25 <b>newly</b> 41:1 160:19 <b>news</b> 46:14,19 80:12 183:17 <b>Newsome</b> 50:4,4 <b>Newsweek</b> 40:22 <b>NIAID</b> 1:13,14,15 1:16,17,19,19,25 3:13,25 50:4 <b>nice</b> 57:23 74:5 78:1 86:21 95:6 107:16,17 117:12 124:14 129:18 137:21 168:19 254:8 278:11 279:1 <b>nicely</b> 23:18 48:5 128:23 131:4,24 140:21 211:15 <b>Nichol</b> 11:12 <b>Nichols</b> 20:25 137:20 142:12 <b>Nicole</b> 141:15 <b>night</b> 44:25 <b>NIH</b> 7:24,25 8:6 53:24 86:9 233:8 <b>Nile</b> 24:6 <b>nine</b> 100:4 103:19 <b>nitrate</b> 240:1 274:3 <b>nitrites</b> 274:7,10 <b>nitric</b> 61:24 62:1 274:2,7 <b>node</b> 77:8 107:14 <b>nodes</b> 105:10 130:24 140:20 273:4 <b>noise</b> 48:18 <b>noisy</b> 195:22 <b>non</b> 62:13 72:15
---	--	---	--	--

108:24	<b>normal</b> 16:10 54:6	111:16 117:22	190:17 195:1	92:1,6 145:22
<b>nonhuman</b> 21:15	62:13 69:10 72:9	127:1,15 134:2,8	<b>obtain</b> 148:10	149:12,23 166:10
176:14 177:12	73:24 83:19 85:4	135:16 136:6	<b>obtained</b> 68:6	167:8,14 177:16
178:9,17,20 180:3	86:2 87:8 89:20	140:25 146:3	220:12	183:13,16,19
180:8 182:3	113:1	152:6 153:8	<b>obtaining</b> 65:4	186:1,20 188:19
184:21 186:2,19	<b>normally</b> 88:24	156:16,17 165:13	148:7	189:4 196:11
196:1 197:21	151:17	174:15 193:12	<b>obvious</b> 12:15 50:8	204:6 210:3 212:7
198:23 201:3,10	<b>Norris</b> 142:9	197:9 198:8	112:8 132:13	225:4 234:25
203:13,16 233:20	<b>north</b> 4:9	201:21 206:7	<b>obviously</b> 50:7	235:6,18 256:18
233:21,23 234:4	<b>northeastern</b> 37:15	223:22 241:13	80:20 139:3 143:4	265:2 266:18
236:13,18 239:17	<b>northern</b> 40:11	244:17 254:3	143:13 157:10	279:17 281:8
246:21 250:9	<b>nose</b> 102:6	258:25 259:17	185:2 221:4	<b>Olander</b> 167:20
257:19 258:8,9,14	<b>notable</b> 129:25	264:10 273:11	246:11	<b>old</b> 36:3 101:8
258:18 265:18	130:22 132:5	274:24	<b>occasionally</b> 156:7	118:22 153:12
272:21	137:25	<b>numbers</b> 12:7 35:8	<b>occasions</b> 54:25	157:17
<b>nonidentical</b> 155:1	<b>notably</b> 30:2 201:8	37:23 43:12 45:14	<b>occupied</b> 50:16	<b>older</b> 194:5
<b>nonneutralizing</b>	<b>note</b> 125:10 221:23	60:12,14 106:4	<b>occur</b> 14:11 32:1	<b>oligonucleotides</b>
161:10 177:6	<b>noted</b> 42:13 138:2	151:8,22 234:6	32:25 34:3,12	17:6
<b>nonspecific</b> 41:22	174:3 271:23	240:24 250:8	39:2 40:2,7 50:13	<b>Olinger</b> 164:6
<b>nonspecifically</b>	<b>notes</b> 210:16	264:10	54:20 110:14	174:20
192:16	235:14	<b>numerous</b> 167:24	148:5,5 238:15	<b>once</b> 16:23 29:9
<b>nonsurvivors</b>	<b>nothing's</b> 234:17	<b>nurse</b> 35:4 44:3	275:1	40:15 44:13 62:5
193:25	<b>notice</b> 116:1 132:9	<b>Nuzum</b> 1:20 227:24	<b>occurred</b> 30:1	69:6 70:24 125:1
<b>nonvalidated</b>	142:9 196:25	227:25 235:2,6	33:18 34:7,24	125:2 164:12
223:21,23	273:7	240:13 243:16	36:1 37:15 39:4	232:22 248:13
<b>non-fatal</b> 60:19	<b>noticed</b> 119:17	246:6 250:22	39:11 40:8,11,14	<b>ones</b> 34:24 55:10
61:23,25 62:14	209:7 211:17	251:13,20,22	40:21,24 42:25	96:18 97:10 152:8
<b>non-human</b> 2:12	<b>novo</b> 207:8	252:4,23 255:12	43:25 44:11,15	172:11,13 174:5
2:14 24:9,23,25	<b>NP</b> 152:18,25	261:2 262:23,25	45:10 46:2	180:7 183:10,11
26:23 32:14,16,19	153:10 175:17	263:2 264:24	<b>occurrence</b> 8:24	234:3 271:21
38:24 41:2 43:19	181:8,11 185:23	265:2 266:18,24	34:21 40:17 50:12	<b>ongoing</b> 31:21,22
45:25 55:3 64:9	196:19 198:10,15	267:19 275:22	<b>occurring</b> 45:12,20	45:20 125:6 183:9
64:15 66:1 67:22	<b>NPC-2</b> 237:1	277:15 279:7	146:14,15	<b>onset</b> 49:20 142:24
67:25 68:3,12,20	<b>NP-only</b> 153:17	280:13	<b>occurs</b> 16:9 39:19	219:7
68:23 77:21 78:4	<b>nuclear</b> 6:10 12:11	<b>N-linked</b> 154:16	56:25 65:22 140:3	<b>onsite</b> 28:19
81:1,20 84:19	15:2	<hr/> <b>O</b> <hr/>	146:12 147:11	<b>open</b> 9:16 14:13
86:5 90:12 91:3	<b>nucleoprotein</b>	<b>O</b> 154:16	155:8 267:3 279:4	45:21 96:13
92:4,9,18 93:14	13:21,23 152:11	<b>objective</b> 33:3	<b>offense</b> 23:4	163:16 191:10
93:20,25 95:10	153:22 169:10	<b>objectively</b> 54:5	<b>offer</b> 121:10 163:24	236:16 238:1
107:3,6 114:8	173:8 184:4,12	<b>objectives</b> 127:23	<b>office</b> 3:5,25 8:2	254:10
119:8,21 127:5,24	<b>nucleoside</b> 17:5	<b>observation</b> 32:15	210:25 227:15,15	<b>opened</b> 20:20 63:17
128:15 129:9	<b>number</b> 5:18 7:25	121:11	227:17 252:14	<b>opening</b> 4:1
140:24 168:16	8:9 33:20 34:21	<b>observations</b> 35:22	<b>Officer</b> 3:4	<b>opinion</b> 122:15
206:13	36:12,22 38:2	55:7 63:3 129:19	<b>official</b> 11:17 37:22	176:13 177:7
<b>non-specific</b> 57:12	39:25 40:13 42:6	130:7,8 139:18	<b>offline</b> 227:18	215:6 231:11
123:15	42:8 43:9 49:18	238:14	<b>off-license</b> 231:3	250:23
<b>non-specificity</b>	70:20 88:12 98:6	<b>observe</b> 43:3	<b>oh</b> 59:7 92:9	<b>opinions</b> 176:21
123:8	98:23 103:14	258:22	<b>okay</b> 9:7 10:1 34:4	<b>opportunities</b>
<b>non-structural</b>	104:10 107:7	<b>observed</b> 189:7	59:24 60:9 63:3	54:18
56:4	109:9 110:4,12		65:17 67:8 90:9	<b>opportunity</b> 4:3

33:8 43:2 44:17 88:25 89:7 166:2 210:22 211:1 246:9 <b>oppose</b> 23:23 <b>opposed</b> 52:5 115:12 <b>opposite</b> 206:9 <b>opt</b> 54:23 <b>optimism</b> 162:24 <b>optimize</b> 186:9 <b>optimized</b> 178:22 <b>option</b> 230:4 <b>options</b> 229:21 <b>oral</b> 117:22 120:22 127:13 266:2 268:21,22 274:18 <b>orally</b> 206:18 270:24 <b>orange</b> 105:16 <b>orchard</b> 42:7 <b>order</b> 6:19 11:1 12:10 22:14 67:22 151:8 156:23 183:6 187:2 262:18 265:12 266:7 <b>ordinarily</b> 157:24 158:22 <b>organ</b> 62:4 105:6 106:16 <b>organization</b> 126:18 <b>organizers</b> 3:11 92:7 125:19 <b>organizing</b> 236:4 255:18 <b>organs</b> 62:11 75:24 75:25 109:4 <b>orifices</b> 81:19 <b>origin</b> 95:23,23 96:5 97:19,21 214:19 <b>original</b> 35:13 98:3 101:1 164:9 260:12 264:14 <b>origins</b> 97:22 <b>ought</b> 88:15 161:14 167:3 <b>ourfibrin</b> 273:10	<b>outback</b> 32:1 <b>outbreak</b> 4:21 12:4 31:21,24 32:21 33:4,13,24 34:2,6 34:12 35:1,10,15 36:1,8 37:9,14,21 37:24 38:16,22 39:10,18 40:23,25 41:17 42:2,4,15 42:25 43:2,7 44:15,17,21 45:1 45:11,12,14,20 46:10,10 49:8,8 50:14 51:9,19 52:17,18,23 53:11 53:15 55:19 59:16 63:13,16 69:1,22 81:15 98:17 102:23 103:3 121:7,9 126:7 129:6 147:12 225:11 230:25 232:4 250:11 254:16 257:2,2 264:9 272:14 280:20 <b>outbreaks</b> 31:25 32:11,24 33:9,15 33:17,20,20 34:18 34:22 35:25 36:9 37:6 39:2,3,6,16 39:21 40:2,6 41:16,17 43:24 44:6,7,22 45:3,16 45:18,19 46:2 48:6,8,10 49:2 54:19 55:6 65:7 94:18 121:6 126:6 140:3 145:8 146:5 238:19 267:3 <b>outbred</b> 182:4 <b>outcome</b> 24:11 112:23 150:15,19 218:20 223:6 <b>outcomes</b> 58:13 60:19 61:15 <b>outer</b> 185:12 <b>outlier</b> 97:8 241:11 <b>outliers</b> 166:23 <b>outline</b> 151:6	<b>outpacing</b> 91:8 <b>outright</b> 80:14 <b>outside</b> 36:14 39:11 67:11 107:18 113:25 148:16 218:2 <b>outward</b> 56:11 <b>overall</b> 79:14 150:9 <b>overcome</b> 157:22 <b>overhead</b> 95:18 <b>overlap</b> 12:20 197:1 <b>overlapping</b> 12:21 172:5 191:9 <b>overlaps</b> 12:15,22 <b>overnight</b> 132:20 <b>override</b> 159:20 262:18 <b>overt</b> 160:11 <b>overtly</b> 153:18 <b>overview</b> 149:9 171:15 <b>overwhelming</b> 105:19 <b>oxide</b> 61:24 62:1 274:2,7 <b>o'clock</b> 53:4	111:16 237:13 265:10 <b>papers</b> 15:22 237:12 <b>paracrine</b> 90:20 <b>paradigm</b> 187:9 <b>paradoxical</b> 264:13 <b>paragraphs</b> 211:8 211:10 <b>parainfluenza</b> 161:4 <b>parallel</b> 6:18 186:5 216:7 <b>parallels</b> 115:20 118:13 205:6 <b>parameter</b> 208:16 209:23 245:16 <b>parameters</b> 27:15 64:14 273:22 <b>paramyxovirus</b> 161:2 <b>parenteral</b> 128:5,8 128:13,22 129:11 129:25 130:4,16 130:21 131:23 134:14 135:13 139:11,16 143:21 143:25 167:1 230:22 231:2 270:5 273:20 275:15 <b>parenthetically</b> 161:16 202:5 <b>parking</b> 4:7 <b>parlance</b> 64:23 <b>part</b> 13:20 14:7 22:11,18 33:25 50:23 62:20 65:16 76:12,21,23 127:2 128:2 138:16 143:7 174:20 209:15 239:23 245:19 246:24 251:13 253:12 256:8 <b>partial</b> 79:7 162:18 <b>PARTICIPANT</b> 29:4,18,25 48:5 66:16 86:21 87:5 87:20 88:1,20	120:3 121:10 122:2,7 123:22 142:17 143:3,11 144:3,11,18 145:14,18,22 163:20 165:8 177:3 178:24 181:4 182:24 205:7,11 207:3,12 207:18 208:2,5,10 208:15,19 210:2 228:6,11 230:7 232:8,11,14 233:19 234:12 239:21 242:5 244:5 245:21 246:19 247:16,24 248:17 249:21 251:11,16,21 252:1 254:2 261:10 262:3 264:12 265:1,3 272:18 273:24 274:6 278:1 <b>participants</b> 10:7 <b>particle</b> 12:8 14:17 18:19 21:22 169:12 253:13 <b>particles</b> 13:5 17:1 18:25 19:2 22:1 60:6 94:25 159:23 166:21 253:15,17 <b>particular</b> 13:15 29:13 36:16 38:11 42:4 44:5,21 47:7 47:16 70:10 83:14 98:2 121:8 127:19 138:2,22 141:13 142:8 171:12 234:14 236:19 259:16 275:3 <b>particularly</b> 13:18 21:12 48:24 50:22 51:3 88:16 105:14 108:23 114:12 128:15 129:5 130:23 139:19 140:1 216:23 221:16 237:1 269:14 270:4
<b>P</b>				
	<b>package</b> 222:16 226:3 <b>packaged</b> 169:11 <b>page</b> 2:2 3:20 55:25 <b>pahrm/tox</b> 217:17 <b>pancreatitis</b> 62:17 62:21 <b>pandemic</b> 5:22 <b>paneer</b> 122:12 <b>panel</b> 2:24 3:21 166:18 211:15 214:17 233:16,18 235:3,4,6,7,9 238:1 242:6 255:19 268:4 277:16 281:11 <b>panels</b> 235:11 245:18 <b>pantropic</b> 151:12 <b>paper</b> 14:25 46:22 47:14 76:9 100:15			

