

**FOOD AND DRUG ADMINISTRATION (FDA)
Center for Biologics Evaluation and Research (CBER)
Office of Tissues and Advanced Therapies (OTAT)
70th Cellular, Tissue and Gene Therapies (CTGT)
Advisory Committee Meeting**

OPEN SESSION

**Web-Conference
Silver Spring, Maryland 20993**

September 2-3, 2021

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

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Roland Crystal, M.D.	Weill Cornell Medicine
Lindsey A. George, M.D.	The Children's Hospital of Philadelphia
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DAY 22 **OPENING REMARKS: CALL TO ORDER, INTRO OF COMMITTEE**

3

4 MR. MICHAEL KAWCZYNSKI: Good morning and
5 welcome to Day 2 of the 70th meeting of the Cellular
6 Tissue and Gene Therapies Advisory Committee. Let's
7 get started we have a big agenda today. Dr.
8 Butterfield, you ready to take it away? Oh, wait a
9 minute. I think she's connecting. So, she's
10 reconnecting her audio. All right, no problem. Yes,
11 give us one second here. So, again, welcome to the
12 70th meeting of the Cellular Tissue and Gene Therapies
13 Advisory Committee meeting. We are going to get
14 started shortly, we do have a large agenda.

15 Just some reminders to the public in that keep
16 in mind with the storms going around throughout the
17 country sometimes we do run into technical

1 difficulties. Our panelists and members are calling in
2 from around the country and we have people even calling
3 in from around the world. So with that being said, Dr.
4 Butterfield are you ready?

5 DR. LISA BUTTERFIELD: Good morning everyone,
6 thank you very much. I'd like to welcome everyone to
7 today's meeting and I'd like to welcome all of the
8 participants, all of the presenters, our colleagues at
9 the FDA, our audience, and the public who are joining
10 us today online. With that brief welcome, I'd like to
11 turn the meeting over to Jarrod who's our designated
12 federal officer and he will lead us through the initial
13 administrative processes. Jarrod, please.

14

15 **ADMINISTRATIVE ANNOUNCEMENTS, ROLL CALL, INTRODUCTION**
16 **OF COMMITTEE, CONFLICT OF INTEREST STATEMENT**

17

18 MR. JARROD COLLIER: Thank you, Dr.

1 Butterfield. Good morning everyone. My name is Jarrod
2 Collier and it is my pleasure to serve as the
3 designated federal officer for today's 70th CTGTAC
4 meeting. On behalf of the FDA, the Center for
5 Biologics Evaluation and Research and the committee,
6 I'd like to welcome everyone to day two of this virtual
7 meeting. The meeting for today will be to discuss the
8 toxicity risk of adeno-associated virus vector-based
9 gene therapy products. The discussion topics include
10 oncogenicity risk due to vector genome integration and
11 safety issues identified during preclinical and/or
12 clinical evaluation.

13 Today's meeting topic was described in a
14 Federal Register Notice that was published on July
15 26th, 2021. I would now like to acknowledge the
16 contributions of a few other members of the Division of
17 Scientific Advisors and Consultants team, including our
18 Director, Dr. Prabhakara Atreya, Joanne Lipkind, Karen

1 Thomas, Cristina Vert, who will serve as the backup DFO
2 for today's meeting, and Kathleen Hayes, who have all
3 assisted in preparing for this meeting. I would also
4 like to express many thanks to Mr. Michael Kawczynski
5 for facilitating the meeting today.

6 For any media or press-related questions, you
7 may contact FDA's office of media affairs at
8 fdaoma@fda.hhs.gov. The transcriptionist for today's
9 meeting is Mr. Graham Koester. We will begin today's
10 meeting by taking a formal roll call of the committee
11 members and temporary voting members. When it is your
12 turn please turn on your camera and unmute your phone,
13 state your first and last name, expertise, and your
14 organization. When you're finished please turn your
15 camera off and we will proceed to the next person.

16 Please see the member roster slide where we
17 will begin with our chair, Dr. Lisa Butterfield. Dr.
18 Butterfield, please introduce yourself.

1 DR. LISA BUTTERFIELD: Good morning. Lisa
2 Butterfield, I'm at the Parker Institute for Cancer
3 Immunotherapy and also the University of California,
4 San Francisco, and my expertise is tumor immunology and
5 cancer immunotherapy.

6 MR. JARROD COLLIER: Thank you, Dr.
7 Butterfield. Next, we have Dr. Tabassum Ahsan.

8 DR. TABASSUM AHSAN: Hello. I'm Taby Ahsan.
9 I'm head of Analytical Development and Characterization
10 at the Therapeutic Manufacturing facility at MD
11 Anderson. I have expertise in stem cell tissue therapy
12 and am currently focused on ACT for immuno-oncology.

13 MR. JARROD COLLIER: Thank you, Dr. Ahsan.
14 Next, we have Dr. Berns.

15 DR. BARRY BYRNE: Hi, I'm Barry Byrne.
16 Kenneth Berns or Barry Byrne?

17 MR. JARROD COLLIER: Excuse me, we're
18 introducing Dr. Kenneth Berns.

1 DR. KENNETH BERNS: Sorry, Dr. Barry is my
2 namesake at Florida. I'm Kenneth Berns, I'm a
3 professor Emeritus of molecular genetics and
4 microbiology and I work on the molecular genetics of
5 AAV.

6 MR. JARROD COLLIER: Thank you, Dr. Berns.
7 Next, we have Dr. Christopher Breuer.

8 DR. CHRISTOPHER BREUER: Hi my name's Chris
9 Breuer. I'm the Director of Regenerative Medicine at
10 Nationwide Children's Hospital. My area of expertise
11 is tissue engineering and translational research.

12 MR. JARROD COLLIER: Thank you, Dr. Breuer.
13 Next, we have Dr. Benard Fox.

14 DR. BERNARD FOX: My name is Bernie Fox. I'm
15 the Harder Family Chair for Cancer Research, Member and
16 Chief in the laboratory of Molecular and Tumor
17 Immunology at the Early Child's Research Institute,
18 Providence Portland Medical Center in Portland, Oregon.

1 My interests and research background are in tumor
2 immunology and translational clinical trials for
3 patients with cancer.

4 MR. JARROD COLLIER: Thank you, Dr. Fox.
5 Next, we have Dr. Randy Hawkins.

6 DR. RANDY HAWKINS: Good morning, Randy
7 Hawkins. Consumer representative, private practice,
8 pulmonary critical care medicine. University of
9 Arkansas for Medical Science. Good morning.

10 MR. JARROD COLLIER: Thank you, Dr. Hawkins.
11 Next, we have Dr. Jeannette Lee.

12 DR. JEANNETTE YEN LEE: Good morning, my name
13 is Jeannette Lee. I'm a professor of biostatistics and
14 a member of the Windsor P. Rockefeller Cancer Institute
15 at the University of Arkansas for Medical Sciences. My
16 area of expertise is clinical trial design. Thank you.

17 MR. JARROD COLLIER: Thank you, Dr. Lee.
18 Next, we have Dr. Eric Crombez.

1 DR. ERIC CROMBEZ: Good morning, I am Eric
2 Crombez, Chief Medical Officer of Ultragenyx Gene
3 Therapies. And my expertise is in the development of
4 gene therapies for the treatment of rare disorders.

5 MR. JARROD COLLIER: Thank you, Dr. Crombez.
6 Next, we have Dr. Mark Walters.

7 DR. MARK WALTERS: Good morning. Mark
8 Walters, Professor of Pediatrics at University of
9 California, San Francisco, and Director of the Blood
10 and Bone Marrow Transplant Program at Children's
11 Hospital, Oakland. My expertise is in hematology and
12 bone marrow transplantation. Thank you.

13 MR. JARROD COLLIER: Thank you, Dr. Walters.
14 Next, we have Dr. Joseph Wu.

15 DR. JOSEPH WU: Good morning, my name's Joe
16 Wu. I'm a Professor of Medicine and Radiology at
17 Stanford University. I also direct the Cancer in
18 Cardiovascular Institute. My expertise is in cardiac

1 tissue engineering and stem cells in gene therapy.

2 MR. JARROD COLLIER: Thank you, Dr. Wu. Next,
3 we have Dr. John Zaia.

4 DR. JOHN ZAIA: Thank you, my name's John
5 Zaia. I am the Director of the Center for Gene Therapy
6 at the City of Hope Medical Center in Duarte,
7 California. My area of expertise is a clinical
8 trialist. I'm interested in bringing new designs to
9 the clinic. Thank you.

10 MR. JARROD COLLIER: Thank you, Dr. Zaia. Now
11 we'll move on to the temporary voting members.
12 Starting with Dr. Frederic Bushman.

13 DR. FREDERIC BUSHMAN: Good morning, my name's
14 Rick Bushman. I'm a Professor of Microbiology at the
15 University of Pennsylvania and chair of the
16 microbiology department. My lab studies a number of
17 problems in genomics of microbes and gene therapy.

18 MR. JARROD COLLIER: Thank you, Dr. Bushman.

1 Next, we have Dr. Barry Byrne. You're on mute, Dr.
2 Byrne.

3 DR. BARRY BYRNE: There we go. I'm Barry
4 Byrne, a Professor of Pediatrics at the University of
5 Florida and Director of the Powell Gene Therapy Center
6 where we specialize in translational research related
7 to neurovascular diseases and other rare diseases using
8 AAV vectors. I'm glad to be here.

9 MR. JARROD COLLIER: Thank you, Dr. Byrne.
10 Next, we have Dr. LaTasha Crawford. LaTasha, I think
11 you're on mute right now.

12 DR. LATASHA CRAWFORD: All right, is that
13 better? Can you hear me now?

14 MR. JARROD COLLIER: That's perfect.

15 DR. LATASHA CRAWFORD: Okay, great. Good
16 morning, at least I wasn't the first one. My name is
17 LaTasha Crawford, I'm an assistant professor at the
18 University of Wisconsin-Madison School of Veterinary

1 Medicine. I'm a neuroscientist that studies cell-
2 specific mechanisms of pain and peripheral
3 neuropathies. I'm also a board-certified veterinary
4 pathologist with an interest in comparative
5 neuropathology.

6 MR. JARROD COLLIER: Thank you, Dr. Crawford.
7 Next, we have Dr. James DeFilippi.

8 MR. JAMES DeFILIPPI: My names James DeFilippi
9 and I'm a project manager with BR+ A Consulting
10 Engineers focusing on particle therapy. I'm a patient
11 representative and have hemophilia A.

12 MR. JARROD COLLIER: Thank you, Mr. DeFilippi.
13 Next, we have Ms. Peggy DiCapua.

14 MS. PEGGY DiCAPUA: Hi, I'm Peggy DiCapua. I
15 am patient representative. I am not a doctor. I've
16 had several people say that through the thing. I have
17 worked in the pharmaceutical industry for 30 years and
18 I am a former president for New York/New Jersey Chapter

1 of Batten Disease Support and Research. So I guess my
2 expertise is a little bit in rare disease.

3 MR. JARROD COLLIER: Thank you, Peggy. Next,
4 we'll move to Dr. Theo Heller.

5 DR. THEO HELLER: Hi. My name is Theo Heller.
6 I'm a senior investigator and hepatologist and chief of
7 Translational Hepatology Section at the NIH. My area
8 of expertise is liver disease.

9 MR. JARROD COLLIER: Thank you, Dr. Heller.
10 Next, we'll move to Dr. Roland Herzog.

11 DR. ROLAND HERZOG: Hello, I'm Roland Herzog.
12 I'm the director of the Stem Cell Therapy Program at
13 the Herman B. Wells Center for Pediatric Research at
14 Indiana University. My lab is working on AAV vectors
15 for treatment of genetic disease processing.

16 MR. JARROD COLLIER: Thank you, Dr. Herzog.
17 Next, we have Dr. Raymond Roos.

18 DR. RAYMOND ROOS: I'm Raymond Roos, professor

1 in the Department of Neurology at the University of
2 Chicago Medical Center. My expertise is
3 neurodegenerative diseases and virus infections of the
4 central nervous system.

5 MR. JARROD COLLIER: All right, thank you, Dr.
6 Roos. Next, we have Dr. Carlos Sanchez.

7 DR. CARLOS SANCHEZ: Good morning, my name is
8 Carl Sanchez. I'm a pediatric neurosurgeon at
9 Children's National Hospital at George Washington
10 University. My research focus is brain tumors
11 immunotherapy and AAV gene delivery.

12 MR. JARROD COLLIER: Thank you, Dr. Sanchez.
13 Next, we have Dr. Charles Venditti. Dr. Venditti I
14 believe you're on mute right now.

15 DR. CHARLES VENDITTI: Good morning, Chuck
16 Venditti. I'm a senior investigator in the NHGRI in
17 the Intramural Research Program at NIH. I'm a
18 pediatric biochemical geneticist. My research focuses

1 on the clinical characterization of patients and also
2 the development of AAV gene therapy.

3 MR. JARROD COLLIER: Thank you, Dr. Venditti.
4 Next, we have Dr. Charles Vite.

5 DR. CHARLES VITE: Yes, I am Dr. Charles Vite.
6 I'm a veterinary neurologist and the Director of the
7 National Referral Center of Animal Models of Human
8 Genetic Disease at the University of Pennsylvania. My
9 expertise is large animal models in preclinical trials.

10 MR. JARROD COLLIER: Thank you, Dr. Vite.
11 Lastly, we have Dr. Caroline Zeiss.

12 DR. CAROLINE ZEISS: Good morning, I'm
13 Caroline Zeiss. I'm a veterinarian boarded in retinal
14 pathology and in large animal medicine. I'm a
15 professor in comparative medicine at Yale University
16 and the expertise is neuropathology associated with AAV
17 vectors as well as translational animal models. Thank
18 you.

1 MR. JARROD COLLIER: Thank you, Dr. Zeiss, and
2 thank you all for your introductions. At this time I
3 would like to acknowledge our leadership team of the
4 Center for Biologics Evaluation and Research. Dr.
5 Peter Marks, Director, Dr. Celia Witten, Deputy Center
6 Director, Dr. Wilson Bryan, Director of Office of
7 Tissues and Advanced Therapies, who will be providing
8 FDA opening remarks and Dr. Rachael Anatol, Deputy
9 Director for Office of Tissues and Advanced Therapies.
10 Dr. Peter Marks will join later to provide closing
11 remarks.

12 Before we begin reading the Conflict of
13 Interest Statement, I would just like to briefly
14 mention a few housekeeping items. For speakers,
15 members, FDA staff and anyone else joining us in the
16 Adobe room, please keep yourself on mute until you are
17 speaking to minimize the feedback. If you have raised
18 your hand or called upon on the chair, Dr. Lisa

1 Butterfield, please speak slowly and clearly so that
2 your comments are accurately recorded for transcription
3 and captioning. Lastly, for all presenters, please try
4 and stay within your allotted presentation times so
5 that we can stay on schedule for today. I will now
6 proceed with Conflict of Interest Statement.

7 The Food and Drug Administration is convening
8 virtually today, September 2nd and 3rd, 2021 for the
9 70th meeting of the Cellular, Tissue, and Gene
10 Therapies Advisory Committee, under the authority of
11 the Federal Advisory Committee Act of 1972. Dr. Lisa
12 Butterfield is serving as the chair for today's
13 meeting.

14 Today on September 3, 2021, the Committee will
15 meet in open session to discuss the toxicity risk of
16 adeno-associated virus (AAV) vector-based gene therapy
17 products. The discussion topics include oncogenicity
18 risk due to vector genome integration and safety issues

1 identified during preclinical and/or clinical
2 evaluation. This topic is determined to be a
3 particular matter of general applicability (PMGA). With
4 the exception of the Industry Representative member,
5 all standing and temporary voting members of the CTGTAC
6 are appointed as special government employees (SGEs) or
7 regular government employees (RGEs) from other Agencies
8 and are subject to Federal conflict of interest laws
9 and regulations.

10 The following information on the status of
11 this committee's compliance with federal ethics and
12 conflict of interest laws include, but are not limited
13 to, 18 U.S. Code Section 208, is being provided to
14 participants in today's meeting and to the public.
15 Related to the discussions at this meeting all members,
16 RGE and SGE consultants, of this Committee have been
17 screened for potential financial conflict of interest
18 of their own as well as those imputed to them,

1 including those of their spouse or minor children, and
2 for the purposes of 18 U.S. Code Section 208, their
3 employers.

4 These interests may include investments,
5 consulting, expert witnesses testimony, contracting
6 grants, cooperative research and development agreements
7 (CRADAs), teaching, speaking, writing, patents and
8 royalties and primary employment. These may include
9 interests that are current or under negotiation. FDA
10 has determined that all members of this Advisory
11 Committee, both regular and temporary members, are in
12 compliance with Federal ethics and conflict of interest
13 laws.

14 Under 18 U.S. Code Section 208, Congress has
15 authorized FDA to grant waivers to Special government
16 Employees who have financial conflicts of interest,
17 when it's determined that the agency's need for special
18 government employees services outweighs the potential

1 for conflict of interest created by the financial
2 interest involved, or when the interest of regular
3 government employee is not so substantial as to be
4 deemed likely to affect the integrity of the services
5 which the government may expect from the employee.

6 We have the following consultants serving as
7 temporary voting members. Dr. Frederic Bushman, Dr.
8 Barry Byrne, Dr. Latasha Crawford, Mr. James DeFilippi,
9 Ms. Peggy DiCapua, Dr. Theo Heller, Dr. Roland Herzog,
10 Dr. Raymond Roos, Dr. Carlos Sanchez, Dr. Charles
11 Venditti, Dr. Charles Vite, and Dr. Caroline Zeiss.

12 Based on today's agenda and all financial interests
13 reported by committee members and consultants, there
14 have been four conflicts of interest waivers issued
15 under 18 U.S. Code Section 208 in connection with this
16 meeting.

17 Dr. Kenneth Berns is a committee member and
18 Dr. Charles Vite, Dr. Roland Herzog, and Dr. Barry

1 Byrne are special government employees who have been
2 issued a waiver for their participation in today's
3 meeting. The waivers are posted on the FDA website for
4 public disclosure. Dr. Eric Crombez, of Ultragenyx
5 Gene Therapies, will serve as the alternate industry
6 representative to the committee. Industry
7 representatives are not appointed as special government
8 employees and serve as non-voting members of the
9 committee.

10 Industry representatives act on behalf of all
11 related industry and bring general industry perspective
12 to the committee. Industry representatives on this
13 committee are not screened, do not participate in any
14 closed sessions if held, and do not have voting
15 privileges. Dr. Randy Hawkins is serving as the
16 consumer representative for this committee. Consumer
17 representatives are appointed special government
18 employees and are screened and cleared prior to their

1 participation in the meeting. They are voting members
2 of the committee.

3 The following guest speakers for this meeting
4 have been screened for conflict of interest and cleared
5 to participate as speakers for the Day Two meeting.
6 Dr. Deepa Chand, Executive Director of Patient Safety,
7 Novartis Gene Therapies, Bannockburn, Illinois. Dr.
8 James Wilson, Director of Gene Therapy program at
9 Perelman School of Medicine, Philadelphia,
10 Pennsylvania. And Dr. Roland Crystal, Professor, and
11 Chairman, Department of Genetic Medicine, Weill Cornell
12 Medical College, New York, New York.

13 Disclosure of conflict of interest for guest
14 speakers follows applicable federal laws, regulations,
15 and FDA guidance. At this meeting, there may also be
16 regulated industry speakers and other outside
17 organization speakers making presentations. These
18 participants may have financial interests associated

1 with their employer and support from other regulated
2 firms. The FDA asks in the interest of fairness that
3 they address any current or previous financial
4 involvement with any firm whose products they may wish
5 to comment upon.

6 These individuals were not screened by the FDA
7 for conflict of interest. FDA encourages all meeting
8 participants, including open public hearing speakers,
9 to advise the committee of any financial relationships
10 that they have with any affected firms, its products,
11 and if known, its direct competitors. We would like to
12 remind members, consultants and participants that if
13 the discussions involve any of products or firms not
14 already on the agenda, for which the FDA participant
15 has a personal or imputed financial interest, the
16 participants need to inform the DFO and exclude
17 themselves from such involvement and their exclusion
18 will be noted for the record.

1 Additionally, I would like to provide specific
2 guidance regarding the CTGTAC September 2nd - 3rd, 2021
3 meeting. Please note that the topic of this meeting,
4 the Toxicity Risk of Adeno-associated Virus Vector-
5 based Gene Therapy Products is determined to be of
6 particular matter of general applicability and as such
7 does not focus its attention on any particular
8 products, but instead focuses on the classes of
9 products under discussion.

10 Therefore, CTGTAC's role is to advise and
11 inform the FDA, CBER, and OTAT on strategies to
12 evaluate and mitigate risk associated with AAV vectors
13 used for gene therapy. Speakers will describe safety
14 issues identified during preclinical or clinical
15 evaluation of various AAV-based gene therapies. Those
16 issues include hepatotoxicity, thrombotic
17 microangiopathy, neurotoxicity and oncogenicity due to
18 vector genome integration.

1 The examples presented by the speakers
2 facilitate the committees' discussion of each risk and
3 possible mitigation strategies. This CTGTAC meeting is
4 not being convened to recommend any action against or
5 approval of any specific AAV-based gene therapy product
6 or clinical trials. Furthermore, this CTGTAC meeting
7 is not being convened to make specific recommendations
8 that may potentially impact any specific party, entity,
9 individual or firm in a unique way, and any discussion
10 of individual products will be only to serve as an
11 example of the product class.

12 Additionally, this meeting of the CTGTAC will
13 not involve the approval or disapproval, labeling
14 requirements, post-marketing requirements or related
15 issues regarding the legal status of any specific
16 products. This concludes our reading of the Conflict
17 of Interest Statement for the public records. At this
18 time I'd like to hand the meeting back over to Dr. Lisa

1 Butterfield. Thank you.

2

3

FDA OPENING REMARKS

4

5 DR. LISA BUTTERFIELD: Terrific. Thank you
6 very much, Jarrod. So now to kick us off I'd like to
7 welcome Dr. Wilson Bryan, the Director of OTAT at CBER
8 with our opening remarks.

9 DR. BRYAN: Can you hear me? Yeah, but do you
10 hear?

11 DR. LISA BUTTERFIELD: Dr. Bryan.

12 DR. BRYAN: Good morning. On behalf of the
13 FDA, I want to thank the members of this advisory
14 committee for your efforts to help us detect the safety
15 of study subjects and patients. Yesterday's discussion
16 was thoughtful and comprehensive. You have set a high
17 standard for this committee on a very important topic,
18 that is the risks associated with AAV vector-based gene

1 therapy products.

2 I'm very much looking forward to your
3 discussions and recommendations today. I also want to
4 express appreciation for our guest speakers, for the
5 public comments submitted to the docket and for the
6 opinions expressed in the open public hearing. The FDA
7 recognizes that we must listen to many voices as we
8 work together to advance the field of gene therapy.
9 I'll stop there and turn it back over to Dr.
10 Butterfield.

11

12 **SESSION 3: THROMBOTIC MICROANGIOPATHY**

13

14 **INVITED SPEAKER PRESENTATION: CLINICAL FINDINGS OF**
15 **THROMBOTIC MICROANGIOPATHY (TMA)**

16

17 DR. LISA BUTTERFIELD: Thank you. To start
18 session three we're going to welcome the Executive

1 Director of Patient Safety from Novartis, and invited
2 guest speaker, Dr. Deepa Chand, who will talk about
3 related findings for thrombotic microangiopathy.
4 Please.

5 DR. DEEPA CHAND: Thank you, everybody, thank
6 you, Dr. Butterfield. And I'd like to thank the
7 organizers for inviting me today to deliver this
8 presentation. As mentioned, I am the lead safety
9 physician for pediatric gene therapy at Novartis Gene
10 Therapies. I am also a practicing pediatric
11 nephrologist at St. Louis Children's Hospital in
12 Washington University Medical Center, where I do manage
13 patients with TMA from a clinical vantage point.

14 Today I'll provide a brief introduction to
15 thrombotic microangiopathy with an overview of the
16 pathogenesis as well as a clinical course of TMA. This
17 will be followed about with some comments regarding the
18 clinical management of TMA, with some specificity to

1 TMA experience after gene therapy, as well as a
2 discussion regarding mitigation and monitoring
3 strategies. We'll also discuss some research
4 considerations for the future, and then finally leave
5 with some summary and conclusion statements.

6 First, I think it's very important to
7 recognize that TMA is a clinical diagnosis. It's a
8 triad that consists of thrombocytopenia, which is
9 defined as platelet aggregation consumption,
10 microangiopathic hemolytic anemia, which involves the
11 destruction of red blood cells within the
12 microvasculature as well as acute kidney injury, which
13 is a possible organ dysfunction that can occur which
14 includes acute kidney injury. But it's important to
15 recognize that other organs may be involved.

16 TMA is a rare disorder and really the
17 fundamental is that this occurs as a result of
18 microvascular damage. It can occur in adults or

1 children, and the occurrence in children approximates 1
2 to 3.3 cases per million per year. As mentioned
3 yesterday, there are other terms that are often used to
4 describe TMA which includes hemolytic uremic syndrome.
5 From a nephrology perspective, we often use the term
6 hemolytic uremic syndrome, specifically atypical
7 hemolytic uremic syndrome, when defining a complement-
8 driven pathway.

9 Other entities such as thrombotic
10 thrombocytopenic purpura can also fall under the
11 broader TMA category. Therefore it's really important
12 to recognize that this is a clinical diagnosis that's
13 comprised of those three entities. When we talk about
14 the pathogenesis of TMA, what we need to understand on
15 a fundamental basis is that endothelial injury occurs
16 as part of this process. And this can either be due to
17 or be a result of dysregulation of the complement
18 pathway. It can involve either the classical or the

1 alternative pathway of complements.

2 Because this can be complex and actually be a
3 cause as well as an effect, the cause for this
4 complement dysregulation may not be evident in all
5 situations. It's important to remember that TMA can be
6 acquired and it may have genetic components or it may
7 be genetic in origin. In acquired situations, it can
8 occur in association with a wide range of infections
9 including viral, bacterial, fungal, or even parasitic
10 etiologies.

11 It's not often clear if this is as a direct
12 effect of the pathogen, or if the pathogen acts as a
13 trigger that can unmask a latent complement defect.
14 Specifically encapsulated organisms have been
15 identified as a trigger. And the reason for this is
16 that the capsular polysaccharide, which is a critical
17 virulence factor, can enable the immune system evasion.
18 It's important to note that TMA can also be associated

1 with medicinal products, and we'll dive a little bit
2 deeper in a moment regarding this.

3 Finally, underlying disease states may also
4 pose a risk factor predisposing to the development of
5 TMA. Underlying coagulation abnormalities, for
6 example, may be present in some disease states for
7 which gene therapy is used. Finally, it's important to
8 understand that gene abnormalities resulting in
9 uncontrolled complementing activation have also been
10 described.

11 When we talk about complement activation, as I
12 mentioned, complement activation does occur in
13 thrombotic angiopathy cases. Specifically, we need to
14 focus a bit more, I think, on the tissue level.
15 Complement pathway activation can occur, and this on
16 the left-hand side describes some of the complement
17 components, but I'd like to focus a bit more on the
18 endothelial activation. When endothelial injury

1 occurs, this can cause a thrombocytopenia, again,
2 through the cellular injury level. It can also cause
3 release of complement into the bloodstream and we can
4 monitor these as biomarkers that are collected through
5 blood draws.