275:13	117:20,24 122:8	<b>pentamer</b> 12:17,20	57:10 63:22 67:6	103:19,23,25
<b>particulars</b> 158:11	122:10 128:11	<b>pentavalent</b> 181:20	200:17 203:13	104:3,5,22 112:1
<b>partner</b> 15:6 46:7	129:20 137:24	<b>people</b> 3:16 4:8,23	207:25	114:19 116:10
201:7	140:15,20 142:10	10:18 11:3,8	<b>performance</b>	120:8 127:8 128:4
<b>parts</b> 165:23,24	213:17 218:19	21:21 30:14 31:20	200:15	133:22 168:22
<b>pass</b> 256:12	221:3,4 225:22	32:18 41:9 43:21	<b>performed</b> 58:2	247:1,3,4 250:14
<b>passage</b> 69:3,24	242:8,25 271:21	48:13,24 50:9,15	61:17 167:16	254:4,5,9,17
72:25 73:19,24	<b>pathomechanistic</b>	73:11 76:16 80:22	200:13,18 201:9	260:14,24 262:24
100:24 101:1,3	25:17	81:18 82:1,18	<b>performing</b> 63:20	<b>pfus</b> 134:6,12 135:1
120:7,8 121:4,13	<b>pathophysiological</b>	85:8 102:10 119:2	<b>performs</b> 163:22	139:5 141:5
121:23,25 129:13	213:14	125:22 141:12	<b>perfume</b> 144:8	166:20 167:7
129:16 134:8	<b>pathway</b> 16:3 26:2	160:17,18 163:13	<b>period</b> 36:13 38:15	246:23,23 259:12
140:13 253:10	89:15,18 111:7,7	176:22 182:6	103:2,4,11 105:17	260:14 262:15,23
256:17,23 257:20	111:12,16 112:12	201:2,9,13 210:20	189:9 206:3,9	272:25 273:2
258:16,16 261:22	186:4 189:18	212:16 214:7	218:19 229:13	<b>pfu's</b> 132:4
<b>passaged</b> 120:20	<b>pathways</b> 108:21	215:4 216:17	<b>peritoneal</b> 74:12	<b>pH</b> 15:19,20 23:24
<b>passages</b> 21:7	137:18 139:23	217:4 231:13,17	<b>peritoneally</b> 74:25	<b>pharmacodynam...</b>
253:11 258:19	<b>patience</b> 9:24	232:1,18 244:14	<b>permeability</b> 25:9	215:13
<b>passaging</b> 73:5	<b>patient</b> 46:10 55:11	255:16 269:4,18	26:13 83:6,10	<b>pharmacokinetics</b>
<b>Passing</b> 161:7	58:10,25 63:2	269:21 275:12	<b>permeabilize</b>	246:13
<b>passive</b> 178:8	81:16 92:20	277:17 281:3	191:11	<b>pharmacological</b>
<b>passively</b> 170:6	<b>patients</b> 29:14 39:9	<b>peptide</b> 22:10,10	<b>persistent</b> 69:16	54:7
<b>Pasteur</b> 42:22	43:3,9,16 52:22	172:7,9,20,21	83:1	<b>pharm/tox</b> 217:13
<b>path</b> 267:24 275:23	54:22,24 55:16	180:13 191:10	<b>person</b> 88:3 146:17	<b>phase</b> 59:24 163:6
<b>Pathogen</b> 46:4	56:13,15 59:1,11	<b>peptides</b> 17:1,6,13	166:7	186:12 215:25
<b>pathogenesis</b> 2:4,8	59:17,19 60:4,11	171:21 172:4,6,7	<b>personal</b> 52:16	216:3,3,4,8,11,21
9:9 25:19 45:24	60:14,15,16,18,21	172:12 173:3	163:7 177:19	217:15,18,18,20
56:17,25 63:8	60:23 61:3,15,17	<b>percent</b> 12:1 35:7,8	178:9 215:6 231:5	224:8,9,9 232:24
70:24 79:17 82:10	62:6,14,18,22	35:18 36:6 38:18	231:10 241:7,8	<b>phenomena</b> 82:24
98:24 104:17	66:13,23 69:24	44:5,9 93:19	<b>personally</b> 241:5	<b>phenomenon</b> 75:11
120:5 128:21	80:20 81:13 119:4	96:11 100:2,12,12	<b>personnel</b> 40:1,4	81:21
129:10 137:19	180:21 248:18	102:25 103:4,10	49:1 52:13,19	<b>phenotype</b> 24:17
138:13 140:14	271:24 272:5	104:21 108:22,25	169:18 280:21	73:6
213:17 234:13	<b>pattern</b> 131:13	111:18 112:6,7,22	<b>person-to-person</b>	<b>phenotypes</b> 194:7
<b>pathogenic</b> 41:2,6	154:1 155:10	112:24 115:2,22	146:6	<b>phenotypic</b> 86:23
54:6 89:19 129:5	<b>Pau</b> 201:8	115:23 138:8	<b>perspective</b> 31:13	<b>Philippine</b> 42:2
263:24	<b>paying</b> 95:19	151:9 170:20	33:17 36:21 37:13	45:17 96:20,25
<b>pathogens</b> 31:9,13	<b>PBMCs</b> 60:11	190:7,7 193:1,2	39:5 210:25	97:6 241:19
32:4 34:5 41:5	121:17 191:8	195:18 197:6,14	214:20 215:16	<b>Philippines</b> 41:16
180:14 184:20	<b>PCR</b> 47:23 49:24	199:19 250:10,11	<b>perspectives</b> 2:22	41:18,20 42:7
265:11 278:4	51:12 58:6 153:25	250:18,19 264:1,7	210:12	44:14
<b>pathognomonic</b>	259:18	264:8,11,15 272:8	<b>pertain</b> 144:1	<b>philosophy</b> 163:8
109:7	<b>PDX</b> 24:4	272:9	<b>Pete</b> 201:5 203:9	261:23
<b>pathologist</b> 137:20	<b>peak</b> 61:8 133:11	<b>percentages</b> 191:20	<b>petechial</b> 129:25	<b>phone</b> 3:9
137:22 272:20	206:4	191:21	<b>Peter</b> 1:17 4:5 9:11	<b>phospho</b> 14:2
<b>pathologists</b> 130:19	<b>peaks</b> 59:10 133:5	<b>perenchymal</b> 75:24	9:13 41:4 53:1,23	<b>phosphor</b> 12:12
<b>pathology</b> 2:9	133:9,13	<b>perfect</b> 234:18	68:5 92:14,25	<b>photo</b> 76:19
53:20 54:15 57:24	<b>peculiarity</b> 74:11	<b>perfectly</b> 102:11	93:15 125:22,24	<b>photographs</b> 39:22
61:10 94:16	<b>pedigree</b> 263:15	<b>perforin</b> 60:17	189:23	<b>photos</b> 77:21
104:19 117:3,11	<b>pending</b> 219:6	<b>perform</b> 54:23 57:8	<b>pfu</b> 99:21 103:18	<b>photo-mike</b> 77:17

<b>physically</b> 97:9	237:8 259:1	175:22 176:4,10	230:13 238:21	<b>portable</b> 61:18
<b>physician</b> 35:15,17 44:1	<b>piling</b> 258:25	185:3 203:24,25	247:18,24 248:14	<b>portion</b> 150:18 151:19 203:10
<b>physicians</b> 36:15	<b>pillar</b> 213:23	204:8 208:24	249:1 256:18	<b>Porton</b> 40:9 98:4
<b>physicist</b> 141:18	<b>pillars</b> 213:6	<b>plausible</b> 147:18	259:13,16 261:4,8	<b>position</b> 235:2
<b>physiological</b> 29:8 129:1 168:15	<b>pilot</b> 197:25 222:17 245:3	<b>play</b> 26:15 134:9 147:7 171:10 179:2	263:2,4 265:23	<b>positive</b> 10:21 46:25 59:5 99:25 171:17 172:3,11 172:17,19,20,20 180:9
<b>physiologically</b> 166:16	<b>Ping</b> 1:14 3:3 4:2 9:5	<b>player</b> 75:20	270:17 273:15	<b>possibilities</b> 89:16 151:11 172:17
<b>Ph.D</b> 1:13,14,15,15 1:16,17,17,18,19 1:19,20,21,22,25	<b>PITT</b> 266:11,17	<b>players</b> 82:11	279:20 280:25	<b>possibility</b> 29:17 90:1 147:15 157:12,15 256:21
<b>Piccolo</b> 61:18	<b>pivotal</b> 216:12,19 222:14,23 234:15	<b>playing</b> 72:14 183:7	<b>pointed</b> 38:19 60:5 75:16 106:23 109:10 123:6 174:17 249:14 267:12	<b>possible</b> 7:4 50:10 63:23 126:8 145:6 146:15,16 147:2 177:23 180:12 193:11 223:2,19 250:16
<b>pick</b> 3:19 77:25 86:2,3 127:7 240:15 257:13 259:4	<b>PK</b> 7:9 99:7	<b>plays</b> 50:12 80:3 237:2 240:2	<b>pointing</b> 171:16	<b>possibly</b> 168:16 268:21 278:18
<b>picked</b> 96:14 187:18	<b>place</b> 16:5 30:4 56:23 61:12,12 62:19 63:15 70:8 73:21 77:5 224:1	<b>pleasant</b> 164:25	<b>points</b> 111:21 114:7 134:17 153:13 186:22 195:21 206:22 208:9,14 210:17 217:25 219:4 236:15 276:13	<b>possibly</b> 225:24
<b>picking</b> 173:17	<b>places</b> 32:1 51:5 212:21 221:8	<b>please</b> 9:15 23:3 29:3 177:1 201:16 232:25 249:2	<b>pointing</b> 171:16	<b>post</b> 42:17 119:1 131:17 145:24 209:24 217:20 219:19 228:7 229:9,13 244:20 244:23 247:18 251:3,4,4,5,5,14
<b>picnic</b> 53:6	<b>plague</b> 218:8 231:12,14,18	<b>pleased</b> 64:25	<b>Poisson</b> 260:22	<b>postal</b> 268:16
<b>picture</b> 54:14 62:22 67:7 80:21 137:19	<b>plan</b> 6:12,16 216:15 224:1 243:21	<b>pleasure</b> 10:3	<b>policy</b> 165:15 272:16	<b>posted</b> 272:21
<b>pictures</b> 75:16 130:2	<b>Plank</b> 22:19	<b>PLoS</b> 265:11	<b>polio</b> 34:7 41:24	<b>postulated</b> 146:21
<b>piece</b> 74:5	<b>planned</b> 9:13	<b>plot</b> 155:1	<b>political</b> 9:2	<b>post-exposure</b> 112:8,14 115:25 122:13
<b>pieces</b> 156:7	<b>plans</b> 7:15	<b>plotted</b> 116:8	<b>politically</b> 37:19	<b>post-marketing</b> 7:12
<b>Pierre</b> 188:21 201:5	<b>plant</b> 34:8 41:24	<b>plus</b> 16:2 79:23 222:5 224:16	<b>politics</b> 160:23,24	<b>post-onset</b> 59:7,11
<b>pig</b> 14:24 68:25 69:23 70:25 127:9 127:24 141:16 142:7 236:12 253:19 258:4 265:17	<b>plaque</b> 27:7 121:23 134:5 253:3,9,12 253:16,17,22 258:12	<b>pneumonia</b> 117:9 140:18	<b>polychromatic</b> 192:10	<b>potency</b> 157:12 158:1 159:3,10 160:21 199:15
<b>pigs</b> 64:9 67:24 69:3,4,21 70:15 70:19,22 72:21 73:2 74:9 75:8,22 76:5,14,25 78:6 78:17 79:5,8,10 80:18 83:8 84:3 84:22 94:7 124:2 126:14 127:4 141:7 145:7 152:5 152:6,13,16,18 153:10 158:3,14 161:3 175:9,22 184:19 201:21 202:3 236:22	<b>plaques</b> 73:7,9	<b>pneumonic</b> 231:13	<b>polyclonal</b> 57:3 178:11 202:10	<b>potent</b> 27:2 74:21 85:14 160:3 188:14 257:6
	<b>plaque-forming</b> 153:16,23 252:25 253:6,20	<b>podium</b> 9:4	<b>polyfunctional</b> 193:18	<b>potential</b> 8:14 13:17 20:20 22:22 25:7 88:2 126:17 126:20 144:25
	<b>plaquing</b> 259:14	<b>point</b> 9:18,19 10:24 11:12 20:16 22:2 23:3,6,9 25:15 29:24 32:24 39:8 45:21 55:14 58:16 66:5 68:12 75:13 92:11,19 94:11 96:17 99:20 101:4 101:13 102:12,18 112:10 116:13 119:14 120:11 123:5,17,24 124:3 132:7 137:9 148:4 158:18 171:12 174:9 184:8 187:25 190:9 193:4 195:20 197:16 202:12 209:17 217:3,11 217:21 219:23 224:14 225:7 226:15,22 230:11	<b>polymerase</b> 12:14 14:15 30:3,8 153:1	
	<b>plasma</b> 16:5 90:23 91:3,4 116:15	<b>podium</b> 9:4	<b>pool</b> 155:19 172:9,9 256:13 261:13	
	<b>plate</b> 208:3,6,8	<b>point</b> 9:18,19 10:24 11:12 20:16 22:2 23:3,6,9 25:15 29:24 32:24 39:8 45:21 55:14 58:16 66:5 68:12 75:13 92:11,19 94:11 96:17 99:20 101:4 101:13 102:12,18 112:10 116:13 119:14 120:11 123:5,17,24 124:3 132:7 137:9 148:4 158:18 171:12 174:9 184:8 187:25 190:9 193:4 195:20 197:16 202:12 209:17 217:3,11 217:21 219:23 224:14 225:7 226:15,22 230:11	<b>pools</b> 172:5,7,8,10 172:17,21	
	<b>plateau</b> 75:11	<b>plot</b> 155:1	<b>poor</b> 37:20 40:3 63:1 84:14 112:23	
	<b>Platelet</b> 102:7 137:3	<b>plus</b> 16:2 79:23 222:5 224:16	<b>Popp</b> 127:3 164:21	
	<b>platelets</b> 65:15,15 65:18,22 107:22	<b>pneumonia</b> 117:9 140:18	<b>pops</b> 105:8	
	<b>plates</b> 59:15	<b>pneumonic</b> 231:13	<b>population</b> 70:9,13 187:12 192:4,5,19 192:23 258:19 268:12	
	<b>platform</b> 115:12 116:21 199:22 203:20 228:18	<b>podium</b> 9:4	<b>populations</b> 48:15 191:6,14 192:13	
	<b>platforms</b> 164:14 167:25 175:6,19	<b>point</b> 9:18,19 10:24 11:12 20:16 22:2 23:3,6,9 25:15 29:24 32:24 39:8 45:21 55:14 58:16 66:5 68:12 75:13 92:11,19 94:11 96:17 99:20 101:4 101:13 102:12,18 112:10 116:13 119:14 120:11 123:5,17,24 124:3 132:7 137:9 148:4 158:18 171:12 174:9 184:8 187:25 190:9 193:4 195:20 197:16 202:12 209:17 217:3,11 217:21 219:23 224:14 225:7 226:15,22 230:11		

161:24 185:19 224:2 230:20 250:4 <b>potentially</b> 89:3 162:9 184:12 <b>potently</b> 26:18 <b>powerful</b> 18:15 19:10 20:18 <b>pox</b> 147:21 156:11 207:5,6,12 <b>practical</b> 260:8,17 261:20 265:24 267:19 <b>practically</b> 257:18 <b>practice</b> 195:8 <b>practices</b> 39:17 52:21 222:2 267:13 <b>pre</b> 206:5 207:15 217:17 222:1 251:5 <b>preaching</b> 221:13 <b>prebleed</b> 202:9 <b>precautions</b> 39:24 <b>precedence</b> 124:7 <b>prechallenge</b> 188:13 <b>precise</b> 197:20 <b>precisely</b> 277:5 <b>precision</b> 195:11 204:1 <b>preclinical</b> 198:25 217:13 <b>precludes</b> 212:14 <b>precursor</b> 22:7,11 22:15 <b>predetermined</b> 223:8 <b>predicator</b> 112:22 <b>predict</b> 101:24 155:14 161:13 187:8 197:4,13 209:24 223:6 <b>predictable</b> 257:24 <b>predicted</b> 131:15 173:20,23,24 <b>predicting</b> 173:16 180:1 <b>prediction</b> 33:24 <b>predictive</b> 197:2	214:1 233:22 <b>predictors</b> 84:14 <b>preimmune</b> 202:6 207:20,22 <b>preimmunity</b> 206:22 <b>preincubate</b> 277:9 <b>preliminary</b> 243:24 <b>prelude</b> 87:22 <b>premature</b> 5:7 <b>preoccupied</b> 276:23 <b>prep</b> 73:11 266:16 269:19 <b>Preparation</b> 223:9 <b>preparations</b> 56:1 <b>prepared</b> 6:9 <b>preparedness</b> 31:15 <b>presence</b> 52:22 57:10 179:13 200:19 205:18 249:10 <b>present</b> 1:12 33:22 51:2 52:5 64:15 76:13 89:15 124:13 149:10 172:22 194:12 211:5,11 214:12 222:4 272:5 <b>presentation</b> 27:17 54:3 58:22 64:3 75:5 80:11 88:21 92:2,4 93:11 125:6,8 185:3 215:22 272:23 <b>presentations</b> 210:10 <b>presented</b> 95:6 113:15 124:14 176:23 178:25 190:25 229:16 243:25 <b>presenters</b> 148:14 <b>presenting</b> 171:19 187:18 207:10 <b>presents</b> 183:1 <b>pressing</b> 109:12 <b>pressure</b> 25:3 <b>Preston</b> 81:18	<b>presumably</b> 78:21 <b>presume</b> 205:4 258:15 <b>presumes</b> 276:25 <b>pretend</b> 162:22 <b>pretty</b> 32:1 34:14 38:1 39:6 41:8 44:7 51:13 64:3 100:16 103:16 106:9,16 115:2,3 118:8 126:20 137:11 138:17 152:10 153:24 166:21 189:8 195:13 196:17 202:2 226:23 239:9,17 240:16 241:16 247:12 248:6 259:5 261:3 263:21 267:13 268:19 271:13 <b>prevalence</b> 48:7 <b>prevalences</b> 48:18 <b>prevent</b> 28:4 246:2 <b>prevented</b> 260:20 <b>preventing</b> 90:22 <b>prevention</b> 1:3 66:20 213:15 214:25 <b>preventive</b> 116:20 269:8 <b>prevents</b> 85:10 <b>previous</b> 48:13 101:14 217:12 <b>previously</b> 129:3 <b>pre-challenge</b> 255:8,9,11 <b>pre-existing</b> 166:11 199:11 200:16 205:12,15,23 206:18 207:4 <b>pre-exposure</b> 27:3 <b>pre-immune</b> 195:25 196:5 <b>pre-IND</b> 212:5 216:8 232:20 <b>pre-pre-IND</b> 232:21 <b>primarily</b> 58:8 95:25 96:18	105:10,20 125:24 127:17 171:17 <b>primary</b> 15:1,25 22:7 33:3 36:10 42:9 45:6 75:18 87:25 107:1 118:5 134:1 227:11 <b>primate</b> 25:1 26:23 32:14,19 38:21 40:23 42:9 45:5 45:25 55:3 64:15 67:23 68:12 78:4 81:1 84:19 93:20 94:1 95:10,15 107:6 126:21 127:24 140:24 141:11 145:6 168:16 176:14 186:2,19 196:1 197:21 198:23 201:3,10 203:3,16 233:21,21,23 234:4 246:21 250:9 258:14,18 <b>primates</b> 2:12,14 21:15 24:10,23 32:17 34:8 38:25 41:3,8,25 43:19 43:19 64:9 66:2 67:25,25 68:4,20 68:24 72:16 77:22 81:20 84:8 86:3,4 86:5 89:4,19 90:6 90:12,15 91:3,5 92:5,10,18 93:14 107:3 108:25 114:9 119:8,21 125:17 126:14 127:5 128:16 129:9 141:6 142:11 177:13 178:9,17,20 180:4 180:8 182:4 184:22 203:13 206:13 236:13,18 239:17 257:19 258:8,9 265:19 272:21 274:19 <b>prime</b> 12:12 21:13 185:4 188:22	189:7 199:16 202:1,14,21 204:9 204:12,17,19,20 <b>priming</b> 159:9 198:19 <b>principal</b> 157:7 <b>principally</b> 271:25 272:1 <b>prior</b> 49:13 129:6,9 129:10 131:16,25 170:21 199:14,17 217:15 227:5 <b>prioritizing</b> 262:17 <b>priority</b> 258:9 <b>probably</b> 4:8 5:20 9:12 33:14 35:19 38:20 39:23,24,25 43:17 45:6 49:19 50:25 51:1 68:16 69:19 71:8,12 72:21 74:13,25 79:23 80:2 83:8 84:3 90:21 94:18 97:5 99:15 102:10 106:24 108:25 110:4 114:20 115:10 118:10 120:20 129:7 147:24 151:1,22 151:25 155:17,24 164:9 165:4,18 166:9,10 179:20 185:1 214:8,13 221:8 226:13,15 227:23,25 229:15 234:8,10 239:5,16 240:18 241:3,8 255:22 262:7 264:13,18 269:2 270:9,14 272:21 275:4,14,17,23 276:14 279:4 <b>problem</b> 6:3 11:4 57:12 62:11 64:16 79:11 98:6 113:8 113:15 114:3 122:12 143:17 162:2,6 195:11 205:23 206:19,24 239:23
---	---	---	---	--