6 But on a molecular level what we also see is
7 that this platelet dysregulation also affects other
8 cellular activities, namely the leukocyte aggregation
9 as well as leukocyte transmigration. This can lead to
10 some of the features that we see clinically in
11 thrombotic microangiopathy, including the hemolytic
12 anemia as well as the acute kidney injury. When we
13 focus just a bit more on drug-associated TMA, it's been
14 described with over 75 different agents across a
15 variety of drug classes. The mechanism can vary quite
16 a bit, and really a better understanding of that drug-
17 associated mechanism can provide a basis for better
18 diagnosis as well as treatment options.

1 In describing some of the drug-associated
2 TMAs, I think we can speak a little bit more about gene
3 therapies in our experience with them. AAV-based gene
4 therapies are currently intended for monogenic
5 disorders, two of which include Spinal Muscular Atrophy
6 as well as Duchenne Muscular Dystrophy. In both of
7 these indications, the gene therapy is administered
8 using a viral vector that delivers the replacement
9 gene. Also in both of these therapies, it's
10 administered as a single dose.

11 To focus a little bit more on spinal muscular
12 atrophy, Onasemnogene abeparvovec is the only approved
13 systemically administered AAV9-based gene therapy.
14 It's designed to deliver the SMN gene to treat Spinal
15 Muscular Atrophy. TMA is a risk that was identified
16 not in our preclinical studies, not in our clinical
17 trials, but rather based on post-marketing safety data.
18 In other words, it was described in commercial use in

1 the real-world setting.

2 When we talk about Duchenne Muscular Dystrophy
3 gene therapy, specifically AAV9-based gene therapy, it
4 is currently in clinical development through two
5 compounds. This gene therapy delivers the
6 microdystrophin gene for patients with Duchenne
7 Muscular Dystrophy. In the clinical trials, there have
8 been patients who have been reported to have acute
9 kidney injury and thrombocytopenia, which have been
10 associated with a TMA-like complement activation.

11 In focusing a bit more on each of those
12 indications, let's talk a bit about the Spinal Muscular
13 Atrophy data. And in over 1,400 patients dosed,
14 through July of 2021, nine cases have been reported.
15 As mentioned yesterday, in October of 2020, we did
16 publish our findings of three cases that were reported
17 as of that date. The information I'm going to provide
18 you today is through the end of July 2021.

1 In these patients that have been dosed, there
2 have been some themes that have emerged. The time to
3 onset of TMA, after the administration of gene therapy,
4 has ranged from 6 to 12 days, most commonly within one
5 week of dosing. The patient age at the time of dosing
6 has vary greatly ranging from four months of age to
7 four years of age, with this occurring in girls.

8 The treatments that were administered by the
9 managing physicians included fluid and electrolyte
10 management, in some cases platelets and/or red blood
11 cell transfusions, dialysis was utilized in two cases
12 and plasmapheresis was administered in one patient.
13 Eculizumab, which is a humanized monoclonal antibody
14 that inhibits complement-mediated TMA was administered
15 in four of nine patients described.

16 With respect to the outcome in these patients,
17 eight of these nine patients were described as
18 recovering or improving. In one patient, there was

1 evidence of recovery from TMA as determined based on
2 laboratory values as well as clinical course. For
3 instance, the evidence of hemolysis had completely
4 resolved with disappearance of schistocytes from the
5 peripheral smear, as well as resolution of the acute
6 kidney injury and normalization of serum creatinine.
7 Unfortunately, that patient did pass away due to sepsis
8 which occurred later in the patient's clinical course.

9 Potentially confounding variables were
10 identified in many of these cases. Nusinersen, which
11 is an oligonucleotide that is also used for the
12 treatment of SMA, sometimes after the treatment with
13 Onasemnogene abeparvovec, was given in seven of these
14 nine patients. It's important to note that Nusinersen
15 has been associated with acute renal injury as well as
16 thrombocytopenia and are known adverse drug reactions
17 of that oligonucleotide. Concurrent infections with
18 encapsulated organisms were identified in five of nine

1 of these patients. Underlying coagulation
2 abnormalities, while not specifically evaluated for in
3 these patients, have been associated with SMA.

4 When we look at the published literature with
5 reference to gene therapy use in Duchennes Muscular
6 Dystrophy, a total of 15 patients have been described
7 to have been dosed in the clinical trials with four
8 cases of TMA having been reported across two compounds.
9 Here what's described is a time to onset of two weeks
10 after dosing. Again, dosing with the gene therapy.
11 The patient age at the time of dosing ranged between 7
12 and 12 years, with all patients described as being
13 males.

14 Treatments that have been used include fluid
15 and electrolyte management, corticosteroid use in some
16 cases, platelet and/or red blood cell transfusion, as
17 well as dialysis. Eculizumab, again the humanized
18 monoclonal antibody, was administered in three of the

1 four patients, and it's unknown if it was administered
2 in the fourth patient. With respect to the outcome in
3 these patients, three out of the four patients have
4 been described as recovered or improving with the
5 disposition unknown in one patient. Potential
6 confounding variables have not been described in the
7 literature and remain unknown.

8 With respect to mitigation strategies of TMA,
9 at this time there are no known preventative measures
10 that would obviate the risk of TMA, hence early
11 clinical detection of TMA is really key. And that it
12 should be based on the recognition of signs and
13 symptoms of TMA. Initial presentation of TMA can
14 include fever, vomiting, hypertension -- again, due to
15 the microangiopathic process that underlies the TMA --
16 as well as the potential decreases in urine output.

17 From a renal perspective, I would also like to
18 mention that renal involvement may range greatly and be

1 isolated hypertension to full kidney injury, including
2 oliguria/anuria as well as edema. If TMA is suspected,
3 based on that clinical presentation and routine
4 monitoring of platelet counts has shown
5 thrombocytopenia present, then a focused diagnostic
6 evaluation for both hemolytic anemia as well as
7 possible renal dysfunction should be initiated.
8 Consultation with a pediatric hematologist and/or a
9 pediatric nephrologist should be undertaken
10 expeditiously.

11 From a company perspective, it's very
12 important that the product labeling ensure appropriate
13 monitoring as well as mitigation strategies as data
14 emerges. The theme at this time should really be
15 though the anticipation and early recognition are
16 imperative to ensure the optimal clinical outcome in
17 these patients.

18 Other questions that I often get asked are

1 regarding additional laboratory evaluations, and should
2 there be evaluation and ongoing monitoring of
3 hemoglobin? This is a bit challenging because anemia,
4 as defined as low hemoglobin for age, can be seen in up
5 to 20 percent of all children at any given point in
6 time. The anemia that's associated with TMA is a
7 specific type, namely that it's hemolytic in nature and
8 would not necessarily be identified by monitoring
9 isolated hemoglobin values.

10 Similarly, serum creatinine, which is a
11 typically used marker of renal function, is a product
12 of creatine metabolism. It's released from muscle. In
13 these children who have neurodegenerative diseases that
14 result in poor muscle mass and progressive muscle
15 weakness, these patients may not have creatinine values
16 that accurately represent their renal function.
17 Therefore, routine monitoring really does not have that
18 high yield as opposed to using clinical acumen.

1 When we talk about management of TMA, really
2 the therapy must be individualized based on the
3 clinical presentation and the clinical course of each
4 patient. The mainstay remains supportive management,
5 namely fluid and electrolyte management and correction
6 of any abnormalities that may occur. Platelet and/or
7 red blood cell transfusions may be required, however,
8 it's important to note that there are no absolute
9 numerical threshold values indicated for transfusions.

10 The management of hypertension should be
11 undertaken using subspecialty consultation and
12 sometimes may require intensive care monitoring. Fresh
13 frozen plasma and/or plasma exchange may be warranted
14 in certain circumstances. As I described, genetic
15 component may play a role; however, genetic testing
16 should not necessarily be implemented globally, but
17 rather should be focused and considered to evaluate for
18 any abnormalities for underlying causes. Eculizumab,

1 which is of the humanized monoclonal antibody, has been
2 used to inhibit complement-mediated thrombotic
3 microangiopathy.

4 With respect to the unknowns, as I mentioned,
5 it's really not very clear as to the full trigger and
6 mechanisms for thrombotic microangiopathy in these
7 patients. While we know that histologically
8 endothelial injury can occur and theologically
9 complement pathways aberrancies can occur, the
10 interplay between these have not been fully elucidated.
11 Exploratory biomarker evaluation may help us in
12 understanding these connections as well as help us to
13 identify potential contributing factors.

14 In summary, gene therapies have been developed
15 for rare, life-threatening conditions with great
16 potential benefit. As with any therapy, individual
17 patient benefit/risk should be considered when
18 administering this gene therapy. TMA has been

1 identified after AAV gene therapy, yet the pathogenesis
2 remains unclear.

3 But what we do know is that TMA is a clinical
4 diagnosis which should be identified early through a
5 focus diagnostic evaluation. And contributing factors
6 really should be considered including disease state
7 comorbidities, specifically infections as well as
8 coagulation abnormalities. Other contributing factors
9 can include concomitant medications as well as genetic
10 contributors. Supportive treatment should be
11 instituted immediately with subspecialty consultation
12 obtained promptly.

13 The clinical characteristics of TMA, as well
14 as monitoring and potential mitigation strategies,
15 should be detailed in product labeling and educational
16 materials for both the healthcare providers as well as
17 patients and their families. Thank you very much for
18 your time and attention.

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INVITED SPEAKER PRESENTATION Q&A

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DR. LISA BUTTERFIELD: Terrific, thank you very much, Dr. Chand. So we have now about 12 minutes for questions of our invited speaker, so first let's move to Dr. Kenneth Berns, please.

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DR. KENNETH BERNS: Yes, thank you for that very clear presentation. In terms of the SMA patients, I was struck with the gender difference. And secondly, where the patients were treated with the oligonucleotides, was that treatment before they developed the TMA or after?

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DR. DEEPA CHAND: With respect to your first question regarding the gender, we've really not seen any predilection in terms of the TMA literature. And I say that as a pediatric nephrologist in terms of gender predisposition. While all of the patients with SMA

1 described with TMA were female, all of the patients in
2 the DMD program, who were described, were all boys. So
3 I think that leaves us --

4 DR. KENNETH BERNS: But that (inaudible) by
5 requirement, by definition (inaudible).

6 DR. DEEPA CHAND: Correct. Exactly. By
7 definition, exactly. But that, I think, begs the
8 question really is this something that has a gender
9 predisposition? And at this juncture we really don't
10 believe there is anything to support that from a data
11 perspective.

12 With respect to your second question regarding
13 the Nusinersen use and the oligonucleotide, it was
14 actually quite variable. In some patients, the
15 oligonucleotide had been administered a few months
16 before the gene therapy. In some situations, it was a
17 week before gene therapy, and in certain situations it
18 was continued after gene therapy. So a great deal of

1 variety there which I think raises questions as well.

2 DR. KENNETH BERNS: Yeah, I guess the question
3 to me is to what extent can we attribute the TMA to the
4 AAV vector versus the oligotherapy, in these cases?

5 Anyway, thank you very much.

6 DR. LISA BUTTERFIELD: Thank you, Dr. Berns.

7 Next question from Dr. Wu.

8 DR. JOSEPH WU: Dr. Chand, that was a great
9 talk. I had a similar question as Dr. Berns but you
10 kind of answered it with regards to the gender
11 difference. Another related question would be, the
12 prevalence of the TMA, is it higher or lower in adult
13 patients who undergo the AAV gene therapy? Meaning,
14 there are many adult patients that have undergone AAV
15 gene therapy for cardiac disease and other diseases.
16 What is the prevalence between adult versus the
17 pediatric patients that you presented for the SMA and
18 DMD?

1 DR. DEEPA CHAND: Thus far based on the
2 Novartis experience as well as the Duchenne's
3 experience, it's only be described with those two
4 conditions in children. So we really don't have
5 comparative data in adults at this time.

6 DR. JOSEPH WU: Thank you.

7 DR. LISA BUTTERFIELD: Thank you. Dr. Raymond
8 Roos, please.

9 DR. RAYMOND ROOS: Just two questions. I was
10 a little confused at the frequency of TMA in Duchenne.
11 It looked like it was something like four out of 15
12 patients, or is that frequency really unclear? And
13 second with respect to the (inaudible) oligonucleotide
14 treatment in SMA, if one sees that treatment alone has
15 TMA been described?

16 DR. DEEPA CHAND: So with respect to -- maybe
17 I'll take your second question first. With respect to
18 the oligonucleotide, based on the prescribing

1 information for Nusinersen, both thrombocytopenia and
2 acute kidney injury have been described. TMA has in
3 itself not been described. It's important to note,
4 with respect to frequencies, Nusinersen has been dosed
5 in many patients who have also not developed these
6 findings. Similarly, there are many patients with
7 Onasemnogene who have been dosed that have not
8 developed TMA. So, to your point with respect to the
9 interplay, it's not very clear at this time.

10 Going back to your original question regarding
11 the frequency in the Duchenne Muscular Dystrophy. What
12 I've presented to you is what's described in the
13 literature and what's available through public forums
14 with respect to the DMA program without first-hand
15 knowledge. And what we've seen through those forums is
16 that there've been descriptions of four patients with
17 TMA after the therapy.

18 DR. RAYMOND ROOS: We don't really know the

1 denominator, is that right?

2 DR. DEEPA CHAND: What I've seen in the
3 clinical trials, this was based on 15 patients, but I
4 could not firsthand speak to the denominator.

5 DR. RAYMOND ROOS: Thanks.

6 DR. LISA BUTTERFIELD: Thank you very much.
7 Dr. Venditti next.

8 DR. CHARLES VENDITTI: Good morning. Thank
9 you for that excellent presentation. I'm wondering if
10 you could comment on a few things that I guess came up
11 during the topic. One of them is related to renal
12 biomarkers. It seems that we should be, as you pointed
13 out, looking beyond creatinine. This is sort of a
14 well-known thing in the pediatric arena. And should we
15 be measuring cystatin C and potentially lipocalin 2 in
16 these patients that are at risk? Will that help us?
17 That'll be my first question.

18 The second question would be with respect to

1 (audio skip), it looked like of the number of patients
2 that developed the TMA-like syndromes was a very small
3 fraction of the total patients treated. And I can't
4 remember the exact numbers you showed. Some of them
5 seemed like they could have a concomitant infection.
6 But you also mentioned something, that I think is near
7 and dear to the people that practice in the field of
8 metabolic genetics and inborn areas of metabolism,
9 which is host genetics.

10 Whether or not that is telling us in these
11 patients who have received similar doses we presume,
12 and only a small number of them develop the TMA,
13 there's a hope that the host genetic factors, plus or
14 minus environment, should be scrutinized in these
15 patients. And so with your background, with respect to
16 the TMA genetics which we didn't talk a lot about, I'm
17 wondering if you could address or make comments about
18 what we should be doing for biomarkers, A and then B,

1 what about host genetics and what should we do about
2 that and what has been done.

3 Again, I understand a lot of this information
4 is going to be public domain potentially and not
5 obtained, but if you could comment on that I think it
6 would help the discussion. Thank you.

7 DR. DEEPA CHAND: Absolutely, happy to do so.
8 With respect to other renal biomarkers, as you
9 mentioned creatinine isn't always the best renal
10 biomarker. With respect to cystatin C it becomes a bit
11 complicated, in that there are other factors that can
12 also give us abnormal cystatin C. Namely, the biggest
13 one that comes to mind is prednisolone use, it can
14 produce inaccuracies.

15 Given that all patients in the Onasemnogene
16 program receive prednisone prophylactically, it becomes
17 a challenge to see if this is, in fact, a reliable
18 biomarker in this population. And there remain a lot

1 of biomarkers that are being evaluated from a renal
2 space. And as a pediatric nephrologist, this is a
3 great area of interest because we look at things like
4 chem 1, we look at (inaudible), we look at all those
5 biomarkers. And which one is the best in this
6 population has really not been fully elucidated.
7 Again, I think we need a biomarker that would not be
8 influenced by concomitant therapies such as
9 prednisolone. So I do think that that's an area of
10 research.

11 I would tell you from a clinical standpoint,
12 the one thing that is often helpful is a change from
13 baseline. And while elevations in creatinine in terms
14 of routine monitoring can be difficult, if there's a
15 sudden change that would be indicative of acute kidney
16 injury with respect and in conjunction with clinical
17 signs and symptoms, then I think it provides guidance
18 to the clinicians in terms of how to manage TMA on that

1 individualized level.

2 With respect to your second question, in terms
3 of underlying genomics and host factors, I think that's
4 terrific. And as for an example, one of the patients
5 who did have TMA after Onasemnogene abeparvovec, was
6 found to have a genetic abnormality that would be
7 associated with nephrotic syndrome. So your point is
8 very well taken. And I think with respect to what do
9 we do about it as a community, it's very challenging to
10 make blanket statements when this has only been seen in
11 nine patients out of 1,400 dosed.

12 I think in terms of individual evaluation of
13 each patient, it's exactly that. It's individualized
14 based on how the patient presents and any other host
15 risk factors. Environmental risk factors have been
16 laid out in terms of infection risk and really having
17 the patient free of infection in the Zolgensma product
18 labeling as well. So I think as much as can be

1 controlled for we would try. But I do think it's
2 something that needs to be considered and explored
3 further as we gain more information.

4 DR. LISA BUTTERFIELD: Thank you very much.

5 DR. CHARLES VENDITTI: Thank you, I just
6 wanted to follow up. I had one point of clarification
7 which is respect to that influence of steroid treatment
8 on cystatin C. Can you comment whether or not the
9 patients you described -- the Zolgensma that had TMA?
10 Did they have ambiguous cystatin C measurements? Was
11 it measured? Or was it not measured because people
12 said, well maybe there could be interference. And then
13 therefore it wasn't done?

14 DR. DEEPA CHAND: As these cases came from the
15 post-marketing setting we received the data really
16 after the fact, if you will. And we did not see any
17 evidence of cystatin C collection. I had no values of
18 cystatin C to assess.

1 DR. CHARLES VENDITTI: Thank you.

2 DR. LISA BUTTERFIELD: Thank you. We have
3 time for one, or maybe two more questions. Dr.
4 Hawkins.

5 DR. RANDY HAWKINS: So, thank you, Dr. Chand.
6 I'm having trouble with my cursor. I'm a physician in
7 private practice and consumer representative. And my
8 question is if you know your experience with product
9 availability? So those with a diagnosis who otherwise
10 qualify, what about the availability to receive this
11 product? Do you have any experience with that? Can
12 folks who need it, get it?

13 DR. DEEPA CHAND: Sure. To my knowledge, yes.
14 We've not had any product shortages or anything like
15 that. There's certain, of course, eligibility criteria
16 which are described in the product monograph, namely
17 testing for AAV antibodies, et cetera. But, yes,
18 product availability is certainly there.

1 DR. RANDY HAWKINS: Okay, and financial
2 support if needed?

3 DR. DEEPA CHAND: Yes, there are various
4 patient support programs that Novartis offers.

5 DR. RANDY HAWKINS: Thank you very much.

6 DR. LISA BUTTERFIELD: All right, let's
7 squeeze in a final question from Dr. Mark Walters,
8 please.

9 DR. MARK WALTERS: Thanks very much. That was
10 a very nice talk. So I have a related question and it
11 has to do with my experience with transpondence-
12 associated (inaudible). It happens 20 to 40 percent of
13 the time depending on what (inaudible) often related to
14 the endothelial injury associated with conditioning
15 regiment.

16 So we've developed weekly screening studies.
17 For example, urinary protein to creatinine ratio of LDH
18 and looking at the blood for schistocytes because of

1 the high incidence. And that seems to have help us
2 then go on to more detailed evaluations in early
3 institution of Eculizumab and included outcomes.

4 I don't know if you thought about how to adapt
5 that kind of approach in the patients, or if the AAV
6 incidence is just too low to not warrant that.

7 DR. DEEPA CHAND: As a clinical nephrologist,
8 certainly I've seen bone marrow transplant induced TMA.
9 And you're absolutely right, we see that in much
10 greater prevalence, and we respond to that as well.
11 And as you also know, that therapy is individualized.

12 With respect to being able to apply that to
13 Onasemnogene abeparvovec or any of the gene therapies,
14 I think it becomes a bit more challenging because of
15 the other risk factors that we don't know about. And
16 given that this is such a young field, and that this
17 was really just described starting in fall of last
18 year, I think it's very difficult to make any type of

1 those routine monitoring that's implemented with bone
2 marrow transplant.

3 For example, we do know that the immune
4 response associated with adeno-based therapies can
5 include fever as a presentation. And protein to
6 creatine ratios, for example, can become elevated in
7 the setting of fever.

8 So given that this matrix is really
9 complicated, it's really difficult to come up with any
10 type of blanket statements or monitoring at this
11 juncture. We are continuing to monitor this, and
12 certainly as more data becomes available perhaps we can
13 continue to have that dialog.

14 DR. MARK WALTERS: Thank you.

15 DR. LISA BUTTERFIELD: All right, well thanks
16 very much for all the great discussion. We're now at
17 time. We're going to take a quick five-minute break
18 which brings us back at four minutes after the hour,

1 and then we'll go to the open public hearing. Thank
2 you very much.

3 [BREAK]

4

5 **SESSION 3: OPEN PUBLIC HEARING**

6

7 MR. MICHAEL KAWCYNski: All right. Welcome
8 back from that quick little break. We are now going to
9 start our OPH session. Dr. Butterfield, would you like
10 to take it away?

11 DR. LISA BUTTERFIELD: Thank you very much.
12 So welcome to the Open Public Hearing session. Please
13 note that both the Food and Drug Administration, FDA,
14 and the public believe in a transparent process for
15 information gathering and decision making. To ensure
16 such transparency, at the Open Public Hearing session
17 of the Advisory Committee meeting, FDA believes that
18 it's important to understand the context of an

1 individual's presentation.

2 For this reason, FDA encourages you, the Open
3 Public Hearing speaker, at the beginning of your
4 written or oral statement to advise the Committee of
5 any financial relationships you may have with a
6 sponsor, its product, and, if known, its direct
7 competitors, for example, if the financial information
8 may include the sponsor's payment of expenses in
9 connection with your participation at this meeting.

10 Likewise, FDA encourages you at the beginning
11 of your statement to advise the Committee if you do not
12 have such financial relationships. If you choose not
13 to address this issue of financial relationships at the
14 beginning of your statement, it will not preclude you
15 from speaking. And with that, I turn it over to Jarrod
16 to run the OPH session.

17 MR. JARROD COLLIER: Thank you, Dr.
18 Butterfield. For Session 3, we have one OPH speaker,

1 and that is Dr. Dan Levy representing Pfizer. Dr.
2 Levy, if you can introduce yourself and begin your
3 presentation. You have five minutes.

4 DR. DAN LEVY: Hi. Thanks to the Committee.
5 I'm Dan Levy, and I serve as the team lead for Pfizer's
6 gene therapy program for Duchenne muscular dystrophy.
7 Slide 2, please.

8 Our candidate gene therapy is an AAV9-based
9 vector. The transgene contains a miniaturized
10 dystrophin gene that's driven off of a muscle-specific
11 promoter. And I'll be sharing data from our Phase 1b
12 trial, which has treated 21 boys, aged 5 through 14.
13 In this trial, we have had three serious adverse events
14 that are consistent with TMA as manifested by an
15 atypical form of hemolytic uremic syndrome. None of
16 the boys have had any genetic defects identified
17 related to atypical hemolytic uremic syndrome. Slide
18 3, please.

1 All the data I'm showing you has been
2 presented publicly, so I'll move through this very
3 swiftly: three cases, aged 9 through 14, weight between
4 23 through 41 kilograms, all presenting with different
5 degrees of hemolysis, thrombocytopenia, and renal
6 dysfunction.

7 These boys had initial manifestation starting
8 about one week after infusion of gene therapy, and they
9 all recovered fully, generally resolving within a few
10 days to a few weeks. Slide 4, please. So most
11 participants in -- I'm going to assume we're Slide 4.
12 I have a little bit of a lag here.

13 Most participants in the study had a
14 characteristic drop of platelets later in between Days
15 8 and 10 as shown here by the central lab values. Now,
16 neither hemolysis nor renal dysfunction were seen in
17 any of the participants besides the three boys that I
18 mentioned, even though relative thrombocytopenia was

1 seen promptly.

2 Based on the safety profile of our gene
3 therapy, we're now actually sampling at least every
4 other day during the first two weeks and actually daily
5 between Day 6 through 10. And it is required in all of
6 our protocols. Yes? So are we on Slide 4?

7 MR. MICHAEL KAWCZYNSKI: Dr. Levy, we are on -
8 - we have Case 1, 2, and 3 up.

9 DR. DAN LEVY: Okay. So, if you look in the -
10 - oh, I see they're all showing zero. Okay. So you're
11 seeing Case 1, 2, and 3, and I apologize. That was the
12 slide in which I just mentioned the weights, the ages,
13 and the different extent of hemolysis,
14 thrombocytopenia, and renal dysfunction. If we could
15 advance to the next slide, which shows platelets in the
16 upper-left corner and complement in the lower-left
17 corner? Thank you.

18 So there I was speaking about a pretty typical

1 drop of platelets between Days -- with nater between
2 Days 8 and 10. And what you're seeing in that slide
3 should be the central lab values. And I had mentioned
4 that there was no hemolysis nor renal dysfunction seen
5 in any of the other participants in the trial besides
6 the ones that I had mentioned from the previous slide.
7 And we are measuring local labs on a daily basis
8 between Days 6 through 10.

9 You can also see in the lower-left corner that
10 complement, here at C4 is being shown, declines also
11 pretty characteristically. But there doesn't seem to
12 be any relationship between the extent of either C3 or
13 C4 decline with development of HUS -- or severity of
14 HUS. If we could advance to the next slide, please,
15 and that has a colored vector genome figure on the left
16 side.

17 So we've shared that the vector genome
18 concentration is very high in the first week after

1 infusion with a 3 log drop between Days 5 and Day 7, so
2 there is a fair bit of vector out there in the
3 beginning. And, if we look on the right, we have also
4 presented previously that antibody-antigen complex is
5 necessary and sufficient for complement activation in
6 an ex vivo study with healthy volunteers. And, when I
7 say antigen-antibody, I'm referring to AAV capsid and
8 anti-AAV-IgG.

9 Next slide, please. Actually, could we skip
10 to Slide 7, which has the neutralizing antibody
11 figures, the large neutralizing antibody on the left?

12 So, in the boys with serious adverse events,
13 they've had a rapid rise in neutralizing antibodies
14 during the first week or two. We don't know at all
15 what accounts for the more intense rise in those
16 stations, but it does seem to be a critical component
17 in the development of TMA, presumably contributing to
18 the pool of antibody-antigen complex.

1 I'm going ask to skip to Slide 10, please,
2 which is the one -- a few more down, which has a
3 histogram on the right side of the figure. It's the
4 second to last slide, Slide 10.

5 So, this is a study in Cynomolgus Monkeys.
6 They were all negative at baseline for AV neutralizing
7 antibodies, and we treated them with empty AAV9 capsids
8 plus glucocorticoids in some groups and different doses
9 of sirolimus on top of that.

10 I'm not sure that the correct slide is being
11 shown. Yes, okay. Thank you. Yes, so the one with
12 the gray and black histograms. Thank you.

13 So we found the addition of Sirolimus
14 significantly reduced the elaboration of neutralizing
15 antibodies. And the dose of high Sirolimus is roughly
16 equivalent -- or the levels of Sirolimus -- were
17 roughly equivalent to what's used in current-day
18 transplantation for Sirolimus.

1 And so we do think that there's potential to
2 treat patients with sirolimus for the first few weeks
3 after gene therapy to hopefully reduce the likelihood
4 or severity of TMA, and we do plan to study this in DMD
5 patients very soon. One final slide, please. Next
6 slide. Conclusion slide.

7 So, in the case of very high-dose AAV gene
8 therapy, we recommend frequent monitoring of clinically
9 relevant labs in the early stages in development to
10 determine whether there's evidence of complement
11 activation and potential for TMA. As far as immune
12 profiling goes, we believe it's important to collect
13 these samples, but there's some unclear clinical
14 utility there. Certainly, the turnaround time really
15 prevents any impact on any individual patient in real
16 time.

17 And finally, we recommend against capping the
18 vector load of gene therapy. And let me say why.

1 There are clear cases of dose dependency with gene
2 therapy treatments of life-threatening disorders, even
3 in the E14 vector genome per kilogram range. And, at
4 least in our hands, we feel that we have been able to
5 manage the likelihood and severity of TMA.

6 So thank you to the Committee, and I am
7 certainly available to respond to questions offline if
8 there are any follow ups.

9 MR. JARROD COLLIER: All right. Thank you,
10 Dr. Levy. And this concludes the OPH speaker for
11 Session 3, and at this time I will turn it back over to
12 Dr. Butterfield.

13 DR. LISA BUTTERFIELD: Terrific. Thank you
14 very much, Jerrod. So, with that, we now begin the
15 Committee discussion of the questions for Session 3.

16

17

COMMITTEE DISCUSSION OF QUESTIONS

18

1 DR. LISA BUTTERFIELD: So, again, Session 3 is
2 on Thrombotic Microangiopathy, and we have three
3 questions. We'll follow the same format that we had
4 yesterday, so we'll go question by question. I'll read
5 the questions. We'll discuss those. I'll sum up, and
6 we'll move to the next one to make sure we remain
7 focused and give the feedback that the FDA would like.

8 So our first question is, "Please discuss
9 factors that may increase the risk of TMA following AAV
10 vector administration." And let's start with our
11 discussant for this session, Dr. Barry Byrne. We can't
12 hear you yet.

13 DR. BARRY BYRNE: Thanks very much. I wanted
14 to start by bringing up some of the points in Dr.
15 Chand's presentation that may come up during the course
16 of the open session.

17 Because one of the -- despite the large number
18 of patients treated with SMA, there is not a REMS

1 program for this product. So my concern is that,
2 although it's important that these nine cases have been
3 brought forward, that's only the responsibility of the
4 treating physician then to provide that information to
5 the safety team and Novartis.

6 And, in our own experience, doing immune
7 profiling in now out of 24 patients that receive
8 systemic AAV9 gene therapy, the majority of them with
9 SMA, in fact all cases, show laboratory evidence of
10 activation in its pathway. Luckily, because of their
11 early diagnosis with now 80 percent of states do
12 newborn training for SMA, the total vector capsid
13 burden is low compared to the subjects with DMD. They
14 are somewhat described by Dr. Levy.

15 There was emphasized this -- this is the
16 clinical finding. Those things that might be
17 clinically relevant are really the physical signs and
18 symptoms, but, as Dr. Chand pointed out, these are

1 really laboratory findings of thrombocytopenia, anemia,
2 and acute kidney injury. So it's really important as I
3 think as was emphasized in these open public hearing
4 sessions that frequent and early laboratory assessments
5 are necessary to anticipate the clinical (audio skip)
6 outcome with more severe manifestations. And it's a
7 unique thing about AAV gene therapy (audio skip). It's
8 very difficult to reverse or mitigate those genes, so
9 (audio skip) two medical therapies.

10 DR. LISA BUTTERFIELD: Sorry, Dr. Byrne, to
11 interrupt.

12 DR. BARRY BYRNE: Yeah.

13 DR. LISA BUTTERFIELD: You keep cutting out.
14 Is that the same for everyone? Michael?

15 MR. MICHAEL KAWCZYNSKI: Sorry, go ahead,
16 Lisa.

17 DR. LISA BUTTERFIELD: Is the audio cutting
18 out for just me or for everyone?

1 MR. MICHAEL KAWCZYNSKI: Well, I hear you.

2 Let's see here.

3 DR. LISA BUTTERFIELD: For Dr. Byrne.

4 MR. MICHAEL KAWCZYNSKI: Oh, you mean for Dr.
5 Byrne?

6 DR. LISA BUTTERFIELD: Yes.

7 MR. MICHAEL KAWCZYNSKI: Dr. Byrne, go ahead
8 and talk again.

9 DR. BARRY BYRNE: Okay, Michael, thank you.
10 Can you hear me?

11 MR. MICHAEL KAWCZYNSKI: Yep. You're fine.

12 DR. BARRY BYRNE: Okay. (audio skip).

13 MR. MICHAEL KAWCZYNSKI: I see. Oh yeah. I
14 hear it now. Okay. Dr. Byrnes, I do hear it. Okay.
15 Dr. Byrnes, what I'm going to have you do momentarily -
16 - and we're going to go to somebody else -- just hang
17 up your phone and reconnect and maybe you just have a
18 bad connection with your current phone line.

1 DR. BARRY BYRNE: Will do. Yep.

2 MR. MICHAEL KAWCZYNSKI: Okay. So let's move
3 on to the next person, Dr. Butterfield.

4 DR. LISA BUTTERFIELD: Because these are --
5 the opening remarks of the discussant before the
6 discussion, I'd rather --

7 MR. MICHAEL KAWCZYNSKI: Oh, okay. Fair
8 enough. We'll just wait till he reconnects. All
9 right. He's coming in right now.

10 Like I said, with all the storms around the
11 country, I'm expecting some people to have some issues,
12 you know, with audio and all that stuff. There you go,
13 Dr. Byrne. You're back.

14 DR. BARRY BYRNE: Hi there. Can you hear me?

15 MR. MICHAEL KAWCZYNSKI: Yes, go ahead.

16 DR. BARRY BYRNE: Is this better? Okay. Just
17 trying to emphasize a point related to the presentation
18 on SMA that's relevant to the questions we have to

1 address. Dr. Chand emphasized that clinical findings
2 in TMA. Often we would consider those clinical
3 findings physical signs and symptoms, but the
4 laboratory data's really what matters in the early
5 identification of this pathway being active.

6 Since Zolgensma is not managed through a REMS
7 program, it may be that the ascertainment of cases with
8 TMA is greater than -- is less than expected and having
9 -- the great value of having 1,400 patients chose would
10 be to really understand how frequently these findings
11 might exist within the patient population.

12 So, about two years ago, having done the first
13 commercial infusion of Zolgensma, we started to
14 investigate the daily time course of immune profiling
15 following systemic AAV9 therapy and actually found that
16 the pathway is active in all of the 17 patients we have
17 treated to date. And this is not ameliorated by
18 prednisone therapy.

1 So we'll get into, I think, this further in
2 the course of the discussion. But, to address the
3 first question, the factors that increase risk of TMA
4 following AAV vector administration, in our
5 observations in these patients and others, the first
6 clinical finding -- or laboratory finding -- is IgM
7 presence in the serum and response to the vector that's
8 administered. Particularly, the magnitude of the IgM
9 increase is greater in those that are truly naive just
10 by the ability to task for seronegative activity to be
11 included in the study.

12 And then following that, the D-dimer becomes
13 positive, and this precedes the depletion of C4 and the
14 activation in the membrane attack complex.

15 So these are important laboratory values. The
16 findings include diminished platelets, anemia, and
17 acute kidney injury, then follow as a response of the
18 classical complement pathway activation. So just start

1 with that regarding Question 1 and open it up for
2 further discussion.

3 DR. LISA BUTTERFIELD: Great. Thank you so
4 much. Thanks for working through those AV issues. So
5 Dr. Venditti, please.

6 DR. CHARLES VENDITTI: So common knowledge I
7 was going to mention to the group for discussion -- and
8 that's very interesting what, Dr. Byrne, what you
9 mentioned about finding the evidence of this immune
10 activation phenomena in the patients -- is whether or
11 not we should also consider -- again, this is to harken
12 back on the conversation we had previously -- which
13 group of patients is going to be at the most risk for
14 progression to the TMA syndrome and renal injury.

15 And one of the things I wondered about -- and
16 again, part of this is colored from my clinical
17 activities -- are micronutrient considerations and
18 vitamin metabolism. And it is known that there are

1 certain patients, particularly those that have an
2 intrinsic inherited defect in cobalamin uptake
3 utilization, can develop -- HUS -- can develop TMA.
4 And in those patients, you usually work them up, and we
5 do a genetic workup to see if there's a second risk
6 factor.

7 But it's also the case that vitamin B12
8 deficiency -- and again, this is more recognized in
9 adults and elderly patients -- that that in itself can
10 be a previous dosing factor.

11 So, you know, I wonder as we think about risk
12 mitigation, we go and -- again mining what data we have
13 and be mindful about going forward to consider things
14 like a patient that is a young patient may be not
15 feeling well with a neurologic syndrome. They may be
16 someone who's predisposed to develop micronutrient
17 problems that could impact risk with the right exposure
18 of environment, which could be the capsid and/or a

1 genetic component, which is maybe a polymorphism in a
2 gene, a complement gene, and/or an infection.

3 It's a complex genetic interaction that we're
4 sort of thinking about, but, as we maybe try to dissect
5 out what could be the risks, returning back to sort of
6 the host genetics and micronutrients, which is
7 something you can intervene. We can fix someone's
8 micronutrient imbalance if we knew it was out of
9 alignment. And this is a routine clinical activity.

10 And then I guess the more theoretical comment
11 I would offer, and again related to this, would be
12 whether or not -- and again with the data sets we have
13 available but going forward -- this is something we
14 discuss, you know, again in the academic NIH setting,
15 whether or not we should be offering and/or obtaining
16 and/or consenting patients their whole genome
17 sequencing and whole axon sequencing.

18 So, when we see the extreme risk manifest of

1 these artifacts into the real severe clinical syndrome
2 of a renal failure, what are the underlying genetic
3 factors? And we learned about this from using genomic
4 techniques. And you could even expand that to talk
5 more about these other approaches one could use. But,
6 in clinical genetics, it is I would say rather routine
7 in some settings to obtain such data. It's done every
8 day when we work up patients. We get genomes and
9 exomes and we usually do triage. And I just wonder
10 whether or not, as we think about risk mitigation for
11 some of the things we talked about during this session,
12 that could be an adjunct to help us determine at using
13 a personalized genetic medicine approach, what is the
14 risk profile per patient?

15 And then there's a separate consideration
16 about capsids, which I think we could get to maybe
17 after Dr. Wilson's talk. About, you know, is it the
18 capsid load, what exactly is it that's producing this?

1 And are some capsids much -- you know, the high-risk
2 profile due to AAV9 as the one we've been talking
3 about. But what about other capsids and what can we
4 learn from the capsid biology? And how does that
5 translate to lose mitigation? That's the comment that
6 I want to offer for discussion. Thank you.

7 DR. LISA BUTTERFIELD: Perfect. Thank you.
8 Dr. Roos, please.

9 DR. RAYMOND ROOS: Yeah. Maybe that's
10 directed at Dr. Barry Byrne, and it seems like it was a
11 big disconnect between the very low frequency of the
12 TMA and SMA patients versus Duchenne dystrophy
13 patients. And I wondered if it's a reflection of
14 dosage, or is it in fact more frequent than SMA and
15 we're just not identifying them? I wondered about the
16 answer here. Thanks.

17 DR. LISA BUTTERFIELD: Let me ask Dr. Barry
18 Byrne to please step in and reply.

1 DR. BARRY BYRNE: Okay. Thanks, Lisa. So,
2 yes, I think you're correct in assuming that frequency
3 is related to the total viral capsid exposure because
4 the amount of the antibody formed in any individual
5 when they're immunized or exposed to the antigen -- in
6 this case, a vector capsid -- can be intimate. And the
7 antigen-antibody complexes that then trigger the
8 classical pathway will be a greater severity with
9 greater antigen-antibody complex formation activation
10 of C1Q.

11 That can be measured both in the magnitude of
12 the D-dimer response, the magnitude of the C4
13 depletion, and the amount of membrane attack complex
14 that accumulates until C4 and C3 are depleted. And, in
15 some cases when there's a -- these cases associated
16 with SAEs do appear to fully deplete C4 and activate as
17 much of the membrane attack complex as possible. And
18 that may pertain to our discussions yesterday related

1 to the acute changes in the liver because the C3a
2 fragment and the C5a fragment are potent
3 immunostimulants.

4 This is really how the innate pathways are
5 connected to adaptive antibody responses and the
6 adapted cell-mediated responses that are reflected in
7 liver injury. So I think you're notion is correct.

8 In fact, I can provide some direct evidence of
9 that because in two additional studies we evaluated 11
10 patients who had, through CD20 antibody depletion of B
11 cells, elimination of all antibody responses in a
12 preventative strategy before AAV9 vector
13 administration. And in those cases there was no C3
14 activation, no membrane attack complex and no findings
15 of AST, ALT elevation.

16 So there are preventative strategies that can
17 be employed to block this pathway, and this may come up
18 further as we continue down the list of questions. So

1 I'll stop there.

2 DR. LISA BUTTERFIELD: Thank you. Dr. Kenneth
3 Berns, please.

4 DR. KENNETH BERNS: Okay. My questions are
5 like the ones that have already been asked but in a
6 different sense. And that is you indicated, Barry,
7 that there seemed to be a dose-response effect. And I
8 just want to make sure that that's correct what I
9 heard. And the second question related to that is,
10 when would it make a big difference in terms of your
11 vector preparation as to how many empty capsids you had
12 versus vector carrying capsids?

13 DR. BARRY BYRNE: No. Yeah, I can answer.
14 Yes, you're correct that the last thing that (phonetic
15 01:46:11 on the mixer BU) the total (audio skip) would
16 be adventitious, but I think there's a very high
17 empty/full ratio in Zolgensma, for example, so it's
18 hard to improve on that. And even in three-kilo

1 patients this pathway is active.

2 So, even in the case of the Duchenne
3 population or even in adult patients, there's going to
4 be a 10- or 20-fold higher capsid exposure even if your
5 preparations are completely full capsids to full capsid
6 to empty capsid ratio. So, yes, less is better, but
7 it's not possible to eliminate the capsids and maintain
8 the active agent, which is the genomes within the AAV.
9 So that's the challenge I think we face in the field.

10 DR. LISA BUTTERFIELD: Thank you for that
11 discussion. So, Dr. Roos and Dr. Barry Byrne, your
12 hands are still up. Are there new comments? So, Dr.
13 Roos? All right. So are there additional comments
14 from other members of the Committee on Question 1 or
15 shall I go ahead and sum up?

16 DR. BARRY BYRNE: Dr. Butterfield, yeah, I do
17 want to make one more clarifying point is that in terms
18 of factors that increase the risk. So obviously,

1 patients are screened for preexisting antibodies to
2 AAV. As was alluded to yesterday, some of those assays
3 require or use a neutralizing assay which one can't
4 differentiate the immunoglobulin class between IgM and
5 IgGs. So, using only that assay, it's not possible to
6 determine whether someone who's seronegative was
7 actually naive. And this is, again, a limitation of
8 the qualifying assays, whether it's the neutralizing
9 assay or the total binding assay.

10 Post exposure, it is important to measure
11 class switching because it's the IgM that creates most
12 of the greatest magnitude of complement activation
13 because of its pentameric nature fixes complement very
14 effectively. And that's how we connect the innate
15 immune system to the adaptive antibody formation.

16 And it's interesting that, during the open
17 public hearing, Dr. Levy presented the effects of
18 sirolimus which does prevent class switching and would

1 then influence the ability to form IgG from those pre-B
2 cells that become plasma cells.

3 So the antibody status is important. The way
4 it's measured afterwards is also going to help unravel
5 the time course, and these early time points are
6 critical. Many studies, many clinical programs that
7 measure anti-AAV capsids following dosing are not
8 looked at the time course in the first week. And, in
9 some cases, the first time point is two weeks or one
10 month post-dosing when many of these events have
11 concluded. So that would just be a point about the
12 nature of testing for inclusion and studies and the
13 type of follow-up assessments that are needed.

14 DR. LISA BUTTERFIELD: Great. Thanks for
15 those points. And so it looks like we'll have a final
16 comment from our invited speaker, Dr. Chand, please.

17 DR. DEEPA CHAND: Thank you, Dr. Butterfield.
18 I think, you know, just to comment as a clinician, as a

1 pediatric nephrologist, I kind of would like to say
2 maybe we could take a step back. I think this is a
3 wonderful discussion regarding the clinical pathways
4 that might be activated and implicated with TMA.

5 I think when we talk about risk factors and
6 patient risk factors, I think certainly infection
7 status becomes very important and really recognizing
8 any other potential triggers that may occur. I think,
9 it's certainly very important to understand the
10 pathways and see if they can be mitigated. From a
11 clinical, practical standpoint at the bedside, I think
12 really optimizing the patient's health and really
13 considering that as well as the monitoring, as we just
14 stated, for both clinical signs and symptoms as well as
15 the platelet abnormalities that may occur.

16 DR. LISA BUTTERFIELD: Thank you. All right
17 then. With regard to the first question, I'll
18 summarize some of the key points of the discussion.

1 Immune activation is likely a very common
2 event and that this antigen-antibody complex and
3 complement triggering is critical. So the magnitude of
4 IgM versus the AAV vector and how that's measured,
5 total antibodies, neutralizing IgM specific or IgG
6 total is really critical to differentiate those that
7 are seronegative from those that are truly naive. And
8 the timing of that testing to catch that potential
9 class switching is important for that determination.

10 Another early test of D-dimer, those are
11 earlier lab testing that could be utilized earlier to
12 catch things before they progress to kidney
13 dysfunction.

14 Other than also general health, the notion
15 that micronutrients could play a role, and that could
16 be treatable early on. While there doesn't seem to be
17 data about gender across different trials that are not
18 gender-specific trials, there may be information in

1 genetic predisposition that should be investigated with
2 whole exome or whole genome sequencing. And it's also
3 noted that different capsids used in different disease
4 treatments do get different immune activation levels.

5 So I think those are the major points about
6 factors that could increase the risk, steps that could
7 be screening tests before, which also touches on the
8 second question, that could be employed early. So
9 anything else? Dr. Chand, your hand is still up. Did
10 you have something to add to that quick summary for
11 Question 1? No. Okay. Well, thank you, everyone.
12 Let's move to Question 2.

13 So this is really expanding on the same
14 discussion. "Please provide recommendations on
15 strategies that could be implemented before and/or
16 after AAV vector administration to prevent or mitigate
17 the risk of AAV vector-induced TMA."

18 So let's now ask Dr. Barry Byrne to initiate

1 this discussion as our lead discussant. Not hearing
2 you yet.

3 DR. BARRY BYRNE: All right. Let's begin.
4 Now, so in order to consider mitigating strategies,
5 obviously, it's important to recognize that the pathway
6 predominately involves a classical complement
7 activation. And, as was shown during Dr. Levy's
8 presentation, a vector genome concentration in the
9 blood immediately post-dosing is around ten to the
10 eleventh per mil so very high and falls -- so really a
11 two-compartment model that leads to liver transduction
12 and then subsequent exposure to the rest of the tissues
13 and predominately then to the musculature after dosing.

14 And the time course between that decline in
15 vector genomes and the onset of the antibody formation,
16 is probably really one of the critical events that
17 determines when the peak antigen-antibody complexes are
18 formed and how that leads to complement activations.

1 So the risks mitigation strategy that involve a
2 downstream pathways would seem to be -- at the terminal
3 part of this pathway have been employed. And Dr. Chand
4 mentioned that some of the patients in the Zolgensma
5 group that she described received eculizumab.

6 I'll also note that one of the patients had
7 sepsis and died following these events. And five of
8 the nine had infections with encapsulated
9 microorganisms. So, when considering the anti-
10 complement therapies that might influence the ability
11 to respond to encapsulated microorganisms, we have to
12 keep that in mind.

13 So the approach we had taken is to be very
14 proximal in the pathway and use anti-CD20 therapies in
15 conjunction with sirolimus to block the activation of
16 pre-B cells to plasma cells. So that's one of the
17 strategies that could be implemented.

18 And I'll just mention one other thought is

1 that there are other drugs under development for
2 paroxysmal nocturnal hemoglobinuria, which involves
3 complement. So particularly C3 convertase inhibitors
4 may be very useful in this setting if there wasn't a
5 need for prolonged depletion of antibodies. These
6 drugs are under development. They may be useful in
7 this setting, and those are things that maybe the group
8 can discuss further.

9 DR. LISA BUTTERFIELD: Great. Thank you for
10 that excellent initiation to our discussion. So I'm
11 watching the Committee for raised hands for additional
12 comments on this key question on strategies for
13 prevention or mitigation. Dr. Breuer, please.

14 DR. CHRISTOPHER BREUER: I really like and
15 agree with Dr. Byrne's comments. I think a more
16 targeted approach makes a lot of sense. But the one
17 caveat I would place is that any prophylaxis needs to
18 be considered in light of the risk of the problem.

1 For example, in the SMA population, it looks
2 like the risk of this -- the serious complication was
3 less than one percent, so any prophylactic strategies
4 are also going to have risks involved. You have to
5 then start to weigh those risks. So my point is the
6 risk of the adverse event needs to be considered when
7 considering the need for prophylaxis.

8 DR. LISA BUTTERFIELD: Thank you. Dr. Herzog.

9 DR. ROLAND HERZOG: Yeah. I just wanted to
10 add to what Dr. Byrne was saying about the prophylactic
11 use of B cell depletion along with giving rapamycin.
12 And just to say that the reason why it's critical to
13 add the rapamycin or some other drug in this approach,
14 is like when you deplete B cells with, for example,
15 rituximab, then as the B cell population eventually
16 recovers it comes with a very high production of
17 cytokines and growth factors that could really get the
18 immune system (audio skip) back and you dampen that by

1 adding the rapamycin to- (audio skip).

2 So, if you do consider preemptive B cell
3 depletion as part of your strategy, then you have to
4 consider that you probably need to (audio skip) drug
5 company. (Audio skip). So I think (audio skip) is
6 doing the right thing. It's just (audio skip) how to
7 design (audio skip).

8 DR. LISA BUTTERFIELD: Thank you. So, if
9 you're done with your questions and comments, go ahead
10 and put your hand down. And meanwhile, Dr. Roos.

11 DR. RAYMOND ROOS: If the reason for what
12 looks like an increased frequency of TMA in the
13 Duchenne clinical trial is related to increased dose,
14 perhaps anti-capsid, one might suggest backing off or
15 some additional purification of the AAV vector in the
16 case of the Duchenne clinical trial. I don't know
17 whether that's been tried. Maybe this dosage is needed
18 to really replace the mini dystrophin into muscle.

1 DR. LISA BUTTERFIELD: Thank you. So, Dr.
2 Herzog, did you have another comment? Your hand is
3 still up. No. Okay. Dr. Barry Byrne, please.

4 DR. BARRY BYRNE: Thanks. I just wanted to
5 ask maybe that Roland would comment on -- we focused a
6 lot on capsid proteins, but there is important work
7 done by his lab to understand the impact of CpG islands
8 on activation of TLR9, which does directly influence
9 the magnitude of the antibody response.

10 That can be from the vector genome itself or
11 from adventitious DNA which travels along with AAV.
12 Some of that may be on the capsid surface and some may
13 be encapsidated, but maybe Roland can speak to that
14 because that's his specific expertise.

15 DR. ROLAND HERZOG: Yes, so obviously, I don't
16 know to what extent TLR9 activation plays a role in
17 this particular phenomenon. But AAV vectors, they will
18 activate TLR9, and that does contribute to innate

1 immunity (audio skip). (Audio skip) activate certain
2 subsets of dendritic cells and, for example, lead to
3 type one difuran production. Complement plays a role
4 in antibody formation as well, so (audio skip) and
5 immunity could increase potentially the risks that
6 (audio skip) associated with activating complements.

7 We found that the TLR9 activation that results
8 from the AAV vector genome itself that's packaged, it
9 is impacted by the CpG content. So, if you use vectors
10 in which the genome is edited to reduce the CpG
11 contents, you're going to not eliminate, but you will
12 reduce TLR9 activation and reduce that arm of the
13 immune response.

14 It has a greater impact on the T cell
15 response, (inaudible) response it appears (inaudible)
16 into the capsid then on antibody formation. It has
17 more of a modifying effect on the (audio skip) how much
18 of which (audio skip) for example (audio skip).

1 At the same time, we found that exogenous DNA
2 that outside the AAV capsid when (audio skip) mixed
3 that in with the vector that that can enhance antibody
4 formation (audio skip) or it can enhance cell -- it can
5 enhance the activation of inflammatory dendritic cells
6 and thereby increase germinal center formation. So but
7 again, what I don't know is to what extent that would
8 actually enhance the IgM production that you're
9 concerned about, which is probably not going to the
10 germinal (audio skip).

11 So I would say in general, whatever you can do
12 to reduce TLR9 activation would (audio skip) good thing
13 to do. It would reduce innate immune activation, but I
14 don't know exactly to what extent it would eliminate
15 (audio skip) complement (audio skip) related toxicity.
16 I think that that needs to be studied further. Really
17 IgM (audio skip) whether TLR9 activation enhances that
18 or not (audio skip).

1 DR. LISA BUTTERFIELD: Great. Thank you for
2 adding that immunologic color to this. Dr. Barry
3 Byrne, a final word on this question? And you're
4 muted.

5 DR. BARRY BYRNE: Thanks. So not to totally
6 implicate IgM, Dr. Levy alluded to one of the cases in
7 their case series that had complement activation, or at
8 least the renal insufficiency was first noted at two to
9 three weeks post-dosing. When it appears that if there
10 is an anamnestic response in a patient who may have
11 been seronegative but having either cross-reactive or
12 secondary antibody formation, this will be
13 predominately IgG that can also activate the classical
14 pathway as it does in many cases. A higher level of
15 IgG is required and that could happen through the
16 pathway that Roland just mentioned.

17 And then just to reemphasize, I think the
18 primary risk to patients in these situations is that

1 the early cleavage of C3, it says 3Ca and C5a, are
2 potent anaphylatoxins. And so they cause direct tissue
3 injury, capillary leak and can cause in themselves
4 cardiopulmonary insufficiencies.

5 So that's been the most challenging part of
6 this problem is to combat that aspect, and that's why
7 we think -- I think it was alluded to this has to be
8 tailored to the right patient population. Those
9 aspects have not been observed -- those really more
10 acute problems have not been observed in the SMA
11 patient population as much as they have in the larger
12 subjects with Duchenne or in adults who've received
13 AAV9 therapy for forms of cardiomyopathy.

14 DR. LISA BUTTERFIELD: Great. All right.
15 Well, thank you to the group for the great discussion,
16 and here's what I heard about recommendations on
17 strategies to implement before and after vector
18 administration to prevent or mitigate these effects.

1 The focus is on the classical complement
2 pathway, and so, following the time course, vector,
3 copy number decline and IgM increase is critical. And
4 as just noted, IgM is not the only player but it's the
5 more efficient player in setting off these cascades.

6 B cell-targeted mitigation would be perhaps
7 most efficient. Sirolimus or similar to stop class
8 switching, anti-CD20 depletion of B cells, that kind of
9 targeted approach may be more effective than a broader
10 less specific approach.

11 New specific complement inhibitors are being
12 developed. These could be tested in the future, and
13 also it's important to look at the potential role of
14 CpGs that are present in the vector sequence. If
15 they're shown to play a role in some of these effects
16 and initiation of complement, they could then be edited
17 in a specific way to reduce the number of CpGs. And
18 then overall, thinking about the two settings that

1 we've seen, in particular SMA and DMD, there is a
2 different frequency, so the setting of the disease is
3 important. There may be less need for prophylaxis, for
4 example, in SMA.

5 So that's what I heard. Dr. Barry Byrne, did
6 you have something to add to that before we move to the
7 third question?