<b>problems</b> 62:19 122:3 158:12 181:7 261:17	<b>proforin</b> 60:19	152:8,18,19 153:7	174:13 177:18	169:2 173:4
<b>probs</b> 58:9	<b>progeny</b> 106:3	158:3,17,19 159:8	178:3 185:22	178:13 194:6
<b>proceed</b> 143:11,14 235:3	<b>prognosis</b> 63:1	165:2,14 167:6	186:19 188:8,17	<b>provides</b> 190:13
<b>process</b> 9:25 54:10 55:13 70:6 73:20	<b>prognostic</b> 55:10	170:23,25 181:1	188:18 262:8	<b>providing</b> 55:15
106:4,5 136:4	<b>program</b> 3:4 178:15 181:19,19	183:6 189:5,21	<b>protects</b> 116:20	<b>proving</b> 202:16
186:10 187:14,15	268:10,11	225:17 240:18	245:5 270:20,22	<b>provocative</b> 92:2
<b>processes</b> 54:6	<b>programs</b> 269:22	243:6 267:1,17	<b>protein</b> 12:11,12,13	<b>proximity</b> 269:3
<b>processing</b> 17:10 65:4,9	<b>progress</b> 4:18 65:1 74:3,5 130:25	271:7,19 275:8	13:9,10,15 14:2,2	<b>pro-inflammatory</b>
<b>produce</b> 22:25 79:21,24 81:6	133:13 138:16	276:3	14:7,16,19 15:2	79:10 80:7 83:4
100:7 117:1	<b>progressed</b> 9:24	<b>protected</b> 111:19	19:25 78:20 108:2	87:17 88:13
122:16 127:4	<b>progresses</b> 273:19	112:13 153:9,22	108:6 111:14	110:11
188:15 191:17	<b>progression</b> 24:11 61:4 119:23	159:6 165:3 190:2	112:18,19,22	<b>Prudence</b> 162:24
<b>produced</b> 82:16 83:11 191:5	218:19	225:4 262:16	113:5,8 116:4	<b>pseudotype</b> 17:24
<b>produces</b> 22:21 24:16	<b>projects</b> 56:11	270:25	169:7,20 172:4,5	<b>pseudotypes</b> 18:7
<b>producing</b> 80:7 193:6,7,7,20	<b>proliferation</b> 111:3 175:12,22,25	<b>protecting</b> 83:22 158:17	179:6 181:8,9	19:12
<b>product</b> 5:25 7:15 7:23 8:7,10,15	<b>prolonged</b> 61:5 79:7	<b>protection</b> 2:15,19 2:21 40:4 52:14	185:1 187:17	<b>PT</b> 137:13
9:18 22:7 108:19	<b>prominent</b> 59:25 60:5 76:1 78:13	52:16,17 111:18	<b>proteins</b> 13:8,16,20	<b>PTH</b> 273:10
212:24 213:4,16	102:4 105:22	112:6 115:22,23	15:10 16:4,7 19:1	<b>PTs</b> 79:1
215:13 220:5	109:3 110:15	124:17,17 125:13	20:1,6 22:9,25	<b>PTTs</b> 79:1
224:3,20 225:12	118:7 236:20,22	127:21 144:15	23:2,2,6,10 55:22	<b>public</b> 3:14 9:16
227:4 233:11	<b>prominently</b> 77:7	149:3 152:10,11	55:24,25 56:5	11:22 36:14 59:20
234:22 243:19,21	<b>promise</b> 43:14	152:17 153:8	57:2 108:7 169:6	143:5 163:5 250:3
250:4 251:10	<b>promising</b> 21:25 194:2 200:25	154:8 163:21	169:8,24,25 173:9	256:9
252:14 255:14	211:19 278:13	164:13 167:12	174:7,16 175:15	<b>publication</b> 150:7
267:24 275:23	<b>prone</b> 39:15 41:22	169:22,23 170:1,7	178:4,5,6 180:10	<b>publications</b> 11:8
<b>production</b> 14:5 34:10,11 61:2	<b>proof</b> 157:2 216:9	170:8,19,20	180:15 181:13,21	29:16 46:18
82:21 85:10 89:23	<b>proofs</b> 156:8 157:15	171:10,10 173:4	182:2,10 207:13	<b>publish</b> 202:15
162:9	<b>properly</b> 108:10	173:20,24 174:17	<b>protein-based</b>	<b>published</b> 11:6
<b>productive</b> 4:4	<b>prophylactic</b> 219:9 219:19	174:23 176:12	160:3	15:18 20:11 38:10
<b>products</b> 8:14 58:6 95:15 186:22	<b>prophylaxis</b> 27:3	177:19 178:1,13	<b>prothrombin</b> 79:7	47:2,14 84:12,13
214:8 217:10	<b>proposal</b> 11:20	179:2,12 180:1,23	<b>protocol</b> 189:19	88:12 96:6 97:17
223:23 234:9	<b>propose</b> 209:16	181:6,16 182:20	226:25	98:21,22 104:18
<b>professional</b> 44:1 149:19	<b>proprietary</b> 9:19	183:11,15,21,25	<b>protocols</b> 233:1	111:16 115:15
<b>profile</b> 132:24	<b>prospective</b> 216:15 224:1	184:17,22 185:11	<b>prototype</b> 41:7	127:1 156:14
<b>profiles</b> 142:19 237:19	<b>protease</b> 17:11	186:14 187:6,8,12	<b>protracted</b> 131:22	157:5 164:15
<b>profitable</b> 88:19	<b>protect</b> 40:1 43:18 83:23,25 84:19,21	188:1,4,7,12,24	132:2 135:5	173:8 185:25
	84:22 85:5 89:18	189:15 190:7,13	<b>proved</b> 147:17	212:20 237:13
	89:21 93:18,24	195:3 197:15,24	<b>proven</b> 144:20	239:20 258:6
	123:14,14 151:13	199:3 203:25	<b>provide</b> 55:20 63:8	264:10
		209:16 215:17	142:6 168:4	<b>PubMed</b> 49:10
		220:11,12 244:9	174:22,24 176:12	<b>puddling</b> 109:2
		250:18,19 254:8	178:16 181:6,16	<b>pull</b> 113:11
		254:20,23 265:9	181:24 185:11	<b>pulmonary</b> 130:14
		270:15	187:1 197:19	273:3
		<b>protective</b> 153:10	199:7 213:5 251:1	<b>pulp</b> 94:4,25
		167:4 168:9	255:25 256:19	<b>pump</b> 114:1
		170:11 171:4,7	276:20	<b>puncture</b> 109:3
		173:15,24 174:2	<b>provided</b> 32:14	<b>purification</b> 258:12
			34:15 44:17 55:6	<b>purified</b> 55:25
			55:12 168:17	<b>purpose</b> 220:13

224:20 228:19  
266:25  
**purposes** 93:11  
255:15 264:4  
**pursue** 82:9 230:15  
278:6  
**pursued** 88:15  
157:9  
**push** 31:5 229:14  
**pushed** 18:21 27:22  
**pushing** 26:1  
**put** 7:14 21:14 24:9  
28:17 30:20,25  
40:21 68:25 70:25  
71:9 73:4,24  
92:20 100:20,24  
100:25 113:22  
121:21 142:17  
147:14 155:3  
171:22 172:8  
200:24 213:10  
216:2 221:3 223:3  
226:3,4 227:24  
229:19 235:19  
251:8 261:24  
273:14,25  
**putative** 155:6,7  
**putting** 24:22  
67:22 69:14,19,19  
70:8 144:8 163:3  
167:20 214:25  
**P-R-O-C-E-E-D-...**  
3:1  
**p.m** 148:19 149:2  
210:6,7 281:13

## Q

**quadratic** 208:17  
**qualitative** 204:16  
**qualitatively**  
202:22  
**quality** 162:23  
**quantify** 131:11  
**quantitation** 55:9  
58:8,11  
**quantitative** 187:5  
204:15  
**quantities** 269:4  
**queried** 151:2  
**question** 51:15

56:24 65:24 71:6  
79:13 81:10 82:3  
82:8 85:6 88:22  
88:23 89:25  
109:22 122:6,13  
122:21 139:25  
145:4 146:11,14  
146:19 148:1  
150:23 151:3,11  
165:17,20 166:4  
166:11,13,14  
177:4,11 179:16  
179:19 182:12,17  
192:3 205:10,11  
206:14 207:19  
212:4 228:6 234:1  
235:11,13,15  
236:7,8 242:4,6  
243:2,15 244:10  
245:3,9 247:8,16  
247:23 248:18,23  
249:21,22 250:1  
254:2,9,19 256:1  
257:16 263:6  
264:24 266:18,23  
269:22 273:24  
276:8

**questions** 3:21  
28:25 29:2 45:22  
63:24 64:1 79:3  
86:20 90:10,11  
118:16,19 137:25  
140:9 142:15  
156:19 163:17  
177:2,3 201:14,17  
204:3 211:6,12  
213:12 228:3,5  
233:16 235:9,12  
243:16 244:4  
246:7,16,20 256:8  
271:14 274:23  
281:7  
**queue** 221:19  
**quick** 20:22 91:10  
116:18 152:8  
182:11,17 210:17  
210:18 235:18  
246:16,18,20  
248:6  
**quicker** 135:11

247:10 264:1,2  
**quickly** 10:13  
19:15 22:4 24:21  
80:23 198:2  
212:17 215:21  
248:24 256:5  
279:18  
**quite** 4:17 5:10  
8:16 15:16 23:17  
24:17 36:20 38:20  
38:24 41:2 69:12  
70:22 89:1 95:4  
106:11,14 110:15  
110:17 118:6  
128:14,23 131:3,4  
131:24 135:2  
140:21 164:15  
198:15,16 200:13  
201:11 205:19  
217:6 236:21  
239:10 247:22  
271:3 274:19  
**quote** 144:24  
205:17

## R

**rabies** 115:14  
**race** 79:18 122:19  
122:21 123:19  
248:13  
**radar** 125:14  
**radiological** 6:10  
**rags** 269:23  
**raise** 89:9,11  
270:11 276:13  
**raised** 202:25  
219:17 275:18  
**raising** 250:2  
**ramped** 262:16  
**range** 12:6 38:13  
62:10 100:4,19  
133:24 134:14,23  
135:12 141:4  
156:20 168:23  
176:5 191:22  
192:1 260:7  
261:16 265:14  
**ranged** 272:24  
**ranging** 11:25  
254:4

**rapid** 160:8,10  
254:25  
**rapidity** 108:6  
**rapidly** 75:10 80:5  
82:13 133:6 266:6  
**rare** 32:25 248:19  
**rarely** 250:11  
**rash** 98:8,12 102:5  
117:13,14 129:25  
130:2,6 131:1,6  
139:19 248:7  
249:1,10  
**rashes** 98:14,15,16  
102:5,7  
**rate** 121:7 147:19  
198:16 199:18  
272:7  
**rates** 12:5  
**ratio** 179:7 253:13  
**rationale** 2:17  
149:8 243:20  
255:17  
**rationaly** 161:13  
**rats** 69:20  
**Ravn** 100:24,25  
101:1 141:5  
154:13  
**reach** 62:5 75:11  
248:14  
**reached** 105:10  
276:10  
**reaches** 63:1  
**react** 48:20 214:1  
**reactant** 57:12  
**reaction** 41:22  
**reactive** 48:20  
**reactivity** 57:13  
**read** 66:11 81:16  
221:23 227:25  
266:22  
**reading** 53:9  
191:10  
**readout** 191:20  
197:3  
**ready** 132:10  
**reagent** 245:13  
**reagents** 57:5,11  
71:20 79:12  
168:13 174:11  
179:22 245:13,20

**real** 7:16 8:2 15:22  
31:16,19,19 55:10  
58:7 85:16 97:14  
113:15 135:7  
147:16 210:18  
235:18 239:19  
246:16 254:16  
269:20  
**realistic** 166:21  
167:5 228:16  
254:11  
**reality** 163:5  
219:25 228:15  
229:15  
**realize** 221:7  
269:18 277:3  
**really** 6:1 12:25  
14:9 15:11,14  
18:12 29:12 31:1  
35:13 36:17 39:25  
47:6,21 49:3  
52:15 63:19 64:25  
67:25 70:7,18  
71:6 74:4,14 75:4  
78:23 79:1,25  
81:22 83:9 88:8  
90:18,24 91:20,25  
93:8 96:1,8 98:10  
102:2 103:25  
107:16,23 109:15  
113:16 117:3,10  
117:18,19,21,23  
118:11 119:12,23  
123:17 124:14  
134:20 142:23  
147:11 150:11  
159:2 161:12  
162:12 164:6  
192:3 198:16  
199:17 209:6,9,25  
210:10,21 214:10  
230:13 231:25  
236:7 237:7,18,21  
241:14 247:6  
249:5,15 250:20  
252:25 261:7,18  
264:17 267:9  
270:11 271:15  
275:1 277:12  
278:5,11 279:14

280:1,4,21,23 281:1 <b>reason</b> 90:4 115:19 134:1 137:5 164:1 189:18 204:11 212:12 216:1 258:20 <b>reasonable</b> 49:4 189:14 213:4 224:24 <b>reasonably</b> 135:1 187:1 213:13,18 213:20 <b>reasons</b> 30:6,6 38:3 166:19 180:6 189:18 229:20 249:2 <b>reba</b> 124:7 <b>rebel</b> 44:24 <b>rebut</b> 257:16 <b>recall</b> 41:13 46:9 152:25 264:15 <b>recap</b> 15:7 <b>receive</b> 141:24 <b>received</b> 177:13 <b>receiving</b> 198:7 <b>receptive</b> 226:24 <b>receptor</b> 15:5,8,12 15:13,17 16:23,24 71:24,25 155:7 <b>receptors</b> 15:14 85:13 <b>recess</b> 148:19 <b>recessed</b> 210:7 <b>recipients</b> 182:9 <b>reckon</b> 51:13 <b>recognition</b> 6:16 <b>recognize</b> 73:7 230:3 <b>recognizing</b> 65:2 178:11 <b>recombinant</b> 18:21 19:8 27:1 111:14 160:13 161:1 <b>recombinants</b> 265:11 <b>recommendation</b> 162:25 <b>recommendations</b> 162:21 218:4	<b>reconquer</b> 158:11 <b>reconvened</b> 210:7 <b>record</b> 37:14 67:13 129:1 248:24 <b>records</b> 96:4 97:3 <b>recover</b> 42:21 <b>recoverable</b> 51:11 <b>recovered</b> 42:21 43:11 49:13 153:19 <b>recovers</b> 58:25 <b>recruitment</b> 82:18 <b>red</b> 49:17 94:4,25 105:16 116:11 131:16 136:10 191:24 <b>redefined</b> 13:11 <b>reduce</b> 39:25 185:21 199:11,14 <b>reduced</b> 65:19 73:21 199:18 <b>reduces</b> 24:6,7 <b>reducing</b> 185:23 260:6 <b>reduction</b> 1:2 205:25 213:16 <b>Reed</b> 1:20 125:15 125:18 142:20 143:10,20 144:10 144:17 145:5,17 145:20,23 146:10 147:3 148:11 243:25 265:25 <b>Reed's</b> 272:20 <b>reemphasize</b> 10:15 <b>refer</b> 35:23 71:8 151:22 214:7 246:23 278:25 <b>reference</b> 261:10 261:13 <b>references</b> 35:23 169:17 <b>referred</b> 49:9 <b>referring</b> 4:13,19 <b>refine</b> 141:21 <b>refinement</b> 208:20 <b>refining</b> 8:14 <b>reflect</b> 78:7 222:7 245:5 <b>reflecting</b> 78:21	<b>reflection</b> 121:8 <b>reflects</b> 188:16 <b>reforms</b> 9:1 <b>refreshments</b> 67:11 <b>regard</b> 4:16 51:16 110:24 178:8,22 280:8 <b>regarding</b> 205:11 236:18 254:2,9 <b>regardless</b> 242:22 <b>regards</b> 23:2 28:13 262:3 <b>regimen</b> 188:23 189:16 <b>regiment</b> 112:14 <b>regiments</b> 111:20 <b>region</b> 56:10 59:10 197:1 <b>Register</b> 221:24,25 <b>registered</b> 3:16 <b>regs</b> 222:6 <b>regular</b> 216:25 <b>regulate</b> 111:1 <b>regulates</b> 110:2 <b>regulation</b> 12:24 26:3 115:6 <b>regulations</b> 212:21 221:22 <b>regulatory</b> 2:22 210:11,12 212:2 219:23 249:22 <b>related</b> 66:18 86:24 146:7 207:5 214:23 257:10 259:6 261:3 <b>relates</b> 102:22 103:13 <b>relative</b> 179:23 <b>relatively</b> 34:11 45:11 108:2 <b>release</b> 59:19 109:23 160:22 162:7 171:25 172:25 173:23 230:21 278:12 279:1 <b>released</b> 6:11 22:19 83:12 <b>relevance</b> 257:1 260:8 261:5	<b>relevant</b> 2:16 8:13 63:10 128:19 139:24 149:4 166:16 187:22 192:5,18 214:16 215:13 246:1,10 247:17 259:23 262:6 280:24 <b>relying</b> 195:19 <b>remained</b> 189:8 <b>remains</b> 75:13 108:2 254:10 <b>remarkably</b> 160:6 <b>remarks</b> 4:1 <b>remember</b> 29:6 43:9 118:25 203:9 254:5 <b>remembering</b> 224:19 244:22 <b>remind</b> 162:13 166:23 236:1 <b>remote</b> 32:2,25 44:22 54:20 <b>remove</b> 72:9 <b>removed</b> 41:13 70:1 171:21 <b>renal</b> 62:19 109:19 <b>reoccurrence</b> 42:24 <b>repeat</b> 41:23 72:4 114:4 206:2,12 <b>repeated</b> 111:22 140:10 257:20 <b>repeats</b> 40:13 41:14 <b>repetition</b> 75:3 <b>replaced</b> 18:23 169:7 <b>replaces</b> 160:14 <b>replicase</b> 14:4,15 <b>replicate</b> 19:7,8 23:19 28:1 70:10 70:13 75:19 79:20 87:1,6 88:6 89:10 91:17 106:2 122:16,23 247:14 <b>replicates</b> 123:1,4 <b>replicating</b> 77:11 122:18 <b>replication</b> 13:22 13:25 14:10 16:1	16:9 17:4,14 18:12,13 19:2,14 20:3,17 21:23 24:5,21 30:10 63:7 75:6,18 77:5 79:18 87:21,23 152:4 156:9 169:13,15 <b>replications</b> 86:25 <b>replicon</b> 152:2,3 156:10 158:15,23 159:15 163:21 168:4,25 169:5 170:2 174:18 178:19 <b>replicons</b> 169:2,20 <b>report</b> 46:14 73:14 <b>reported</b> 18:5 19:24 37:22,24 38:23 45:15 53:10 69:1 76:8 98:15 126:11,12 129:24 131:23 136:22 141:8 144:2 154:3 154:18 157:13 159:7 175:25,25 <b>reports</b> 4:22 98:7 130:9 140:4 175:10,11,16,21 <b>represent</b> 52:15 62:11 145:16 261:18 <b>represented</b> 47:22 <b>representing</b> 277:1 277:13 <b>represents</b> 186:25 187:9 214:4 261:17 <b>reproducible</b> 219:11 <b>Republic</b> 45:3 <b>require</b> 7:8,9,16 141:11 160:4,5 243:17 <b>required</b> 176:16 188:15 269:14 <b>requirement</b> 222:10 233:25 <b>requirements</b> 213:1,7 221:12
---	--	---	---	---