8 DR. BARRY BYRNE: No, nothing to add. That
9 was great. That was great. Thank you.

10 DR. LISA BUTTERFIELD: All right then. Then
11 let's move to the third question. And this is a
12 similar question to the one that we addressed yesterday
13 afternoon when we were talking about hepatotoxicity.

14 Considering the risk of TMA observed in
15 clinical trials with high doses of AAV vectors. A)
16 "Please discuss whether an upper limit should be set
17 for the total vector genome dose per subject. And
18 given that many AAV products contain significant

1 amounts of empty capsids, please discuss whether an
2 upper limit should be set on the total capsid dose."

3 And so I'll remind you as we begin this where
4 we landed regarding hepatotoxicity. It may be different
5 in this setting, but the recommendation was to perhaps
6 not set an upper limit of total vector genome dose per
7 subject but instead focus more on per kilogram or BMI
8 in a more -- but not subject.

9 And then the empty capsids, there was a lot of
10 discussion last afternoon about not arbitrary capsid
11 limits but rather focused on standards in vector QC
12 including titer-ing approaches and the measurement of
13 empty capsids or whatever the capsids contain.

14 So that's where we left it yesterday
15 afternoon. And so to initiate the discussion here, Dr.
16 Barry Byrne, please.

17 DR. BARRY BYRNE: Thanks, Dr. Butterfield. So
18 yeah, the same principles apply. I think that what's

1 driving these reactions are total protein exposure from
2 the capsid component. And obviously, as was said
3 yesterday, you can only limit that to the degree in
4 which you have nearly a hundred percent of full capsids
5 containing AAV genomes.

6 It's important to differentiate though that
7 there are -- the proper terminology. So full capsid
8 terminology should really be referred to those that
9 contain complete AAV genomes with two Igs. DNA-
10 containing capsids are a proportion of those that are
11 not empty. And those may contain partial AAV genomes
12 or fragments of other DNA that's adventitiously
13 packaged. This is not the majority of preparations.

14 And then depending on the method used to
15 isolate the AAV, these critical quality attributes can
16 be assessed and determine what proportion of the vector
17 preparation are truly empty capsids: those that are
18 DNA-containing with any partial genomes or those that

1 contain full AAV genomes with two IGRs.

2 And there are now importantly improved methods
3 for assessing through next-gen sequencing and other
4 molecular methods to characterize the capsid -- the
5 proteomic aspect of the capsid as well as the DNA
6 content of the capsid. So these will influence the
7 response in a given individual.

8 There will be obviously some individual
9 variability, but the more we do to standardize -- and
10 this goes back to again another point from yesterday --
11 the absence of true reference standards inhibits our
12 ability to make adequate comparisons between different
13 sponsored products and in different clinical studies.
14 Mostly, it related to dose, but also in these important
15 characteristics of the product quality.

16 So I would say one couldn't set an upper limit
17 if you wanted to, given the inability to characterize
18 these in a uniform way. So that's the first step in my

1 view is to establish some standards in which sponsors
2 can either provide using their methodology
3 quantification related to a reference. This is common
4 in clinical laboratory medicine. We could establish
5 this in the field.

6 It's been important to have the sponsor's
7 ability to determine the concentration potency of their
8 own products. But having some knowledge about how that
9 compares to a widely distributed reagent would be, I
10 think, useful before trying to go down this road where
11 you would be artificially limiting based on some assays
12 that are not standardized. That's my starting point on
13 that point.

14 DR. LISA BUTTERFIELD: Terrific. Thank you.
15 All right. I am watching for those raised hands from
16 members of the Committee who would like to comment.
17 Dr. Roos.

18 DR. RAYMOND ROOS: All right. Yeah. Here's a

1 question for Dr. Barry Byrne. And that is whether the
2 risk of TMA changes with particular AAV vector
3 serotypes. Or some engineered AAV vector serotype?

4 DR. BARRY BYRNE: That's a great question. I
5 think we have limited experience to date outside of the
6 AAV9. Predominately in this area that we're discussing
7 these evolving AAV9 products, there have been --
8 obviously was also mentioned yesterday -- a substantial
9 experience in the hemophilia field, both with AAV5
10 products and the AAV8 products that are given at a much
11 lower dose because they're liver-directed therapies.

12 So when there's either the CNS targeting or
13 muscle targeting, that's what led to the use of more
14 muscle tropic capsids like AAV9. So we don't have a
15 lot of experience outside of that, but it's an
16 important question that needs to be raised whether
17 there are capsid-specific influences on this process
18 that influenced the complement activation.

1 DR. LISA BUTTERFIELD: Great. Thank you for
2 that. Any other comments from around the virtual table
3 on these last two questions for this session on TMA?
4 All right.

5 Then with that, to revisit the notion of total
6 vector genome dose, I think we left that where we left
7 it last afternoon in this setting as well. That
8 perhaps not per subject but per kilogram body weight or
9 BMI, or a patient-specific measure instead of per
10 subject of any type, would be more appropriate to
11 consider for that upper limit.

12 Most of our discussion today was around the
13 capsids, and we're now being able to employ better
14 next-gen sequencing and proteomic methods to
15 characterize those better. But that being said, there
16 is still -- it's a dearth of standards against which
17 any one sponsor's measures could be compared to give
18 greater insight into these critical quality attributes

1 of a complete AAV vector genome in the capsid versus
2 partial genomes, other contents and truly empty
3 capsids.

4 So all of those we would benefit from having
5 more full data disclosure, more standardized data to
6 decide whether there should be an upper limit set on
7 capsids. And that right now not an arbitrary limit
8 being set because the needs of vector directed
9 therapies, those doses are different from perhaps
10 higher doses needed to be efficacious in muscle-
11 directed therapies. So anything to add to that summary
12 to these last two questions? Not seeing any hands.
13 All right.

14 So that concludes Session 3 and those
15 questions for the Committee. And I believe we're a bit
16 ahead of schedule, but we're going to take a 35-minute
17 lunch break. So, I'll let the powers that be advise me
18 if that's going to be different than the 35-minutes we

1 would normally schedule. It looks okay.

2 So I have 9:03, which is kind of like 9:05
3 here in San Francisco for the San Franciscans/Los
4 Angelenos and Portlandians, just afternoon for the east
5 coasters. So let's come back at 40 after the hour.
6 Again, 40 after the hour, 12:40 on the east and then we
7 can begin Session Number 4. Thank you very much for
8 everyone's participation.

9

10 [LUNCH BREAK]

11

12 **SESSION 4: NEUROTOXICITY: DORSAL ROOT GANGLION (DRG)**

13 **TOXICITIES**

14

15 **MR. MICHAEL KAWCZYNSKI:** All right. Welcome
16 back. Thank you for those who hung around with us or
17 just stepped away for a break. Again, welcome to the
18 Center for Biologics Evaluation and Research 70th

1 meeting of the Cellular, Tissue, and Gene Therapy
2 Advisory Committee meeting. We are now going to begin
3 Session 4, and I'm going to hand it off to our Chair,
4 Dr. Butterfield. Dr. Butterfield, take it away.

5 DR. LISA BUTTERFIELD: Thank you very much.
6 Again, welcome back everyone, and now we're moving to
7 our fourth of five sessions on this two-day meeting.
8 Now we move to neurotoxicity.

9 First, we'll begin with the presentation from
10 our invited speaker Dr. James Wilson, the director of
11 the Gene Therapy Program at the Perelman School of
12 Medicine to present on Nonclinical Findings of Dorsal
13 Root Ganglion, Spinal Cord and Peripheral Nerve
14 Toxicity. Dr. Wilson, please.

15

1 **INVITED SPEAKER PRESENTATION: NONCLINICAL FINDINGS OF**
2 **DORSAL ROOT GANGLION, SPINAL CORD AND PERIPHERAL NERVE**
3 **TOXICITIES**

4

5 DR. JAMES WILSON: Thank you. These are my
6 disclosure statements. And I'd like to recognize those
7 at the Gene Therapy Program at Penn who contributed to
8 this work, in particular, Juliette Hordeaux, Liz Buza,
9 Cecilia Dyer, and Christian Hinderer.

10 So we're going to start with respect to
11 neurotoxicity as it relates to two cases that Dr.
12 Sherafat had shared with us at the beginning in which
13 there was evidence at autopsy of degradation in what
14 are called the dorsal root ganglia. These are nerves
15 in which the cell bodies preside outside of the central
16 nervous system in the so-called ganglia with
17 projections of axons, both into the cord and centrally
18 up the spinal cord and then peripherally -- as

1 peripheral nerves.

2 And she described two patients, one with giant
3 axonal neuropathy that died of the disease after gene
4 therapy in which the vector was delivered into the CSF
5 through the lumbar space. And also a patient with ALS,
6 in which in both cases there was evidence for
7 degeneration of the dorsal root ganglia. In the case
8 of the ALS patient, there was also evidence of a
9 neuropathy including paresthesia and neuropathic pain.

10 So prior to that, we, during the conduct of a
11 GLP study, in which we were delivering an AAV vector
12 into the CSF through the cisterna magna, we read the
13 histology out as normal. But an internal peer-review
14 pathologist saw these little ghost-like figures in the
15 dorsal column of the spinal cord. And they relate to
16 what would be a cross-section of axons that are no
17 longer there. So he called it an axonopathy.

18 It turns out we had not recovered DRGs because

1 it's not normally part of the necropsy list. But, in
2 subsequent studies where we evaluated this in more
3 detail, we were able to show that along with the
4 degeneration of the axons up the spinal cord, that
5 there was a primary lesion in the DRGs, which are shown
6 here. And these are the large cell bodies showing the
7 progression in nonhuman primates where the study was
8 done to the point where a subset of the DRGs no longer
9 exist. That presumably then would lead to, with the
10 DRG gone, a degeneration in both proximally and the
11 dorsal column and as we showed distally in the
12 peripheral nerve.

13 So we saw this in a few (audio skip) spine --
14 in a few studies, which then led our team to develop a
15 grading system to quantify the extent of both the axon
16 degeneration and also the DRG pathology based on a
17 percent of cells or axons that were affected. They're
18 graded one through five, five being the worst.

1 So, with that as sort of the sentinel case, we
2 continued to evaluate a number of products in which the
3 vector was administered either into the CSF through a
4 cisterna magna or IT approach that is to the lumbar
5 space or intravenously. In this case, every animal
6 that received vector, we harvested DRG and evaluated
7 animals for the axon findings and the DRG findings.
8 And, at the time at which we published this over a year
9 ago, we had evaluated 205 monkeys receiving CSF vector,
10 the majority of which showed these lesions.

11 We also evaluated animals that received vector
12 intravenously, and most of them did not. The only ones
13 that did show evidence for this pathology were those
14 that received a very high dose vector at the level of
15 around $1e14$ per kilogram. That is the realm where the
16 other pathologies have been described earlier in this
17 meeting.

18 Now, what about background lesions which are

1 important? So, if we just showed vehicle controls,
2 animals that either were untreated or received the sham
3 control, that there is a pretty low background, but
4 there's a low level of background. So this seemed to
5 be more a function of the platform than a specific
6 product

7 In this retrospective analysis of the data,
8 we've evaluated a number of key parameters that are
9 relevant to evaluating the safety of a product
10 delivered in this way. And I'll just show a few that
11 we thought were more important.

12 The first is dose. So, in this panel, we
13 evaluate the pathology score; remember, it's zero
14 through five. And I will say at the outset, that most
15 of the pathology that we see is in the minimal to
16 moderate. We rarely see severe pathology.

17 As a function of dose, less than 312, 312 to
18 313, and greater than 313, this is total dose. When we

1 scale, we scale per gram of brain wave, which in a
2 macaque is about a hundred grams. And evaluating the
3 dorsal root ganglia pathology as well as the spinal
4 cord pathology, so it's dose-dependent, the meta-
5 analysis. But what we've observed is we cannot find a
6 dose usually that is low enough to not demonstrate
7 pathology but high enough to be probably efficacious.

8 And how about the time course, which is really
9 important in terms of the potential for evaluating this
10 preclinically, and also what is the trajectory? We
11 have the pathology scores as the function of Days 14,
12 21, 60, 90, 120, 196, and 180. And what we see is this
13 occurs later and not at an early time point. So, if
14 you look early, you miss it at Day 14. But
15 importantly, it doesn't progress. It may peak
16 somewhere around 90 days or so, and when you follow out
17 later it actually begins to recede. That's probably
18 the cells that were damaged are not just being cast

1 away, and it's not a progressive finding.

2 And how about capsid, can we engineer our way
3 around this? And this is a variety of capsids, and
4 it's somewhat capsid agnostic.

5 One parameter that seemed to make the most
6 difference in our primate studies with respect to the
7 severity of the finding -- and this is sort of on an
8 average pathology score -- is the transgene itself.
9 These are data with 20 different transgenes showing the
10 average pathology of the axonal degeneration, which is
11 the most sensitive, ranging from one case that was
12 undetectable to another case where it was very high.

13 One of these two high ones are GFP, green
14 fluorescent protein, that's very toxic and also
15 immunogenic. So our sort of meta-analysis indicated
16 that the transgene plays a role in this.

17 So does it affect the animals clinically? One
18 challenge in animal studies is eliciting a clinical

1 history. Which you can but trained vet pathologists,
2 who've been trained by vet neurologists, can perform a
3 pretty detailed neurologic eval which we do routinely
4 on our animals. At cage side it's mentation, posture
5 and gait. And the animals are restrained and you can
6 actually do a more detailed neurologic exam.

7 We've now dosed 483 animals through either a
8 lumbar or an ICM infusion with vectors at doses that
9 should be therapeutic based on our scaling. We did
10 find neurologic findings in five animals, and they were
11 limited to those that expressed the green fluorescent
12 protein at a very high dose 1e13. There were findings
13 of the hindlimb ataxia and tremors and possibly
14 paresis. But these were all consistent with the
15 pathology and the DRG. So there's a clinical
16 correlation. Four recovered, and one we had to
17 euthanize because the symptoms progressed.

18 There's a way to evaluate a DRG toxicity or

1 peripheral neuropathy, which is noninvasively, which is
2 called a nerve conduction velocity measurement, which
3 we did not have available to us at the time we dosed
4 those animals.

5 So I want to share with you data that we've
6 now incorporated into all of our studies, sort of
7 noninvasively measure using a standard clinical
8 technique called nerve conduction velocity in our
9 primates.

10 What is done -- I'll just go to the next slide
11 and we can come back to this. This is an example in
12 our macaques in measuring the median nerve in and the
13 sural nerve. And what is done is that we measure the
14 conductance through a nerve that's elicited through
15 this device here, and then we measure the conductance
16 with this detector. And there are other things that we
17 need to control for. But the readout, of which you can
18 capture in a recording, is the stimulus latency, but

1 what's called the SNAP. And the SNAP is a function of
2 how many nerves still persist and have synapses. So,
3 if there's axonal degeneration, there will be a
4 decrease in the nerves and a decrease in the SNAPs.

5 So we -- let me just go back, sorry. We've
6 conducted these studies in 23 studies, some of them
7 GLP, some of them not, 183 animals and there were 714
8 measurements. Again, not the GFP animals. And out of
9 all of those studies we only had evidence for NCV
10 abnormalities in one study, that I'll share with you,
11 but it's important with respect to correlation.

12 I apologize for this being so small, but these
13 are sequential NCV measurements in one animal here,
14 left and right, and another animal here, left and
15 right. This is baseline. You see the SNAP, this
16 little wave. And, in this animal, the SNAP is not
17 affected following vector. But in this animal, by Day
18 28, we lose the amplitude that persists throughout the

1 duration of the study bilaterally.

2 And, when you look at all the animals in that
3 study as shown here, this is the peak amplitude
4 measured over time in animals that were either vehicle
5 or three different doses. And one of the animals,
6 which is the mid-dose animal, here showed a decline
7 that persisted. And there was only one other animal
8 that showed a decline that persisted, and that was a
9 high-dose animal. But pretty consistent measurements
10 as you can see longitudinally.

11 So, we also then necropsied the animals and
12 conducted histopathology and evaluated the DRG
13 degeneration, the spinal cord axonopathy, the median
14 nerve axonopathy and periaxonal fibrosis in the
15 cervical, thoracic, and lumbar. And this is the kind
16 of spectrum of data that you would see across many
17 animals. It's just a total of 22 animals.

18 But interestingly, the two in which we saw

1 evidence, noninvasively, for more severe disease are
2 noted in red. And they are the ones that had the more
3 severe pathology, especially evaluating the peripheral
4 nerve. So I realized it's anecdotal, but in this one
5 study it seems that the NCV may be a good measure of
6 what actually is going on from a pathologic standpoint.
7 But again it's fairly rare because in all of those
8 other studies there were no NCV abnormalities.

9 So we're going to talk briefly about the
10 mechanism and then conclude. But one thing that was
11 really curious is why are the DRGs so affected but
12 other neurons -- other cellular components of the brain
13 aren't? When you capture DRGs following infusion of
14 vector into the CSF of monkeys, and evaluate them for
15 transgene or protein expression, the level of
16 expression is extraordinarily high. In fact, I think
17 it's probably one of the most transducible cells in the
18 body at least when delivered in this way or even at

1 high dose AAV.

2 But there's variation from cell to cell as you
3 can see, pretty significant, but some are highly
4 overexpressing. When you evaluate actually many -- and
5 there are many DRGs up your spinal cord -- in three
6 different animals and ask the question, what percent of
7 the neurons in the DRGs are positive, there's also
8 quite a variation between DRGs as well. So we have a
9 situation where we're transferring genes into this
10 cellular component, but there are a subset of cells
11 that may be expressing very high levels of protein.

12 So one of the questions we asked very early
13 when we saw this -- actually the first study -- is, is
14 this due to a T cell response, a cytotoxic T cell
15 response? So in that first study, what we did is we
16 injected vector into the CSF in rhesus macaques. But
17 we also treated some of the animals with prednisolone
18 to see if prednisolone could suppress this finding.

1 And this is the summary. This is published.
2 It's an evaluation of the axonal degeneration, the DRG
3 degeneration, and median nerve findings with AAV alone
4 or with steroids. And you can see that steroids do not
5 ablate or prevent this from occurring. We tried more
6 severe or potent immune suppression in treating animals
7 with MMF and rapamycin and necropsying animals at the
8 end of the study.

9 Now, I'm just going to highlight one thing
10 here, and what we have are DRG-cumulative scores and
11 axonopathy over time. But this is an example of an
12 animal at Day 14, high dose, Day 90 and Day 180 showing
13 again the time course, which is important, nothing at
14 Day 14 and doesn't progress.

15 But let's get on with the point of the study.
16 Does day timing and suppression help? This is with,
17 without, and with. It didn't help much. Without,
18 with, maybe slightly down. Without, with, without,

1 with, could have made it worse, I doubt it. And then
2 in this other study a different transgene, no effect of
3 immune suppression on reducing this. So we believe
4 that, at least in these studies, it was more than just
5 T cell immunity.

6 So what is the mechanism of DRG toxicity that
7 we see is going on here? And it relates to a mechanism
8 that was proposed earlier, which is we think in some
9 neurons and some DRGs, there's a very high load of
10 vector that then leads to high expression of mRNA and
11 protein that could then lead to stress of the cell and
12 primary degeneration of the cell. Alternatively, it
13 could be proposed that this also sets the cell up for a
14 T cell response, but at least in those experiments that
15 we conducted this can't all be explained by T cell
16 immunity.

17 One mechanism that we propose to eliminate
18 this, is to decrease the expression of the transgene

1 protein in the neurons of the dorsal root ganglia. And
2 you can do that by lowering the dose, you can do that
3 with a promoter. And the way that we decided to do
4 that was to take advantage of the fact that in the
5 neurons of the dorsal root ganglia, in terms of the
6 central nervous system, there's a subset of microRNAs
7 that seem to be specifically expressed in the DRGs.
8 And how could that be helpful?

9 Well, what we propose -- and Juliette and her
10 team propose this -- is that why don't we incorporate
11 into the transcriptional unit a target for this
12 microRNA, so that when this vector enters the DRG in a
13 cell that expresses the microRNA, that it will
14 recognize this as a template and it will shred the RNA
15 and decrease expression? Whereas, in cells not
16 expressing the microRNA there shouldn't be an impact on
17 expression.

18 And so the initial study of this was an enzyme

1 that leads to lysosomal storage disease. In which
2 Juliette made a vector expressing that transgene with
3 or without the microRNA and then evaluate it in
4 monkeys' expression of IDUA, and sure enough it
5 suppressed it in DRGs but not other cells.

6 But how about a histology? Without the
7 microRNA as we showed before there's low level, minimal
8 to moderate evidence of pathology, but with the DRG it
9 was significantly reduced. We've now evaluated this in
10 nine other transgene settings. In seven out of nine,
11 we see a reduction, a significant reduction, but in two
12 of the transgenes the results were mixed.

13 I want to talk about one other component that
14 is worth considering. This is an example of a study
15 where we injected vector either ICM or intravenously at
16 a high dose. Then we evaluated the level of DRG
17 degeneration in various different combinations. We've
18 seen this now on three occasions where there's a marked

1 variation in the level of pathology, and in some
2 animals quite severe. So these are different routed
3 administration groups. But there's a subset that
4 really are quite high, out of the ordinary, for what we
5 normally see, and also associated with axonopathy.

6 So, if you evaluate the animals for T cell
7 responses against a transgene, which is shown here,
8 while it's not a great correlation, the animals that do
9 have greater pathology happen to be the ones that mount
10 an adaptive immune response to the transgene.

11 And why the variation from animal to animal?
12 Well, we did HLA typing on these animals or MHC, and
13 the ones that had the worst pathology and the higher
14 ELISPOT actually carried a non-frequent MHC haplotype
15 allele. While we can't prove that's the reason, I do
16 think it's an interesting consideration when you think
17 about clinical trials.

18 So just to conclude, we do believe the

1 mechanism of toxicity as a disproportionate transection
2 of a subset of neurons that leads to ER stress and
3 degeneration. But it's possible that if the transgene
4 is immunogenic, this could also set up an associated T
5 cell response that will make this and exaggerate the
6 response. We haven't seen it in many of the monkeys.

7 So just to conclude, very high levels of DRG
8 neuron transduction are observed following delivery of
9 vector into the CSF or a high dose IV. A consequence
10 of high transduction is a toxic insult, which then
11 degeneration of the neurons. The vector-induced DRG
12 pathology is consistent with what are called the human
13 sensory ganglionopathies, which is a primary insult to
14 the cell body rather than the distal neuron. This is a
15 function of AAV, and I don't think there are ways to
16 completely engineer around it.

17 It may be potentiated if there's an adaptive
18 response. But importantly in most -- in virtually all

1 cases, the DRG pathology occurs in the absence of
2 clinical sequelae. And the nonhuman primate is the
3 most sensitive animal model for studying this, although
4 other models, if you go to very, very high doses you
5 begin to see it.

6 Also maybe for purposes of discussion today,
7 how should you evaluate it? I think our data would
8 suggest that nerve conduction velocity is a sensitive
9 and specific non-invasive method for detecting more
10 severe occurrences of DRG toxicity even before it's
11 clinically relevant. And anything we can do to
12 decrease expression in DRGs, if it does become a
13 problem, may help mitigate it.

14 So I think that's it, and I'm happy to answer
15 questions.

16 DR. LISA BUTTERFIELD: Terrific. Thank you
17 very much for that really fascinating data
18 presentation. So we have a little over 13 minutes for

1 Q and A, and our first question is from Dr. Vite.

2

3 **INVITED SPEAKER PRESENTATION Q&A**

4

5 DR. CHARLES VITE: Thank you, Dr. Wilson.
6 That was really excellent and helpful for all of us. I
7 just had two questions. One, you'd talk about dosing
8 both at lumbar at dosing at the cerebellomedullaris
9 cistern, I wondered if that changed the location of the
10 DRGs that were affected where these are more cervical
11 or sacral DRGs that were affected which might then lend
12 to more specific signs of potentially neuropathic pain
13 either in the sacral area or in the arm.

14 Then the second question was, if you saw
15 anything related to the afference coming from some
16 autonomic system -- more basically, I guess, if you saw
17 a sympa- (audio skip) ganglia affected?

18 DR. JAMES WILSON: Yeah, I know that's an

1 excellent point, Charles, because you could get --
2 through the autonomic nervous system you could get
3 other gut abnormalities and other. And we didn't see
4 that in the monkeys at all.

5 And then your first question was -- oh yeah,
6 so whether you inject a vector in the cisterna magna or
7 the lumbar space, it ends up being primarily in the
8 lumbar space. But, when you inject the vector in the
9 cisterna magna you get better distribution in the
10 cervical region, and the thoracic, no matter what,
11 seems to be less. So it would be lumbar greater than
12 cervical and then thoracic for an ICM injection.

13 If it's a lumbar infusion, you get a lot in
14 the lumbar and little in the cervical. And most of the
15 neuropathies, sensory neuropathies, want transduction
16 all the way up the cord.

17 DR. CHARLES VITE: Okay. Thank you.

18 DR. LISA BUTTERFIELD: Thank you. Next is Dr.

1 Crombez.

2 DR. ERIC CROMBEZ: Hi, Dr. Wilson. Good to
3 see you. I think I saw some evidence in the data you
4 presented, but I guess I wanted to be clear as this is
5 a little bit outside of my area of expertise. But,
6 given that at least at this point it's a single dosing
7 of gene therapy, would we expect these findings to
8 resolve over time or would we think there would be any
9 potential for irreversible damage?

10 DR. JAMES WILSON: Yeah. Eric, when they're
11 asymptomatic, then all we can rely on is serial
12 pathology. And I think if there's a degeneration of a
13 cell, its associated axons, then that pathology is
14 eventually going to clear. What we don't see is we
15 don't see progression of the pathology itself. And
16 there's one experiment that I didn't have a chance to
17 present, but this was dosing newborn monkeys that were
18 (audio skip).

1 DR. LISA BUTTERFIELD: (Audio skip) if you've
2 made your comments, and so we'll move next to Dr. Barry
3 Byrne.

4 DR. BARRY BYRNE: Thanks very much. Jim,
5 that's an elegant set of experiments, so thank you for
6 presenting that.

7 I just wanted to bring up one point related to
8 the immunosuppression studies that you did with
9 predominately targeting the T cell activation. And I
10 know Dr. Sherafat presented the findings of NALS. And
11 in addition to that one subject that was described, Bob
12 Brown (phonetic) reported actually two subjects that
13 were in that expanded access study, the second of which
14 received that same pretreatment I described for the
15 cases where we have an observed complement activation.
16 And in fact, that second patient didn't develop
17 neuritis and had what appears to have been a persistent
18 beneficial effect.

1 So there are complex interactions, obviously,
2 between the B cell compartment and T cells and what
3 chemoattractants may be elaborated by the early
4 activation of interferons and the other complement
5 fragments that contribute T cell activation. So I'm
6 curious if you can comment on that relationship in
7 these primate studies.

8 DR. JAMES WILSON: Yeah, I mean, when we first
9 approach it, it was rather simple-minded. We wanted to
10 go after killer T cells. But, you know, we do see
11 infiltrating B cells, Barry, so there could be a
12 component of that in the animals where we see more of a
13 cellular infiltrate. I wouldn't rule that out.

14 DR. BARRY BYRNE: Okay. Helpful. Thanks.

15 DR. LISA BUTTERFIELD: Okay. Next, Dr. Roos.

16 DR. RAYMOND ROOS: Just with respect to ALS,
17 there is some literature that sensory abnormalities can
18 be seen, any pathology, and of course the

1 comorbidities. But I wondered, Dr. Wilson, whether you
2 have any experience with respect to humans that had
3 gene therapy with respect to nerve conduction studies
4 or pathology?

5 DR. JAMES WILSON: Very limited with the
6 sponsors that I've supported their research. But I do
7 think other sponsors, after this has been raised as a
8 potential concern, have begun to incorporate NCV
9 evaluations in their studies. But, Dr. Roos, where all
10 of our studies in which we give vector into the
11 cisterna magna or centrally, we incorporate NCV
12 evaluations as part of the experiment.

13 DR. RAYMOND ROOS: And how efficient is it as
14 far as demonstrating abnormality?

15 DR. JAMES WILSON: How what?

16 DR. RAYMOND ROOS: How efficient is it in
17 demonstrating the abnormalities in DRGs?

18 DR. JAMES WILSON: Oh yeah. Well, I could say

1 that in virtually all of our animals, in which we've
2 measured NCV, that we haven't had symptomatic animals.
3 And the animals -- that one study where the pathology
4 was about as bad as it gets, the NCVs went down. So
5 it's pretty anecdotal. But the absence of findings
6 where you have a positive control so that -- the thing
7 that was fortunate about that experiment with those
8 monkeys is when the pathology was bad, in that same
9 animal and those same nerves, we had an NCV
10 abnormality. Which would then suggest that when the
11 NCVs are normal, that you don't have severe pathology.
12 Because we can't measure clinical endpoints because the
13 animals don't develop -- they'll be detectable
14 neurologic abnormalities. If they had a mild
15 paresthesia, it would be hard to know.

16 If they had severe neuropathic pain, we'd --
17 the behavioralist who study the primates, they'd be
18 able to see that.

1 DR. LISA BUTTERFIELD: Great. Thank you.
2 Professor Fox.

3 DR. BERNARD FOX: Okay. I turned off my own
4 camera. So, Jim, very nice presentation. Thanks for
5 your commitment to this area, it's impressive. A
6 couple of questions on your immune response evaluation.
7 The ELISPOT you did, do know that those are CD4s or for
8 CD8s that are making the cytokines?

9 DR. JAMES WILSON: Those were gamma positive
10 ELISPOTS, and we didn't deplete CD4s or CD8s. So they
11 could be -- we don't know for sure. But I bet they're
12 CD4s, but that's just a guess.

13 DR. BERNARD FOX: And so, I guess, you know,
14 part of it is kind of what is the profile of cytokines?
15 And you probably haven't done all that, but, I mean,
16 that's part of it.

17 The other thing we've noticed in our (audio
18 skip) doing some multiplex is these nerve bundles.

1 And I've never looked at a dorsal root ganglia, so I
2 don't really know. But in nerve bundles we see very
3 high expression of PDL1. And I just wonder if DRGs
4 actually express PDL1 and whether or not that might
5 conflict with a T cell-mediated mechanism of
6 construction that there's a high level of PDL1 in those
7 cells.

8 DR. JAMES WILSON: Yeah, it could, Bernie. I
9 mean, a killer T cell response is really not a
10 component of the pathology in the overwhelming majority
11 of settings. So the evidence for that is only that --
12 you know, in vivo gene therapy when you have outliers
13 of sort of inflammatory pathology, I always believe it
14 has nothing to do with the immune system. And one
15 thing that varies between us is our HLA composition.

16 But see we don't have enough sort of
17 experimental substrates where we have findings in the T
18 cell response to even begin to study that. But, as you

1 can tell, we're committed to continuing to evaluate
2 this. So we're going to try to learn more about the T
3 cell response. But now that I'm thinking about it,
4 there was in those two groups where there was clearly
5 an inflammatory response, that didn't respond as well
6 as the miR-183, we actually mapped epitopes in those
7 animals, and they were CD8 epitopes.

8 DR. BERNARD FOX: Great. Thank you.

9 DR. LISA BUTTERFIELD: Okay. A couple more
10 minutes for questions. Dr. Crawford, please.

11 DR. LaTASHA CRAWFORD: Thanks. That was a
12 wonderful presentation. Thank you for that.

13 I had a quick question for you. So it sounds
14 like you've got a really great amount of compelling
15 evidence, that nerve conduction velocity and also
16 seeing lesions that are in the dorsal column, maybe
17 kind of implicated really heavily myelinated neurons in
18 at least some aspect of the pathology that you're

1 seeing. But nerve conduction velocities don't do a
2 great job at picking up pathology that's in very
3 unmyelinated neurons. And I was wondering if you have
4 any evidence, or if you guys have started to look to
5 see if there are things that are happening potentially
6 in -- for example, a lot of our pain neurons, not
7 receptors, are not very heavily myelinated so it would
8 not be picked up really by nerve conduction or in
9 looking at the dorsal column.

10 DR. JAMES WILSON: Right.

11 DR. LaTASHA CRAWFORD: So I was wondering if
12 you have any insight as to whether those are also
13 playing a role or any ideas, I guess, for measurements
14 whether it be nerve fibers in the skin or kind of
15 targeted measures to look at those kinds of readouts
16 that might not show up in what you've presented thus
17 far. Thanks.

18 DR. JAMES WILSON: That's a good question, and

1 are you referring to clinical measures or
2 histopathologic analysis?

3 DR. LATASHA CRAWFORD: I think either. I
4 think that I was kind of thinking more kind of on the
5 clinical side of things or measurements that could be
6 kind of useful. But we could certainly discuss some of
7 the other possibilities now or later during this
8 discussion.

9 DR. JAMES WILSON: Sure. We haven't, and I'll
10 talk to Liz and Juliette about it, but it's a good
11 suggestion. I'd just like to if I could, Chair, make
12 one other comment. That we've often -- the field
13 that's been criticized for not developing reference
14 standards, not sharing pre-competitive space. The
15 society of toxicology pathology convened a working
16 group soon after this was observed. All the companies
17 that are involved in gene therapy, in which this may be
18 affected, are working together to develop a consistent

1 grading system. And I think that this is an example of
2 sort of how it should go forward, so kudos to all of
3 those. And it came around as society that brought them
4 all together.

5 DR. LISA BUTTERFIELD: One of the benefits and
6 the roles of professional societies. Next question
7 from Dr. Zeiss.

8 DR. CAROLINE ZEISS: Hi, Dr. Wilson. First of
9 all, I'd like to commend you for finding the problem,
10 understanding it and fixing it in a very short period
11 of time. That's really impressive.

12 My question also pertains to pain. I
13 completely agree with the previous speaker. I wonder
14 if one way you could get that is to look at makers of
15 more susceptible neurons in the material you already
16 have and try to follow that through. I think the
17 problem with clinically assessing pain in monkeys is
18 they're notorious for hiding pain until the bitter end.

1 DR. JAMES WILSON: Right.

2 DR. CAROLINE ZEISS: So clinically, I don't
3 know if you're really going to get a good handle on
4 that. But a retrospective analysis of all these stored
5 materials you have might be really useful.

6 DR. JAMES WILSON: No, I agree with you about
7 the clinical assessment, not being a primatologist but
8 what little I know on monkeys. But that's a great
9 suggestion and I'll talk to the team about it. Thank
10 you for that.

11 DR. LISA BUTTERFIELD: All right. And the
12 final question from Dr. Barry Byrne, please.

13 DR. BARRY BYRNE: Thanks. A quick question
14 regarding other biomarkers that might be relied on to
15 consider. Were there CSF evaluations at the same time
16 blood was drawn in these animals over the course of the
17 follow up. And any values to work around NFL or other
18 inflammatory markers in the spinal fluid?

1 DR. JAMES WILSON: Yeah, Barry, yeah. The
2 initial evaluation was basic sort of cytology. There
3 is a variable amount of pleocytosis that happens in the
4 first sort of 14 days that resolves. But Juliette just
5 reported the first wave of evaluating all those CSF
6 samples, all of them, or other biomarkers, such as NFL.
7 It's preliminary, so I don't feel comfortable sharing
8 it with you, but I think we may have some very
9 interesting data. And not only in CSF, but in serum,
10 that may be another biomarker for those. We just
11 didn't get the story together enough to present it here
12 but stay tuned, but it's a good idea.

13 DR. LISA BUTTERFIELD: Terrific. Well, thank
14 you for the great presentation, Dr. Wilson, and all the
15 discussion. So now we have a ten-minute break before
16 the open public hearing for Session 4. So I have 20
17 after the hour, so we'll reconvene at 30 after the
18 hour, 10:30 or 1:30. Thank you very much.

1

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[BREAK]

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SESSION 4: OPEN PUBLIC HEARING

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Mr. MICHAEL KAWCYNKI: Hello and welcome back from that short little break. We are in Session 4 and we are about to jump into our OPH Session. Dr. Butterfield, are you ready?

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DR. LISA BUTTERFIELD: Yes thank you very much. Welcome to the Open Public Hearing Session. This is for Session 4 on Neurotoxicity. Please note that both the Food and Drug Administration, FDA, and the Public believe in a transparent process for information gathering and decision making. To ensure such transparency at the Open Public Hearing Session, at the advisory committee meeting, FDA believes it is important to understand the context of an individual's

1 presentation. For this reason, the FDA encourages you,
2 the Open Hearing Speaker, at the beginning of your
3 written or oral statement to advise the committee of
4 any financial relationship you may have with the
5 sponsor, it's product or, if known, its competitors.
6 For example, this financial information may include the
7 sponsors payment of expenses and connection with your
8 participation at this meeting. Likewise, the FDA
9 encourages you at the beginning of you statement to
10 advise the committee if you do not have any such
11 financial relationships. If you choose not to address
12 the issue of your financial relationships at the
13 beginning of your statement, it will not preclude you
14 from speaking. Thank you and now let me turn this over
15 to Jarrod to run the OPH.

16 MR. JARROD COLLIER: Thank you very much, Dr.
17 Butterfield. For Session 4 we have two speakers ready
18 to present. And we will start with Dr. Nicholas Buss,

1 representing REGENXBIO Incorporated. Dr. Buss, you
2 will have five minutes. Thank you.

3 DR. NICHOLAS BUSS: Thank you very much. I'm
4 Nicholas Buss, I represent pre-clinical development,
5 here at REGENXBIO, and I'm grateful for the time today.
6 If you can go to slide 2 please.

7 As presented and published, DRG findings have
8 been well described in preclinical studies following
9 either IT or ID administration across multiple AAV
10 serotypes. We also acknowledge that this demonstrates
11 the importance of correct DRG sample collection and
12 appropriate neuropathological evaluation in our
13 preclinical studies.

14 In our preclinical studies which have
15 primarily been done in primates and mice, we have shown
16 that the DRG findings are general observed in non-human
17 primates, but not consistently in mice. They've been
18 observed with or without immune suppression and

1 generally observed between two weeks and six months.
2 They have not been associated with any clinical
3 observation, in NHPs or mice, and they were not present
4 in juvenile primates after four years as presented by
5 Dr. Valdez (Phonetic) paper.

6 We have also shown in this work that has been
7 published, that's this is not being attributed to the
8 purification methods and is not influenced by the
9 empty/full capsid ratios. This is either (inaudible)
10 or colon purification. And importantly, at equivalent
11 doses, DRG finding were not seen in primates
12 administered a non-expressing AAV9-null capsid.
13 Indicating and supporting the hypothesis that these
14 findings are primarily mediated by transgene
15 overexpression with a secondary immune component.
16 Slide 3 please.

17 While these findings have been well described
18 in primates, we recognize there is lack of measurable

1 and translatable endpoints. Therefore, we included
2 additional nonstandard endpoints in our four-week
3 primate studies.

4 In these studies, where we collected CFS and
5 serum from day four onward to the end of the study, end
6 of week four, the changes were not associated with any
7 changes in pro-inflammatory cytokines, chemokines or
8 neurodegenerative biomarkers, and this did include a
9 Quanterix neurofilament light chain evaluation.

10 In addition to this, we did a thorough
11 evaluation of peripheral nerve conduction in primates
12 using methods adapted from neurological clinical
13 protocol. There were no known effects on nerve
14 conduction suggesting any functionally relevant
15 adenopathy or demyelination was minimal.

16 Furthermore, we looked to the findings using
17 MRI to correlate to what we saw histopathologically.
18 And we noted there was an increased signal intensity in

1 the spinal nerve and dorsal spine root, both on T2-
2 weighted and STIR images. There were slight decreases
3 in FA in all spinal nerve and dorsal spinal cord.
4 Decreases in axial diffusion in most nerve pairs and
5 radial diffusion in the spinal cord. There was slight
6 increase in radial diffusion in all nerve pairs. Slide
7 4.

8 So we concluded that DRG changes in NHP's were
9 not associated with any adverse clinical findings, CSF
10 or serum biomarkers or effects on nerve conductions
11 that were detectable using MRI's of the regions of
12 interest which are the lumbosacral regions.

13 The DRG changes were not observed in the AAV9-
14 null treated animals, which further supports the data,
15 the hypothesis of an overexpression-related injury, not
16 necessarily capsid mediated. We feel the DRG findings
17 are well described in NHP'S and the field is getting
18 clinical experience. Sufficient data and understanding

1 exists where it may not be necessary to request
2 additional animal studies to characterize risks. Slide
3 5.

4 So our recommendation for panel consideration
5 are to continue to perform neurological exams,
6 assessment of nerve conduction and consider MRI for
7 regions of interest in the L/LS region in clinical
8 studies where appropriate. And I think this is
9 important because clinical significance in MRI remains
10 to be defined.

11 To utilize robust risk mitigation and clinical
12 monitoring strategies to build long-term clinical
13 safety profile. To consider a benefit/risk
14 consideration on a case-by-case basis. And also we
15 encourage stakeholder collaboration to develop an open
16 access premarket and post-market database to monitor
17 report potential AEs. And consider establishing an
18 independent review board of experts to assess potential

1 AEs across trial.

2 And we would like to encourage the last two
3 point, because of recent peripheral neuropathy
4 described in the LS trial did not necessary correlate
5 with the findings in NHP and was more indicative of an
6 active inflammation. And this highlights the need for
7 stakeholder collaborations to support the development
8 for gene therapy for patients. Thank you.

9 MR. JARROD COLLIER: Thank you very much, Dr.
10 Buss. Lastly, we have Michael Lehmicke, representing
11 Alliance for Regenerative Medicine. Mr. Lehmicke, if
12 you can start please?

13 MR. MICHAEL LEHMICKE: Hello can you hear me
14 ok?

15 MR. JARROD COLLIER: Yes we can.

16 MR. MICHAEL LEHMICKE: Thank you. My name is
17 Michael Lehmicke, I am the Director of Science and
18 Industry Affairs at the Alliance for Regenerative

1 Medicine ARM for short. I will be speaking on behalf
2 of ARM today and not on behalf of any individual ARM
3 member. My oral comments are synopsis of ARM's,
4 written comments which have been posted in the public
5 docket. ARM thanks the FDA for organizing this
6 advisory committee meeting and providing the docket for
7 public comments.

8 ARM is the leading international advocacy
9 organization dedicated to realizing the promises of
10 regenerative medicines and advance therapies. ARM
11 promotes legislative regulatory reimbursement and
12 manufacturing initiatives to advance this innovative
13 and transformative sector. In our 12-year history, ARM
14 has become the voice of the sector representing the
15 interests of 400 plus members worldwide. Including
16 small and large companies, academic research
17 institutions, major medical centers, and patient
18 groups.

1 Given how critically important these topics
2 are for the field, and the limited time provided for
3 responses from the general stakeholders, ARM request
4 that the docket for public comments be kept open for 60
5 days after the advisory committee meeting in order to
6 allow the broader community time to reflect on specific
7 issues that are raised during the meeting.

8 We have summarized our perspective on each of
9 the five topics selected by FDA, based on the
10 experience of our members who are actively developing
11 AAV based therapy. I'll speak briefly to each topic
12 now.

13 AAV gene therapies have tremendous potential
14 to treat many serious diseases with unmet medical needs
15 and these potential benefits must be considered
16 alongside the possible risks. AAV toxicity risks is
17 depending on several variables. FDA should take a
18 balance and pragmatic approach to evaluating and

1 monitoring the safety and toxicity risk based on AAV
2 serotype, vector design, vector dose, targeted tissue
3 or cell type and disease specificities. Neurotoxicity
4 risk of each gene therapy should be independently
5 evaluated, taking into consideration these variables.
6 Regarding vector integration and oncogenicity risks,
7 given that AAV exposure is very high with the US
8 population, and the extent of nonclinical and clinical
9 data generated up to date, ARM believes that the data
10 does not support a link between AAV integration and
11 recombinant AAV oncogenicity risk in humans.

12 Further, the translatability of vector-mediate
13 insertional mutagenesis and hepatocellular carcinoma
14 observed in mouse models, to larger animal models and
15 humans is unclear.

16 Regarding hepatotoxicity issues, sponsors
17 should mitigate the risk of liver disorders to
18 appropriate patient selection, post treatment

1 monitoring of liver function and appropriate choice of
2 immunosuppression protocols. Dose escalation,
3 especially in pediatric patients with underlying
4 comorbidities associated with the condition, could be
5 carried out after balancing the risks with the
6 potential benefits. ARM is not in favor of
7 implementing a uniform suppression protocol or a
8 uniform dose cap, because both may be product and/or
9 patient population specific.

10 Regarding TMA issues, ARM recommends that
11 patients are carefully monitored for symptoms of TMA
12 using routine test. And investigators should be made
13 familiar with the risks of TMA and have access to
14 expert advice on its treatment. The risk/benefit of
15 dose escalation should be made on a case-by-case basis,
16 based on the specifics of the product. ARM is not in
17 favor of implementing a uniform immunosuppression
18 protocol or a uniform dose cap at this time.

1 Regarding nonclinical findings and DRG
2 toxicity. Based on the review of the literature, ARMS
3 believes the De facto transferability of DRG toxicity
4 between nonhuman primates and human patients should not
5 be assumed. Positive findings of preclinical modules
6 should not necessarily preclude human benefits.
7 Rather, preclinical results should inform a
8 risk/benefit analysis as one part of an overall
9 assessment for each patient.

10 Regarding Neurotoxicity findings based on
11 brain MRI scans, there's been limited use of this
12 procedure and a limited amount of data generated to
13 date to draw a meaningful conclusion. ARM position is
14 to evaluate use of this procedure on a case-by-case
15 basis in a context of benefits verses risk. Any
16 postoperative changes from baseline should be followed
17 with the need of long-term imaging and the absence of
18 acute abnormalities or clinical symptoms is unclear.

1 Lastly, ARM urges the agency and the advisory
2 committee that the toxicity risk of AAV vector-based
3 gene therapies should be put into perspective against
4 the backdrop of thousands of patients who could benefit
5 from genome with AAV vector-base gene therapy products
6 over the next decade.

7 Many diseases with gene therapy product and
8 development are serious, life threatening or fail. The
9 FDA should weigh theoretical risks against the
10 underlying pathology of the disease and potential
11 benefits to patients to address unmet medical needs.
12 While some conditions may also have available
13 treatment, AAV gene therapy has demonstrated the
14 potential to provide transformative clinical benefit.
15 This benefit to patients should not be ignored, based
16 on risks which can be managed with appropriate labeling
17 or (inaudible), or risks which are considered
18 theoretical.

1 ARM would like to conclude the remarks by
2 emphasizing the value of facilitating informed patient
3 choice. So the patient can weigh potential risks and
4 benefits of the gene therapy product in the context of
5 the burden of their disease and the burdens or absence
6 of currently available therapies. I thank you for the
7 opportunity to speak today on these important issues.

8 MR. JARROD COLLIER: Thank you very much
9 Michael Lehmicke. At this time that concludes the, OPH
10 speaker for Session 4. This actually concludes the OPH
11 session for the entire meeting. And at this time I
12 will turn it back over to Dr. Butterfield.

13

14 **COMMITTEE DISCUSSION OF QUESTIONS**

15

16 DR. LISA BUTTERFIELD: All right, thank you to
17 the speakers, thank you Jarrod. Now we have our
18 committee questions to discuss for Session 4. Again,

1 on neurotoxicity specific dorsal root ganglion
2 toxicities. So the same format as before. I'll go
3 through each of the questions in turn. We have three
4 questions for this period that we'll be discussing.
5 The first of which is, "Based on the published data,
6 please discuss the relevance of nonhuman primate cases
7 of DRG toxicity to human subjects." And to set the
8 stage our discussant for Session 4, is Dr. Raymond
9 Roos.

10 DR. RAYMOND ROOS: (Audio skip) since. It's
11 important to do preclinical studies, for example, in
12 nonhuman primates prior to humans. But there are
13 limitations in extrapolating some of that data. For
14 example, we heard that mice have a locus in which AAV
15 can integrate, but the locus is not present in humans.

16 With respect to nonhuman primates, it sounds
17 like there is minimal to mild or moderate DRG
18 pathology, generally without any symptoms. And as Dr.

1 Wilson presented, the pathology doesn't seem to be
2 affected by immunosuppression. But the transgene
3 appears important and knocking down the transgene may
4 be useful as far as decreasing DRG pathology.

5 He mentioned that the high level of the
6 transgene may be affected by ER stress resulting either
7 from messenger RNA at the transgene or the protein
8 itself. It may be of interest in investigations to
9 perturb the integrated stress response which result
10 from ER stress to see whether perturbation of the ISR
11 can modulate DRG pathology. And there are many
12 reagents that target the ISR. And, for example, one
13 might knock down a key part of the ISR with perhaps a
14 DRG-specific promotor. So these may be interesting
15 investigations for the future to give us some idea
16 about the mechanism.

17 So there's been many hundreds of clinical
18 trials with AAVs, but symptoms of DRG abnormalities

1 appear to be very rare. And from the literature,
2 Sarepta used 1.33 time 10 to the 14dg per kilogram of
3 AAVRH74. But "neurotoxicity" has not been observed
4 either preclinical or in clinical studies. What I
5 think might be important in these investigations is to
6 make sure that a skilled Pathologist even
7 Neuropathologist is involved.

8 Nonetheless, it appears that DRG toxicity in
9 humans generally has low severity, appears to be
10 nonprogressive as well. And also, remember
11 risk/benefit is an important issue, especially because
12 the diseases that we're using AAV for are serious and
13 in many cases devastating ones.

14 Still, one must be cautious about the
15 situation in humans because we have different AAV
16 serotypes, different promoters, different dosages and
17 different expression levels. It makes thing
18 complicated and all the more reason to have some

1 cooperation between different groups involved in gene
2 therapy trials.

3 There remains still open questions regarding
4 the mechanisms and clinical relevance of DRG toxicity
5 in humans. And for this reason further investigation,
6 especially involving the mechanism, would be extremely
7 helpful. Thanks.

8 DR. LISA BUTTERFIELD: Thank you, Dr. Roos.
9 And if I can ask you, first, a point of clarification.
10 When you suggest further investigation of the ER stress
11 modulation, transgene modulation, is that in human
12 settings or as additional mechanisms in nonhuman
13 primates?

14 DR. RAYMOND ROOS: I would be reluctant to get
15 involved in human situations without some initial
16 animal studies. I was a little surprised about the
17 mouse being not such a good subject for DRG
18 investigation. If that's in fact true, it could be

1 that some of the reagents that modulate the ISR could
2 be used on nonhuman primates.

3 DR. LISA BUTTERFIELD: Thanks for that
4 clarification. Let's open this up to committee
5 commentary and discussion. Let's start with Dr. Vite,
6 please.

7 DR. CHARLES VITE: I think those questions are
8 important ones. I'll share some data that -- in dogs
9 and cats who have been treated intrathecally with AAV
10 we also see DRG changes. I will also qualify them as
11 mild to moderate. The nerve conduction velocity has
12 not been as sensitive a method. The amplitude in the
13 dogs and cats we see it, it's the pathology. We often
14 don't pick it up in doing sensory nerve conduction
15 velocity studies at least on the amplitude of the wave.

16 I can tell you that we've also seen dorsal
17 root ganglia abnormalities in small molecules that's
18 been administered intrathecally. So it's not something

1 that just alone set to AAV, but we do see it with also
2 some small molecules. And again, we've been looking at
3 them primarily because of the work that Dr. Wilson did.

4 I will also add that neurogenic pain is very
5 difficult to assess in animals unless it's extreme. So
6 again, determining whether these animals feel
7 paresthesia is very, very difficult to do. So we don't
8 know the symptomatic episodes that are present in these
9 dogs and cats.

10 And my final comment would be that since the -
11 - it seems to be primarily the lumbar and the sacral
12 dorsal root ganglia that are effected, one of the
13 clinical aspects that probably could be looked at more
14 would be urination incontinence studies to determine if
15 there's any problems with the lower spinal cord. And
16 those usual show up as a urinary reflex problem. So
17 either by excessive holding or excessive dribbling of
18 urine.

1 That might be something that shows up. I can
2 say that we have seen it in some of the dogs we looked
3 at, but it's also a product of the diseases that we're
4 studying so we haven't looked in normal animals to see
5 if those develop as well. But I think there are a lot
6 of preclinical studies that can be done. But the
7 paresthesia studies may be the most difficult to may go
8 back to what was brought up on histopathology pain
9 fibers, which I think would be an important accept.
10 Thank you.

11 DR. LISA BUTTERFIELD: Thank you. Next, Dr.
12 Zeiss.

13 DR. CAROLINA ZEISS: As you're contemplating
14 more intrathecal administrations to people, I think
15 this is an important issue to address. Because pain is
16 so difficult to assess in preclinical modules, I do
17 think that nerve conduction studies, as a surrogate as
18 overall sensory retention would be valuable and I think

1 that it is a worthy goal to eliminate toxicity.
2 Assuming that if we can effectively eliminate toxicity
3 in dorsal root ganglia, that we are likely to improve -
4 - or mitigate the risk of sensory neuropathy.

5 You know, we do see some toxicity in two human
6 cases. And if you consider that some of the disease
7 that would warrant intrathecal administration could be
8 affecting motor function and proper accepted deficits
9 arising from DRG toxicity may be obscured by the
10 primary disease. And yet people may still suffer pain
11 which would greatly diminish the quality of their life.
12 I do think this is an important issue to address.

13 DR. LISA BUTTERFIELD: Thank you. Next, Dr.
14 Kenneth Berns. We're not hearing you.

15 DR. KENNETH BERNS: Hello?

16 DR. LISA BUTTERFIELD: There you go.

17 DR. KENNETH BERNS: I think y'all hear me now.
18 There's an intermediate connection that somehow I had

1 to overcome. Anyway, I was quite impressed by Dr.
2 Wilson's presentation. And one thing that really
3 struck me is that as far as I can remember, in all the
4 sessions that we've had over the last two days, there's
5 always been the sense that the basic problem might be
6 the capsid as opposed to the DNA component of the
7 vector.

8 His data clearly showed that the transgene was
9 a serious consideration. So it really got to a
10 different dimension of potential problems. And which
11 transgene was the problem -- what kind of transgene was
12 the problem, I thought it was really interesting and
13 probably deserves a lot more investigation. Initially,
14 we were always very worried about whether there was
15 going to be a reaction to transgene expression. We
16 really hadn't seen too much of that. But I think in
17 his studies right now, that's extremely (audio skip).

18 The second part and related to that -- and

1 again that depends on what you (audio skip) sensitivity
2 or measure- (audio skip) is the notion that you have
3 very distinctive aberration at the cellular level. But
4 the clinical consequences of these changes there not
5 very evident.

6 And I think that it's probably going to be
7 very important to investigate that issue further
8 because you really would like to know were there may be
9 a tipping point between cellular abnormalities and
10 clinical consequences. Thank you.

11 DR. LISA BUTTERFIELD: Thank you very much.
12 Next Dr. Barry Byrne.

13 DR. BARRY BYRNE: Thanks very much. I just
14 wanted to raise one other point about this transgene-
15 mediated effects. Because if I correctly recall, that
16 in Dr. Wilson's presentation, in the (inaudible)
17 example these were expressing the human protein.