222:2 244:25	122:24 131:7,10	<b>Reston</b> 11:16 20:11	<b>re-interested</b> 43:21	<b>rip</b> 113:23
<b>requires</b> 7:17 52:4	131:11 132:14	20:14 40:24 41:2	<b>rhesis</b> 237:20	<b>rise</b> 58:23 78:6
163:5 185:6	134:24 135:23	41:23 42:4 44:10	<b>rhesus</b> 27:8 92:22	<b>rises</b> 75:10 218:10
254:23	155:15 161:13	44:11,12 45:17	95:12,17,19,22,25	<b>risk</b> 44:23 122:15
<b>rescue</b> 14:11	165:23 175:20	56:2 66:24 67:2	96:7,10,11 98:1	<b>risks</b> 162:25
<b>research</b> 3:5,25	176:9 177:7,8,8	126:7 213:21	98:25 99:3,8,9,11	<b>risk/benefit</b> 163:1
7:22 71:21 82:8	177:10,14 179:1,1	<b>restricted</b> 20:16	99:24 100:1,3,5	<b>RNA</b> 12:10 13:7,22
86:9 92:23 96:1	179:6,7,8,14	62:7 170:16	100:18,20,25	14:14 22:13 58:11
122:9 158:10	181:10,14 182:2,8	<b>restrictions</b> 7:13	102:2,15 104:3	59:2 66:2 169:10
221:10 277:19	182:21 183:7	<b>result</b> 24:18 27:21	111:17 114:10	<b>rNAPc2</b> 26:21
<b>researchers</b> 63:19	184:13,23 185:19	59:5 60:16 76:2	116:9,13 118:8	111:13
<b>resemble</b> 214:21	186:18 187:7,19	81:24 88:9 111:23	121:2 127:12	<b>RNAs</b> 55:22
215:2	187:21 188:11,14	112:5 114:23	128:16 130:15	<b>RNA-dependent</b>
<b>reservoir</b> 45:7	193:5,15 198:16	213:3 250:3	131:4,21 133:11	14:14
<b>reset</b> 33:1	199:18 200:6	<b>resulted</b> 24:23	134:23,24 135:13	<b>RNP</b> 13:21 14:7
<b>resident</b> 50:20	204:15,16,22	<b>results</b> 47:2,9,18,23	136:9,17,17 137:1	20:2
<b>residents</b> 41:4	205:17,20 206:4	48:22 49:14 68:2	137:16 138:10,17	<b>road</b> 40:25
<b>resist</b> 72:12 74:22	206:11 207:9	68:3 69:20 84:10	139:5,7,15 140:11	<b>robustness</b> 216:20
<b>resistance</b> 72:14	209:9,14,15 214:1	85:4 158:13 160:7	158:20 238:12	<b>rodent</b> 14:23 68:16
85:8	214:20 215:17,20	195:10 197:11	240:14,19,25	68:17 82:4 83:13
<b>resistant</b> 60:6	223:20 237:19	256:19 260:2	241:2,4,8,15,17	86:8,16 92:18
68:23 69:8,12	246:12 254:23,25	<b>resume</b> 129:22	242:4 245:9,11,12	93:13,24,24
72:9 83:15 84:1,7	255:5,11 271:14	<b>resumed</b> 67:14	245:15,16,18	126:21 143:17
85:1 139:8 199:23	271:15 280:10	<b>retain</b> 43:15	249:18,19 258:7	159:13,25 184:17
200:4,11	<b>responses</b> 54:7	<b>retrospective</b> 39:8	261:25 266:2	203:1,4,7,14
<b>resistance</b> 74:20	63:8 79:8 82:12	<b>retrovirus</b> 18:3	<b>ribosomes</b> 16:4	233:20 234:7
<b>resolution</b> 194:4	83:22 85:15 87:4	<b>returned</b> 35:2	<b>rich</b> 88:21 105:9	257:14,22 258:3,7
196:9	87:8,10 90:11,17	<b>reuse</b> 272:16	<b>Richard</b> 76:7 81:18	258:11 270:4
<b>resolved</b> 276:14	168:6,6,10,14	<b>reusing</b> 102:24	<b>rickettsial</b> 108:4	<b>rodents</b> 80:25 82:7
<b>resource</b> 40:3	169:9 172:14	<b>revaccinate</b> 206:7	<b>rid</b> 10:19 71:21	83:14 84:10,13
<b>resources</b> 40:19	173:16,18 174:15	<b>reverse</b> 13:19	72:7,13	93:19 107:3 160:7
276:6	175:13 176:2,7,8	19:20 20:5 23:12	<b>Rift</b> 270:3 271:12	220:20 236:23
<b>respect</b> 67:2	176:14 179:24,25	85:22 88:16 99:1	<b>right</b> 5:1 15:13	240:11 257:19
<b>respirator</b> 52:14	180:3,24,25 183:3	157:22 261:12,19	31:22 33:11 53:14	258:25 274:18
<b>respiratory</b> 125:11	183:12,24 187:22	<b>reversion</b> 25:1	53:18 57:11 71:11	<b>Roederer</b> 192:9
275:9	188:5 189:6,8	157:20	78:1 83:11 88:1	<b>role</b> 2:18 14:22
<b>respond</b> 43:5 85:2	190:21,23,24	<b>review</b> 33:15 47:3	92:11 93:22 102:5	26:15 50:12 56:17
114:24	191:3,4,17,25	150:6 157:5	108:14 113:21,21	59:25 60:5 61:3
<b>responded</b> 114:25	192:3,7,12,21,22	161:17 174:25	123:18 124:23	72:14 80:3 167:11
<b>responding</b> 33:13	192:25 193:1,14	227:8 233:1,5	125:18 129:23	179:2 183:6 237:2
33:19	193:23 194:10,17	<b>reviewed</b> 68:14	134:13 136:25	240:3 275:16
<b>response</b> 2:9 3:14	194:18,19,25	226:25	138:19 143:10	<b>roles</b> 33:11 161:9
31:15 33:11 36:18	198:11,13,15	<b>reviewer</b> 227:11	144:10,17 145:23	<b>Rollin</b> 188:21
37:20 53:21 59:1	204:10,21 205:3	<b>reviews</b> 150:8	146:10 148:11	<b>room</b> 126:10
60:9 71:22 72:10	209:8,12,21 220:8	163:10 274:24	151:1 192:11	132:16 150:2
72:20 80:18,22	225:2 246:4	<b>revisit</b> 13:13	232:6 248:3 255:3	230:12 238:4
81:6 83:18 84:1,2	<b>rest</b> 133:7 137:9	<b>revolution</b> 37:17	261:9 263:8 271:1	<b>rooms</b> 130:9
85:3,18 86:24	262:12	<b>revolves</b> 5:24	279:9 280:13,22	<b>Rosettas</b> 47:17,21
88:14 91:9,19	<b>restimulated</b>	<b>revolving</b> 210:11	<b>rigid</b> 109:12	<b>Rose's</b> 18:23
93:24 95:7 122:17	171:21	<b>re-emerging</b> 6:2	<b>rigorous</b> 196:18	<b>rotation</b> 206:5

<b>roues</b> 274:18	168:2 256:2	<b>Sarah</b> 142:9	261:23	98:10,12,19 99:1
<b>rough</b> 275:12	264:17	<b>SARS</b> 5:20 26:7	<b>scientific</b> 32:16	100:8,18 101:13
<b>roughly</b> 70:4	<b>running</b> 67:9 115:9	<b>satisfy</b> 233:24	33:6 46:18 218:4	104:2 106:5,18,19
207:13	116:17 161:17	<b>save</b> 52:19 149:12	252:10 276:15	106:21 107:5,20
<b>round</b> 70:2 169:13	246:15 256:4	<b>saved</b> 92:16	<b>scientists</b> 86:8,15	107:21,22 108:1
<b>rounds</b> 169:15	<b>Russia</b> 46:11	<b>saw</b> 25:1 98:14	127:11 130:8	108:14 109:4,6,8
<b>route</b> 50:6 52:9	<b>Russian</b> 98:15	117:10 129:21,23	156:23	109:17,20 110:5
71:1 72:16 74:10	101:8	133:12 137:14,18	<b>screen</b> 17:23	110:16 112:18,23
74:23 102:20	<b>Russians</b> 66:24	138:9 169:23	125:15 215:23	112:24 113:25
117:24 125:11	92:24 104:8	170:1,8,19,24	<b>screening</b> 8:13	114:6 116:10
147:16 167:1	126:13 127:1	172:18 174:17	20:25 30:21 47:12	117:3,8,10,15,16
215:24 218:25	<b>rVSV</b> 27:23 28:3	175:24 201:22,25	203:5,6,15	117:19 118:6
223:8 224:3 225:8	270:19	202:14 262:21	<b>se</b> 247:21	119:6,9,10,12
225:13 226:2	<b>R0</b> 147:24	<b>saying</b> 14:25 52:9	<b>second</b> 13:8 17:7	120:1 121:20
229:6,13,23 231:6		83:9 217:16	31:8 34:20 89:14	122:18 123:24
231:9,9 233:7	<b>S</b>	218:10 222:4	109:22 164:8	130:2,3,9,20
246:25 250:14	<b>Sabin</b> 86:12	237:7 256:10	177:11 211:4	131:2,4,4,6,18,22
251:11,19 252:2,3	<b>sac</b> 107:10 119:15	<b>says</b> 252:16	213:23 232:6	131:24 132:5
252:20 266:19	<b>sacks</b> 273:18	<b>scale</b> 126:3	277:17	133:4,21 134:12
267:16 270:8,13	<b>safe</b> 186:11 226:1	<b>scale-up</b> 160:9	<b>secondary</b> 34:15	134:20 135:1,8,16
272:6 274:14	244:5	<b>scar</b> 277:23	147:19 155:25	135:23 136:6,8,18
275:3,22	<b>safely</b> 54:20 63:23	<b>scatter</b> 141:24	171:8 232:3	136:23 137:1,9
<b>routes</b> 50:25	<b>safety</b> 7:10 19:17	<b>scattered</b> 105:18	267:14	138:8,8,20,21
218:22 225:14	30:6 54:23 143:5	<b>scenario</b> 66:20	<b>secondly</b> 90:1	139:11,19 140:16
247:6,10 250:16	150:10 157:11,19	144:7 229:17	191:21 206:2	140:18 142:23
250:19 266:24	157:25 159:2,2,19	267:4,8	243:3	143:24 146:6,23
268:14 269:1	160:20 162:10	<b>scenarios</b> 147:22	<b>Secretary</b> 257:3	149:15,22 154:13
270:15 274:15,20	166:8 199:6	219:16	<b>secrete</b> 193:9	156:2 162:11
276:2	212:25 217:17	<b>schedule</b> 92:3	<b>secreted</b> 22:9,12	167:8 169:22
<b>routine</b> 263:5	224:7 249:24,25	<b>scheduled</b> 67:8	51:7	170:7 171:4,10
<b>row</b> 40:9	<b>SAH</b> 17:4	<b>scheme</b> 7:23	<b>secreting</b> 50:24	172:8,16 173:2,21
<b>RSV</b> 157:17	<b>salad</b> 268:20	<b>schemes</b> 8:7	192:16 193:18	174:3 175:19
<b>RT-PCR</b> 58:2 59:8	<b>saline</b> 114:17	<b>Schmaljohn</b> 1:22	<b>secretion</b> 26:11	177:25 180:6
<b>rubella</b> 160:20	<b>salt</b> 138:18	149:6,12 163:18	<b>section</b> 3:4 6:15	183:1 190:9,11
<b>rule</b> 6:23 7:2,12,17	<b>sample</b> 54:21	164:1,19 165:13	94:24 212:22,23	191:18,25 193:22
8:20 64:23 127:19	121:24 202:8	166:17 201:18	222:6	198:8,20 203:2
147:4 176:18	260:10,11	230:18 231:16	<b>sections</b> 216:1	205:22 206:1,15
179:17 186:5,20	<b>samples</b> 55:16	238:3 239:18	<b>security</b> 44:24	206:23 208:10
211:7 212:9,12,19	65:10 75:7 136:5	245:8 256:2,4	<b>see</b> 5:4 6:3 21:11	209:8,9,10,14
213:1,7 214:6	136:6 196:1,21	261:9,15 262:11	23:16 25:12 29:13	211:13 214:2,21
215:5 216:3,24	242:10	265:22 266:4,15	35:6 39:23 44:6	215:6 219:23
217:14,19 221:24	<b>Sanchez</b> 1:21 22:13	271:17 272:12	53:4 55:17 58:23	225:20 228:9,18
224:9,10,16,20	53:21,23 64:1,8	277:17	59:4,12 62:6,12	228:23 229:1,3,4
226:20 227:14	65:18 66:7,22	<b>school</b> 1:24 36:3	64:25 66:7,13	229:8 238:7,11
231:7 232:23	94:11 107:4	149:7,21	67:24 68:11 69:11	239:7,11 240:1,4
234:2 242:24	125:10 188:21	<b>SCID</b> 71:10 72:6	74:22 75:9 76:22	244:2 245:4 249:1
245:1 252:7,7	248:12 252:24	73:19 183:9	77:6,24 78:8,15	249:12 252:16,18
278:8 279:22	253:18 273:25	<b>SCIDs</b> 72:12 73:18	79:9 82:4,25 83:2	258:23 265:9,17
<b>rules</b> 6:25	280:12	73:23	83:3,6 86:6 94:8	272:8,25 273:5,9
<b>run</b> 113:16,20,21	<b>Sans</b> 43:4	<b>science</b> 88:5 96:6	94:12,25 95:1,3	273:18 274:1,5

276:12 277:5,10 280:22 281:12 <b>seed</b> 100:1 253:15 253:16 256:17 260:12 <b>seeds</b> 258:14 266:6 <b>seeing</b> 46:9 66:10 69:2 106:10 107:13 109:20 155:4 175:8 182:20 204:9 205:6 206:24 248:7 278:11 280:7 <b>seemingly</b> 38:24 155:14 <b>seen</b> 5:18 6:7 30:18 39:9 40:2 46:13 46:17 53:13 59:7 81:15 83:8 98:16 98:17 106:15 108:22 119:20 130:15,18 157:14 163:11 178:3 205:3 206:12 208:23 215:2 229:10 233:19 237:20,24 248:3 249:11 264:6 269:12 273:9,13 278:5 <b>sees</b> 165:5 <b>select</b> 143:15 217:24 221:12 <b>selection</b> 70:8 215:15 218:1 257:21 <b>selfishly</b> 228:17 <b>Senate</b> 9:1 <b>send</b> 255:18 <b>sense</b> 12:18 16:2 25:12 38:15 166:25 216:5 239:13 245:10 261:4 <b>sensitive</b> 51:13 103:7 150:20 152:14,15 153:24 190:23 200:10 250:6	<b>sensitivity</b> 173:10 <b>sent</b> 49:14 264:19 <b>separate</b> 21:3 41:16 114:15 209:25 251:3 <b>separately</b> 165:25 224:12 <b>sepsis</b> 26:8 76:8 108:23 112:21,22 112:25 113:2,4 <b>September</b> 1:7 4:14 <b>septic</b> 54:16 62:24 <b>sequence</b> 12:18 56:11 96:11 151:10 172:6 <b>sequenced</b> 42:21 47:15 96:7 <b>sequences</b> 19:24 <b>sequencing</b> 11:14 <b>sequential</b> 72:25 104:17 <b>sequentially</b> 73:19 104:23 <b>sera</b> 48:16 174:22 178:12 197:21 207:20,22 <b>serial</b> 69:3 107:10 119:15 273:18 <b>series</b> 36:9 42:12 43:23 45:9 235:23 235:24 <b>serine</b> 17:11 <b>serious</b> 32:5 <b>seriously</b> 5:14 <b>seroconverted</b> 49:5 59:19 <b>seroconverting</b> 59:21 <b>seropositivity</b> 48:8 <b>serotype</b> 206:5 <b>serotypes</b> 66:19 206:8 <b>serum</b> 61:22 87:13 90:18 116:14 136:3 170:5 188:9 188:12 202:6 <b>service</b> 268:16 <b>serving</b> 39:19 <b>SES</b> 55:24	<b>session</b> 2:4,15 9:5,9 28:17 148:14 149:3 211:16 214:18 <b>sessions</b> 269:17 <b>set</b> 6:25 29:21 34:18 35:23 62:17 62:21 209:6 211:5 211:18 223:15,17 232:17 244:25 278:17 <b>setting</b> 56:25 145:13 146:7,20 146:24 148:6 219:15 232:4 <b>settings</b> 150:19 275:13 <b>setup</b> 20:6 169:1 <b>seven</b> 43:9 45:18 59:12 71:2 72:1 73:3 100:5,21 102:15 152:1 163:12 171:22 180:11 <b>Seventy-six</b> 272:12 <b>sever</b> 108:23 <b>severe</b> 39:22 41:6 54:15 55:18 56:25 58:17 66:12 68:22 76:7,12,24 78:19 109:1 112:21,22 112:25 113:2,4 117:9 130:3 131:3 137:18 219:7 229:7 244:13 <b>severely</b> 72:18 102:7 <b>severity</b> 58:24 81:10 134:16 135:15 136:2 139:20 142:25 <b>sGP</b> 22:10 25:6,8 25:10 29:6,19 56:4,16 <b>shape</b> 13:5 <b>share</b> 256:16 <b>Sharif</b> 94:20 118:23 <b>shed</b> 139:25 <b>shedding</b> 50:23	161:23 269:4 <b>shift</b> 202:2 258:19 <b>shipment</b> 44:12 <b>shipping</b> 95:17 <b>SHIV</b> 113:18 <b>shock</b> 54:16 62:23 62:24 81:23 83:6 <b>short</b> 19:23 37:1,5 75:5 84:24 103:12 109:25 113:8 161:17 <b>shortcut</b> 224:16 <b>shorter</b> 135:15 143:1 <b>shortest</b> 139:14 <b>shortly</b> 42:15 166:12 <b>short-change</b> 36:11 <b>shot</b> 78:4 141:24 159:16,17 160:8 164:13 165:1 185:6 189:25 <b>shots</b> 164:25 <b>show</b> 12:3 22:20 29:18,22 60:24 64:12 71:12 75:15 76:19 77:21 78:6 79:6 90:2 110:11 110:23 115:5 117:8 118:15,16 127:21 129:15 130:13 132:12 134:18 137:21,24 144:19 145:3 156:15 164:2 179:12 181:13 184:19 185:9,21 186:11,17 188:13 198:3 199:25 205:21 211:12 223:3 224:23 229:7 231:8,15 237:1,7 238:23,25 244:8 250:15,23 251:25 <b>showed</b> 60:16 75:17 79:16 104:8 107:25 118:23 120:14 122:7 126:13 130:2	131:3 138:6 139:22 141:15 142:18 181:5 192:20 200:12 239:25 241:13 242:7 273:5 <b>showing</b> 55:25 77:9 78:9 88:7 99:16 107:4 111:17 115:19 127:2 131:10,13 140:15 140:19 172:4 173:6 187:11,13 188:22 189:22 190:10 196:21 197:11,12 231:20 272:23 <b>shown</b> 14:4 16:22 25:15,17 26:12 27:1,4 29:15 51:17 56:3 60:7 76:2 84:18 116:11 141:16 142:12 161:2 169:1,17,20 170:11,13,14 171:6,11 172:11 172:14 174:15 175:19 176:1 180:14 184:15 185:23 193:16 198:12 200:11 206:12 231:7 240:11 254:7 263:25 <b>shows</b> 14:1 21:9 56:12 59:2 62:24 74:24 87:11 99:14 103:12 105:13 108:19 109:17 112:10 116:7 118:3 125:2,3 131:1 132:23 133:2 144:4 145:5 181:12 194:4 236:18 276:20 <b>showstopper</b> 157:21 159:1 <b>showstoppers</b> 157:8 <b>shut</b> 268:16
---	--	--	---	--

<b>sick</b> 27:13,20 35:3 36:4,16 42:20 69:17 70:1 71:10 71:11,25 73:3 132:18 153:18 265:7	278:21 <b>simpler</b> 194:19 <b>simplifies</b> 185:24 <b>simply</b> 72:4 76:7,12 80:25 81:2 87:24 186:6 194:22,24 246:2 263:5	152:1 165:10 169:8,23,24 180:11 210:17 237:14 247:3 <b>sixth</b> 4:14 5:15 <b>sizable</b> 37:7,21 39:7	277:21 278:3 <b>smarter</b> 114:11 <b>Smith</b> 169:2 <b>snapshot</b> 168:5 174:25 <b>sneezing</b> 51:20 <b>sockets</b> 97:12 <b>solely</b> 268:23 <b>solid</b> 45:14 154:8 <b>solidify</b> 240:8 <b>solidly</b> 153:10,22 165:3	261:14 264:12 268:25 269:2 274:16,24,25 275:10,12,19,20 <b>sorted</b> 166:12 <b>sorting</b> 203:5 <b>sorts</b> 6:9 52:21 71:20 <b>sounded</b> 10:6 <b>sounds</b> 187:3 <b>source</b> 35:14 36:24 42:3,10 51:8 83:11
<b>side</b> 24:15,16 37:18 64:24 180:9 265:16 <b>sign</b> 69:11 <b>signal</b> 12:16 48:17 49:24 59:9 193:6 <b>signaling</b> 56:19 85:12 <b>signals</b> 12:19 191:14 197:22 <b>significant</b> 4:15 111:24 112:19 114:25 117:19 124:18 130:5 134:18,21 135:7 136:13,18 137:1,4 138:24 140:25 160:22 170:8 196:23 205:19 225:22 230:22 240:3 <b>significantly</b> 5:8 136:11 139:14 <b>signs</b> 71:12 78:19 132:13,14 139:18 229:7	<b>simultaneous</b> 39:3 <b>simultaneously</b> 45:13 <b>single</b> 13:13 18:10 40:10 42:16 79:23 79:23 97:21 120:8 150:17 151:12 158:17 159:16,17 160:8 165:1 169:13 185:5,10 186:24 189:20,25 191:20 194:1 195:20 198:18 199:21 207:25 214:4 256:12,13 257:24 259:7 270:20 <b>singling</b> 14:20 <b>sinus</b> 77:8 <b>sir</b> 49:7 206:22 <b>sIRNA</b> 124:19 <b>sit</b> 211:15 <b>site</b> 23:14 54:3 75:18 87:23 109:3 <b>sites</b> 154:17 <b>siting</b> 25:3 <b>sits</b> 211:7 <b>sitting</b> 140:8 178:15 226:7 <b>situation</b> 16:11 25:2,25 57:17 66:3 98:18 250:13 252:9 277:1 <b>situational</b> 160:25 <b>situations</b> 32:21 52:17 <b>six</b> 4:16 43:9 95:24 99:15 102:9 103:17 104:23 120:8 133:1 135:17 138:10,10 138:11 141:7 145:20,22,24	<b>skinned</b> 49:12,19 <b>skins</b> 51:10 <b>skip</b> 21:19 <b>skipped</b> 44:16 <b>Slavin</b> 227:13 <b>sleeping</b> 228:1 <b>slide</b> 5:5 26:24 63:12 65:13 101:14 116:7 118:1 142:18 150:14 156:1 169:18 171:15 172:23 201:19 220:15 227:20 236:9 274:6 <b>slides</b> 17:19 75:4 137:21 145:14 170:15 236:10 241:13 276:12 <b>slight</b> 23:16 136:20 137:1 <b>slightly</b> 139:8,15,17 154:23 222:18 258:20 271:3 <b>slow</b> 71:13 <b>slower</b> 24:25 <b>small</b> 2:11 34:12,22 36:9 38:6 43:8,12 44:5,10,14 45:3 67:17 68:11 74:4 82:24 94:7,17,18 108:7 124:9 147:21 196:13 221:13 233:2 269:3 <b>smaller</b> 34:23 214:3 255:5,7,22 <b>smallpox</b> 231:18	<b>soluble</b> 13:2 22:8 22:22 23:2,6 <b>solved</b> 64:25 <b>somebody</b> 33:1 118:1 144:7 154:20 251:18 256:15 267:12 268:18,20 <b>somewhat</b> 33:9 45:1 70:19 216:6 217:2 225:2 <b>soon</b> 4:9 100:16 <b>sorry</b> 9:7 11:18 29:1 65:17 67:16 72:24 86:4 115:24 122:21 125:9 172:7 208:4 220:21 272:12 273:25 <b>sort</b> 5:6,16 6:25 33:12,18 34:16,18 35:8,21 39:11,18 44:23 45:23 46:19 48:17 50:16,21 52:5,14,19 70:16 71:13,16 73:1,9 86:15 91:24 190:12 193:21 210:17 211:2,12 211:17 213:10 214:17 215:24 216:20 217:25 218:10,10 219:17 220:3,15,23 221:2 221:9,12 223:13 224:6 225:24 229:11 230:15 234:17 261:12,13	<b>space</b> 52:13 74:15 <b>span</b> 191:10 <b>spark</b> 43:21 <b>sparse</b> 4:5 <b>speak</b> 10:3 26:21 28:11 125:19 163:9 164:2 258:25 <b>speaker</b> 9:21 31:8 149:6 167:10 <b>speakers</b> 9:6 10:7 151:25 211:18 281:11 <b>speaking</b> 276:8 <b>speaks</b> 276:24 277:18 <b>special</b> 31:9,13 32:4 34:5 41:5 46:4 257:22 <b>species</b> 2:13 11:5,9 11:11,14,15 12:24 38:12 39:5 41:1 42:16 46:24 47:4 47:7,17,20,22 48:1 56:2 68:21 78:13 89:8 92:21 95:12,25 98:23 100:9 114:8 125:16 128:14 129:24 130:11 133:2 134:19

138:4,14,25 139:4 139:12,21,24 143:1,6,15 145:7 151:21 155:3,11 155:12 165:14,16 165:16 186:24 214:1,4,9,13,14 214:15 217:22,23 218:1 220:9,19,21 220:22 226:12,16 233:23,24,25 234:3 235:16 236:13 237:17 238:9 259:5,7 281:1	253:3 <b>spoken</b> 28:16 160:21 <b>sponsor</b> 218:11 230:14 251:9 267:22 279:11,15 <b>sponsored</b> 269:21 <b>sponsors</b> 211:13 217:20 220:16 224:5 226:24 243:19 255:23 <b>sponsor's</b> 233:10 <b>sporadic</b> 49:8 <b>spray</b> 272:22 <b>spraying</b> 144:8 146:17 <b>spread</b> 39:15 79:19 143:16 146:6 260:5 <b>spreads</b> 80:5,5 <b>spring</b> 6:11 <b>squeeze</b> 113:10 <b>squint</b> 156:20 <b>stability</b> 254:15 <b>stable</b> 21:5 268:19 <b>staff</b> 221:14 <b>stage</b> 8:3,3 119:1 119:13,22,24 216:6 225:20 247:20 248:10 252:9 <b>stages</b> 8:10 <b>stain</b> 107:16 191:12 273:10 <b>staining</b> 57:25 117:13 140:19 172:23 191:19 <b>stamp</b> 63:13 <b>stand</b> 193:25 211:22 <b>standard</b> 39:24 179:9,11 190:12 195:8 247:13 256:14,15 <b>standardization</b> 256:18 <b>standardize</b> 261:12 263:17 <b>standardized</b> 174:10,11	<b>standing</b> 9:11 106:20 <b>staph</b> 18:10 <b>start</b> 12:16 17:23 84:5 101:11,11 107:21 108:15 112:9 120:12 123:23 185:20 194:2,23 211:19 214:17 216:5 219:12 220:3,24 221:6,7 248:7 265:6 268:14 280:22 <b>started</b> 5:17 39:14 58:4 71:18 73:17 86:9 93:14 99:5 114:18,20 118:15 154:12 235:5,22 245:11 263:21 <b>starting</b> 12:11 19:20 30:12 72:23 73:1 94:13 107:22 118:15 124:8 141:2 232:8 <b>starts</b> 229:6 <b>STAT</b> 182:19 <b>state</b> 28:3 34:14 58:10 61:6 269:21 <b>stated</b> 143:7 258:15 <b>statement</b> 145:3 205:17 <b>states</b> 63:18 93:9 <b>state-of</b> 156:4 <b>station</b> 40:11 <b>stations</b> 65:8 <b>statistical</b> 216:15 224:1 <b>statistically</b> 60:24 136:13 196:23 <b>statisticians</b> 233:4 <b>statistics</b> 142:10 <b>STAT-1</b> 85:18,23 <b>stay</b> 75:12 92:3 <b>steal</b> 6:24 <b>stemming</b> 61:3 <b>step</b> 21:10 155:25 195:6 197:8 201:16 208:22 <b>Stephen</b> 19:21	<b>stepped</b> 167:21 <b>steps</b> 18:11 21:23 154:9 <b>sterile</b> 154:5 <b>Steven</b> 76:3 <b>Stewart</b> 20:25 <b>stick</b> 146:8 166:22 230:12 247:14 254:11 259:24 272:7,9,14 <b>sticky</b> 48:16 <b>stimulate</b> 204:20 <b>stimulating</b> 172:14 191:7 <b>stimulation</b> 275:21 <b>stimulatory</b> 124:10 <b>stinal</b> 273:2 <b>stock</b> 70:24 121:11 121:15 253:24,24 261:14 <b>stockpile</b> 30:25 <b>stocks</b> 121:14 253:2,24 <b>stolen</b> 167:22 168:8 <b>stop</b> 12:16 28:21 250:20 <b>storm</b> 25:25 <b>story</b> 39:1 47:8 93:1,7 109:25 <b>straight</b> 68:25 193:23 271:22 <b>strain</b> 100:20 127:3 129:13 140:12 144:25 152:5,6,14 152:16,17 153:7 220:23 223:8 243:4,12,13 245:4 256:6,11,25 257:6 261:13 263:24 <b>strains</b> 11:11 41:7 151:13 152:13 154:11,24 165:13 174:13,16 179:5 185:15 213:20 214:16 235:16 238:9 257:8 261:11 263:12,16 264:14,17 281:2,2 281:3,6 <b>strain-like</b> 34:17	<b>stranded</b> 12:10 <b>strange</b> 10:6 47:9 120:5 <b>strategies</b> 17:17 104:13 111:11 123:24 124:24 149:10 201:21 <b>strategy</b> 6:8,9 22:5 70:3 73:16 115:22 171:16 <b>stratify</b> 206:22 <b>stream</b> 75:9 <b>streams</b> 169:21 <b>strictly</b> 145:12 <b>Stroher</b> 24:2 <b>strong</b> 59:9 152:21 175:8,19 241:17 <b>strongly</b> 237:5 <b>structural</b> 23:9,24 55:25 161:24 169:6 <b>structurally</b> 162:1 <b>structure</b> 12:8 25:14 97:12 150:23 155:23,24 <b>struggling</b> 5:9 278:3 <b>Stuart</b> 11:12 <b>stuck</b> 222:7 230:11 <b>studied</b> 88:11 <b>studies</b> 7:13 8:20 22:3 25:18 41:3 55:5 60:3,7 63:19 63:23 87:12 93:16 95:21 97:1,23 98:2,12,25 99:2,5 99:12,19,20 101:12 102:13 103:20 104:18 113:12 114:15 115:10 118:23 119:2,15 120:13 120:17 123:16 127:1,10,15,17 128:21 129:11 140:11,24 141:11 141:14 168:24 169:4,16,17,19 170:4 171:14,18 171:19 176:14,15
--	--	---	---	--