18 And we have some evidence that even at doses

1 four times higher in intrathecal administration with a
2 (inaudible) transgene. So, expressing the cynomolgus
3 gene in the CSF, we don't see the same level of
4 inflammation as was described in these experiment.
5 Their smaller groups, but nonetheless I think that's
6 going to be an important consideration.

7 And this is, of course, challenging for
8 sponsors who want to test clinical products made in a
9 way that's representative of the final clinical
10 material. So they're going to use a clinical vector in
11 the studies. But it may be an important consideration
12 to express either syneresis equivalence in those
13 experiments as a control.

14 DR. LISA BUTTERFIELD: Thank you. So, Dr.
15 Zeiss, your hand is still up. Did you want to add
16 anything up or is that a lingering hand up?
17 Lingering, all right.

18 We still have time for additional comment.

1 Otherwise, I can summarize what I think I've heard to
2 this first question. We also want to make sure that we
3 here from the full array of committee members of
4 course. I can give a couple of comments and questions
5 before summing up while the other committee members
6 consider additional comments.

7 I have not heard anyone say yet that the non-
8 human primate DRG toxicity is not relevant to humans.
9 I want to make sure we cover everyone's thoughts based
10 on the data. So any other hands up from the group on
11 this question?

12 Okay. So I'll sum up and then we want to make
13 sure that our FDA colleagues can also ask for
14 additional clarity given that we have some time. So,
15 what I've heard is that the nonhuman primates cases of
16 DRG toxicity are relevant to human subjects, although
17 it is noted that this is perhaps one of the most
18 sensitive models to detect this toxicity.

1 The mouse is not the good model, but this is
2 also seen in a perhaps milder form in dogs and cats,
3 and can also be studied there for intrathecal
4 delivery of AAV vectors. And then there's also a
5 general call for additional mechanistic studies to
6 further understand how this is operating particularly
7 in the nonhuman primates, looking at ER stress and the
8 role of the transgene alone- (audio skip) syngeneic in
9 that model.

10 So looking forward for additional comments.
11 All right then, being none, we'll go through the other
12 two questions. We'll have the committee comment and
13 discussion and then we'll give some time to our FDA
14 colleagues to be sure that all of their questions and
15 need for clarity has been achieved.

16 The second question is, "Please provide
17 recommendations on preclinical study design elements
18 such as the animal species, disease models, age, in

1 license postmortem assessments, and duration of follow
2 up post-dose, that may contribute to the further
3 characterization of DRG toxicity." And again, let's
4 begin with our discussant, and let's hear from Dr.
5 Roos.

6 DR. RAYMOND ROOS: All right, thanks.
7 Preclinical studies involving animal models are
8 valuable. And ideally, one could use normal animals in
9 addition to animals that manifest the disease of
10 interest. For example, there's a mouse model that has
11 genetics very similar to SMA in patients.

12 In addition, having more than one animal
13 species is valuable. So, after investigations of AAV
14 in mice or dogs or cats, one can then go on and
15 investigate the AAV with respect to DRGs in non-human
16 primates. Injections of animal at an age that is
17 similar to what one plans in patients would be valuable
18 since the effect may very considerable depending on

1 maturity of the host. One could also use animals for
2 dose escalation. And then that dose escalation would
3 be involved in clinical trials with patients.

4 Ideally, patients would be selected with no
5 underlying disease that will contribute to AAV toxicity
6 and that's also hopefully true of animals that are
7 tested with the AAV. The endpoint depends to some
8 extent on the particular disease that's being treated
9 as well as the history of AAV treatment. So, if
10 hundreds of patients have already been investigated,
11 some parameters tested could be relaxed. However, a
12 new AAV vector gene treatment should investigate
13 toxicity carefully. How long? Certainly weeks,
14 months. And I think new vector gene treatment would be
15 valuable to test over years. And in the case of
16 humans, nerve conduction velocities or MRIs might be
17 valuable to test before as well as after AAV delivery.

18 So, these are important issues with respect to

1 preclinical studies. One concern of mine has to do
2 with investigations in animals that are used in these
3 studies and even in humans. DRGs that are effected may
4 be scattered and for that reason it's valuable to
5 target the DRGs and to make sure that you have the
6 pathology expertise to interpret the pathology.

7 Thanks.

8 DR. LISA BUTTERFIELD: Thank you very much, Dr.
9 Roos. That was a fairly detailed list of
10 recommendations and thoughts on question Number 2. And
11 so, now we have time for the committee and we'd really
12 like to hear broad views from the committee members on
13 their thoughts and recommendations. And let's start
14 with Dr. Crawford.

15 DR. LATASHA CRAWFORD: Sure. Thank you.
16 Thanks for that. I just wanted to add into the
17 discussion a couple of quick points. One thing, I
18 think, that's going to be useful for us to keep in mind

1 is that for any given SNAP chart that we're looking at
2 in terms of histopathology of dorsal root ganglion, if
3 we see a hand full of neurons that are injured, those
4 are not the only neurons that can contribute to
5 neuropathic pain. So, uninjured neurons that are
6 adjacent to them are also neurons that can be
7 hyperexcitable, that have increased excitability or
8 hyperactivity, that do contribute particularly to pain
9 that's the result a stimulus. So, dynamic mechanical
10 allodynia and things of that nature certainly are
11 reliant on some of the uninjured neurons as well.

12 I think that we should be a little bit
13 cautious of just kind of ignoring kind of minimal to
14 mild changes without actually having the data that
15 supports the presence or absence of actual kind of
16 clinically relevant gain of function and lose of
17 function signs.

18 So, I did just want to throw that out there in

1 our discussion so that were not dismissing it simply
2 because it's mild histologic change. If people dive
3 into looking more carefully at, I think, molecular
4 markers and really more careful characterization of
5 sensory function, I think that will provide a lot more
6 reliable evidence for whether or not this really is
7 something that we need to keep in mind or is it
8 something really doesn't have a clinical (audio skip).
9 We need to actually look those clinical (audio skip).
10 That was my perspective comment.

11 DR. LISA BUTTERFIELD: Thank you. All right,
12 Dr. Barry Byrne please.

13 DR. BARRY BYRNE: So, in terms of the models,
14 obviously the findings thus far in NHPs have been,
15 eliminating. And one of things I think we may be
16 seeing, is that because of the redundancy of these
17 system that clinical manifestation don't appear until
18 their thresholds has been reached and a critical number

1 of neurons are present that would result in behavioral
2 findings in the animals.

3 Clearly, it's more of a chronic problem so
4 longer time points would be important. It may be
5 appropriate to consider other purpose-bred animals for
6 these studies, such as K9, because they're more likely
7 to be guaranteed to be naïve to AAV exposure and have a
8 more uniform response. And appears that
9 neurophysiology is maybe a more sensitive measure than
10 the histological findings. So, I think those potential
11 consideration when the principle target in one of the
12 clinical studies is the sensory neuron or other
13 structures that'll be influenced by the AAV exposure
14 into the CFS.

15 DR. LISA BUTTERFIELD: Great, thank you for
16 that. Dr. Vite

17 DR. CHARLES VITE: Thank you. I'd add two
18 more preclinical test that could be useful. One is a

1 difficult test to do sometimes, but the H reflect
2 actually tests the pathway through the dorsal root
3 ganglion and I wonder if that might be something a
4 little bit more sensitive than just looking at the
5 affects once the peripheral nerves been damaged. So, H
6 reflex testing is something that we can start looking
7 at. But I think others might (audio skip). I don't
8 know if Jim has looked at those in the primates, but
9 that might be interesting test.

10 The other -- and it's been brought up before
11 but I think it's essential. For a number of years we
12 sent tissue to different pathologist. And it wasn't
13 until we sent it to people who were really good at
14 neuropathology that they were picking up dorsal root
15 ganglia changes and other spinal cord changes.

16 So, I think the points been brought up by a
17 number of people, in the same way yesterday as the need
18 for hepatologist and some of these boards to understand

1 what's going on. I think the expertise in
2 neuropathology is essential because some of these
3 changes, especially just neuronal necrosis of the DRG
4 without inflammatory cells and some external
5 degeneration, can often be missed. So, I think those
6 are two points in the preclinical test that I would
7 advise.

8 DR. LISA BUTTERFIELD: Thank you. Indeed some
9 of those histology slides did seem subtle, at least to
10 this tumor immunologist. Next we have -- okay, double
11 check your raised hands, make sure that they should be
12 up if their up. Dr. Barry Byrne, you are next on my
13 list.

14 DR. BARRY BYRNE: No additional comments,
15 actually.

16 DR. LISA BUTTERFIELD: Right. Dr. Zeiss.

17 DR. CAROLINE ZEISS: I do wonder if we could
18 use the pig to give us some information in

1 understanding some of the basic physiology underlying
2 cell death, in the DRG, as well as looking at various
3 mechanisms to mitigate that. Because they are
4 resistant to liver pathology even with high IV doses.
5 And they are sensitive to DRG toxicity, and they have
6 clinical phenotype. (Audio skip). And I think that
7 data would probably have to be confirmed in ANHPs
8 (phonetic), but they may be a good place to start.

9 DR. LISA BUTTERFIELD: Great, thank you for
10 that. Dr. Breuer.

11 DR. CHRISTOPHER BREUER: I think the use of the
12 nonhuman primate model, especially longer term, would
13 be necessary using the more sophisticated detection
14 method mentioned. But I also feel like this needs to
15 be prioritized within some of the diseases we've spoke
16 about today. And this seems an order of magnitude less
17 significant than the other things we've been talking
18 about (inaudible) (audio skip).

1 DR. LISA BUTTERFIELD: Thank you. Okay,
2 looking for, new hands up. Additional comments from
3 different members of the panel for -- I don't know if
4 we still have Dr. Wilson here. He can perhaps comment
5 on some of the questions.

6 DR. JAMES WILSON: This is Jim Wilson. I
7 thought they were all really good points about some
8 additional ways that we can evaluate this. In
9 particular, the pig is an interesting idea because in
10 (audio skip) pigs, we actually systemically saw very
11 severe in clinically evident DRG toxicity. All the
12 other comments were helpful about other things that we
13 can do for sure.

14 I do say that having been the lab that brough
15 this up, just reflecting on the point of the last
16 person that commented, is I suspect all stakeholders
17 assess this risk relative to the potential benefit, so
18 it's all a risk/benefit.

1 The one thing that worries me a bit is, when
2 we were doing the studies -- and reenforced by many of
3 those on the committee, are primates may look well but
4 may have tingling or paresthesias and we wouldn't know
5 it. So, I do think maybe that'll get to the next
6 question. I don't think there's more we can do in
7 animals but really carefully monitor this in people.
8 Because we may be seeing first subclinical in animals
9 evidence for sensory neuropathology that we're not
10 seeing -- that may begin to surface in humans.

11 DR. LISA BUTTERFIELD: Terrific. Thanks very
12 much. Okay. Dr. Breuer, do you have an additional
13 comment? That looks like a no. Dr. Crawford.

14 DR. LATASHA CRAWFORD: Sure. I thought that
15 another thing that might be worth bringing to
16 discussion as well is in terms of some potential
17 endpoints to look for. Would be either kind of
18 translation or first translation or things like that

1 from some of the other places where people have done a
2 little bit of a better job in terms of actually
3 characterizing or looking for things like pain in
4 mechanical allodynia as one of the other committee
5 members or folks here said. Looking for neuropathic
6 pain is extraordinary difficult in animal models. It's
7 actually challenging in patients as well. To kind of
8 fully characterize it unless you're looking for it.

9 So things like quantitative sensory testing
10 have been, you know, he has very successfully to be
11 able to kind of characterized entire sensory phenotypes
12 that patients have and cases of neuropathic pain,
13 including some components of sensory dysfunction that
14 the patient may not have even realized was present
15 until they actual looked for it.

16 And that certainly has been something that's
17 been used to some degree, particularly in dogs. And
18 could probably be used to some degree in other animal

1 models, at least some components of that. So, we're
2 looking for things like (inaudible) fray filaments or
3 dynamic brush, (inaudible), things of that nature.
4 Have also been using (inaudible) in primate as well.

5 And so, I think that having very specific test
6 that actually can try to detect pain is going to be far
7 more successful than just doing a neurologic exam and
8 assuming that the animal would show you if they were
9 people. And so, I think that's certainly something to
10 consider in terms of potential endpoints for these
11 models in animal studies or in investigations of
12 mechanisms and that sort of thing.

13 And then the other I was going to also mention
14 was, once we can get a little bit more information on
15 mechanism, I think that may also open up other types of
16 models. Mice may not have the increased cellularity
17 and things like that in the DRG, but there may be other
18 models that exist that have components of

1 hypersensitivity that you don't see on pathology at
2 all. And so, I think that kind of being open to those
3 types of models when it comes time to actually look at
4 mechanisms and look at ways to mitigating those risks
5 or blocking those mechanisms, I think would be really
6 useful as you're moving forward.

7 All that being said, I do think that the
8 sensory signs that folks have described do seem to be a
9 little bit, you know, less severe than some of others
10 potential risks. But I do know that things like
11 mechanical allodynia are certainly limiting factors for
12 other types of drugs that you have (inaudible) DRG, so
13 chemotherapeutic stuff and things like that.

14 So, it is something that certainly is worth
15 investigating and it needs understanding a little bit
16 more so that we can communicate that to patients
17 clinicians can be aware.

18 DR. LISA BUTTERFIELD: Great thanks very much.

1 Dr. Kenneth Berns.

2 DR. KENNETH BERNS: Yeah, I have a question for
3 Dr. Wilson if he's still there. It's a follow up on my
4 earlier comments. And that's a question, Jim, as to
5 whether you are systematically looking at different
6 (audio skip) transgenes in terms of the differences and
7 also levels of expression from a given vector in terms
8 of what you see in the DRG damage in your nonhuman
9 primate model.

10 DR. JAMES WILSON: Yeah. So, Ken, the spectrum
11 of, really, protein that we have evaluated have
12 naturally sort of fell out of programs that we're
13 interested in. But they range from what you know I
14 think Barry had suggested human versions of enzymes or
15 intracellular proteins. We have receptors, we have
16 reporter gene and we also have secreted protein such as
17 the antibody. So it really is the full spectrum of --
18 I mean, not completely -- but that reflected in there.

1 And we've asked the question, is it more severe with
2 the secreted protein verses an intracellular protein,
3 or is it more severe with a receptor? And we haven't
4 sort of grossly seen those associations.

5 DR. KENNETH BERNS: Thank you.

6 DR. LISA BUTTERFIELD: All right. I really
7 appreciate all the discussion and suggestions from the
8 committee. I will take a moment to sum up what I've
9 heard about Question 2.

10 Recommendations on preclinical study design
11 elements that could include age-appropriate models that
12 reflect the disease state. Designed elements that
13 would include, not just histology, but broad functional
14 testing that includes age reflective testing, thinking
15 about specific sensory test and not a more basic work
16 up. Things like dynamic brushing that would ask
17 specific sensory questions.

18 In terms of animals, dogs were raised as a

1 model that would be a naive state to the AAV vector.

2 Porcine models were mentioned that would also be
3 excellent for asking questions of DRG toxicities.

4 In terms of disease models, either healthy or
5 diseased were mentioned matching an age to the desired
6 diseased state, including in life the nerve conduction
7 velocity in addition to what I mentioned about sensory
8 nerve testing.

9 And then for those histology examinations,
10 making sure neuropathology expertise was present in
11 order to see things that might not be seen otherwise.
12 And then longer follow-up for newer vectors, new
13 subtypes, or in this case to fully detect some of the
14 neurologic changes and toxicities that might occur.

15 Any comments, for that sum up to close out
16 Question 2? Dr. Crawford, is you hand up or is it
17 still up from earlier? Still up from earlier.

18 All right. With that then, let's move to the

1 final question, for Session 4 on DRG neurotoxicity.
2 And Questions 3, "In addition to periodic neurological
3 examinations, please provide recommendations on other
4 methods to mitigate the risk of DRG toxicity in
5 clinical trials." Let's start off with Dr. Roos.

6 DR. RAYMOND ROOS: Thanks. Unfortunately, we
7 don't really have optimal test for evaluating the DRG
8 pathology that might be present in patients. A
9 neurological exam is mentioned and I think that's
10 certainly very valuable, especially an assessment of
11 sensory function.

12 A little bit of an issue here is that some of
13 the patients that are being treated may be very
14 immature with respect to their age. They may have
15 problems with cognition or language function.

16 We spoke about neurophysiological test, for
17 example sensory nerve action potentials. That's
18 certainly valuable. However, my impression is that

1 these test aren't as sensitive as we'd might like,
2 especially if the DRG pathology is scattered within the
3 spinal cord -- within the central peripheral nervous
4 system.

5 Mention was made about MRIs. And of course
6 we're dealing with something somewhat expensive here.
7 But I think it might be valuable, especially in a
8 subset perhaps of patients, an MRI with gadolinium to
9 identify enhancements of the DRG or axioms. Again,
10 there may be potential problems here because of
11 pathology in that particular patient's disease.

12 Dr. Wilson mentioned spinal fluid, looking for
13 a biomarker in spinal fluid that might reflect the DRG
14 pathology. Finding this would be wonderful. However,
15 this may be somewhat challenging. Or eventually, if
16 DRGs become available, for example at autopsy, looking
17 for biochemical abnormalities of transduced DRGs might
18 be important.

1 How can we mitigate the risk in DRG pathology
2 in humans? Dr. Wilson mentioned knock down at the
3 transgene with another AAV, but I'm a little concerned.
4 If in fact the DRG pathology we're talking about has no
5 clear clinical effect, I want to be a little cautious
6 about that, but he may want to respond. I think we
7 need to look carefully at the mechanism involved with
8 respect to AAV vector delivery and DRG toxicity.
9 Thanks.

10 DR. LISA BUTTERFIELD: Thank you, Dr. Roos,
11 for setting the stage for our committee discussion.
12 So, a number of discussion points. Here, again, our
13 focus is on recommendations to mitigate the risk of DRG
14 toxicity in clinical trials. And so, I'm watching for
15 hands from our committee members. Dr. Barry Byrne,
16 thank you.

17 DR. BARRY BYRNE: Yeah I can comment. Having
18 seen many of these patient I know that these would be

1 difficult to layer on complex neurophysiologic
2 assessments into many of the assessments that the
3 patient has already received. I think it would be very
4 important to have a biomarker that would assess the
5 clinical relevance or severity if possible,
6 particularly in the peripheral blood and not just in
7 CFS.

8 You know, many patients aside from those with
9 systemic exposures like SMA have received intrathecal
10 gene therapy without. And those that are, you know,
11 verbal and cooperative with a detailed neurological
12 exam like in CLN7 and CLN6 and other types of SMA and
13 SMA 2 and 3. So those evaluation should continue and
14 it's important to probably focus on these additional
15 assessments like the sensory and nerve action
16 potential.

17 And the H reflect would be a really excellent
18 outcome measure to study, in a limited set of patients,

1 to see whether there really is a clinically relevant
2 change here, or mostly, maybe, focusing too much on the
3 early (audio skip). Whereas Jim Wilson pointed out,
4 were low grade findings but present. There's no doubt
5 they're present. But are those clinically relevant, I
6 think, still remains to be determined.

7 DR. LISA BUTTERFIELD: Thank you. Dr.
8 Crawford.

9 DR. LATASHA CRAWFORD: I just wanted to add in
10 -- and actually this may be a little bit relevant to
11 Question 2 as well. But one thing that I think could
12 be a useful thing to think about is some
13 recommendations in addition to looking for, kind of
14 liquid biomarkers. Also, maybe potential looking at
15 intraepidermal nerve fibers and trying to understand if
16 that could be a potential marker. It's certainly
17 something that's a very good diagnostic tool for
18 diagnosing neuropathic genes in neuropathic gene

1 patients.

2 It's something that's very translatable that
3 can be done in animal models, and that can certainly be
4 done in humans as well. To be able to look for things
5 that may be changing. And maybe you might be able to
6 kind of predict that there may be something that comes
7 up that is worth treating that patient to prevent
8 development of neuropathic pain or things of that
9 nature. So that might be also something to consider in
10 terms of employing, first, obviously, in animal models,
11 but if it does become something that useful that can
12 certainly be done in patients as well.

13 And then, I also wanted to put in a plug for
14 potentially looking at quantitative sensory testing as
15 a tool that could be useful for being able to detect
16 changes and potentially hoping to prevent (inaudible)
17 or something that kind of arises at some point in time
18 for a subpopulations of patients.

1 DR. LISA BUTTERFIELD: Great. Thank you very
2 much. Dr. Crombez.

3 DR. ERIC CROMBEZ: Thank you. I just wanted
4 to add and, again, recognize that there is no easy
5 answer here. I think certainly there is additional
6 work that can be done in nonhuman primates or other
7 animal models and that would be very helpful and
8 informative as would the work on additional biomarkers.

9 But I think really to directly answer this
10 question, I think, maybe today with what we know today,
11 and as we wait for further development and work in
12 nonhuman primates and other animal models that a very
13 careful neurologic exam may be the best place to start
14 in clinical trials.

15 DR. LISA BUTTERFIELD: Thank you. I'm seeing
16 some hands still raised from folk we've heard from, so
17 check and see if your hand is really raised. Okay.
18 Other comments from committee members? Recommendations

1 for clinical trials. Recommendations to mitigate the
2 risks,

3 Let me see if I can summarize some of the
4 suggestions that we've heard so far from the different
5 committee member. A note that there's already a lot of
6 assessments, being undertaken, of patients in these
7 trials. And some of these assessments and the data
8 obtain can be complicated by the patients age and their
9 language and communication skills, which may not allow
10 for some of the sensory change questions to be answered
11 with desired clarity.

12 Though there aren't too many good specific
13 test for this yet, other than the neurological exams
14 already happening, but certainly a search for
15 biomarkers in CSF, as in many disease setting
16 biomarkers in the blood are the holy grail. But
17 looking in CSF, perhaps, first.

18 Selective use of MRI testing, while costly,

1 with gadolinium enhancement could be valuable in a
2 subset of patients. And then knowing that some sensory
3 nerve action assessment, quantitative sensory testing.
4 We do have a number of tools that, perhaps, would be
5 tested first in animals for validation, but then might
6 be considered for clinical trials in specific cases.
7 So, that's what I heard. And, Dr. Barry Byrne, you
8 want to add to that?

9 DR. BARRY BYRNE: That's excellent, Lisa,
10 thank you. I want to add one point because there is
11 the temptation to consider that any cellular
12 inflammatory process can be influenced by
13 glucocorticoids. But Dr. Wilson's presentation
14 actually showed that in this setting, steroids actually
15 worsen the finding. So, we should just be cautious not
16 to overuse glucocorticoids to try and suppress
17 something we think is a silent problem, because there
18 are other consequences to that therapy as well.

1 DR. LISA BUTTERFIELD: Great. Thanks for that
2 addition. So, those are the 3 questions. And so, I
3 want to give an opportunity to our FDA colleagues to
4 make sure that we've answer their questions and given
5 some recommendation. Dr. Wilson Bryan.

6 DR. WILSON BRYAN: So, let me call fist on Dr.
7 Urban.

8 MR. JARROD COLLIER: Dr. Urban, you're on mute
9 right now.

10 DR. DANIEL URBAN: Oh mute sorry. Okay.
11 Start over. I just wanted to state that the FDA
12 supports the sharing of these data regarding
13 preclinical findings via the publications and other
14 routes. But I just have a follow-up question. Could
15 the committee please comment on these recommendations
16 for preclinical studies? But take into the account the
17 clinical population and the issue of benefit/risk as
18 well as the importance of judicial use of the cited

1 large animal species.

2 DR. LISA BUTTERFIELD: All right. What I
3 heard -- I'm going to watch for hands to specifically
4 address that. What I heard was that this has not been
5 a major finding to date in humans, and so it may not be
6 as prioritized as other toxicities in terms of
7 risk/benefits. But, let me defer to some of the
8 clinical colleagues who see these patients to address
9 that question. Watching for hands. Dr. Roos.

10 DR. RAYMOND ROOS: If I understand the
11 question it had to do, perhaps, a little bit with a
12 money and an ethical issue in the sense that DRGs don't
13 seem to be an overwhelming clinical issue in humans
14 with respect to gene therapy at present. And working
15 with nonhuman primates is expensive. And in addition,
16 one may not have all the controls that one wants.
17 Certainly much easier to work with mice.

18 I think these are important issues, complex

1 ones. I worry a little bit about issues that seems
2 perhaps not so important now. But if gene therapy is
3 successful, with age one loses neurons, and maybe some
4 of these issues become important in the future. So, I
5 certainly agree one has to be judicious in
6 investigations, especially with nonhuman primates that
7 are expensive. But I think understanding the
8 mechanisms (audio skip) investigations are important.
9 And also, clarifying the situation in humans.

10 I don't think we actually have a very good
11 handle on that. And I certainly think that's an
12 important priority. There are probably many autopsies
13 that have been done on patients that have had gene
14 therapy, especially related to ALS is my guess. I
15 don't know how many of them have been investigated in a
16 really expert fashion with respect to DRGs. So, I
17 think there are other priorities here that are
18 important before we try and close the door.

1 DR. LISA BUTTERFIELD: Thank you. Other
2 questions from our regulatory colleagues?

3 DR. WILSON BRYAN: Let me check with the Dr.
4 Sherafat.

5 DR. ROSA SHERAFAT-KAZEMZADEH: Good afternoon.
6 Thank you for discussion. So for children who are
7 unable to provide information and are receiving product
8 intrathecally or cisterna magna, would you recommend
9 this nerve conduction study to be performed under
10 anesthesia, at baseline, and at regular intervals? And
11 how often would that be recommended even (audio skip)
12 done (audio skip).

13 DR. LISA BUTTERFIELD: Looking for clinical
14 hands to be raised from people who see those pediatric
15 patients. Dr. Byrne.

16 DR. BARRY BYRNE: The clinical obviously don't
17 require any form of sedation, and nor does the sensory
18 nerve action potential. So if they need to run a few

1 test, it can be performed in (audio skip) patients to
2 determine clinical risk/benefit.

3 DR. LISA BUTTERFIELD: Thank you. Other
4 clarifying questions?

5 DR. WILSON BRYAN: That's all the questions
6 from the FDA. Thank you. We really appreciate the
7 discussion.

8

9 **SESSION 5: NEUROTOXICITY: BRAIN MRI FINDINGS**

10

11 DR. LISA BUTTERFIELD: Terrific. Thank you,
12 Dr. Bryan. I believe that closes Session 4. We now go
13 immediately to Session 5, our final session on this
14 two-day meeting. And this session is going to be
15 neurotoxicity on brain MRI findings.

16 Here we begin with a presentation from our
17 invited speaker, DR. Ronald Crystal, Professor and
18 Chairman of the Department of Genetic Medicine at Weill

1 Cornell Medical College. Thank you very much, Dr.
2 Crystal.

3

4 **INVITED SPEAKER PRESENTATION: CLINICAL AND NONCLINICAL**
5 **CONSEQUENCES OF DIRECT CNS PARENCHYMAL ADMINISTRATION**
6 **OF AAV VECTORS**

7

8 DR. RONALD CRYSTAL: Thank you. If we could
9 have the slides. What I'd like to present is or
10 experience in terms of direct intraparenchymal
11 administration of vectors to humans and to nonhuman
12 primates. Next slide has my disclosures.

13 The disease that we studied is late infantile
14 neuronal ceroid lipofuscinosis. It's one of the forms
15 of Batten disease now referred to by the gene CLN2
16 disease. It's autosomal recessive, it's 400 to 600
17 cases worldwide.