178:19 183:9	241:19 242:11	244:7,8	<b>suit</b> 74:15 164:4	196:5 203:9
184:1,1,6,7 185:8	263:7 266:2	<b>subtypes</b> 89:8	272:4	206:25 207:11
186:6,7,8,16,16	272:20	165:10 185:13,16	<b>suitable</b> 248:25	247:22 258:21
189:17 195:4	<b>studying</b> 68:20	244:18	<b>suits</b> 52:13	279:9
196:17 197:25	<b>stuff</b> 211:24 225:24	<b>subunit</b> 177:5	<b>Sullivan</b> 1:25 96:16	<b>Surely</b> 118:19
198:3,4,17,21,24	226:8 261:11	<b>subway</b> 144:7,8	159:12 183:14,19	<b>surface</b> 13:14 19:1
199:1,5,13,15	266:7	<b>sub-cu</b> 113:10,14	202:24 204:6	214:17
200:11 201:3,4,5	<b>Styrt</b> 227:16	128:5	206:20 207:11,17	<b>surfaces</b> 50:25
201:6,10,11	<b>sub</b> 95:24	<b>sub-species</b> 95:23	207:22 208:4,7,13	254:14
206:20 212:13	<b>subcutaneous</b>	96:18	208:17,19 209:18	<b>surge</b> 136:25
213:3 216:3 217:1	74:23	<b>success</b> 158:14	245:17 246:24	<b>surprise</b> 24:10
217:4,13,14,17,17	<b>subject</b> 7:18 64:6	<b>successful</b> 74:1	253:21 255:3	164:25 165:1
217:19,20 219:14	191:2 199:1	<b>successfully</b> 258:24	276:10 277:25	195:3
220:6,6 221:18	207:25	<b>succumbed</b> 132:6	278:22	<b>surprised</b> 132:17
222:1,14,17,17	<b>subjected</b> 258:11	188:25	<b>Sullivan's</b> 241:18	132:22 203:8
223:11,15,17	<b>subjective</b> 57:7	<b>succumbing</b> 132:1	<b>summarize</b> 26:4	<b>surprising</b> 27:18
224:9 226:9,10	187:3	<b>suckling</b> 69:7	185:7 197:18	27:21 117:21
232:24 240:7,21	<b>subjectivity</b> 101:22	<b>Sudan</b> 11:15,24	198:25 279:18	<b>surprisingly</b> 76:13
241:12,18,18,20	102:1,18	39:4 40:12 41:7	<b>summarized</b> 48:5	<b>surrogate</b> 278:16
241:23 243:18,25	<b>subjects</b> 186:7,11	44:14,15 45:10	<b>summarizes</b> 35:25	<b>survey</b> 152:9
244:23 245:4	188:9,24,25	51:4 56:1 58:18	<b>summarizing</b> 110:7	<b>surveys</b> 48:14 49:2
254:4 255:9	189:24 190:2,3	59:6 60:18,21	<b>summary</b> 75:2,4	<b>survivability</b>
259:11 263:3,5	197:14 198:8,10	70:17 100:11	133:14 168:4	145:10
270:19 275:3	198:21 199:17	141:2 170:18	174:1 188:20	<b>survival</b> 27:16
276:1,4,4 278:20	224:8	185:13 198:13	235:13 236:10	60:25 101:24
279:24 280:2	<b>submission</b> 229:19	199:2 238:24	237:23 267:25	115:21 116:2
<b>study</b> 2:23 18:15	<b>submit</b> 226:5	239:1,15 243:7	276:13	147:6,10 173:12
19:11 47:6 54:18	232:25	<b>sudden</b> 264:8	<b>summation</b> 134:16	190:11 197:5,6,14
63:7 88:13 96:6	<b>submitting</b> 8:20	<b>sufficient</b> 134:9	<b>summer</b> 46:15	214:24
97:16 98:15 99:10	<b>subsequent</b> 46:3	163:7 165:2	<b>superior</b> 233:22	<b>survive</b> 80:21
101:13,19,19,20	48:24 173:5	177:11 181:5	<b>supervisor</b> 227:10	116:2 121:1
102:3,10,14,17	<b>subset</b> 60:20	215:9	<b>supplemental</b>	190:20,21 209:1,2
105:2,4,7 107:10	<b>subsets</b> 191:13	<b>sufficiently</b> 186:25	276:4	265:7
111:22 114:4,13	278:19	214:4	<b>suppliers</b> 96:20	<b>survived</b> 27:11,13
115:14 117:8	<b>subspecies</b> 241:14	<b>sugar</b> 93:5,5	<b>support</b> 20:6 28:19	27:19 35:5,17
120:6,22,24	242:22 243:12	<b>suggest</b> 39:7,23	40:19 43:6 47:19	38:17 44:4 49:12
123:10 129:10	<b>substance</b> 213:15	124:4 181:15,17	52:3 127:18	49:23 60:12
133:16,21 134:24	<b>substances</b> 54:9	182:12 199:16	169:17	102:11 115:1
135:23 137:20	55:17 62:1 109:24	<b>suggested</b> 45:7	<b>supported</b> 24:2	120:21,23,24
140:14 177:12	<b>substantial</b> 7:12	51:20 64:11 195:5	<b>supporters</b> 46:3	133:24 135:6
187:12 188:20,23	36:12 202:2	220:15	<b>suppose</b> 271:24	<b>surviving</b> 60:13
189:22 190:17	213:16	<b>suggesting</b> 51:21	<b>supposed</b> 28:25	66:14
194:17 195:1	<b>substitute</b> 259:18	51:25 205:15	54:1 90:2	<b>survivor</b> 139:5
196:12 197:11	<b>subtle</b> 173:17	206:17 220:16	<b>supposition</b> 256:6	<b>survivors</b> 27:14
198:5,10 210:13	<b>subtract</b> 195:25	<b>suggestion</b> 31:21	<b>suppression</b> 80:2	44:20 191:23
214:23 216:3,3,4	196:5 207:19,24	41:19 50:1,15	82:12	193:25 196:23
216:8,12,19,21	<b>subtracted</b> 202:7	85:16	<b>sure</b> 11:12 17:6	<b>susceptible</b> 68:22
217:16,19 218:25	<b>subtracting</b> 202:9	<b>suggestions</b> 199:12	47:21 74:6,16	70:19 71:23 72:6
222:13,15,23	207:24	<b>suggests</b> 97:21	82:9 106:11	72:11 74:10 83:17
223:5,11 226:11	<b>subtype</b> 243:9	269:13	150:25 183:7	83:20



256:25 257:1,6 260:8,17 269:7 270:10 272:15 277:21 <b>terribly</b> 149:14 <b>terrific</b> 67:11 158:16 160:7 <b>terrorist</b> 126:18 <b>test</b> 41:21 48:16 49:21 84:11 88:4 196:18 243:13 244:7,8 <b>tested</b> 55:17 68:21 169:19 172:18,24 203:11 245:6 252:14 <b>testing</b> 54:22 55:8 64:10 67:6 78:24 84:6,15 85:20,25 85:25 87:22 99:7 218:23 276:19 <b>tests</b> 50:3 <b>tether</b> 113:19 <b>th</b> 114:17 <b>thank</b> 4:2 9:8,14,23 10:2 28:21 31:7 52:24 53:2 86:18 86:21 92:1,7 118:16 142:13 146:1 148:13 167:9,14 176:24 177:1 183:13,19 227:17 228:3 234:25 281:8,11 <b>thanks</b> 4:3 53:23 125:18 163:16 177:15 208:19 210:5 230:7 <b>theme</b> 112:17 <b>theoretically</b> 157:21,23 <b>theories</b> 24:1 <b>therapeutic</b> 28:10 54:7 219:8 229:10 247:19 248:1,15 251:5,7,17 276:18 <b>therapeutics</b> 6:19 7:8 63:9 127:18 148:7 245:23 246:10,13	<b>therapy</b> 28:13 89:24 90:7 227:16 <b>the-art</b> 156:5 <b>thick</b> 59:3 <b>thing</b> 3:17 10:13 25:6 31:25 39:20 43:17 58:21 69:9 71:16 73:2 76:6 84:3 90:15 91:12 99:11 104:2 105:7 110:3,5 115:10 116:1 129:11 150:7,10 190:22 193:4 203:1 204:5 208:22 211:4,17 211:21 224:13 239:11 240:2 244:21 253:12 258:13 260:3 263:11 269:15 273:7 274:5 275:10 279:9,19 280:9 <b>things</b> 5:8 29:10 37:7 45:1 52:14 64:19,22 75:2 77:19 78:15 80:20 88:4 99:19 101:24 104:9 106:25 107:8 117:18 124:23 134:8 141:21 144:1 150:17 152:23 159:5 178:18 185:20 191:18 192:16 209:7 232:20,21 237:10 239:6 240:20 251:6 255:21 259:22,25 261:21 263:3,14,18,19 267:21 274:16 278:14 280:16 <b>think</b> 4:15 10:16 11:3,23 12:1 15:2 15:12,24 16:19 17:18,20 18:17 19:21 20:19,25 21:14,19,24 23:3 25:20,21 26:20	27:16 28:8,18,19 28:20 29:6,15 30:9,11,19,22 31:1,3,15 32:4,7 32:13,15 33:14 37:7,8,12 38:20 42:14 43:18 44:3 47:6,20 51:9,11 52:24 54:13 63:18 63:19 64:18 65:6 68:6 69:18 72:15 75:2 77:21 79:17 80:19 81:11 83:10 84:17 86:7 88:2,7 88:15,24 89:8,14 90:9 93:22,23 95:24 96:8,9 97:24,25 98:8 102:9 103:6 107:2 110:2 111:21 112:7,10 115:5 116:8 117:25 118:7 120:3,10 121:3 123:22 124:3 125:1,10 126:20 134:9 142:20 144:5,6,13 145:2 146:20 147:3,16,17,18,23 150:3 151:22 154:25 159:7 160:21 164:4 166:10,19 176:13 177:16,23 178:1 178:17 179:4,15 179:21 180:9,18 181:2,18,23 182:12 185:18 187:20,21 190:25 194:4,18 197:25 200:21 203:3,15 203:17 204:4,7 205:6,13 208:17 210:19 211:14 213:7,12 216:18 216:19 217:8 219:16 220:4 221:6,22 223:1,2 223:12,13 224:14 226:1,4,17,23	228:17,25 229:14 229:18 230:11,13 230:14 232:5,6 234:3,5,8,21,23 235:1,5,6 236:17 236:23,24 237:9 237:10,15 238:17 238:20 239:13,15 239:24 240:2,4,5 240:6,8,11,18,19 241:5,9,10,11 242:2,4,17 243:16 243:21,23,25 244:3 245:9 246:9 247:1,12 248:2,4 248:6,8,10,14,25 249:3,9,18,19 250:5 252:4,5 253:6,11 254:19 255:1,12,16 257:17 261:7 262:4,5,9,21 263:13,19,20 264:4,5 267:5,12 267:19 268:3,6,6 268:12,13 269:15 269:17,20,21,25 271:11 272:3,10 272:14 273:15 274:13,18 275:11 275:16,18,22 276:11,14,19 277:1 278:4,6 279:4,8,19,20 280:15,16,25,25 281:1,10 <b>thinking</b> 90:8 133:19 154:19 162:23 198:4 215:4 216:8 217:8 219:9,12 225:5,13 231:21 232:19 243:4 261:10 268:14 277:12 <b>thinks</b> 244:15 <b>third</b> 40:9 112:13 153:19 214:22 <b>Thirty</b> 228:15 <b>thorough</b> 50:14 <b>thought</b> 4:12 92:17	110:24 118:12 126:4,8,19 132:21 154:10 163:18 264:7 267:9 280:9 <b>thoughts</b> 166:14 229:3 <b>thousands</b> 79:22 <b>thread</b> 38:8 <b>threat</b> 1:2 32:6,9 166:24 230:22 257:1,4 <b>threats</b> 6:10 147:21 185:15 <b>three</b> 2:13 12:12,23 21:13 31:22 35:7 47:20 56:2 71:2 71:14,25 95:11 98:22 101:2,15 104:24 111:19 114:14,16 116:23 125:16 138:13 139:3 142:18 143:1,15 153:18 165:19 170:15,24 171:22 173:3,10 176:5 179:12 193:18 196:13 198:6,6 202:22 206:3,8,11 208:1 218:5 221:23 228:21 236:13 246:16 256:25 258:6 266:19 <b>threefold</b> 258:12 <b>threshold</b> 124:22 164:10,11,12 177:18,25 <b>thresholds</b> 62:25 <b>threw</b> 63:12 268:20 <b>thromboblasic</b> 109:24 <b>thrombocytopenia</b> 65:22 78:12 108:18 110:16 <b>thromboplastin</b> 79:7 <b>throughput</b> 30:20 <b>throw</b> 127:6 140:6 <b>thunder</b> 6:24 <b>Th1</b> 176:9
--	---	---	---	--

<b>Th2</b> 188:9	<b>tink</b> 88:18	263:11 266:20	106:22 107:4,25	<b>traditional</b> 73:9
<b>tie</b> 250:12	<b>tip</b> 124:20	268:1 269:13	108:11 116:6	128:4 134:25
<b>till</b> 109:7	<b>tippling</b> 158:18	278:5 279:13,19	118:14 137:23	277:8
<b>Tim</b> 227:11	<b>tissue</b> 21:5 24:18	279:24	140:7 142:13	<b>traditionally</b> 96:25
<b>time</b> 8:1 19:15 32:7	26:2 34:10 57:25	<b>toe</b> 41:12	154:19,21 188:21	<b>traffic</b> 4:6
33:2 34:13 36:13	77:13 80:7 81:7	<b>toes</b> 33:23	201:4 238:22	<b>trailer</b> 19:24
38:15 40:22 41:1	82:14,21 109:24	<b>told</b> 4:11 12:3	239:25 240:6,10	<b>train</b> 91:22
42:23 43:18 47:22	110:2 111:1,5,6	131:8 140:2	241:25 246:25	<b>transcribe</b> 266:23
52:25 58:4,7	111:15 112:12	246:25	248:11 252:23	<b>transcribed</b> 9:20
59:11,13,21 61:8	121:13	<b>Tom</b> 1:16,18 21:14	253:21 258:15	<b>transcriptase</b> 14:3
66:5 72:4 73:19	<b>tissues</b> 57:22 62:9	25:4 26:20 27:2	280:9	14:15
73:21 74:7 79:8	76:21 94:4,17	28:20 31:8,9	<b>Tony's</b> 94:12	<b>transcription</b> 12:25
80:15 92:16 93:19	105:9 117:17	38:20 46:9,21	118:12 125:2	13:25 14:8 15:1
93:21 94:16 100:2	138:21 139:1	48:5 50:4 51:17	238:18,24 247:7	16:1 17:4 18:11
100:18 102:14	260:1 273:11	52:24 53:20 54:13	248:2 259:13	18:13 20:3,17
103:10 104:4	<b>titer</b> 106:16 194:22	54:17 55:4 64:11	<b>tool</b> 20:18	<b>transcriptional</b>
112:9 115:9	195:23 197:4,5,15	65:25 68:4 76:4	<b>tools</b> 17:20,22	13:22 21:3
117:25 118:9	197:24 198:12	76:18 77:21 82:22	18:15 19:10 28:8	<b>transfected</b> 169:11
119:14 122:17,22	<b>titers</b> 24:7,7,17	84:12,12 90:13	94:21 153:5	<b>transfer</b> 178:8
123:23 124:4,13	105:6,10 106:15	92:4 94:10 100:13	179:22	182:18 183:1,2
126:4 134:13	121:22,25 175:8	120:3 122:7	<b>top</b> 97:14 218:10	<b>transferred</b> 170:6
135:5,10,11,25	175:14,17 176:4	123:22 125:22	<b>topic</b> 50:6 101:6	170:21 172:25
136:24 139:9,13	177:24 189:12,13	126:20 127:11	161:8	173:4,21
141:8,22 143:16	195:7,9,25 196:4	128:3,13,24	<b>topics</b> 31:4	<b>transfers</b> 182:25
149:25 153:4	196:8,22,24 197:1	129:12,17,24	<b>total</b> 4:24 33:20	<b>transfusion</b> 50:8
156:18,19 158:4	197:23 201:23,25	130:1 131:2,23	36:5 62:12 78:7	<b>transient</b> 209:8
158:12,23 161:17	203:12,24 208:25	132:6 136:22	78:20 94:19 108:2	<b>transition</b> 184:21
163:12 165:5	225:1	137:23 138:6	108:6 114:15	257:14
168:7 182:14,23	<b>title</b> 149:7	140:13 141:8,10	136:16 138:25	<b>transitional</b> 154:10
189:1 190:3	<b>titrate</b> 121:14,14	142:5 145:13	198:8	<b>translate</b> 127:7
207:13 210:4	121:15 277:4	189:2,23 201:5	<b>totally</b> 54:24 69:12	203:16 205:5
214:12 219:18	<b>titrating</b> 122:3	203:8 223:13	83:17 250:1	<b>translates</b> 145:7
222:4,12 223:5,15	<b>titration</b> 195:14	230:8,24 232:2	<b>totals</b> 45:16	<b>transmembrane</b>
225:21 226:13	<b>titrations</b> 195:11	235:16 238:3	<b>tote</b> 39:12	22:15,21,23
229:6,12 230:10	197:19	242:7 245:10	<b>totes</b> 45:17	<b>transmission</b> 36:11
235:13 238:25	<b>TM</b> 192:19	247:4 248:23	<b>touch</b> 129:22 161:7	36:22 50:10 51:16
246:15 256:2	<b>TNF</b> 25:9 26:11,15	264:23 265:20	202:25	51:20,21 52:3
260:3,5 264:2	26:15 29:19,22	266:19 272:4	<b>touched</b> 46:21	126:5,8 144:13
270:14 276:11	79:9 110:13	273:5	<b>tough</b> 114:12	147:19 148:4
<b>timeline</b> 229:4,9	191:17 193:5,6,7	<b>tomb</b> 47:17	<b>town</b> 37:16	232:5 254:13
<b>timely</b> 65:7	<b>today</b> 3:22 4:14 5:1	<b>tomorrow</b> 3:22	<b>toxic</b> 56:9	268:2
<b>times</b> 42:6 54:23	50:5 68:8 125:19	245:22 246:7,11	<b>toxicity</b> 162:8	<b>transmit</b> 144:21
64:17 70:4,21	128:1 129:14	281:12	213:15	<b>transmittable</b>
111:22 114:5	140:1 146:4	<b>tomorrow's</b> 247:17	<b>toxicologist</b> 227:11	144:19
120:20 137:13,17	179:20 211:5,18	<b>tone</b> 34:18	<b>tracheal</b> 273:3	<b>transmitted</b> 52:1
139:6 145:3 146:3	211:22 212:6	<b>Tony</b> 1:21 18:17	<b>track</b> 151:1 224:10	144:5,22
208:1 211:9	215:19 219:10,17	22:13 27:16 29:15	<b>tracked</b> 29:9	<b>travel</b> 44:25
227:22,23 228:1	219:21 223:13	49:7 53:21 65:12	<b>tract</b> 271:23	<b>traveled</b> 44:1
235:1 274:24	225:9,25 226:6	67:19 68:14 78:14	<b>trade</b> 113:5	<b>traveler</b> 35:2
<b>timing</b> 219:13	229:25 246:8	80:19 94:11 95:6	<b>tradition</b> 127:8	<b>treat</b> 25:8 83:19