18 The disease onset is ages 2 to 4. These

1 children develop, starting about age 3 or so, cognitive
2 impairment, visual failure, seizures and a
3 deteriorating motor development leading to a vegetative
4 state and death by age 8 to 12. The MRI of that child
5 in the picture is shown below with a huge amount of
6 brain loss.

7 The disease is caused by mutations in the CLN2
8 gene which is a lysosomal peptidase -- tripeptidyl
9 peptidase. From a gene therapy point of view, it is
10 advantageous because precursor form of the enzymes is
11 secreted and taken up by the mannose-6-phosphate
12 pathway of neighboring cells. So that an individual
13 cell that's transfected can leverage that in terms of
14 correction of neighboring cells as well.

15 Also, another advantage of studying this
16 disease is that once these children start deteriorating
17 they fall off a cliff, clinically, over a period of 18
18 months. And this is clinical data from combined

1 studies of -- a natural history study carried out in
2 Europe, primarily by a group in Hamburg, combined with
3 our natural history data, motor plus language
4 assessment.

5 The score is on the Y axis and as you can see
6 at age around 24 and 36 months or so, these children
7 deteriorate rapidly. But from a clinical research
8 point of view this provides the ability to see whether
9 or not you can level up the rate of decline of these
10 children, and there's a robust clinical measure of that
11 as well. The gene in the vector that we use was
12 serotype AAVrh.10, nonhuman primate serotype, at least
13 an active CAG promotor and human CLN2 cDNA.

14 The study was a screening study and then
15 children were identified, and the families decided to
16 treat or not treat. We treated over 18 months. I
17 followed them over 18 months, a total of 8 children, 7
18 with follow-up. And we had two controlled natural

1 history studies. On our own of 12 children, they were
2 followed up without therapy. And then our
3 collaborators in Europe, called "DEM Child Natural
4 History Study" followed up 41 children. So, two
5 natural history studies to compare the therapy to.

6 Around administration and the focus of what
7 we're going to discuss is directed to intraparenchymal
8 administration. This was done with flexible glass
9 catheters that we developed. It circumvents the blood-
10 brain barrier. It had not only been used by us, but in
11 studies of other lysosomal storage diseases as well.
12 In our trial we used 6 burr holes per catheter,
13 administered for about an hour at one site and then
14 pulled out a centimeter. So, we got two sites per
15 catheter and per burr hole.

16 The total dose was 9.0 times 10 to the
17 eleventh genome copies, and that was reduced after we
18 saw the lesions. And I'll show you the 2.9 -- about a

1 half gc down -- 2.9 times 10 to the eleventh. For each
2 catheter site -- as I mentioned there were two levels
3 per site per burr hole. It was administered at 2
4 microliters per minute, a total of 300 microliters per
5 burr hole, 150 microliters each of the 12 sites. And
6 the dose per site was 2.5 to 7.5 times 10 to the tenth.
7 That's an important number because as you'll see, going
8 beyond that is probably not acceptable in terms of
9 toxicity.

10 In terms of expression of the transgene over a
11 period of time, the western blots as shown on the left.
12 This is from cerebral spinal fluid. This is the
13 enzyme, the TPP1 enzyme level. We were able to get
14 cerebral spinal fluid from follow-up on 5 of these
15 children. One at 6 months and the other 4 at 12
16 months. And as you can see, in all of the children
17 that we were able to sample, at follow-up there was an
18 increase in the TPP1 levels of each of these children.

1 And there also was evidence of clinical
2 efficacy in the motor plus language assessment. All
3 these children were assessed periodically by a
4 pediatric neurologist. We also videotaped the
5 children's examination. And then three pediatric
6 neurologists, who were blinded to the therapy, also
7 scored them. And the averages were used to determine
8 over time, the rate of decline, of motor plus language.

9 In the data, the rate of decline per year is
10 show on the Y Axis. Zero would be no rate of decline
11 and then -1, -2, -3 increasing rates of decline. In
12 blue, the first dot, as you can see, the 12 children in
13 our natural history study declined at about -1.65 units
14 over a period of a year. The DEM child, 41 children
15 that were followed in Europe, declined at -1.81 units
16 per year. There was no difference in the two natural
17 history studies.

18 As you can see in red, we had an improvement.

1 Not complete correction, but improvement compared to
2 both of the control types. So we did see some evidence
3 that (audio skip) see compared to two different
4 controlled groups on a clinical level.

5 However, there were lesions that were seen and
6 that's the subject that we want to talk about. What we
7 noticed was that there were MRI abnormalities at the
8 site of the catheter tip. The observation was over 18
9 months. In many subjects an example of T2 flair is
10 shown on the right. There was also abnormalities in
11 the diffusion-weighted images and the apparent
12 diffusion coefficient.

13 These were hypersensitivities and they were
14 localized at the site of administration. There was no
15 clinical correlates but, as I'll show you, it's
16 consistent with localized inflammation and edema.
17 There were no other significant adverse events that we
18 could directly relate to these vectors. Now these

1 children of course went through a neurologic procedure
2 and they -- one of the aspect of the disease is chronic
3 seizures and so it was difficult to be able to, in the
4 short term at least, determine significant adverse
5 events directly to the vector. But as you can see,
6 later on, there were none.

7 Here are a few other examples of the lesions.
8 One of the subjects, up at the top, we did an MRI
9 within 48 hours. And that's shown at the first image.
10 And then 6 months and 12 months. And you can see in
11 that child these abnormalities on T2 flair persisted
12 over the period of a year. In contrast, in the subject
13 below, we saw abnormalities initially but then they
14 disappeared over time. Here's a few other examples of
15 the T2 flair. Just in panel A, B, C and D, a T2 flair
16 up to 18 months or so. And then the diffusion wave in
17 panel E and the ADC at panel F.

18 Those were the lesions that we saw. This is

1 the complete chart of all the data. We actually had
2 two cohort. What I talked about before was the eight
3 children that were our therapy cohort. Because we
4 required for the therapy cohort that they have either
5 mild or moderate disease. However, we had five other
6 children who had severe disease whose families agreed
7 to be part of a second cohort that we use for -- not
8 for an efficacy assessments, just for safety. So
9 that's the group -- so a total of 13 children.

10 The pre-transfer in T2 weighted average and
11 DWI and ADC is show. In red are the abnormalities so
12 there was nothing initially. Postoperatively, we saw
13 abnormalities in almost all of the children. This
14 disappeared in some of the children, as you can see at
15 month six. Some of the children that were from Eastern
16 Europe we were not able to get follow-up MRIs on. But
17 as you can see there was persistence in a significant
18 number the children, as shown in red, over a period of

1 time.

2 In regard to other parameters, we know in the
3 left-hand panel is serum neutralizing antibodies
4 against the AAVrh.10 capsid. And all the children had
5 neutralizing antibodies. However, on the right-hand
6 panel, in several of the children we were able to look
7 at CSF in terms of neutralizing antibodies and we could
8 not detect any. We also did blood interferon gamma
9 ELIspots against the capsid. There were mild sporadic
10 positives to the capsid, but there was no correlation
11 at all to time of administration nor to neutralizing
12 antibodies.

13 Going through what's been public, as well,
14 this has also been seen with AAV2 in Parkinson's
15 disease by Voyager and Neurocrine. Lysogene/Sarepta
16 have said, publicly, they've seen this in MPS IIIA
17 study. And in an academic study, metachromatic
18 leukodystrophy was also seen. And there may be other

1 examples as well.

2 So what can we say about the CNS
3 intraparenchymal route? It clearly is effective, as I
4 showed you, with two control groups in slowing -- not
5 curing -- but slowing down the disease progression.
6 But it cannot provide even distribution of the vector
7 throughout the CNS. Doses limiting second degree. The
8 problem is the high concentration of the catheter tip.
9 Distribution, of course, is limited by the number of
10 burr holes. We and others have tried increasing flow
11 rate and there are various strategies to try to do
12 that, but we've not been able successfully to improve
13 that very much by flow rate. And of course it requires
14 general anesthesia and hospitalization.

15 We also have carried out studies. So, when we
16 spoke with the FDA with another study for another
17 product, they recommended that we do MRI studies in
18 nonhuman primates, so let me show you that data. So

1 this is the same vector AAVrh.10 but now coding for
2 hARSA. It had a FLAG tag on it. And was administered
3 to the cortex of African Green nonhuman primates. The
4 doses were 2.85×10^{-10} gc total, 2.4×10^{-9}
5 gc per site, and another series of
6 animals at a higher dose of 1.5×10^{-10} gc total,
7 1.3×10^{-9} gc per site. And
8 nonhuman primates were evaluated at 0, 13, 26 and 52
9 weeks.

10 And we saw similar lesion we saw in humans,
11 but we also saw it with the capsid itself, that's on
12 the second row. That's a Null vector, an AAVrh.10Null
13 vector, at 1.5×10^{-10} gc total. And we saw
14 lesions. And then the row after that is the lower dose
15 of the hARSA vector, and then the higher dose is shown
16 next. So, it's dose dependent and seen in nonhuman
17 primate as well as humans.

18 In terms of histology, the PBS control, you

1 can see the catheter track. So, there is some
2 inflammation following the catheter track, that was at
3 one week. In the next panel, C and D, is a Null
4 vector, and you can see inflammation, lower power and
5 then higher power. And then the next two rows are a
6 lower dose and then the higher dose. And you can see
7 clearly there's inflammation associated, but it's very
8 localized to the tip of the catheter.

9 We also looked in the nonhuman primates at
10 what kind of inflammation it was. So, this is set up
11 in the same way as the panel (audio skip) I just showed
12 you. The top row is saline, next row is a Null vector,
13 the third row is the low dose of the AAVrh.10hARSA
14 vector and the last row is the highest dose. As you
15 can see, it's scattered but clearly inflammation with
16 macrophages, T cells and some B cells as well.

17 So, to put it all together, the MRI in humans,
18 there are lesions localized to the site of

1 administration. We quantify that as a percentage of
2 the total brain volume and it was less than 0.3 percent
3 in all the patients. It is plus/minus reversible, more
4 nonreversible than reversible. However there are no
5 clinical correlates either neurologically or in blood
6 assessment.

7 In regard to nonhuman primates with a
8 different transgene to the same vector, again, the
9 lesions were localized to the site of administration
10 depending on dose. It was dose dependent, 0.5 to 2
11 percent of the total brain volume. The higher doses
12 showed more abnormalities and it was not reversible.
13 The catheter itself, they are minimal to mild, and
14 these are reversible abnormalities. In terms of vector
15 related, it's dose related with higher doses and not
16 reversible in the nonhuman primates.

17 And we videotaped these animals and we
18 periodically assessed them for behavior over time with

1 observers that are blinded. There were no clinical
2 correlates that we could detect. The animals had no
3 weight loss, they had normal hematologic and chemistry
4 parameters. So, probably similar than -- as much as we
5 could do in nonhuman primates, similar to the humans,
6 that we couldn't detect any clinical correlates with
7 this.

8 The problem with it, thinking as to the use of
9 direct parenchyma administration, is the highest
10 concentration are at the catheter tip. So, we know for
11 CLN2 disease, what I showed you was the
12 intraparenchymal catheters can deliver sufficient
13 vector to slow down but not stop disease progression.
14 However, I'd say the levels are at the highest at the
15 catheter tip and the result is localized inflammatory
16 and immune response. It's observed with different
17 capsids, and to our knowledge this cannot be
18 circumvented by modifying the catheter, the volume or

1 the mode of administration.

2 So in conclusion, catheter-based AAV vector
3 administration to the CNS can be safely used to treat
4 localize CNS disorders but it's dose limiting. And one
5 of the problems for many of the disease that many of us
6 are studying are diffuse CNS disorder. And as I showed
7 you, it's limited in regard to how much you can get
8 into the brain and doses they can use.

9 The physical constraint of high concentrations
10 at catheter tip can't be circumvented. And multiple
11 catheters in different sites can be used to circumvent
12 the dose limitation per catheter, but this strategy of
13 course is limited by the number of burr holes.

14 So in our opinion and recommendation, this can
15 be useful, particularly, for the localized disorders.
16 It will be necessary, however, to use other delivering
17 modes of administration such as directly to the CSF,
18 which of course is the direction we and many are using

1 to safely administer vectors in sufficient amounts to
2 effectively treat the diffuse CNS disorders.

3 I also want to pick up to just finish
4 something that both Jim and Barry mentioned in terms of
5 systemic immunosuppression. Because in our study we
6 did not use immunosuppression. And as I'm sure
7 everybody is aware there's varying degrees of
8 immunosuppression that has been used in the field.

9 But at least in our conclusion, it's not known
10 whether systemic immunosuppression can suppress the
11 inflammatory/immune response such as the type we saw,
12 and/or improve vector delivery and efficacy to
13 catheter-based delivery. So, I think that's something
14 that needs to be studied in the future. Thank you and
15 I'm pleased to answer any questions.

16 **INVITED SPEAKER Q&A**

17

18 DR. LISA BUTTERFIELD: Terrific. Thank you

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1 very much, Dr. Crystal. Very interesting. Thank you
2 for sharing those data. We now have about 16 minutes
3 for questions from our invited speaker. Let's start
4 with Dr. Roos.

5 DR. RAYMOND ROOS: All right. Thanks so much,
6 Dr. Crystal for the discussion. I wondered whether
7 (audio skip) your now treating (audio skip) neuronal
8 ceroid lipofuscinosis with spinal fluid (audio skip)
9 delivery. (Audio skip). Was there reasons why you
10 wanted to go this intraparenchymal route?

11 DR. RONALD CRYSTAL: (Audio skip) gene therapy
12 in humans. And at the time we (audio skip) thought
13 intraparenchymal was the best route to go. So, what
14 you saw is an integration through many, many CSF
15 administrations. But that does not mean that
16 intraparenchymal cannot be useful. I think that it has
17 limits disorders with the caveats of the problems
18 (audio skip) of the highest concentrations at the

1 vector tip it still maybe useful.

2 DR. LISA BUTTERFIELD: Thank you. Next up, Ms.
3 Dicapua, please.

4 MS. PEGGY DICAPUA: Hi Dr. Crystal. (Audio
5 skip) more burr holes, correct?

6 DR. RONALD CRYSTAL: If you were going to use
7 this methodology, that would be one strategy. But of
8 course that's very limiting in terms of the number of
9 burr holes that you can do in humans.

10 MS. PEGGY DICAPUA: And then the -- oh my
11 gosh, what the word? At the sites. That
12 nonreversible? So, are the children passing before you
13 can --

14 DR. RONALD CRYSTAL: Well, some of the --

15 MS. PEGGY DICAPUA: -- I'm sorry. I guess my
16 other question goes to the follow-up. Are the children
17 passing away before you can carry out the long term
18 follow-up to see what happens at the sites where the

1 catheter tip goes in?

2 DR. RONALD CRYSTAL: Well, in any of these
3 studies it is an advantage to treat the children as
4 soon as you possibly can. In our experience with this
5 disease, the diagnosis takes about a year from the time
6 of initial symptoms. Usually these children will go to
7 pediatricians and it's rare diseases and it's
8 recognized and it takes about a year before they
9 eventually get the proper diagnosis. So, as I showed
10 you in those curves, these children with this disorder
11 are deteriorating quite rapidly. And I think all of us
12 would agree that the earlier that we possible treat
13 we'd be much better off. But we're not recommending
14 that this approach be used, you know, in the future. I
15 think that this approach can be used for localized
16 disorders, Huntington's, Parkinson's disorders, like
17 that. But not for the diffused disorders as well.

18 MS. PEGGY DICAPUA: All right. Thank you for

1 your research continuing. I lost my son to CLN2 17
2 years ago, so thank you.

3 DR. LISA BUTTERFIELD: Thank you for those
4 questions. Next we have Professor Fox, please. We
5 can't hear you yet, Professor Fox.

6 DR. BERNARD FOX: Sorry, Professor
7 Butterfield. Thank you, Dr. Crystal, for that nice
8 presentation. The areas of inflammation that you
9 showed, and the diagram you put up with the
10 distribution from the tip of the catheter, I'm
11 expecting that the transgene expression would mimic
12 that distribution. Is that correct? Does that overlay
13 with the information?

14 DR. RONALD CRYSTAL: That's correct.

15 DR. BERNARD FOX: So, I wondered -- in some
16 other drug studies that were done where they were
17 trying to be toxins in the brain they used convection-
18 enhanced delivery, and I just wondered if the

1 convection enhanced delivery in parenchymal was ever
2 tried or considered as a way to potentially distribute
3 the vector more evenly? Do you know?

4 DR. RONALD CRYSTAL: We've tried that. And so
5 we're aware of that methodology and catheters that are
6 used. And we tried it with those approaches. And at
7 least in the nonhuman primates that we saw, we did not
8 see any difference between that and the flexible glass
9 catheters that we used. And no advantage of the
10 convection-enhanced delivery. Perhaps in other studies
11 you may see that, but we did not.

12 DR. BERNARD FOX: Thank you.

13 DR. LISA BUTTERFIELD: All right. Next, Dr.
14 Kenneth Berns, please.

15 DR. KENNETH BERNS: Hello Ron. Am I on yet?

16 DR. LISA BUTTERFIELD: Yes we hear you, but we
17 don't see you yet.

18 DR. KENNETH BERNS: Good afternoon, Ron. The

1 question that I have, which is completely naïve, is
2 even though you probably want a focal delivery of the
3 vector, is it not possible to consider IV
4 administration? Or is that just not feasible?

5 DR. RONALD CRYSTAL: Well, clearly IV
6 administrations works in the CMA, so that's not
7 something to -- you know, it's certainly something to
8 consider. We've done considerable studies in nonhuman
9 primates in older animals. And (inaudible) barrier has
10 closed. And we see very little at doses that we think
11 are safe to be able to get into the brain. We've also
12 carried out studies with IV 124, label capsules above
13 (audio skip) AAVrh.10 and AAV9 given intravenously.
14 I124 is a (inaudible) and we can do pet scan, so we can
15 see where the vector go in one 1hr, 24hr, 72hrs. At
16 least within the limitations of technology, we don't
17 see any in the brain at all, so we haven't pursued
18 intravenous strategies.

1 DR. KENNETH BERNS: Thank you.

2 DR. LISA BUTTERFIELD: Thank you. And so while
3 I ask the previous commentors to check their still
4 raised hands, I'll ask, Mr. DeFilippi to speak next.

5 MR. JAMES DeFILIPPI: HI, I'm a patient
6 representative so my apologize, Dr. Crystal, if this an
7 uninformed question. But, did you consider, given that
8 the catheter based method is dose limiting, trying to
9 use multiple routes of infusion of the AAV therapy, so
10 both by catheter and cerebral spinal fluid?

11 DR. RONALD CRYSTAL: Yeah. So, we certainly
12 considered that. I mean, the goal for these diseases,
13 like CLN2 disease and of course many others are like
14 it. These are diffuse diseases. You want to able to
15 get you therapy throughout the brain.

16 And so, I think that's something to consider,
17 is to use multiple routes of administration. We've,
18 like others, have evaluated multiple different routes

1 of administration. And clearly, CSF, intracisternal,
2 or C1C2 intrathecal, it's the most affective
3 methodology, I think, we have in field for it right
4 now. But the concept of using more than one route is
5 something, I think, at least we should consider.

6 MR. JAMES DeFILIPPI: Thank you.

7 DR. LISA BUTTERFIELD: Terrific. So let me see
8 if there are any other questions from the committee for
9 our invited speaker, Dr. Crystal. All right. Well,
10 very much appreciate your sharing these data with us.
11 So next up, we have a 10 min break and when we return
12 we'll go directly to the committee discussion of the
13 questions to us. So, I have, I have about 12:09 or 09
14 after. So, let's 20 after. Let's come back at 20
15 after the hour, 12:20 pm or 3:20 pm, and we'll get into
16 the discussion. Thank you all very much.

17

18 [BREAK]

1

2

COMMITTEE DISCUSSION OF QUESTIONS

3

4

MR. MICHAEL KAWCZYNSKI: Welcome back. Dr. Butterfield, are you ready?

6

DR. LISA BUTTERFIELD: Yes. Welcome back everyone. What we have now is a full 90 minutes to discuss our final group of questions for Session 5 under Neurotoxicity: Brain MRI Findings. We'll follow the same format as before. I'll read each question, we'll discuss it, I'll sum up the key points and we'll move to the next. We have three or four -- four questions for this section. Let's start with the first question.

15

16

17

18

Please provide recommendations for any preclinical in-life and post-mortem assessments, behavioral and neuropathological assessments, and duration of follow-up, post-dose, to identify and

1 further characterize the risk of neurotoxicity
2 following intraparenchymal administration of AAV
3 vectors. That is our first question, and again, for
4 session five, we'll be helped by our discussant, Dr.
5 Roos.

6 DR. RAYMOND ROOS: Thanks. As Dr. Ron Crystal
7 noted, many of the diseases that we can think about as
8 being treatable with AAV vector delivery are diffuse
9 ones. For example, SMA has abnormalities both in the
10 brain and really from the rostral to quarter part of
11 the spinal cord. In these cases it's probably not
12 advisable to think about intraparenchymal injections,
13 especially in the spinal cord, but even in the brain.

14 Injections are going to cause damage from the
15 needle track, the vector and the transgene. Blood
16 vessels could be damaged resulting in hemorrhage. A
17 large dose could cause swelling, inflammation could be
18 a problem.

1 When we talk about intraparenchymal injections
2 of AAV vectors, we're probably dealing with a more
3 localized disease. For example, perhaps one might
4 think of Parkinson's disease. But even there, it seems
5 like we need new innovative, technical approaches with
6 respect to delivering the AAV vector.

7 Before we get to humans, it's going to be
8 valuable to carry out studies initially in animals,
9 including, if available, an animal that might model the
10 disease of interest. For example, if one was
11 considering intraparenchymal injections of AAV vectors
12 in Parkinson's, we have mouse models involving MPTP
13 toxicity, which cause abnormalities in similar
14 structures that are targeted in Parkinson's. What's
15 important in the animal studies is that you have
16 controls available, so that you could delineate how
17 much problem you might get from the AAV vector versus
18 the transgene.

1 Also, these animals could have imaging
2 studies, even small animals, and autopsy's as well.
3 So, you get very important data with respect to
4 pathology and also you could do a number of behavioral
5 studies. For example, you could hang mice on a wire to
6 see how long they sustain their grip, or you could
7 analyze the behavior in a maze, et cetera. So, these
8 animals will provide behavioral and pathology studies.

9 Then, one could move into more appropriate
10 animal species such as non-human primates to be
11 investigated. Although there are clearly expense
12 problems related to the number of nonhuman primates
13 that could be evaluated. It would be important to look
14 over time with respect to these preclinical studies,
15 with respect to behavior and neuropathology.

16 One, in all of these cases, must keep in mind
17 issues related to risk/benefit. And that might be
18 especially the case in certain progressive, difficult

1 to treat human central nervous system diseases. But I
2 think we're dealing primarily with diseases that have
3 relatively localized pathology. Thanks.

4 DR. LISA BUTTERFIELD: Thank you, Dr. Roos,
5 for getting us started. We're looking for
6 recommendations on preclinical assessments, duration of
7 follow-up, pre- and post-dose follow-up, to further
8 characterize this risk of neurotoxicity following
9 intraparenchymal administration.

10 Looking now for hands up on the committee, and
11 your comments, questions and, importantly, your
12 recommendations. Still watching for those hands up. I
13 think we've started with Dr. Roos and some suggestions
14 of imaging assessments, pathologic assessments and
15 behavioral, including starting from murine models with
16 a grip test, maze tests and then perhaps moving, after
17 murine models, to nonhuman primates. Thoughts from the
18 committee? Dr. Vite, please.

1 DR. CHARLES VITE: Perhaps it's a little
2 different than the question's worded, but I think that
3 some of the methods used to assess developing methods,
4 I think, still overall perfects transgene function,
5 whether it's cat, whether it's some other kind of
6 mechanism. Then, correlating that with the MRI changes
7 would be useful science, and it's being done. I think,
8 though, it's still away from being a useful clinical
9 tool and is more of experimental, not even preclinical,
10 for understanding how much of a transgene expression is
11 occurring in an area. I think that's something that
12 still requires a lot of work and a lot of science, MRI
13 always being based on the changes that occur to the
14 parenchyma and the water composition later on, after
15 some things happened.

16 I can say that when we have done these studies
17 in large animals over time, we've sort of set a pattern
18 of every six months imaging them. There's nothing

1 magic about six months, but most of these dogs are
2 around for 10 years, so we can watch them every six
3 months and see what changes that are occurring. I
4 think perhaps the most important would be seeing
5 recovery of the lesions over time, as was brought up
6 before.

7 When most of these intraparenchymal injections
8 are being done you're treating a disease process that's
9 ongoing in the brain, so you have to remove that away
10 from the pathology associated with the vector. That
11 also takes some time, some skill to sit and do. The
12 diffusion-weighted imaging is always interesting. I
13 think, however, it always goes back to the pathology
14 you see on conventional MRI that ends up being that
15 sort of hallmark for what radiologists and clinicians
16 like to see for what's going on.

17 I guess, in trying to add to the discussion, I
18 think beyond the toxicity question, the idea of

1 developing methodology to sort of measure transgene
2 expression in specific brain areas when it's there, and
3 correlating sort of the activity and the damage that's
4 going on I find the most appealing science question.
5 But I think we're still some way away from turning that
6 into a real tool. That's all I have.

7 DR. LISA BUTTERFIELD: Thank you for that
8 input and some suggestions about follow-up in canine
9 models. All right, other thoughts from the committee
10 members on this first question? Dr. Sanchez.

11 DR. CARLOS SANCHEZ: I would just speak to the
12 use of nonhuman primate models as being helpful for two
13 different methodologies. One is advancing some of the
14 more sophisticated surgical application of convection-
15 enhanced deliveries, specifically, looking more for
16 movement disorders, for adult disease. Using
17 interoperative MRI in nonhuman primate models and
18 applying more complex MRI-guided, gadolinium-guided CED

1 is something that really require nonhuman primates
2 model to truly be able to practice to a point where we
3 can then apply to human situations. I would say that
4 there's a particular advantage for NHPs for that.

5 Then the second question, which I think still
6 has significant potential outside of movement
7 disorders, is this idea of utilizing axonal transport
8 with either anterograde, retrograde or both transport
9 where we could inject into a deeper nucleus and then
10 use axonal transport to extend the distribution of the
11 gene in the transgene expression beyond the vector
12 distribution. I hope that in the future the nonhuman
13 primate model can help us answer both of those
14 questions better.

15 DR. LISA BUTTERFIELD: Thank you very much.

16 Dr. Fox?

17 DR. BERNARD FOX, JR.: Mute. Yeah, it's
18 probably obvious, but I think some of the post-mortem

1 characterizations of the immune infiltrates in the
2 preclinical models, what is the nature of those
3 inflammatory cells? Particularly in the adapted immune
4 cells, and it may give insights into things that could
5 be done to avert that.

6 I just wonder about the gene, particularly,
7 depending what the mutation is, whether these represent
8 a really neoantgenic epitopes that the patients have
9 not previously seen, and what's known about that. I
10 don't know that literature, but that would seem to be
11 something. Then what other mechanisms might be adopted
12 in the process to maybe avert that development? Thank
13 you.

14 DR. LISA BUTTERFIELD: Great, thank you for
15 that perspective. All right, we have several thoughts
16 about models and assessments. Other thoughts from the
17 committee? I know it's, especially for the east
18 coasts, it's the afternoon, but we want to make sure

1 that we give in-depth coverage to all of these
2 questions and give our colleagues as much input and
3 discussion as possible, broadly across all the
4 committee members. We're not looking for consensus
5 necessarily, we're looking for a lot of different
6 opinions to make sure that everyone's thoughts are
7 heard. Dr. Roos.