112:4 113:4,7  
 124:15 228:16  
 247:21 248:15  
**treated** 27:9 35:16  
 42:20 49:23  
 111:25 112:2  
 114:16,17  
**treatment** 16:22  
 27:5,12 28:18  
 93:18 99:2,2,5  
 101:19 102:3  
 113:12 114:18,20  
 115:13,16 123:23  
 123:25 247:19  
 248:9  
**treatments** 93:17  
 104:13,13 112:8  
 115:24 124:5  
**tree** 275:9  
**tremendous** 68:1  
 224:18 229:25  
 244:18  
**trends** 157:14  
**trial** 166:9 179:11  
 206:15 212:15  
 227:6  
**trials** 163:6 179:3  
 199:6 201:12  
 206:21  
**trick** 70:16  
**trickle** 137:10  
**tried** 26:19 71:5  
 78:25 113:6  
 141:21 156:21  
 180:7 182:24  
 206:5 213:10  
 262:18 264:22  
**tries** 7:24  
**trigger** 110:4 111:6  
 162:6  
**triggers** 109:23  
**tropism** 19:5 27:25  
 121:18  
**trouble** 163:9  
**true** 74:19 75:21  
 84:3 105:21 140:1  
 196:20 206:10  
 209:3 238:15,16  
**truly** 122:12  
**try** 30:4 53:5 94:6

101:6 111:12  
 115:20 121:16  
 124:20 133:16  
 143:23 168:4  
 178:16 183:23  
 184:23 202:24  
 211:24 225:6  
 264:21 265:12  
 275:8 280:5,6,22  
**trying** 6:24 8:23  
 29:5 33:5 64:21  
 64:22 66:10 68:10  
 88:6 93:21 99:18  
 101:12,23 104:12  
 104:25 107:7  
 114:4 120:11  
 123:6,18 132:19  
 147:3 150:17  
 211:12 226:19  
 246:2 248:15  
 270:7,12 279:13  
**TUESDAY** 1:7  
**turn** 9:4 52:25  
 59:13 204:22  
**turned** 4:13 84:15  
 253:19  
**turns** 70:15 93:3  
 96:24 200:6  
 205:23 272:6  
**Twenty** 115:18  
**two** 11:2 12:23  
 14:14,20 15:22  
 21:2 22:9 29:15  
 35:5 36:15 38:16  
 39:3 40:6 43:1  
 49:9 53:5 62:1  
 85:9 89:15 90:10  
 95:22,25 101:2,15  
 103:24 104:17  
 107:8 111:19  
 115:1 116:24  
 120:21 121:14  
 123:2 127:5  
 131:25 132:21  
 133:22 135:3  
 136:6 138:10  
 139:21 140:24  
 149:22 153:17  
 164:7 165:17,19  
 169:21 170:16,23

171:3 172:8  
 174:13,16 177:3  
 180:10 185:3  
 190:15 193:20  
 195:12 196:3  
 198:7 202:4  
 205:24 206:3,11  
 212:20 214:6,9,14  
 218:5 219:16  
 221:3 225:22  
 226:16,21 228:21  
 232:16 233:15,23  
 233:24 234:4  
 241:1,23 245:24  
 245:24 251:6  
 256:18 258:18  
 259:9 268:24  
 270:18  
**type** 11:15 19:4,7  
 21:11 24:24 66:17  
 66:17 71:9,22,23  
 72:2,10,12,13,19  
 72:22 80:1,2  
 82:12 83:15,16  
 84:2 85:21 86:25  
 89:10 90:14 91:6  
 91:19 123:1,4  
 127:10 156:12  
 181:22 212:9  
 232:16,24 245:3  
 262:2  
**types** 24:12 43:20  
 75:19 162:4 177:4  
 193:9 277:2  
**typical** 12:9,11  
 78:3 94:4 106:9  
 106:16 117:11  
 131:13,18 156:23  
 258:13 271:24  
**typically** 75:9  
 259:1 273:5

---

**U**


---

**UAAUU** 12:18  
**Ud** 24:2  
**Uganda** 34:9 35:12  
 38:7 48:2 60:21  
 61:17 63:13  
**Ugandan** 37:23  
**Uige** 37:16

**ultimate** 279:12  
**ultimately** 163:2  
 186:3,15 206:16  
 262:5,9 267:20  
**unanswered**  
 255:13  
**unclear** 56:15,16  
 61:4,13  
**Uncoating** 15:24  
**unconfirmed** 42:11  
**undergoes** 169:13  
**underlying** 188:16  
 188:18  
**understand** 8:17  
 66:11 82:6 104:14  
 123:7 213:17,18  
 213:22 215:1  
 247:6,23 249:24  
 251:16 268:8  
 277:24  
**understandable**  
 250:1  
**understanding**  
 183:23 278:23  
**understated** 163:14  
**understood** 12:25  
 14:1,10 15:24  
 213:14  
**underway** 178:15  
**undoubtedly**  
 267:14  
**unexpectedly**  
 200:17  
**unfinished** 149:17  
**unfortunate** 10:18  
 32:22  
**unfortunately**  
 32:12 36:13 37:16  
 40:2,6 184:19  
**unhappy** 11:21  
**unhealthy** 62:22  
**unhelpful** 260:7  
**uniform** 133:9  
 141:24 185:11  
 190:13 197:19  
 254:7  
**uniformly** 27:8  
 75:14 247:3  
 257:18  
**Union** 126:16

**unique** 36:23,25  
 271:21  
**uniquely** 257:6  
**unit** 21:4 31:9  
 252:25 253:20,23  
**United** 63:17  
**units** 27:8 158:20  
 159:15 164:23  
 253:5,6 254:21  
**units/ml** 153:16,23  
**universal** 194:12  
 195:9  
**University** 1:22  
 149:7  
**unknown** 257:3  
**unmet** 6:20  
**unnecessary** 217:3  
**unpredictable**  
 257:5  
**unpublished**  
 201:19  
**unregulated** 61:11  
**unresponsive** 61:6  
**unsegmented** 12:10  
**unstable** 22:19  
**unsuccessful** 43:20  
**unsure** 53:25  
**untreated** 218:20  
**unusual** 23:20  
 102:2  
**updates** 4:6  
**upper** 265:24 266:8  
**upstairs** 148:17  
**urban** 144:12  
**USAMRID** 68:5  
 167:16 183:22  
**USAMRIID** 1:20  
 46:5 49:7 73:11  
 92:15 94:23 95:18  
 96:5 98:14 99:16  
 101:23 117:23  
 125:15 126:12  
 127:11 133:19  
 146:3 149:16  
 176:23 177:24  
 256:11 268:9  
**use** 2:22 5:24 7:13  
 19:15 21:6 25:10  
 29:3 33:22 43:8  
 43:15 57:5 61:18

71:21 90:14 93:3 95:12 99:10 101:22 113:10 115:4,13 122:14 123:15 127:6,7 128:15,16,16,17 129:1 141:22 150:15,25 160:5 160:25 176:19 180:4 184:7 185:10 197:8 199:4 202:6 205:24 206:10,17 207:22 210:13 211:23 215:24 217:22,23 222:15 223:19 224:4 225:19 228:23 229:1,2,13 230:5 231:3,6,9 233:5,8 233:12,23 234:3,7 240:16 242:21 243:3,5 246:21,22 258:9 261:14 265:25 266:12 271:12 272:17 278:17 <b>useful</b> 51:4 57:18 73:7 203:4,14 218:6 223:22 226:11,19 250:4 <b>uses</b> 113:4 158:23 185:4 192:11 <b>usual</b> 190:3 <b>usually</b> 18:4,18 44:8 45:5 48:16 132:4 146:7 210:16 248:19 <b>utility</b> 96:9 160:24 178:21 179:25 228:24 237:8 249:7 <b>U.S</b> 221:17,20 267:13	176:10 188:24 189:24,25 190:2 <b>vaccinating</b> 231:1 231:1 <b>vaccination</b> 66:20 122:13 206:2 277:22 <b>vaccinations</b> 28:15 <b>vaccine</b> 2:17,21 19:16 34:7,11 41:24 54:10 84:11 84:14 85:25 89:10 90:8 93:18 98:1 99:3,12 115:12,16 115:18 116:1,20 120:6 122:14,21 122:23,25 123:2 129:10 133:20 149:8,10 150:16 150:25 151:12,12 152:3,4 153:20,21 156:4 157:1,8,10 157:11 158:2,2,12 158:18,24 160:11 160:12,17 161:5 163:2 164:14 165:11,12 166:15 167:6,25 168:1 176:4 177:9 178:2 179:16 180:19 181:12,16,20,22 181:25 182:9 183:15 184:3 185:10,24 186:10 187:9,13,16 190:9 190:12 193:22 194:13 196:17,18 197:9 198:9,14,18 199:10,12,15 201:21,22 203:5 205:18,19 206:1 208:23 213:21,22 214:12,20 215:16 219:16,21 225:1 229:9 230:10 235:3 241:23 244:6,8 245:22 246:1 248:15 250:16,21 251:1 251:17 252:17	254:6 257:8,8 262:6,8 264:4 265:8 266:25 267:16 270:19 275:21,24 276:18 277:20 <b>vaccines</b> 2:15,23 6:18 7:7 28:16 43:20,22 63:10 84:6 85:4 86:4 89:24 93:16 99:1 116:16 127:18 149:3,11 154:2 157:19 158:5 160:3,18 163:13 166:5 167:18 177:4,5,5 179:5 180:10 184:9 186:2,11,13 187:10 197:7 208:12 210:13,25 218:6 227:12 243:14 246:8,12 270:3 271:12 274:22 <b>vaccinia</b> 157:24,25 158:5 <b>vagaries</b> 260:22 <b>vague</b> 23:6 26:5 <b>valid</b> 7:5 8:19 50:3 <b>validate</b> 222:21,22 <b>validated</b> 216:16 222:24 223:19 <b>validation</b> 177:10 221:15 <b>Valley</b> 270:3 <b>value</b> 55:10 163:14 197:2 228:23 <b>values</b> 55:9 66:9 131:16 196:1,5,7 197:21 207:20 <b>variability</b> 100:9 102:17 119:10 154:13,15 155:8 155:13,22 238:19 241:12 256:24 260:2,3 <b>variable</b> 38:20 154:11 181:8 200:8,9	<b>variables</b> 223:17 247:12 271:5 <b>variance</b> 155:12 <b>variant</b> 164:21,22 165:17 <b>variants</b> 71:20 151:14 <b>variation</b> 37:9 161:21 259:8 <b>variations</b> 120:5 175:7 <b>varies</b> 196:2 238:7 <b>variety</b> 76:10 84:20 192:8 278:3 <b>various</b> 8:10 43:4 43:20 48:6,8 57:22 62:25 82:18 85:21 98:19 109:1 147:22 156:9 166:15 254:20 <b>vary</b> 44:8 48:10 197:16 <b>vascular</b> 26:1,4 78:22 82:1,20 83:5,10 108:8 240:3 <b>vasculature</b> 108:8 <b>vast</b> 238:6 <b>vector</b> 18:14 93:2 123:13,20 156:12 157:25 158:21,24 159:19,21 164:7 184:25 199:14 200:3 206:10 207:6,7,7 209:3 <b>vectored</b> 157:25 158:5 177:9 <b>vectors</b> 19:16,18 27:24 28:3 123:16 156:10 181:23 184:5,6,16,20,24 184:25 199:23 200:1,13,16,17,18 200:23 203:21 205:13 207:1 <b>VEE</b> 158:15,23 163:21 168:3 169:12 178:19 271:12 <b>vehicle</b> 39:19	<b>vein</b> 74:15 <b>vena</b> 109:2 <b>Venezuelan</b> 152:2 168:25 270:2 <b>verified</b> 56:13 <b>Vero</b> 21:6 23:17 121:22 134:7 258:19 259:14 <b>Veros</b> 121:23,24 <b>version</b> 19:23 20:12 56:5 57:15 <b>versions</b> 21:2 <b>Versteegen</b> 201:8 <b>versus</b> 47:5 92:18 97:10 101:25 106:12 116:22 117:6 119:18 121:23 132:8 134:7 178:25 179:8 204:8,9 213:21 240:14 242:1 245:9,16 249:19 <b>verus</b> 95:13 <b>vervet</b> 228:2 <b>vessel</b> 107:14 109:19 <b>Vet</b> 142:8 <b>veterinary</b> 137:14 <b>Victor</b> 20:7 24:14 <b>Victoria</b> 11:9 <b>victory</b> 5:7 <b>Vietnamese</b> 96:16 96:20 97:1,6,19 241:20 242:1 <b>view</b> 9:18,19 30:10 31:3 39:8 105:1 217:3 219:23 225:7 226:22 261:5 279:1 <b>viral</b> 58:11 75:6,18 78:1 79:18 116:6 119:3 124:21 125:2 140:18,21 265:14 <b>viremia</b> 27:14,15 27:19 82:25 83:1 108:14 116:14,15 116:15 136:3,10 141:14 153:15
<b>V</b>				
<b>vaccinate</b> 178:6 206:8,12 219:18 251:18 <b>vaccinated</b> 171:20				

154:4 237:10,11 242:20 248:8,22 260:21 271:25 <b>viremic</b> 153:18 <b>Virginia</b> 40:24 <b>virine</b> 124:7 <b>virion</b> 14:2,7 55:25 61:7 79:23 <b>virions</b> 12:3 79:22 <b>virologists</b> 151:2 261:21 <b>virology</b> 260:22 <b>virtual</b> 4:7 <b>virulence</b> 21:13 24:14 25:16 56:19 256:23 257:25 258:2,8 <b>virulent</b> 38:24 53:12 73:25 89:12 129:7,9 139:3 258:5 <b>virus</b> 11:3,3,10,10 11:15,16,16,24 13:4 17:15,25 18:11,12,15,16,24 19:5,11 20:23 21:1,11,14,22 23:14,16 24:6,9 24:16,20,22,24 27:25 34:6,16 36:25 37:3 42:4 42:11,21 44:13 47:15 50:24 51:5 51:7,8,11,18 53:12 55:21 56:1 56:1,3 57:10 58:9 58:12 60:1,8,10 60:12 61:5,13,13 62:10 68:24 69:14 69:19,19,25 70:9 70:12,24 71:10 72:2,24,25 73:5,8 73:13,17,19,23,25 74:9,11,16,19,22 74:23,24 75:9,18 75:22 77:7,11,16 77:24 79:25 80:4 81:3,4 82:11,13 82:15,24 86:11,13 86:22 87:9 88:9	88:17,25 89:4,7 89:10,11,17,19 90:3,3 91:8,24 92:10,20 94:25 103:9 104:14 105:4,5,8,16,18 105:25 106:1,3 120:14 121:13 122:11,17 123:1,5 123:21 127:9,10 129:5,13,13 130:23 138:15,20 138:25 141:14 144:4,24 145:9 147:6 150:23 152:2,3,16 154:12 159:7,8 160:15 161:2,5 162:16,17 166:20 168:20 169:6,8,12 170:16 176:6,6,12 180:12 181:20 183:11 185:14 204:2 205:16,20 207:5,7 207:12 208:11 213:20 223:14 240:22 244:11 253:1 254:11,15 258:3 261:13,25 264:13,20 265:6 266:12 268:19 269:4 271:25 277:4,9 <b>viruses</b> 14:14,22 15:25 16:13 17:15 17:24 18:2,2,3,5 18:22 19:8 20:21 21:5 23:22 24:24 30:3 37:10,12 38:22 51:24 52:11 66:21 70:10,18 89:3 106:13 121:12,21 126:9 126:19,22,25 129:17 131:9 140:5 142:4,7 155:20,20 156:11 161:24 162:3,6 166:23 181:21 206:6 213:19	230:21 254:10 257:9,12,20,23 258:5,11,17,23 259:4,4 270:2 271:18,18 277:2 281:4 <b>Virus-like</b> 159:23 <b>visited</b> 35:11 36:4 <b>visiting</b> 93:1 <b>visuals</b> 259:2 <b>vital</b> 161:25 <b>vitamin</b> 102:24 <b>vitro</b> 21:18 24:4 26:12,18 28:1 87:5,21 171:21 177:22 276:25 277:13 279:3 <b>vivo</b> 21:8,19 25:3 88:6 174:8 274:11 277:1,5,7,11 279:2,4 <b>VLP</b> 21:20 <b>volatile</b> 25:2 <b>Volchkov</b> 24:14 <b>volunteered</b> 63:22 <b>volunteers</b> 63:15 63:16,21 <b>von</b> 107:16 <b>VP</b> 12:13,13,13 <b>VPs</b> 169:10 <b>VP-35</b> 152:12,20 <b>VP-40</b> 154:6 <b>VP24</b> 13:10,12 14:18 21:5 56:18 170:2 172:5 174:19 <b>VP30</b> 12:23 14:11 14:12 <b>VP30s</b> 181:13 <b>VP35</b> 56:17 175:17 <b>VP40</b> 14:16 16:6 <b>VP40s</b> 181:13 <b>VRC</b> 185:4 192:10 198:21,22 201:10 <b>VRPs</b> 174:3 <b>VSL-2</b> 18:9 <b>VSV</b> 18:1,2,19,19 18:22,25 19:4,7 26:25 27:2,10 97:3 115:12,25	116:12,19 122:14 122:15,23,25 123:2 156:12 160:7,14 161:6 228:7 <b>VSVs</b> 27:1 241:22 262:21 <b>VW</b> 147:21 <hr/> <b>W</b> <hr/> <b>waddo</b> 146:15 <b>wait</b> 248:9 <b>waited</b> 112:4 <b>waiting</b> 162:11 230:10 <b>walk</b> 228:8 <b>walking</b> 112:9 132:16 <b>Walskoff</b> 20:7 <b>want</b> 3:17 6:6,13 9:14 10:11,14,15 17:18 26:4 28:17 29:23 32:24 57:9 83:3 86:17 92:11 92:19 99:9,19 102:12,19,20,21 106:25 115:19 141:25 142:9 143:16 151:16 155:15 157:6 164:2 165:14 167:5,15,19 180:4 187:23 190:5 193:4 195:6 197:15 198:2,24 199:21 202:16 210:16,17 211:13 211:23 212:6 217:6 219:23 220:17 222:15 225:16 226:2 228:8 229:8,13 230:5 231:9,22 233:3,18 237:24 237:25,25 238:2 240:13,17 248:11 249:6 250:12,25 251:1,9,22 252:16 252:17 261:17 265:22 267:17,23	271:17 276:7 278:22 281:8,10 <b>wanted</b> 10:21 105:7 128:17,25 151:7 158:10,13 161:16 163:12 189:19 196:13 197:2 226:25 230:18 235:20 236:1,16 249:8 259:9 273:14 279:20 <b>wants</b> 181:25 230:14 <b>war</b> 37:18 <b>Warfield</b> 73:14 159:24 259:1 <b>warranted</b> 176:15 <b>washing</b> 275:12 <b>wasn't</b> 53:20 117:24 158:12 <b>wasting</b> 71:13 <b>Watsa</b> 47:2 <b>way</b> 11:19 16:15 22:24 23:7,10 25:17 33:14,19 36:13 65:5 77:13 112:5,24 126:2 146:19 150:24 154:24 158:3 164:10 168:2 174:7 186:1 191:6 192:13 193:15 194:24 197:17 211:20 212:4 215:5 216:10 219:1,2,3 223:2 229:20 230:4,16 231:18,21 233:14 244:24 253:22 272:4 275:5 <b>ways</b> 14:20 16:12 17:12,14 161:18 161:20 211:9 233:13 277:6 <b>weapon</b> 126:2,20 <b>weapons</b> 126:17 <b>wear</b> 52:13 114:5 144:15,15 <b>weather</b> 4:6,7 <b>website</b> 34:5 35:24
--	--	--	---	--

236:5	107:13 109:20	192:9 195:16	143:6,14 275:19	183:22 217:9
<b>wedding</b> 8:14	123:6 124:22,23	196:3 197:10	<b>wonderful</b> 148:17	230:1 259:11
<b>week</b> 69:7,8 72:19	127:20,20 129:20	198:3 199:13	160:10	277:2
190:15	134:3 135:1 136:4	204:13,17 206:12	<b>wondering</b> 30:5	<b>worker</b> 46:11
<b>weeks</b> 38:5 49:13	145:12 148:1,6,8	208:21,23 209:7	49:14 202:18	<b>workers</b> 144:15
71:14 95:15 145:1	148:15 150:9,11	215:19 219:10,20	232:11 242:12	<b>working</b> 14:12
190:1	150:17,25 153:3	219:25 225:8	245:24 265:23	20:14,24 21:21
<b>weight</b> 108:7	154:2 161:12,15	226:23 231:12,14	<b>word</b> 279:5	25:7 28:6 30:14
<b>welcome</b> 3:13 4:3	162:11 164:10,16	231:21 233:14,19	<b>worded</b> 224:12	30:15 57:20 71:18
9:14 10:17 182:15	164:17 165:18	237:24 239:8,21	<b>words</b> 48:8 90:3	86:10 87:24 94:15
226:4 255:18,25	167:2,2 178:4	241:15,21 245:17	103:24 104:14	99:7 103:22
281:5	179:10,10 182:13	246:17 247:13	105:25 123:13	104:19 124:22
<b>Welfare</b> 222:3	185:24 187:7,14	249:11 253:13	145:16 182:19	131:9 140:23
<b>well-known</b> 84:12	192:11 193:1	256:11 258:17	<b>work</b> 8:9 16:13	151:3,4 159:14
<b>well-taken</b> 29:11	195:17 198:4	261:21 263:21,24	35:20 56:7,15	164:17 166:11
123:17 124:3	199:9,22,24	264:6 269:7,12	68:4,9 69:14,18	217:10 223:25
<b>well-timed</b> 4:11	200:23 203:18,22	270:18 274:13,24	72:23 74:3 76:3	224:15 233:13
<b>went</b> 36:24 39:12	204:9 206:25	276:10	79:3 90:5,6,14,22	255:22 281:4
54:3 67:13 83:8	210:3 211:24	<b>wherewithal</b>	92:8,13,23 93:20	<b>works</b> 57:15 70:17
96:3,24 102:8	216:22 217:7,10	269:11	94:21 96:15,22	123:7 187:13
133:17 153:12	223:16 225:4	<b>whimper</b> 5:17	97:3 98:3 99:2,4,8	213:22 227:3
171:4 172:18	233:16 241:13	<b>white</b> 49:17 78:7	99:14 100:16	228:21
196:11 201:19	243:23 245:21	132:15 136:15,16	113:18 114:4	<b>workshop</b> 1:5,9
202:12 259:13	246:9,15 252:8,9	<b>wholly</b> 5:19	124:1,1,6 125:20	3:14 4:12 5:2,11
<b>weren't</b> 59:21	252:16 255:7	<b>wide</b> 38:13 62:10	128:24 130:24	5:24 6:22 231:12
<b>West</b> 24:5 65:8	257:25 259:19,20	<b>widespread</b> 107:9	137:23 139:3	<b>world</b> 145:1 156:16
<b>western</b> 39:11	262:6,10 274:10	<b>wife</b> 96:14	140:7 141:1,6,12	213:22 214:12
48:20 58:1	275:9 278:2,3	<b>wiggle</b> 238:4	141:16 143:5	219:16 229:15
<b>we'll</b> 4:8 5:10 17:7	280:7,14,23	<b>wild</b> 19:4,7 21:11	148:12 150:5	256:16
34:1 50:5 53:18	<b>we've</b> 5:18 12:1	24:12,23 71:9,23	158:7,9,9 159:12	<b>worms</b> 96:14
55:3 148:17	25:7 26:12 47:24	72:2 83:15 85:20	159:24 163:23	<b>worried</b> 157:11
149:25 151:24	48:12 53:13 59:14	97:13 123:1,4	166:5 167:15,17	<b>worse</b> 135:16
166:12 167:8	64:24 67:19 77:24	127:10 262:2	168:3 185:9	139:21 162:17
170:3 182:16	87:12 92:8 96:4	<b>Willebrand's</b>	203:17 210:23	<b>worst</b> 136:2
183:18 205:25	96:19 97:1,4	107:16	216:1,6,10,10	<b>worth</b> 224:19
210:4,9 229:22	98:12 100:23	<b>willing</b> 74:6 224:17	217:8 219:22	244:22 254:19
235:12 245:23	111:4 113:6,13	<b>Wilson</b> 227:14	220:2 222:20	259:9
256:2 263:3,14	121:1 122:3	<b>win</b> 122:18,21	224:18,22,24,25	<b>worthwhile</b> 82:8
281:11	123:10,10 125:20	<b>window</b> 103:11,16	226:11 227:14	<b>wouldn't</b> 41:15
<b>we're</b> 3:10 4:5,25	127:23 130:15	103:19 104:1,4	228:20 229:18,24	71:11 87:12 93:5
5:9,13 6:21 8:23	131:9 133:14	<b>Winnipeg</b> 73:12	229:25 230:2,2	93:19 146:25
9:22 11:1 20:14	136:5 140:23	235:23,25 270:23	231:14 238:24	167:5 220:14
27:1 28:6,12	141:3,5,7,21	<b>wins</b> 81:4	240:6 243:14	230:9
29:20 30:1 31:14	142:7 143:20	<b>wiped</b> 108:5	244:19 245:18	<b>wrap</b> 139:2 140:22
32:13 33:11 57:16	147:5 150:6 156:8	<b>wiring</b> 82:9	248:9,16 252:11	<b>wrapped</b> 180:22
61:20 64:21,22	156:10 157:14	<b>wisdom</b> 163:24	252:18 253:11	<b>wreck</b> 91:22
66:9 67:8,9 77:5	164:12 167:24	<b>wish</b> 9:23 67:21	<b>workable</b> 18:9	<b>writeup</b> 46:12
89:9 91:13,15,22	169:8 170:12	73:16 76:18	<b>worked</b> 73:5,18	<b>written</b> 174:25
94:12 97:25	171:18 180:13	<b>witchcraft</b> 253:8	84:21 86:2,3,4,4,5	<b>wrong</b> 66:1 89:16
104:25 106:9,11	185:9 190:23	<b>wonder</b> 50:5 64:3	147:22 176:23	91:8

wrote 81:18

---

**X**

---

**x** 164:9 260:12

**Xigris** 113:6,8

114:18 115:25

116:11 237:1

---

**Y**

---

**Yeah** 166:17

**year** 6:7 36:3 95:18

97:18 115:15

167:17

**years** 4:16 5:9

10:16 12:2 15:4

27:1 31:3 42:25

68:6 88:13 92:9

92:13,14 95:13

96:15,19 98:4

99:16 104:11

107:7 111:17

117:22 125:21

126:7 136:6

140:25 149:16

156:2,5 166:7

175:1 183:23

208:21 222:5

224:16 226:8

260:18 264:7

277:21

**yellow** 42:15

173:13 191:23

**yesterday** 4:10

**yield** 79:22 184:25

203:1

**yielded** 203:12

**Yoshi** 23:12 235:22

261:24

---

**Z**

---

**Zaire** 11:15 20:11

39:4 40:10,12

41:7 42:24 44:7

51:4 56:1,5 58:18

60:15 61:15 67:5

69:14 70:16 72:24

100:4,6 102:15

104:22 106:13

112:2 114:19

116:14,24 120:4,7

120:16,22,25

123:14,15 127:3

127:12 128:20

129:4 130:19

131:14,19 133:15

133:17 139:3

141:4 151:23,23

163:22 165:16,18

170:16 182:1

185:13,22 198:14

199:2 213:21

238:6,11,24 239:2

239:12,15 240:25

243:5,6 249:12

253:16,19 256:11

261:25 263:12

**Zaki** 94:20

**Zimbabwe** 35:3

**zone** 77:12 94:5

95:1 109:12

---

**\$**

---

**\$2,500** 95:16

**\$4,500** 95:16

**\$6,500** 95:19

**\$87** 95:20

---

**0**

---

**0.01** 253:20

---

**1**

---

**1** 2:4 21:10 24:3

26:17 71:22 72:10

72:12,13,19,22

80:1,2 82:12

83:16 84:2 86:25

90:14 91:6,19

97:18,20 108:16

137:6 164:9

254:17,17 260:7

**1,000** 27:7 99:21

128:4 132:4 135:1

135:4 166:20

167:7 168:22

246:23 247:4,12

254:9 259:12

260:24 265:19

272:24 273:2

**1:31** 149:2

**10** 2:6 59:12 68:6

71:2 92:9,14

96:17 98:25 100:5

102:16 103:19

104:1,6 106:15

112:3 116:10

133:22,25 134:25

135:2,24 141:5

199:19 246:23

254:21 260:7

266:7 272:24

273:1

**10,000** 262:15

**10-minute** 141:25

**10:00** 53:4

**10:14** 67:14

**10:40** 67:10,14

**100** 3:16 36:6 93:19

100:1,12 102:25

103:10 104:21

112:7 115:21

132:3 135:1

166:19 180:15

190:7,7 197:5

207:13 250:9,11

250:18 259:11

260:24 264:1

272:8,9,24

**1000** 103:18,25

104:3,22 112:1

114:19

**1010** 159:15 165:3

166:6 190:18

**1012** 190:13

**102** 254:21

**103** 254:12,21

266:14

**104** 248:8

**105** 102:9 248:8

266:1,17

**106** 164:10 237:13

**107** 75:12 106:16

123:2 164:23

237:13

**107.5** 106:16

**108** 75:12 153:16

158:19 164:24

165:1 237:11

260:1,13

**109** 106:15 190:18

237:11

**109.8** 106:15

**11** 1:7 100:19,19

102:16 114:16

**11th** 4:14

**12** 71:12 100:19

103:19

**12:22** 148:19

**120** 134:16

**125** 2:14

**13** 113:9,15 152:5

**13s** 152:6,14

**135** 135:16

**14** 102:9 114:15

172:5,7

**140** 53:11

**1400** 197:13

**149** 2:17 103:2

**15** 31:3 36:2 59:9

60:25 71:12 92:9

92:14 112:3

**160** 4:23

**167** 2:18

**18** 94:19 192:11

**183** 2:21

**189** 136:2

**19** 261:19

**19.4** 115:2

**1967** 34:7 69:1

105:3

**1976** 102:22

**1977** 40:11

**1979** 40:14

**1980** 100:17

**1987** 36:1 94:22

**1989** 40:24

**1990** 41:15

**1990s** 71:7 98:13

127:2 212:18

**1992** 41:25

**1994** 42:14

**1995** 42:25

**1996** 44:11 72:24

78:25

**1998** 36:7

**1999** 36:19 48:4

---

**2**

---

**2** 21:24 26:18 75:10

77:5 104:24 105:8

108:16 137:6,12

138:19 152:16

256:1 260:12

**2s** 152:17

**2:59** 210:6

**20** 42:25 45:19 57:9

59:8,9 149:16

192:12 194:14

198:21 240:24

245:16 264:8

272:21

**200** 103:22,24

277:21

**2000** 36:7 48:3

**2000/2001** 61:18

63:13

**2001** 63:13

**2002** 212:20 218:7

**2003** 111:17

**2004** 218:8

**2005** 34:25 37:15

**2007** 1:7

**2008** 236:3,6

**207** 198:21

**208** 198:22

**21** 35:6 45:20

**210** 2:23

**22** 98:25

**23** 11:25 116:9

**235** 2:24

**24** 4:20 12:13,23

112:4 171:1

228:20

**24-hour** 105:17

**25** 100:1 101:16

264:15

**250** 103:22

**27** 236:3 253:16

**270** 4:7 53:6

**28** 164:13

**29** 236:3

---

**3**

---

**3** 75:10 87:13

104:24 108:15

112:19 171:11

264:24

**3:32** 210:7

**30** 12:13,13 14:7

27:9,22 103:15

197:25 198:21 211:8 219:24 227:21,23 228:1,8 235:1 240:25 251:2,14,14 253:15 <b>300</b> 134:12 <b>31</b> 2:8 <b>314</b> 212:23 <b>33</b> 35:7 111:18 112:6 <b>347</b> 53:10 <b>35</b> 12:13 14:2 101:16 <b>3500</b> 197:5 <b>36</b> 99:25 <b>39</b> 134:23	<b>54</b> 2:9 <b>58</b> 222:6,9 <hr/> <b>6</b> <hr/> <b>6</b> 104:4 105:14 107:12 119:14 131:20 133:5,7 136:7 139:17 145:1 247:1 250:14 254:4 <b>6.3</b> 103:2 <b>6.5</b> 134:13 <b>6.6</b> 100:3 103:17 <b>60</b> 114:20 <b>601</b> 212:22 <b>62</b> 135:15 <b>66</b> 97:18 <b>67</b> 2:11 12:4 60:25 69:22 71:5	<b>9</b> 111:9 132:3 133:10 135:23 <b>9.5</b> 103:5 <b>9/11</b> 4:15 5:15,16 5:18 268:7,13 <b>90</b> 12:1 41:16 44:8 108:25 114:20 195:18 197:14 264:11 <b>90s</b> 58:5 73:17 <b>900</b> 166:7 <b>92</b> 2:12 <b>93</b> 96:11 102:9 <b>95</b> 52:15 72:24 108:22 120:25 129:4 240:25 253:16 <b>96</b> 59:14 <b>97</b> 78:25 <b>99</b> 48:3		
<hr/> <b>4</b> <hr/> <b>4</b> 2:3 75:12 77:10 77:13 95:20 105:13,17 107:20 108:15 109:18 112:20 113:13 137:11 276:8 <b>4.2</b> 116:10,15 124:21 <b>40</b> 5:9 22:10 104:5 112:21,24 134:22 135:20 <b>400</b> 4:24 <b>420</b> 37:25 <b>48</b> 4:20 228:20 <b>49</b> 172:7	<hr/> <b>7</b> <hr/> <b>7</b> 104:4 131:20 133:7 136:7 145:1 153:17 <b>7,000</b> 254:5 <b>7.3</b> 135:12 <b>70</b> 111:15 112:12 <b>70s</b> 237:14 <b>75</b> 44:8 <b>76</b> 100:13 120:7,19 129:8 261:25 272:11 <b>78</b> 111:7			
<hr/> <b>5</b> <hr/> <b>5</b> 75:12 105:14,14 105:17 107:11 109:7 133:5 136:7 153:17 260:13,14 <b>5,000</b> 195:14 262:21,23 <b>5:12</b> 281:13 <b>50</b> 35:18 38:17 44:4 100:11 104:5 115:23 138:7 153:23 170:20 250:19 264:7,8 <b>500</b> 197:4 <b>51</b> 171:25	<hr/> <b>8</b> <hr/> <b>8</b> 104:4 132:2 133:6 133:9 135:25 136:7 139:5 <b>8.3</b> 135:25 <b>8.4</b> 100:3 <b>8:30</b> 1:11 <b>8:34</b> 3:2 <b>80</b> 103:4 108:25 134:15 <b>80s</b> 86:10 <b>85</b> 102:23 <b>86</b> 272:8 <b>88</b> 136:1 <b>89</b> 41:16			
	<hr/> <b>9</b> <hr/>			