8 DR. RAYMOND ROOS: With respect to Dr. Fox,
9 who's question, I wondered whether Dr. Ron Crystal
10 might be the best person to talk about the pathology
11 here with respect to immune cells and other elements as
12 a result of the intraparenchymal injections. If he's
13 still on.

14 DR. LISA BUTTERFIELD: Thank you, yes. Dr.
15 Crystal, that would be great to hear your insights from
16 some of those characterizations pathologically.

17 DR. RONALD CRYSTAL: Yeah, one thing I forgot
18 to mention is that in the CSF we didn't see any

1 inflammatory cells, so there was no increase in
2 inflammatory cells. There was not a decrease in
3 glucose or increase in protein. We've not
4 characterized the cells any more than what I showed
5 you. But I think you bring up what's a really
6 important area, is we know very little about the CNS
7 responses to immunity to these vectors.

8 I think that's an area that we all ought to
9 consider in terms of putting more effort into can we --
10 first, as you point out, what are the characteristics
11 of the cells? What's the characteristics of the immune
12 system using much more sophisticated methodologies than
13 I showed.

14 In addition, as I mentioned, the issue of
15 immunosuppression is something, I think, important for
16 the field to assess. Is it useful at all to use
17 systemic immunosuppression and what kind of
18 immunosuppression in regard to CNS administration,

1 independent of the route, or is it not? As we heard,
2 it could make it worse, it could have no effect, or it
3 could be beneficial. I think that's an important area
4 for research, probably in the non-human primate would
5 be most -- or large animals would be most rational.

6 DR. LISA BUTTERFIELD: Great, thank you for
7 raising those important points. Dr. Barry Byrne.

8 DR. BARRY BYRNE: Yeah, just to amplify on
9 Ron's comments. The first patient that we studied with
10 systemic immunosuppression blocking both B cell
11 responses and some T cell aspects with (inaudible) is
12 now four years postdosing. It remains seronegative to
13 AAV and had multiple MRI assessments following a dual
14 route of administration into the lateral ventricle and
15 systemically.

16 And so, we didn't see any signs of
17 neurotoxicity in that patient, nor any of the common
18 biomarkers that would have reflected neurological

1 inflammation. And so, those studies are being expanded
2 upon by another group, but we think that this probably
3 is relevant in some situations, particularly when there
4 is no certainty around the therapeutic dose and the
5 potential for re-administration is warranted. That's
6 just an anecdote that may be relevant to the
7 discussion.

8 DR. LISA BUTTERFIELD: Great, thank you for
9 that. Let me see if I can sum up the discussion and
10 some of the key points raised to this first question
11 about preclinical assessments. As I've already
12 mentioned, I think everyone mentioned, including
13 different types of imaging, pathologic assessments and
14 behavioral tests could be important in both murine
15 models and nonhuman primates. That also dog models,
16 where standardized follow-up of every six months over
17 their lifetime can also be performed. Nonhuman
18 primates do seem to be critical for protocol

1 development in terms of surgical techniques in
2 particular, before translation to humans. And to
3 better understand vector distribution, that may be a
4 unique contribution of non-human primates.

5 Going in a little deeper on the pathologic
6 assessments, better characterization of the immune
7 infiltrate, the cells involved, where they go and then
8 addressing whether or not there's a suggestion that
9 immune suppression may be called for. For example, in
10 (audio skip) Sirolimus is one example of an approach to
11 that. It's noteworthy that, to date, the CSF has not
12 shown a big inflammatory changes. Anything to add to
13 that summary of the first question? Dr. Breuer.

14 DR. CHRISTOPHER BREUER: I would wonder if
15 there would be any role for serial EEGs in these
16 animals too, looking for their focus, in subclinical
17 seizure focuses, given the potential scar formation.

18 DR. LISA BUTTERFIELD: Thank you for that

1 addition. Dr. Zeiss?

2 DR. CAROLINE ZEISS: I just wanted to draw
3 attention to another area of research, which is
4 neuronal tracing in non-human primates. That these
5 vectors, for example, AAV-2, is used at quite high
6 concentration, 10 to the 13, with intraparenchymal
7 injections. Typically no pathology is done in those
8 animals. Really the researchers are focused on the
9 extent of neuronal transduction. I'm sure pathology
10 exists. But that is a pretty large community that
11 would be worth communicating with.

12 DR. LISA BUTTERFIELD: Great, thank you for
13 that suggestion. Taking advantage of existing data is
14 always much more efficient. With that, we're going to
15 move to the second question posed for committee
16 discussion. The second question, "Please discuss the
17 clinical significant, if any, of brain MRI
18 abnormalities observed in clinical trials of AAV vector

1 gene therapies. Please discuss whether the delivery
2 procedure, versus AAV vector, may have contributed to
3 the abnormal brain MRI findings. Again, let's start
4 the discussion off with our discussant, Dr. Roos.

5 DR. RAYMOND ROOS: All right, thanks. I think
6 the delivery and the vector are important. However, it
7 certainly is the case that injections into the central
8 nervous system for varied reasons are not so rare, with
9 respect to many different neurological disorders.
10 Generally, although there's certain pathology after
11 these needle or catheter intraparenchymal deliveries,
12 generally these are subclinical and relatively minor.

13 I think the abnormalities that we saw in Dr.
14 Crystal's investigations are related very much to the
15 AAV vector and it looked even more so by the transgene
16 causing significant pathology.

17 Investigations to clarify this issue probably
18 could be best done in animals and that would be

1 helpful, especially nonhuman primates. It looked like
2 these MRI abnormalities in the human investigation
3 lasted a long time. Nevertheless, I have the feeling
4 that generally there aren't any clear overt symptoms or
5 signs after the intraparenchymal injections in humans.

6 However, many of these patients, I think, have
7 language cognitive problems, communication problems
8 that's going to limit their ability in indicating some
9 significant clinical abnormality. Looking at the MRIs
10 from Dr. Crystal's investigation, one would surmise
11 that there would be some significant clinical
12 repercussions. Thank you.

13 DR. LISA BUTTERFIELD: Great. Thank you for
14 starting us off, Dr. Roos. All right. I'm watching
15 for discussion from our colleagues on the committee.
16 Let's start with Dr. Sanchez, please.

17 DR. CARLOS SANCHEZ: Sorry, I lost you there.
18 I would break down what we see on these imaging

1 findings for all convection enhanced delivery and
2 direct parenchymal injections of the two groups. The
3 first are the less common, which are very obviously
4 related to the actual parenchymal injection, that's
5 contusions and hematoma's. And we see those in the
6 adult CED trials, but we didn't see that evidence
7 presented in the pediatric kids who are shown here.

8 I think the question that we're having here is
9 more for the less severe imaging findings, the T2,
10 diffusion and ADC changes. I think for even both of
11 those, if you look at the adult trials with these T2
12 changes as well as hematomas and contusions, and the
13 pediatric trials with just the T2, diffusion and ADC
14 changes, they're clinically silent.

15 I think these are really more of a
16 ramification of actually doing an invasive procedure,
17 which on the scale of invasiveness is much more
18 invasive than a cisternal tap, and certainly much more

1 invasive than a vector distribution by an LP.

2 So when you look at neurosurgical sort of
3 post-imaging findings, this on a relative scale is
4 quote low, I think, compared to what we could see in
5 other types of procedures. I would argue that any
6 invasive neurosurgical procedure will have some sort of
7 imaging finding. I think we typically try to look at
8 either transient or permanent neurological deficits as
9 the key marker for whether the risk/benefit ratio of
10 the surgery was worth the surgery. And weighing that
11 in context of the natural history of the disease
12 without surgery.

13 But again here, even in these patients that
14 are compromised neurologically in terms of telling us
15 whether they have a deficit or not, if we look at the
16 adult population of patients that have had CED, they
17 appear to be quite silent clinically, the T2, ADC and
18 diffusion changes.

1 MR. MICHAEL KAWCZYNSKI: Hold on one second.
2 Hold on one second, I think -- I just want to make sure
3 we're still broadcasting because we lost it for a
4 second there. Can you give me a sound check again?

5 DR. LISA BUTTERFIELD: We also have a panelist
6 muting to do.

7 MR. MICHAEL KAWCZYNSKI: Yeah, so just a
8 second. Okay, we got the mutes all taken care of,
9 there we go. All right, go ahead. Sorry about that,
10 Dr. Sanchez, and Dr. Butterfield, go ahead.

11 DR. CARLOS SANCHEZ: Sure. Just, lastly,
12 there is an HP data for the catheter tip that shows
13 perivascular cuffing, white matter edema, lymphocyte
14 infiltration that seems to be more specific to the
15 vector distribution versus the actual paths for the
16 catheter.

17 DR. LISA BUTTERFIELD: Okay, and then if I can
18 just confirm what I think I've heard from you, Dr.

1 Sanchez, as I wade through some of the terminology
2 specific to the brain. You're suggesting that it's
3 both a role for the vector, but also a role for the
4 delivery procedure in this case because this is a more
5 invasive delivery procedure than perhaps others that do
6 not result in these MRI findings, is that correct?

7 DR. CARLOS SANCHEZ: I guess what I was trying
8 to say, is if you look at post-operative, post-
9 intervention imaging findings, there are ones that are
10 very obvious in relation to passing a catheter, whether
11 it's a CED catheter or glass catheter, in this case, or
12 other types of catheters for neurosurgical procedures,
13 hematoma, contusion. Those are clearly from physically
14 passing an object through the brain. And those
15 certainly happen, and those certainly happen in CED
16 adult trials. We have several patients who have had
17 those problems. When we look at the imaging findings
18 that I think is more of the question here, the T2, ADC,

1 diffusion changes, those are pretty subtle.

2 The point I was trying to make is that there's
3 always a footprint with the neurosurgical procedure
4 beyond the purpose of the procedure itself. Let's say
5 removing the tumor, the tumors gone. But the frequency
6 of, let's say, T2 changes due to the placement of a
7 retractor or something else like that, are very common.
8 As neurosurgeons we typically measure our success post-
9 operatively, not so much on the asymptomatic
10 radiological changes post-operatively, but really more
11 the symptomatic, transient or permanent neurological
12 deficits.

13 I was just trying to contextualize that, given
14 that this is a much more invasive procedure relative to
15 a cisternal tap or an LP, it's somewhat expected that
16 we'll see changes like this. And the real measure is
17 whether the patient's changed neurologically after this
18 procedure, even despite if they remain on imaging for

1 months or years afterwards.

2 DR. LISA BUTTERFIELD: Thanks very much for
3 that further clarification.

4 DR. CARLOS SANCHEZ: Sure.

5 DR. LISA BUTTERFIELD: All right, other
6 comments, questions and points to be raised by
7 committee members? Dr. Crystal, anything you wanted to
8 add at this point from the first few comments from the
9 committee?

10 DR. RONALD CRYSTAL: We would agree with all
11 the comments that were made. As I said, the
12 methodology, as we just heard from Dr. Sanchez, I mean,
13 putting catheters in the brain is something that's
14 standard in the neurosurgical world. And the issue is
15 really, can we effectively deliver the product of our
16 vectors throughout the brain with this technology? I
17 think the answer is, you can do it in a limited area
18 but not throughout the brain.

1 DR. LISA BUTTERFIELD: All right, thank you.
2 All right, other thoughts? Other questions or points
3 to raise from the committee? If not, I can summarize a
4 couple of points made in response to this question.
5 All right, so the clinical significance to date seems
6 to be very minor. These MRI findings, these T2,
7 diffusion changes seem to be quite minor, clinically
8 silent. And while they have resulted in the lessons
9 learned about gene transfer with the adenoviral
10 vectors, that delivery procedure may not be as
11 efficacious as others under current investigation.
12 Other thoughts to add to that?

13 All right, then we are going to efficiently
14 move to the third question. Our third of four
15 questions for the committee to discuss and provide
16 recommendations on, we're asked to provide
17 recommendations on strategies that could be implemented
18 before and after vector administration to prevent or

1 mitigate the risk of central nervous system injury.

2 We'll start with Dr. Roos to initiate the discussion.

3 DR. RAYMOND ROOS: First, I wonder about
4 features of the vector or the transgene that make them
5 especially prone to trigger pathology, be it
6 inflammatory pathology or edema. Maybe that's
7 something that could be clarified in animal studies
8 because an issue with human intraparenchymal injections
9 is that we invariably lack all the controls that would
10 be appropriate, because we're dealing with human
11 patients. I think obtaining MRIs is clearly important,
12 probably in regular intervals.

13 In addition, I don't know whether the use of
14 immunosuppression has been clarified as well as it
15 could be. And perhaps this should be initiated with
16 intraparenchymal injections in animals and, then if it
17 looks promising, in human studies as well. The issue
18 with respect to strategies in humans really probably

1 relates to localized injections for somewhat localized
2 disease. And so, having animal models that might
3 mirror those particular instances would be valuable in
4 the study. Thanks.

5 DR. LISA BUTTERFIELD: Thank you for starting
6 us off. Now looking to the committee members to weigh
7 in on their thoughts on recommendations on strategies
8 to prevent or mitigate risk of CNS injury. Thank you,
9 Dr. Sanchez.

10 DR. CARLOS SANCHEZ: I would start by saying
11 that any neurosurgeons that are working for a CED trial
12 will apply general principles that we apply for passing
13 a catheter through the brain. So the trajectories that
14 they pick, the avoidance of vessels, the avoidance of
15 sulci where we'll lose the vector, will be in their
16 mind. And then with that would follow routine post-op
17 management with routine post-op imaging and seizure
18 control if needed. Then you can layer on sort of

1 technical aspects, so the convection enhanced delivery
2 that's been worked up by multiple groups, and I would
3 sort of parse that into different cannula designs,
4 different settings for the actual infusion with CED or
5 otherwise, and then use of adjuncts like mannitol for
6 brain swelling if needed.

7 Then I think one thing that some groups are
8 using is the use of interoperative MRI as well as the
9 co-infusion of gadolinium to actually see the infusion
10 in real time. I think that that will provide in the
11 future a lot more safety in terms of seeing where the
12 cannula tip is, where is the infusion starting. It
13 allows us to actually adjust the cannula position in
14 real time as the infusion is occurring. It's seeing
15 our target, it's identifying infusion leak around the
16 catheter, reflux if that happens. Then one of the
17 challenges of injecting deeper spaces is this idea of
18 losing some of the vector with perivascular

1 distribution. If we see that, we can actually advance
2 a catheter as the infusions happening.

3 So, there's more of a cost with that, of using
4 interoperative MRI suite. And there's more of a
5 technical aspect in using MRI-compatible equipment for
6 a procedure like that, but I think the future of CED,
7 again, understanding that it's not a whole brain vector
8 distribution but a localized one, that we'll benefit
9 from using those strategies.

10 DR. LISA BUTTERFIELD: Thanks very much for
11 those specific recommendations. All right, additional
12 thoughts from the committee on recommendations to
13 mitigate this risk? What we've heard thus far are that
14 certainly the surgical techniques in place mitigate a
15 lot of the risk, but there are new approaches to
16 modifying the infusion that can be adopted. And
17 consideration of MRI-guided real-time imaging during
18 infusion could help minimize some of this risk and

1 track the infusion to optimize those outcomes.

2 Then, further study of the viral vector
3 responses and transgene responses in animal models,
4 those could then identify new mechanisms that would
5 then shed light on additional mechanisms that might be
6 employed, including a possible mitigation role for
7 immune suppression. Thoughts in addition to what I've
8 just summed up? Assuming, Dr. Sanchez, that is the
9 previous hand up and not a new hand up. But let me
10 know if you have something to add. Okay.

11 All right, if we have no further
12 recommendations from the committee on question three,
13 then this is our final question for discussion along
14 the committee members. "Please recommend a duration of
15 monitoring for subjects who have abnormal brain MRI
16 findings, or factors to consider for the determination
17 of an appropriate duration of monitoring." We'll start
18 with, again, Dr. Roos, our discussant.

1 DR. RAYMOND ROOS: One has concern about
2 intraparenchymal delivery of AAV vectors after seeing
3 MRIs that Dr. Crystal showed. I think monitoring on a
4 regular basis, patients that have abnormal MRIs, or
5 just intraparenchymal injections would be important.
6 Perhaps 1 month, 3 months, 6 months, 12 months and
7 maybe yearly.

8 The duration of monitoring and interventions
9 might depend a little bit on the MRI abnormality. For
10 example, there's a hemorrhage that's visualized that's
11 of concern, maybe this patient needs to be hospitalized
12 and maybe further interventions might be necessary.
13 The use of steroids or some immunosuppression also
14 might be entertained. I have the feeling that
15 intraparenchymal injections, especially new catheter
16 design, is a little bit of a pioneer area with respect
17 to AAV delivery, and maybe a dynamic and changing one.
18 And for that reason, I think that monitoring patients

1 in a rigorous way would be suggested. Thanks.

2 DR. LISA BUTTERFIELD: Great, thank you very
3 much. We have now some initial thoughts on that
4 monitoring frequency. Professor Fox.

5 DR. BERNARD FOX, JR.: Yeah, I didn't mention
6 it in the last question because it seemed like it'd
7 been covered before, but in thinking about, as Dr.
8 Roos was presenting this data, the idea that when you
9 have these abnormal MRI findings that it's going to be
10 relatively easy to have an overlapping (inaudible)
11 library of the gene of interest and to be able to go
12 back and look at whether or not that correlates with
13 systemic immunity for blood assays. Those are pretty
14 straightforward. And then identifying what were the
15 profiles of those immune cells that might be
16 recognizing it, or if they're not recognizing that,
17 versus the viral or the capsids. Thank you.

18 DR. LISA BUTTERFIELD: Great, thank you for

1 that perspective. I guess, so if I understood Dr.
2 Crystals data correctly, these effects have not been
3 disappearing. So they were not reversible, but they
4 have been clinically silent to date. So I guess the
5 question for Dr. Crystal would be, how long of a
6 follow-up has your group been performing to perhaps
7 guide a recommendation in that area?

8 DR. RONALD CRYSTAL: The longest we've
9 followed the nonhuman primates has been one year. The
10 duration of the study with the children was 18 months,
11 since we had that at 18 months. In a small proportion,
12 the lesions disappear. In most, they continue, but
13 they're not increased in terms of size or extent.

14 In the trial itself we did several months and
15 one year and 18 months, so 6 months, 18 months, done.
16 In terms of the answer to the question of how long you
17 should follow them, probably, if you see the lesions, a
18 yearly basis would be a rational thing to think about.

1 DR. LISA BUTTERFIELD: Great, thank you for
2 that. Ms. DiCapua.

3 MS. PEGGY DICAPUA: I was just going to say,
4 yeah, I would think you would have to come out for a
5 couple of years to see and follow it. In the cases
6 that I know for degenerative, I mean, it also depends
7 on how fast the brain -- there's progression of the
8 degeneration, how fast that's progressing. So, you're
9 looking at that -- so you're seeing deterioration, the
10 MRI, you're seeing that, but possibly you could see
11 where the injections were and you could see that
12 disappearing, I don't know, but, yeah, I would think
13 you'd have to go out at least a couple of years if you
14 can, if the subject is able to.

15 DR. LISA BUTTERFIELD: Yes, thank you for
16 that. Dr. Roos.

17 DR. RAYMOND ROOS: Yeah, I just wanted to get
18 back to the topic of whether these MRI abnormalities

1 are clinically symptomatic. And I wanted to ask Dr.
2 Crystal whether that was clearly the case in these
3 patients with ceroid lipofuscinoses?

4 DR. RONALD CRYSTAL: The question was did we
5 see them progress at all?

6 DR. RAYMOND ROOS: My question was whether it
7 was clear that these MRI lesions from the
8 intraparenchymal injections had no clinical effect on
9 the patients.

10 DR. RONALD CRYSTAL: There's a caveat to my
11 answer, I mean, the answer is we saw no clinical
12 correlates. However, these children clearly have
13 neurologic disease and so, whatever I'm looking at is
14 what you're looking at is something superimposed. And
15 of course, I'm sure you would agree, it's challenging
16 to see new lesions. But as far as we could determine,
17 there was no clinical correlates beyond the primary
18 disease itself.

1 DR. LISA BUTTERFIELD: Thank you. Dr. Roos,
2 any follow-up? Did that answer your question?

3 DR. RAYMOND ROOS: Yes.

4 DR. LISA BUTTERFIELD: All right, thank you.
5 Dr. Kenneth Berns, please.

6 DR. KENNETH BERNS: Well, again, this is just
7 an informational question. Ron, what's the life
8 expectancy of the patients that you're treating this
9 way? How long are they really going to survive? I
10 can't hear.

11 DR. RONALD CRYSTAL: Their after life
12 expectancy is 8 to 10 years.

13 DR. KENNETH BERNS: Okay, thank you. In that
14 case, I guess long-term monitoring might be useful.

15 DR. RONALD CRYSTAL: The one caveat I'd make
16 in terms of trying to follow those children, is that to
17 do an MRI in a child with one of these disorders, you'd
18 have to use general anesthesia. That's another

1 complication that is challenging in terms of how often
2 you want to do that.

3 DR. LISA BUTTERFIELD: All right. Thank you
4 for that discussion. Okay. Let's check the hands up
5 and see if indeed the hands up are current hands up for
6 new comments. All right, so any additional comments
7 from members of our panel today on the topic of
8 duration of monitoring and what factors to consider to
9 determine that? Okay, so what I've heard is that
10 initially perhaps one, three, and six months, but then
11 following annually would be appropriate. One year
12 follow-up in the nonhuman primates has been done in the
13 past, but we have to keep in mind that in a number of
14 pediatric patient settings that the MRI follow-up is
15 challenging in that setting.

16 Then the other note is that if an immune
17 component is found to be important in these MRI
18 findings, that that could be something that might be

1 monitored in the blood, and then to understand that
2 immune mechanism might allow for a blood biomarker to
3 follow that immune response over time.

4 Anything else to add to that quick summary of
5 the recommendations and discussion points? All right.
6 Then, with that, we have these response to the four
7 questions. Now, we have an opportunity, I'll ask Dr.
8 Wilson Bryan and see if our FDA colleagues have other
9 clarifications they would like.

10 DR. WILSON BRYAN: Thank you. The discussions
11 been great. I do have one follow-up question. And I
12 don't like to dwell too much on hypotheticals, but we
13 are really very early, I think, in the therapeutic
14 world for gene therapies. And in the years ahead, I
15 wouldn't be surprised if we do see some patients who
16 have MRI findings with associated clinical
17 manifestations.

18 In that event, any thoughts from the

1 committee? Would their recommendations change in any
2 way with regard to those specific subjects, or to the
3 field? Would the recommendations change in any way if
4 in the years ahead we do have examples of clinical
5 manifestations?

6 DR. LISA BUTTERFIELD: I'm watching for a show
7 of hands from members of the committee that have
8 weighed in on these recommendations. Dr. Roos.

9 DR. RAYMOND ROOS: I think that certainly that
10 would be a concern and perhaps the details would be
11 important with respect to what the disease was, what
12 risk/benefits were involved, was there a mistake in the
13 implementation of the intraparenchymal delivery. It is
14 a bit hypothetical, but I have no question that there
15 would be concerns, and maybe investigations of that
16 particular clinical case. And whether one was dealing
17 with some idiosyncratic reaction rather than something
18 that might recur.

1 I don't know whether I've answered the
2 question, but I think having significant issues with
3 gene therapy approaches is going to impact the field,
4 no question. Looking carefully at that case and the
5 details would be important as far as learning and
6 perhaps modifying the route of entry and distribution
7 and catheter design features.

8 DR. LISA BUTTERFIELD: Thank you. And Dr.
9 Fox, did you also want to weigh in on this question?

10 DR. BERNARD FOX, JR.: Yeah. I think, to Dr.
11 Bryan's point and coming back to Dr. Wilson's comments
12 about GFP being immunogenic. I think in cancer we're
13 really excited to be studying the surfaceome now and
14 the different proteins, whether or not proteins are
15 short-lived or long-lived. If they're short-lived,
16 they tend to end up more into the proteosome and get
17 through the ER to be presented more in class one
18 molecules where they can be targets of immune response.

1 I think as we look at the different gene
2 therapies and the different transgenes, whether or not
3 those transgenes, as part of their function, end up
4 more as a long-lived protein or as a short-lived
5 protein, might end up -- will certainly affect their
6 expression on the surface and their ability to be
7 targeted, as well as the degree of dissimilarity with
8 other gene families in the body.

9 So just thinking about it from the immune
10 side, the potential there is pretty strong to see
11 something that might be a unique epitope and represents
12 a neoantigen that there's a strong response against. I
13 would be looking for those kinds of things as the
14 trials progress and if you saw a series of adverse
15 events.

16 DR. LISA BUTTERFIELD: Thank you. Dr. Bryan.
17 Other questions or clarifications from the FDA to the
18 session, or anything else over our last two days,

1 really?

2 DR. WILSON BRYAN: This is very helpful. The
3 discussion has actually been very invigorating for me.
4 Very thorough and helpful, so I really appreciate the
5 perspective of the committee, and want to re-emphasize
6 how important it is that all the stakeholders
7 communicate with each other as we go forward in
8 thinking about these safety issues. So, thank you very
9 much.

10 DR. LISA BUTTERFIELD: All right then, I will
11 hand the meeting to Jarrod to start the closing
12 procedures, and here we have Dr. Peter Marks.

13

14 **CLOSING REMARKS**

15

16 DR. PETER MARKS: I just wanted to thank
17 everyone on the committee for a really excellent
18 discussion. I was able to actually listen to most of

1 today and at least a part of yesterday. Really, I
2 completely agree with Dr. Bryan, it was an incredibly
3 robust discussion, so I think it was exactly what we
4 were looking for. Thank you so much for that.

5 I also want to thank the staff of the Office
6 of Tissues and Advanced Therapy for preparing
7 everything so well, and also the advisory committee
8 staff for putting this together and all of the work
9 that goes into this.

10 Just to finish up by saying that I think for
11 those who may be listening in who aren't as familiar
12 with gene therapies, some might be concerned that we
13 have all of these different side effects that we've
14 been talking about regarding gene therapies. And they
15 may wonder, are these things going to become a reality?
16 I would say the fact that we're discussing these is
17 evidence that they very much are becoming a reality.

18 And it's actually a good sign because every

1 medical therapy, as it comes along, we have to deal
2 with the side effects that may come up and address
3 them. So, I think that this robust discussion will
4 help us address these. And the fact that we're having
5 to address them is a very good sign that we're actually
6 making progress here.

7 We'll obviously have a lot to take back from
8 the discussion to think about, and we really appreciate
9 that. Given that this is the end a long day prior to a
10 holiday weekend, I'm going to keep my remarks
11 relatively short and just finish by saying, thank you
12 so much to everyone once again, and I wish you a very
13 good holiday weekend.

14

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ADJOURN

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17 MR. JARROD COLLIER: Thank you very much, Dr.
18 Marks, for your closing remarks. At this point that

1 concludes the 70th meeting of the Cellular Tissue and
2 Gene Therapies Advisory Committee. I would like to
3 thank all participants for their time and effort to
4 conduct today's meeting, and I would also like to give
5 special thanks to Mr. Michael Kawczynski for managing
6 the Adobe Connect platform throughout the two-day
7 meeting, and also, our wonderful chair, Dr. Lisa
8 Butterfield, for facilitating the discussion questions
9 and all relevant information regarding this meeting.
10 At this point the meeting is now adjourned. Thank you
11 all for your participation and have a great evening and
12 holiday weekend. Thank you very much.

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[WHEREAS MEETING ADJOURNED FOR THE DAY]